Translating genomic discoveries to the clinic in pediatric oncology

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Purpose of review
The present study describes the recent advances in the identification of targetable genomic alterations in pediatric cancers, along with the progress and associated challenges in translating these findings into therapeutic benefit.

Recent findings
Each field within pediatric cancer has rapidly and comprehensively begun to define genomic targets in tumors that potentially can improve the clinical outcome of patients, including hematologic malignancies (leukemia and lymphoma), solid malignancies (neuroblastoma, rhabdomyosarcoma, Ewing sarcoma, and osteosarcoma), and brain tumors (gliomas, ependymomas, and medulloblastomas). Although each tumor has specific and sometimes overlapping genomic targets, the translation to the clinic of new targeted trials and precision medicine protocols is still in its infancy. The first clinical tumor profiling studies in pediatric oncology have demonstrated the feasibility and patient enthusiasm for the personalized medicine paradigm, but have yet to demonstrate clinical utility. Complexities influencing implementation include rapidly evolving sequencing technologies, tumor heterogeneity, and lack of access to targeted therapies. The return of incidental findings from the germline also remains a challenge, with evolving policy statements and accepted standards.

Summary
The translation of genomic discoveries to the clinic in pediatric oncology continues to move forward at a brisk pace. Early adoption of genomics for tumor classification, risk stratification, and initial trials of targeted therapeutic agents has led to powerful results. As our experience grows in the integration of genomic and clinical medicine, the outcome for children with cancer should continue to improve.

Keywords
genomic alterations, incidental findings, pediatric cancer, personalized medicine, precision oncology, targeted therapy

INTRODUCTION

In 2011, an *ad hoc* Committee of the National Research Council was charged with mapping the future toward a 'new taxonomy of human disease based on molecular biology,' including genomic, epigenomic, and transcriptional data [1]. Clinical application of this multilayered dataset to improve the care of individualized patients was termed 'precision medicine,' and oncology sits at the leading edge of this new movement [2,3]. It has long been recognized that genomic alterations, including point mutations, translocations, amplifications, and deletions, drive the development of cancer with an increased understanding that genomic alterations impact patient prognosis and response to therapy. In fact, the modern era of targeted cancer therapies is predicated on the notion that directing therapies toward somatic genomic alterations unique to cancer cells will improve therapeutic efficacy and decrease adverse effects. In this context, the identification of cancer-specific genomic alterations in adult malignancy (e.g., *HER2*, *BCR-ABL*, *EGFR*, *BRAF*) already provides linkage to upfront, standard of care therapies that specifically target

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KEY POINTS

- The genomic landscape of pediatric cancers is rapidly and repeatedly being described, offering a plethora of new targets for precision oncology.
- Many targeted therapies are now being offered on the basis of genomic alterations found in tumors, although relatively few large-scale and coordinated efforts exist in pediatrics to measure the clinical efficacy of this approach.
- Return of incidental germline findings remains challenging, with ongoing recommendations for return of results continuing to evolve.
- Tumor heterogeneity offers another challenge to the translation of genomic medicine into the clinic, possibly requiring multiple biopsies at both diagnosis and relapse.
- Despite these challenges, clinical genomics offers a promising and educated approach to treating pediatric cancer that now must undergo rigorous trials to prove its benefit.

these oncogenic mutations (e.g., trastuzumab [4], imatinib [5], gefitinib [6,7], and vemurafenib [8], respectively). Data also suggest that adult patients with advanced disease presenting to developmental therapeutics programs have a higher response rate, longer time to progression, and improved overall survival if they receive a phase 1 therapy 'matched' to a molecular aberration in their tumor [9,10▪]. The extent to which this early therapeutic validation of precision medicine transfers to pediatric oncology is largely unknown.

Childhood malignancies are biologically distinct from adult cancers [11,12▪]. The spectrum and frequency of potential actionable somatic genomic alterations has yet to be fully defined. Also unclear is the extent to which children can gain access to targeted agents and how their benefit might be measured among small subpopulations of rare diseases. Finally, inherited cancer susceptibility likely plays a larger role in the etiology of pediatric malignancy and causative germline mutations, now discoverable using comprehensive sequencing approaches, add complexity to the deployment of genomic technologies in the clinic. The present review focuses on the progress, potential impact, and challenges of introducing precision medicine into the pediatric oncology clinic.

