Multicenter Feasibility Study of Tumor Molecular Profiling to Inform Therapeutic Decisions in Advanced Pediatric Solid Tumors

The Individualized Cancer Therapy (iCat) Study

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IMPORTANCE Pediatric cancers represent a unique case with respect to cancer genomics and precision medicine, as the mutation frequency is low, and targeted therapies are less available. Consequently, it is unknown whether clinical sequencing can be of benefit.

OBJECTIVE To assess the feasibility of identifying actionable alterations and making individualized cancer therapy (iCat) recommendations in pediatric patients with extracranial solid tumors.

DESIGN, SETTING, AND PARTICIPANTS Clinical sequencing study at 4 academic medical centers enrolling patients between September 5, 2012, and November 19, 2013, with 1 year of clinical follow-up. Participants were 30 years or younger with high-risk, recurrent, or refractory extracranial solid tumors. The data analysis was performed October 28, 2014.

INTERVENTIONS Tumor profiling performed on archived clinically acquired specimens consisted of mutation detection by a Sequenom assay or targeted next-generation sequencing and copy number assessment by array comparative genomic hybridization. Results were reviewed by a multidisciplinary expert panel, and iCat recommendations were made if an actionable alteration was present, and an appropriate drug was available.

MAIN OUTCOMES AND MEASURES Feasibility was assessed using a 2-stage design based on the proportion of patients with recommendations.

RESULTS Of 100 participants (60 male; median [range] age, 13.4 [0.8-29.8] years), profiling was technically successful in 89 (89% [95% CI, 83%-95%]). Median (range) follow-up was 6.8 (2.0-23.6) months. Overall, 31 (31% [95% CI, 23%-41%]) patients received an iCat recommendation and 3 received matched therapy. The most common actionable alterations leading to an iCat recommendation were cancer-associated signaling pathway gene mutations (n = 10) and copy number alterations in MYC/MYCN (n = 6) and cell cycle genes (n = 11). Additional alterations with implications for clinical care but not resulting in iCat recommendations were identified, including mutations indicating the possible presence of a cancer predisposition syndrome and translocations suggesting a change in diagnosis. In total, 43 (43% [95% CI, 33%-53%]) participants had results with potential clinical significance.

CONCLUSIONS AND RELEVANCE A multi-institutional clinical genomics study in pediatric oncology is feasible and a substantial proportion of relapsed or refractory pediatric solid tumors have actionable alterations.

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In many diseases, molecularly targeted therapies have improved outcomes and decreased treatment-related toxic effects. In oncology, examples such as ERBB2-positive breast cancer and EGFR-mutant lung cancer have led to excitement about the promise of precision cancer medicine. These and other similar treatment advances typically occurred after years of investigation during which (1) a genomic alteration was identified in a reasonable proportion of patients with a particular diagnosis; (2) the alteration was validated to be a central oncogenic event; (3) the activity of targeted therapy was studied in preclinical models and then in early-phase trials; and (4) clinical trials demonstrated activity of the targeted agent only in cancer subtypes with specific molecular alterations. The essential nature of most pathogenic variants has, therefore, been established only in the context of specific disease subsets. Nevertheless, some oncologists have adopted a precision medicine approach for their patients with recurrent or refractory cancers based on the premise that receipt of matched targeted therapy will have superior clinical activity, regardless of diagnosis or patient characteristics.

The implementation of precision cancer medicine in pediatric oncology faces unique challenges. The total number of nucleotide variants per tumor is much smaller for pediatric than for adult cancers. In addition, unmet needs in medical oncology drive the current developmental therapeutics environment such that the availability, formulation, and accessibility of drugs for children may be limited. In general, pediatric cancer is comparatively rare, and most patients are cured with standard therapies, necessitating multicenter collaboration to obtain meaningful cohorts for study. Finally, regulations governing research in children make it difficult to obtain biopsy samples for research purposes.