THE CULTURE OF PEDIATRIC ONCOLOGY

Pediatric oncology is well poised to welcome genomically informed cancer therapies into the clinic. First, the childhood cancer community espouses a rich culture of clinical trial participation in which the procurement of adequate tissue for centralized testing and risk stratification is already engrained. The Children's Oncology Group (COG) in North America and several European consortiums have come to rely on real-time assessment of cytogenetics and copy number alterations (CNAs) to recognize prognostic features and risk-stratify therapy in leukemia, neuroblastoma, and Wilms tumor. Second, a growing number of targeted therapies have completed or are currently under early phase testing in children with cancer (Tables 1 and 2).

Most molecularly targeted agents have been tested in unselected populations, but the few pediatric trials that included genomic biomarkers or distinct molecular cohorts have demonstrated powerful results. For example, COG-AALL0031 combined imatinib with conventional chemotherapy in pediatric patients with Philadelphia chromosome–positive acute lymphoblastic leukemia (ALL) and determined that these formerly ultra-high-risk patients could achieve substantially improved outcomes (disease-free survival $70 \pm 12\%$) at 5 years without bone marrow transplantation [34,35]. Similarly, the phase 1 study of crizotinib (COG-ADVL0912), a MET and anaplastic lymphoma kinase (ALK) inhibitor, included a molecular strata for patients with confirmed *ALK* translocations, amplifications, or mutations and demonstrated an enriched objective response rate with seven complete responses and one partial response among nine patients with anaplastic large-cell lymphoma harboring the nucleophosmin (*NPM*)–*ALK* fusion, and three partial responses among seven patients with inflammatory myofibroblastic tumor with various *ALK* fusion transcripts [23].

THE 'GENOMIC LANDSCAPE' OF PEDIATRIC CANCER

Data from massive parallel sequencing projects suggest that, in general, tumors presenting in childhood have fewer somatic mutations and may be genetically less complex [11]. For example, genomic profiling of rhabdoid tumor, among the most aggressive and chemotherapy-resistant pediatric malignancies, identified recurrent, high-frequency genomic alterations in *SMARCB1*, encoding a component of the critical SWI/SNF chromatin remodeling complex, often the sole somatic mutation present [36]. The Therapeutically Applicable Research to Generate Effective Treatments (TARGET) initiative (https://ocg.cancer.gov/programs/target) is a collaborative project sponsored by the National Cancer Institute (NCI) conceived to

Table 1. FDA-approved molecularly targeted agents with pediatric dose information

Drug	Target	Dose	Reference
Tyrosine kinase inhibitors			
Imatinib	KIT, BCR-ABL, PDGFR	260–570 mg/m^2/day (maximum 600 mg)	[13]
Dasatinib	BCR-ABL, Src, Lyn, FAK	60–85 mg/m^2/dose BID	[14]
Gefitinib	EGFR	400 mg/m^2/day	[15]
Erlotinib	EGFR, ERBB2, *JAK2*V617F (JAK2)	85 mg/m^2/day	[16]
Lapatinib	EGFR, ERBB2	900 mg/m^2/dose BID	[17]
Vandetanib	VEGFR-2, EGFR	145 mg/m^2/day	[18]
Sunitinib	KIT, PDGFR, VEGF, RET, CSF-1R, FLT-3	15 mg/m^2/day	[19,20]
Sorafenib	Raf, PDGFR, VEGFR, Flt-3, KIT	200 mg/m^2/dose BID	[21]
Pazopanib	VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α, PDGFR-β, c-Kit	450 mg/m^2/day	[22]
Crizotinib	ALK, MET	280 mg/m^2/dose BID	[23]
Ruxolitinib	Jak1, Jak2	50 mg/m^2/dose BID	[24]
Other pathway inhibitors			
Everolimus	mTOR	5 mg/m^2/day	[25]
Temsirolimus	mTOR	10–150 mg/m^2/day	[26]
Vorinostat	HDAC	230 mg/m^2/day	[27]
Vismodegib	SMO/hedgeHog (Hh)	150 or 300 mg	[28]
Monoclonal antibodies			
Herceptin	Her-2	4 mg/kg loading 2 mg/kg weekly	[29]
Cetuximab	EGFR	250 mg/m^2 weekly	[30]
Bevacizumab	VEGF	5–15 mg/kg Q2–3 weeks	[31]
Brentuximab vedotin	CD30 (cAC10) conjugated to monomethyl auristatin E	1.8 mg/kg/dose (maximum dose 180 mg)	[32,33]

EGFR, epidermal growth factor receptor; FAK, focal adhesion kinase; FDA, Food and Drug Administration; HDAC, histone deacetylase; mTOR, mammalian target of rapamycin; PDGFR, platelet derived growth factor receptor; SMO, smoothened; VEGFR, vascular endothelial growth factor receptor.

parallel The Cancer Genome Atlas, a genomic catalogue of common adult malignancies. Launched in 2006, the TARGET aims to identify recurring and potentially druggable genomic alterations in ALL, neuroblastoma, acute myeloid leukemia, and, more recently, osteosarcoma and Wilms tumor. Similar academic and international initiatives have developed around Ewing sarcoma, rhabdomyosarcoma, and pediatric brain tumors [37]. Indeed, the results of these large discoveries and recently published datasets describe a 'genetic landscape' of pediatric cancer remarkable for its limited number of genomic alterations. Nevertheless, these efforts have yielded a number of leads to explore rational genomic-based therapies.

LEUKEMIA AND LYMPHOMA

Hematologic malignancies comprise 40% of all of the pediatric cancers. With risk-adapted therapy, outcomes for most pediatric leukemias and lymphomas have dramatically improved. The challenge now remains to better treat refractory and relapsed disease. New therapeutic targets are being identified with advances in pediatric leukemia genomics [38,39]. As described above, the *BCR-ABL1* translocation is diagnostic, prognostic, and predictive of response to treatment with tyrosine kinase inhibitors such as imatinib and dasatinib in B lymphoblastic leukemia as well as CML [5,34]. Deletions of *IKZF1*, a gene encoding the lymphoid transcription factor Ikaros, are present in more than 70% of patients with *BCR-ABL1*–positive ALL and are associated with an unfavorable outcome [40]. Deletions in *IKZF1* may lead to an activated Janus kinase/signal transducers and activators of transcription (JAK-STAT) pathway and an immature B-cell receptor signaling via Bruton's tyrosine kinase (BTK) activation. Thus, JAK or BTK inhibitors may offer an alternative strategy for treatment in *BCR-ABL1*–positive ALL with *IKZF1* deletions [41,42]. A recently described subgroup of Ph-like ALL, which harbor a gene expression signature similar to that programmed by the *BCR-ABL1* transcript but lacking t(9;22)(q34;q11.2), were also found to have kinase-activating genomic alterations anticipated to confer sensitivity to dasatinib or the JAK2 inhibitor ruxolitinib [43]. Similarly, genomic and expression profiling studies have revealed several mutations in the T-cell ALL subtype, including NOTCH pathway activation via *NOTCH1* or *FBXW7* mutations and rearrangements in the *FLT3* and *ABL* kinases [44]. Early T-cell

Table 2. Active multicenter pediatric studies with potential for molecular target matching