To evaluate the feasibility of a precision cancer medicine approach in pediatric extracranial solid tumors, we conducted a multicenter molecular profiling cohort study in children with advanced solid tumors (NCT01853345) (see trial protocol in Supplement 1). We sought to determine whether, in the pediatric setting, it is feasible to identify actionable alterations and make an individualized cancer therapy, or iCat, recommendation using currently available clinical genomic technologies.

Methods

Patients

Patients were eligible if they were 30 years or younger at enrollment and had a recurrent, refractory, or high-risk extracranial solid tumor. Refractory was defined as progression during receipt of standard first-line therapy, and high risk was defined as overall survival for a patient group with the same diagnosis, grade, and stage estimated to be no more than 25%. Patients needed to have sufficient tumor specimen for submission, in the judgment of the enrolling investigator. The institutional review board at each participating site approved the research protocol, and all participants and/or their parent or legal guardian provided written informed consent.

Sample Collection and Tumor Profiling

Submission of 15 to 60 unstained slides containing viable tumor, a paraffin-embedded block, or 0.25 cm³ fresh-frozen tissue was required. All samples were obtained as part of routine clinical care; the protocol did not mandate biopsy for research purposes. Decalcified specimens were considered inadequate for tumor profiling. Tumor tissue obtained following administration of neoadjuvant chemotherapy was permitted if viable tumor was present, although tissue obtained prior to chemotherapy was preferred. Submission of tumor tissue from diagnosis and from recurrence was encouraged. All tumor specimens were reviewed by a single molecular pathologist (M.H.H.) who determined the percent viable tumor nuclei and adequacy for profiling.

Mutation detection was performed initially with a Sequenom assay, OncoMap, detecting 471 recurrent mutations in 41 cancer-related genes. Once available, mutation detection was performed by targeted next-generation sequencing (NGS), OncoPanel, using Agilent SureSelect for target capture and Illumina HiSeq for sequencing. The OncoPanel assay surveys exonic DNA sequences of 275 cancer genes and 91 introns across 30 genes for rearrangement detection. OncoMap and OncoPanel were performed in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory, the Center for Advanced Molecular Diagnostics at the Brigham and Women's Hospital. Blood samples were collected and germline DNA was extracted and banked for future research; however, OncoPanel testing was performed only on tumor DNA. When sufficient tissue was available, copy number assessment was performed with array comparative genomic hybridization (aCGH), using Agilent SurePrint G3 CGH and single-nucleotide polymorphism (SNP) Cancer Microarray 4×180, using Agilent reference DNA as reference. The aCGH analysis was performed in CLIA-certified laboratories at Boston Children's Hospital and Claritas Genomics. Analysis of aCGH data was focused on regions of interest determined prior to study initiation (eTable 1 in Supplement 2). OncoMap, OncoPanel, and aCGH were interpreted and reported by molecular pathologists (M.H.H., A.C., N.I.L.).

Expert Panel Review and Return of Results

Profiling results were reviewed by a multidisciplinary expert panel with the goal of identifying clinically significant results.
In reviewing results, the term actionable alteration was used for a genomic change indicating the potential for activity of a targeted therapy. The term targeted therapy was used in reference to a drug with a specific, limited, and known mechanism of action counteracting an oncogenic process. An iCat recommendation was made if an actionable alteration was present and a matched targeted therapy was available via a clinical trial or as an FDA-approved drug with an age-appropriate dose and formulation, and the patient had not previously been treated with the proposed targeted agent or a biosimilar. More than 1 recommendation could be made per patient. Each recommendation was tiered from 1 (strongest) to 5 (weakest) based on strength of supporting evidence. Tiers 1 and 2 corresponded to evidence from clinical studies, tiers 3 and 4 to preclinical evidence, and tier 5 to consensus opinion (eTable 2 in Supplement 2). If more than 1 iCat recommendation was made, the expert panel made no attempt to rank recommendations; each actionable alteration was evaluated and tiered independently. In some cases, immunohistochemical analysis was performed for validation of genetic findings. Variants of unknown significance (VUS) were evaluated for potential pathologic significance using functional prediction models such as PolyPhen6 or the Sorting Tolerant From Intolerant algorithm (SIFT)7 and/or the medical literature. The expert panel also reviewed identified variants for clinical implications outside the iCat recommendation. In particular, variants suggesting a change in diagnosis or the possible presence of a cancer predisposition syndrome (if also found in the germline) were discussed.