Indication	Phase	Drug	Target	Study [NCT#]
Selected new Dx AML	3	Sorafenib + CTx	*FLT3* ITD	COG AAML1031 [NCT01371981]
New Dx Ph+ ALL	2	Dasatinib + CTx	BCR-ABL	COG AALL1122 [NCT01406756]
Selected new Dx CML, and resistant Ph+ ALL	2	Nilotinib	BCR-ABL	COG AAML1321, CAMN107A2120 [NCT01979536]
New Dx ALCL	2	Brentuximab vedotin, crizotinib + CTx	CD30, ALK	COG ANHL12P1 [NCT01979536]
Relapsed AML	1/2	Decitabine + cytarabine	HDAC	DACOGENAML2004 [NCT01853228]
Relapsed ALL/AML	1/2	Decitabine/vorinostat + CTx	Methylation HDAC	TACL T2009-003 [NCT01483690]
Relapsed ALL	1	Panobinostat	HDAC	TACL T2009-002 [NCT01321346]
Selected relapsed ALL/AML	1	EPZ-5676	DOT1L, *MLL* rearrangement	EPZ-5676-12-002 [NCT02141828]
Selected relapsed ALL/AML	1/2	Midostaurin (PKC412)	*MLL*, *FLT3*	CPKC412A2114 [NCT00866281]
Selected new Dx medulloblastoma	2	Vismodegib in maintenance	SMO/hedgehog (Hh)	SJMB12 [NCT01878617]
New Dx DIPG	2	Bevacizumab, radiation, ±erlotinib, ±temozolomide	MGMT, EGFR	DFCI 10-321 [NCT01182350]
New Dx DIPG	1	MK-1775 + radiation	Wee-1/checkpoint	COG ADVL1217 [NCT01922076]
New Dx DIPG	1	Veliparib (ABT-888), radiation, and temozolomide	PARP	PBTC-033 [NCT01514201]
Relapsed SHH medulloblastoma	2	LDE-225	SMO/hedgeHog (Hh)	CLDE225C2301 [NCT01708174]
Refractory pontine glioma	2	Vismodegib	SMO/hedgeHog (Hh)	NMTRCPG007 [NCT01774253]
Selected recurrent low-grade glioma	1/2	Selumetinib (AZD6244)	MEK (*BRAF*^{V600E}/*KIAA1549* fusion, *NF-1*, MAPK)	PBTC-029 [NCT01089101]
Recurrent/refractory low-grade glioma	2	Everolimus	MTOR	PNOC 001 [NCT01734512]
Relapsed solid tumors	2	Pazopanib	VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α, PDGFR-β, c-Kit	COG ADVL1322 [NCT01956669]
Relapsed solid tumors	1	Cabozantinib	VEGFR, c-Met	COG ADVL1211 [NCT01709435]
Relapsed solid tumors	1	Crizotinib + CTx	ALK	COG ADVL1212
Relapsed solid tumors	1	MK-1775 + irinotecan	Wee-1/checkpoint	COG ADVL1312 [NCT02095132]
Relapsed solid tumors	1	Axitinib	VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α, PDGFR-β, c-Kit	COG ADVL1315
Relapsed solid tumors	1	BMN 673 + temozolomide	PARP	COG ADVL1411 [NCT02116777]
Relapsed solid tumors	1	Vorinostat + etoposide	HDAC	10-096 [NCT01294670]
Selected *BRAF*^{V600E} relapsed solid tumors	1	Dabrafenib	*BRAF*^{V600E}	116013 NCT01677741
Relapsed solid tumors	1	Regorafenib	VEGFR-1, VEGFR-2, VEGFR-3, kit, PDGFRβ, RET, Raf-1	15906 [NCT02085148]
Selected relapsed neuroblastoma	1	Crizotinib	ALK	COG ADVL0912 [NCT00939770]
Selected relapsed solid tumors	1	LDK378	ALK	CLDK378X2103 [NCT01742286]
NMC, relapsed MYCN amplified tumors	1	GSK525762	BET bromodomain	115521 [NCT01587703]

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; DIPG, diffuse intrinsic pontine glioma; MGMT, O^6-methylguanine DNA methyltransferase; NMC, NUT midline carcinoma; PARP, poly (ADP–ribose) polymerase; Ph+, Philadelphia chromosome–positive; SHH, sonic-hedgehog.

precursor (ETP)-ALL, a subtype of T-cell ALL, has recently been identified and represents 10–15% of T-cell ALL with a poor prognosis reported by some [45], but not all, of the groups with the application of contemporary risk-stratified therapy [46]. ETP-ALL has a significant alteration in genes regulating Ras signaling and histone modification with a global transcriptional profile similar to myeloid leukemia stem cells and normal hematopoietic cells, thereby generating interest in treating ETP-ALL utilizing acute myeloid leukemia strategies [37]. Finally, as previously noted, *ALK* translocations represent a unique therapeutic target of crizotinib and will be compared with the CD30-directed antibody–drug conjugate brentuximab vedotin in the current upfront COG trial for anaplastic large-cell lymphoma (NCT01979536) [47].

PEDIATRIC BRAIN TUMORS

A great deal of progress has been made describing the genomic landscape of pediatric brain tumors. This has opened up a 'new horizon' in molecular neuro-oncology in clinical practice [48], although the majority of findings still relate to diagnosis and subclassification rather than prognosis and therapy. Perhaps the biggest discovery over the past year was the description of *ACVR1* mutations in high-grade diffuse (infiltrating) gliomas in children by four separate investigator groups [49–52]. *ACVR1* encodes a bone morphogenetic protein type 1 receptor, and *ACVR1* mutations activate the SMAD pathway, upregulate expression of inhibitors of differentiation protein family members, and lead to cell cycle progression through Rb and p21 [53▪]. As described by Zadeh and Aldape [53▪], these discoveries introduce *ACVR1* and bone morphogenetic protein signaling as future therapeutic targets in diffuse gliomas, a very deadly pediatric brain tumor with exceedingly high mortality.

Further profiling of pediatric low-grade gliomas highlights the presence of *KIAA1549-BRAF* fusions and $BRAF^{V600E}$ mutations leading to constitutively active BRAF in pilocytic astrocytomas and pleomorphic xanthoastrocytomas [54,55]. These studies also describe *MYB* fusions and amplifications, *FGFR1* duplications, *NTRK2* fusions, and *PTPN11* mutations (in a subset *FGFR1*-mutant tumors); the majority of low-grade glioma alterations converge on mitogen-activated protein kinases (MAPKs) including the extracellular signal-regulated kinase (ERK) and phosphoinositide 3-kinase (PI3K) pathways, again opening the door for future therapeutic intervention [56▪]. Sequencing of hindbrain ependymomas reveals a very low mutation rate and absence of CNAs, but these tumors clearly show epigenomic alterations leading to the CpG island methylator phenotype [57]; this finding may lead to the future use of epigenetic modifiers as a therapeutic approach in ependymoma. A second study of supratentorial ependymomas showed that two-thirds of samples contain the novel *C11orf95-RELA* fusion protein that activates nuclear factor-κB target genes [58], introducing a novel fusion and known pathway for future therapy. The majority of papillary craniopharyngiomas contain recurrent *BRAF* mutations ($BRAF^{V600G}$), and nearly all of the adamantinomatous craniopharyngiomas have *CTNNB1* mutations [59], with clear implications for diagnosis and future treatment strategies [60].

In medulloblastoma, discoveries continue to build off the finding of four distinct genomic subgroups [61]. Each subgroup contains its own novel mutations (i.e., epigenetic-related mutations in H3K27 and H3K4 trimethylation in groups 3 and 4 and *CTNNB1*-associated chromatin remodelers in the WNT group) [62]. Enrichment of subgroup-specific structural variations includes recurrent translocations of *PVT1* (*PVT1-MYC* and *PVT1-NDRG1*) along with CNAs in the transforming growth factor beta signaling pathway in group 3, and CNAs in the nuclear factor-κB signaling pathway in group 4, with tandem duplications of *SNCAIP* specifically in group 4a [63]. The genomic subgrouping of medulloblastoma has led to recent insights into tumor formation [i.e., *TP53* mutations and chromothripsis in sonic-hedgehog medulloblastoma (SHH-MB) [64]] and subgroup-specific prognostication [fluorescence in situ hybridization for *GLI2*, *MYC*, chromosome 11, 14, 17p, and 17q to identify very-low-risk and very-high-risk patients in SHH-MB, group 3, and group 4 [65], and poor survival in SHH-MB with *TP53* mutations [66▪]]. Other recent findings include highly recurrent *TERT* promoter mutations in SHH-MB [67] and the use of DNA methylation to better profile and predict clinical outcome using formalin-fixed biopsies [68]. Taken together, the field of medulloblastoma, along with the other pediatric brain tumors, has set the stage for more precise tumor classification with specific genomic alterations to be introduced as new targets in clinical trials.