After expert panel review and panel consensus was reached, the lead reviewer for the case authored a letter to the patient’s primary oncologist (iCat letter). The iCat letter reported the specimen(s) tested, technical performance of profiling tests, and results with clinical implications for diagnosis or that indicated the possible presence of a cancer predisposition syndrome. If the patient had recurrent or refractory disease, the iCat letter also reported the iCat recommendation with tier.

Clinical Data Collection
Clinical and demographic data were collected at the time of patient enrollment and included age, diagnosis, and disease status (newly diagnosed, or refractory or recurrent). If the iCat letter included an iCat recommendation, the primary oncologist was surveyed regarding vital status and initiation of treatment according to the iCat recommendation. In addition, data were collected from the electronic medical record on clinical response to therapy based on iCat recommendations and molecular diagnostic testing performed outside the context of this study.

Statistical Design
Feasibility was defined prior to the study opening as an ability to make an iCat recommendation in 14% of enrolled patients based on published technical failure rates and reported alteration frequencies available at the time of study design.8 A Simon 2-stage design was used to evaluate for early futility. In stage 1, 60 eligible participants were enrolled; if fewer than 6 participants received an iCat recommendation, then accrual would be halted and the approach considered infeasible. If 6 or more received an iCat recommendation, then accrual continued to stage 2, with an additional 40 eligible participants for a total of 100 patients. If at least 14 of 100 participants received an iCat recommendation, then the approach would be considered feasible. This design has a type 1 error of 6.3% and 92% power to test the null hypothesis that the proportion of patients who received an iCat recommendation was 9% or less vs the alternative that it was 19% or more. The rate of accrual was predicted to be 4.2 patients per month, or 100 patients in 2 years. The 95% CIs were constructed on the proportions using the customary normal approximation interval formula.

Exploratory Sequencing
Cases with sufficient tumor specimen remaining after submission for tumor profiling were evaluated for inclusion in exploratory sequencing studies. Cases were submitted for RNA sequencing if sufficient fresh-frozen tumor specimen was available. RNA sequencing data were analyzed using PRADA.9 The list of fusions identified by RNA sequencing was reviewed and fusions with potential clinical significance were validated by targeted NGS in a CLIA-certified laboratory.

Results
Patients and Specimens
Patients were enrolled between September 5, 2012, and November 19, 2013, and clinical follow-up was collected until November 2014 (median [range] follow-up, 6.8 [2.0-23.6] months). Accrual was more rapid than predicted (eFigure in Supplement 2), with 101 patients enrolling in 14 months. One enrolled patient was excluded because the minimum tumor specimen requirement could not be met. The most common diagnosis was nonrhabdomyosarcoma soft-tissue sarcoma (Table 1 and eTable 3 in Supplement 2). Distribution of the remaining diagnoses corresponded to the most frequently occurring pediatric solid tumors. Additional clinical and demographic features are presented in Table 1.

Thirty-one enrolled patients had specimen submitted only from diagnosis and 40 only from recurrence. Eighteen patients had paired specimens from both diagnosis and recurrence. The remaining 11 patients had specimens from local control surgical procedures submitted, 6 of whom had an additional sample submitted from either diagnosis or recurrence.