EMBRYONAL EXTRACRANIAL SOLID TUMORS AND SARCOMA

Neuroblastoma was the first solid tumor to report results from the TARGET initiative. These studies confirm *ALK* alterations in both familial and sporadic neuroblastoma [69–72] as the most frequently occurring somatic mutation (9.2%), suggesting that this small but significant population is well

positioned to pilot upfront screening and stratification to a treatment arm containing an ALK inhibitor such as crizotinib, discussed above [73]. For more than two decades, *MYCN* amplification has been associated with aggressive disease and poor outcome [74]. Using a cell-line screening approach, *MYCN* amplification was identified as a strong genetic predictor of sensitivity to BET bromodomain inhibitors, a new class of compounds that modify epigenetic regulators [75]. These data provide a convincing rationale for phase 1 testing of the first-in-class BET bromodomain inhibitor, GSK525762, in a refractory pediatric cohort selected for *MYCN*-driven disease (NCT01587703) [76].

Comprehensive genome and transcriptome sequencing of rhabdomyosarcoma reported this year continues to document few sporadic genomic alterations and provides convincing evidence that fusion-positive and fusion-negative tumors are biologically distinct subgroups [77▪]. The presence of the *PAX3–FOXO1* fusion has been identified as a prognostic marker of inferior outcome and may be incorporated into the risk stratification of future trials in addition to Intergroup Rhabdomyosarcoma Study TNM stage and age [78,79]. Fusion-positive tumors most commonly demonstrate additional CNAs including amplification of *MYCN*, *CDK4*, and *MIR-17-92*, or loss of *CDKN2A*, whereas fusion-negative tumors have more frequent recurrent somatic mutations most frequently in the tyrosine kinase/RAS/PIK3 axis including *NRAS*, *KRAS*, *HRAS*, *FGFR4*, *PIK3CA*, as well as cell cycle genes *CTNNB1* and *FBXW7*, suggesting that this fusion-negative group may benefit from targeted genomic profiling for directed therapy.

Three new studies on the genetic landscape of the Ewing sarcoma family of tumors confirm that tumors with the *EWSR1* fusion have a very low mutational burden, but do contain frequent cohesin complex subunit *STAG2* loss-of-function mutations (15–22%), *CDKN2A* deletions (12–14%), and *TP53* alterations (6–7%) [80–82]. Cohesin is critical for not only sister chromatid cohesion and chromosome segregation but also DNA replication and repair by homologous recombination. Previous studies in yeast identified synthetic lethality between mutations in the cohesin complex and replication fork stabilizers such as poly (ADP-ribose) polymerase (PARP) [83]. Therefore, cohesin mutant tumors may have increased sensitivity to certain DNA-damaging agents in combination with PARP inhibitors analogous to other tumors harboring *BRCA1* and *BRCA2* mutations [84]. COG-ADVL1411 (NCT02116777) is an ongoing phase 1/2 trial of the oral PARP inhibitor BMN 673 with temozolomide, and further genomic analysis of any extraordinary responders in the present study may provide valuable future insight.

SEQUENCING PLATFORMS AND CLINICAL TRIALS OF GENOMIC PROFILING

Many pediatric cancer programs have begun to clinically screen for genomic alterations in patient tumor specimens using commercial Clinical Laboratory Improvement Amendments-certified targeted sequencing panels or academically based Clinical Laboratory Improvement Amendment and College of American Pathologists-compliant sequencing facilities. Targeted sequencing platforms are high-throughput mutation profiling panels designed to screen for base substitutions, small insertions and deletions (indels), larger gene rearrangements, and CNAs in a predefined set of oncogenes and tumor suppressors associated with response or resistance to targeted therapies important to adult epithelial cancers [85]. The relevance of the pathways on such panels and their downstream analysis, curation, and clinical interpretation to childhood tumors remains unclear. A recent study of 400 pediatric patients profiled by Foundation Medicine (Cambridge, MA) revealed an average 2.3 genomic alterations per patient (range 0–15), most commonly in *TP53*, *CDKN2A/B*, *MYCN*, *ALK*, *MYC*, *KRAS*, and *BRAF*. Using an adult analytic pipeline, 60% were predicted to be 'actionable,' with linkage to a Food and Drug Administration (FDA)-approved drug or investigational agent, although not necessarily a drug in pediatric clinical trials [86]. Eighty percent of these pediatric findings were predicted to be missed by the even more limited 'hotspot' sequencing analysis [87]. Such data can inform and adapt panels and analytic pipelines to better represent, detect, and interpret genomic alterations important in pediatric cancers. Although such predetermined panels allow for depth and therefore fidelity in variant calls, they may also restrict opportunity for discovery. As genomic sequencing becomes more economically feasible, it is likely to be integrated into standard clinical practice.

Despite its inherently rational basis, actual evidence to support the feasibility and clinical utility of precision medicine for pediatric cancer remains in its very early stages. Two groups with separate approaches have shared initial results at national meetings. As part of the National Human Genome Research Institute Clinical Sequencing Exploratory Research program, the Baylor Advancing Sequencing in Childhood Cancer Care study at Texas Children's Cancer Center is evaluating clinical exome sequencing in germline and tumor samples from newly diagnosed patients to determine its impact

on patients, families, and oncologists. The multicentered Individualized Cancer Therapy protocol (NCT01853345) for pediatric patients at the Dana Farber Cancer Institute and three other collaborating institutions utilized OncoMap [87] and the more updated OncoPanel targeted sequencing platforms, in conjunction with array comparative genomic hybridization, to profile archival tumors from patients with high risk, recurrent, or refractory disease and focused on providing a therapeutic recommendation determined by a multidisciplinary expert panel. Both studies used specimens obtained for clinical purposes. Presented at the 2014 Annual Meeting of the American Society for Clinical Oncology, study results seem consistent and promising. Both studies demonstrated an enthusiasm among families with pediatric cancer to participate in clinical genomics research, greater than 80% technical feasibility for profiling samples obtained for clinical purposes, at least one somatic genomic alteration of known or potential clinical relevance in ~30% of participants, and the importance of CNAs [88▪,89▪,90]. The most frequent and potentially impactful aberrations included *MYCN*, cell cycle genes (*CDK 4/6*, *CDKN2A/B*, *CCND1*, *CCNE1*), *FGFR*, RAS pathway (*HRAS*, *NRAS*, *MEK1*, *BRAF*), *CTNNB1*, *ALK*, *KIT*, *PI3K*, and *TSC* [88▪,89▪]. Another recent study focused on relapsed translocation sarcomas [Ewing sarcoma ($n = 18$) and desmoplastic small round cell tumor ($n = 10$)], demonstrating secondary genomic alterations in 6 of 28 (21.4%) tumors, including *KRAS*, *PTPRD*, and *GRB10* mutations predicted to explain sensitivity and resistance to IGFR1-directed therapies, as well as potentially actionable mutations in *MET* and *PIK3CA* [91].

RETURN OF GERMLINE RESULTS

The hereditary basis of pediatric cancer may range from 10% to as high as 33% [92–94], making the discovery of clinically relevant germline mutations all but inevitable during clinical genomic sequencing using germline DNA to distinguish cancer-specific somatic variants from constitutional variants or polymorphisms. In fact, the Baylor Advancing Sequencing in Childhood Cancer Care group reported diagnostic germline findings in 11 patients for pathogenic mutations in cancer susceptibility genes and nononcologic diagnoses [89▪]. In anticipation of unanticipated findings, many groups have tried to tackle the issue of incidental findings in germline DNA and how to return results to patients and their families. Perhaps the best known publication on the subject is the American College of Medical Genetics (ACMG) Policy Statement in July 2013 that includes reporting recommendations for incidental findings in clinical exome and genome sequencing [95▪]; this report recommended the mandatory return of germline results for 56 genes associated with 24 inherited conditions (including cancer predisposition) regardless of patient consent. Following the ACMG Policy Statement, a tremendous backlash erupted from the clinical genetics community about paternalism and lack of patient autonomy [96–101]. A year after the Policy Statement was published, the ACMG revised its recommendation to include an opt-out clause in clinical sequencing consents so that patients could choose not to receive results from the list of clinically actionable results. These discussions remain very relevant to pediatric cancer sequencing because the original ACMG Policy Statement explicitly describes that 'incidental findings should be reported for the normal sample of a tumor-normal sequenced dyad' [95▪]. A recent editorial argues that the ACMG recommendations have important and unanticipated implications for clinical laboratories, oncologists, and patients when clinical sequencing is ordered for tumor information and not germline testing [102▪]; the authors conclude that one way to preserve patient autonomy would be to offer an opt-in approach whereby patients (or their parents) could choose to include germline results with their tumor sequencing results after undergoing pretest counseling with an appropriate genetics healthcare worker [102▪]. The discussions regarding the return of genetic results will undoubtedly continue as the clinical sequencing of pediatric tumors becomes more commonplace.

TUMOR HETEROGENEITY AND EVOLUTION

Also essential to the field of precision oncology is the evidence supporting the molecular heterogeneity of tumors within the same patient: from diagnosis to relapse, between metastatic sites, and even within a single primary tumor [103]. These findings suggest that multiple biopsies may be necessary to identify critical tumor-initiating driver mutations and that Darwinian selection of low-frequency drug-resistant subclones may be at play. Recently, in neuroblastoma, a higher rate of *ALK* mutations was observed at relapse versus diagnosis and in paired diagnostic and relapse samples, clear evidence that both subclonal selection and de-novo *ALK* mutations exist; the authors conclude that their 'findings implicate a change in medical practice in favor of tumor sampling even at relapse, and repeated tumor sampling should become a new standard of care' [104▪]. Similar expansion of loss-of function *STAG2* mutations documented in Ewing sarcoma at relapse substantiates this claim [82]. Such

recommendations for repeat biopsy at relapse solely for the purpose of molecular profiling undoubtedly will generate intense debate for minor patients. This is particularly true because current data suggest actionable genomic alterations may be unlikely to be identified in ~70% of pediatric patients, no firmly established linkage exists between biopsy results and availability of therapeutic options, and the premise that genomic alteration-'matched' therapy in pediatric malignancy imparts true clinical benefit has yet to be demonstrated.

ACCESS TO GENOMICALLY TARGETED AGENTS

For pediatrics, a major obstacle to the implementation of genomic medicine remains access to drugs. Although an increasing number of FDA-approved targeted agents have been studied in pediatrics (Table 1), to date only imatinib, everolimus, and temsirolimus include pediatric labeling information and only the former two have approved pediatric indications (http://www.accessdata.fda.gov/scripts/sda/sdNavigation.cfm?sd=labelingdatabase). Obtaining insurance authorization for off-label use can be problematic, and the lack of available pediatric-friendly formulations presents unique clinical challenges. Ideally, when actionable genomic targets are identified, patients can be directed to one of several available clinical trials (Table 2), but, again, access may be limited because of geography, lack of available treatment slots, or even stringent eligibility criteria. In addition, agents targeting aberrations in certain critical pathways found in pediatric tumors such as PI3K, CDK 4/6, MDM2, FGFR, and *CTNNB1*/WNT are conspicuously absent from the current portfolio.

CONCLUSION

Despite the challenges raised by translating cancer genomic discovery from the research lab to the pediatric clinic, incremental improvements in sequencing technology, understanding of childhood cancer biology, and progress in pediatric clinical trials and drug development have delivered the pediatric oncology community to the current point in the implementation of genomic medicine. General consensus exists that prior selection of patients with somatic genetic vulnerabilities will increase the likelihood of positive efficacy signals for novel targeted agents and be of clinical benefit for individual patients, but this still requires vigorous testing. The NCI has therefore embarked on a plan to launch the Molecular Analysis for Therapy Choice, a master protocol to be made widely available to the major adult clinical trial consortia and utilizing a NGS-targeted platform to distribute patients to multiple small phase 2 trials of a diverse genomically targeted agent portfolio on the basis of genomic alterations rather than diagnosis or histology. Excitingly, the NCI recently announced that they are working with numerous pharmaceutical companies to make the same targeted drug slate available in a parallel pediatric Molecular Analysis for Therapy Choice program for children with relapsed and refractory cancer conducted through the COG (http://www.cancer.gov/clinicaltrials/noteworthy-trials/match). The time for this potentially transformative genomic-based approach has arrived, and offers the promise of a paradigm shift from empiricism to precision in pediatric oncology.

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Conflicts of interest

None.

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