Tumor Profiling and iCat Recommendations
In 5 patients, the required samples were submitted but were determined to be inadequate by central review. In 6 other patients, all profiling tests attempted were technical failures. A total of 89 enrolled patients had at least 1 successfully completed profiling test. Of the 89 patients with technically successful tumor profiling tests, 13 patients had OncoMap as their only mutation detection test, 27 had both OncoMap and aCGH, 25 had OncoPanel and aCGH, and the remaining 24 had OncoPanel only. The 6 technical failures for all attempted profiling tests were due to inability to isolate adequate DNA. In 3
cases (in 2 cases of Ewing sarcoma and 1 case of osteosarcoma) this was likely due to prior decalcification that was not recognized at the time of tissue sample submission. In most cases, aCGH was not possible due to insufficient tissue sample. The expert panel rendered an iCat recommendation in 31 patients (31% [95% CI, 23%-41%]), exceeding the 14 patients required to conclude feasibility. There were a total of 39 iCat recommendations in these 31 patients (3 recommendations [n = 1]; 2 recommendations [n = 6]; and 1 recommendation [n = 24]). Using the highest tier per patient (see eTable 2 in Supplement 2 for tier definitions), 1 patient had a tier 1 recommendation, 2 had tier 2 recommendations, and the majority had tier 3 or 4 recommendations. None of the following factors made it more likely to receive an iCat recommendation: age, type of testing (OncoMap, OncoPanel, or aCGH), or month of enrollment.

The most common actionable alteration leading to an iCat recommendation was a copy number alteration, followed by known or suspected deleterious mutations (Table 2 and eWorksheets 1-3 in Supplement 3). Most copy number alterations resulting in an iCat recommendation were related to alterations of MYC/MYCN or cell cycle–related genes. Ten known or suspected deleterious mutations were detected in 8 genes (Figure). In most patients tested with OncoPanel, results included VUS. The expert panel made an iCat recommendation on the basis of a VUS in only 6 instances. In these 6 instances, PolyPhen-2 or SIFT and/or the medical literature indicated that the variant was likely pathogenic and in 2 cases, the variant would have qualified the patient for a clinical trial.

### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (n = 100)</th>
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<tbody>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>Nonrhabdomyosarcoma soft-tissue sarcoma</td>
<td>27</td>
</tr>
<tr>
<td>Rare tumors*</td>
<td>17</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>14</td>
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<tr>
<td>Ewing sarcoma</td>
<td>12</td>
</tr>
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<td>Osteosarcoma</td>
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<td>Rhabdomyosarcoma</td>
<td>11</td>
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<td>Wilms tumor</td>
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<tr>
<td>Age, y</td>
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<tr>
<td>0-4</td>
<td>21</td>
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<td>5-8</td>
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<td>9-12</td>
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<td>13-17</td>
<td>27</td>
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<tr>
<td>≥18</td>
<td>25</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>60</td>
</tr>
<tr>
<td>Female</td>
<td>40</td>
</tr>
<tr>
<td>Disease status (at enrollment)</td>
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<tr>
<td>Recurrent</td>
<td>72</td>
</tr>
<tr>
<td>Newly diagnosed high risk</td>
<td>19</td>
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<tr>
<td>Refractory</td>
<td>9</td>
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<tr>
<td>Tumor material submitted</td>
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<td>Recurrence only</td>
<td>40</td>
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<tr>
<td>Diagnosis only</td>
<td>31</td>
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<tr>
<td>Diagnosis and recurrence</td>
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<td>2</td>
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<tr>
<td>Local control surgery, recurrence, and diagnosis</td>
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<td>Enrolling institution</td>
<td></td>
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<tr>
<td>Dana-Farber/Boston Children’s</td>
<td>58</td>
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<tr>
<td>University of California–San Francisco</td>
<td>16</td>
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<tr>
<td>Columbia University Medical Center</td>
<td>15</td>
</tr>
<tr>
<td>Children’s National Medical Center</td>
<td>11</td>
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</tbody>
</table>

*Includes adrenal cortical carcinoma, congenital mesoblastic nephroma, germ cell tumor, glomus tumor, hepatoblastoma, hepatocellular carcinoma, melanoma, nasopharyngeal adenocarcinoma, plexiform schwannoma, renal cell carcinoma, renal medullary carcinoma, rhabdoid tumor of the liver, sialoblastoma.

### Table 2. Outcome of Molecular Profiling and Features of 39 Individualized Cancer Therapy (iCat) Recommendations in 31 Patients*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients, No. (% [95% CI])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outcome of specimen profiling and expert panel review</td>
<td></td>
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<tr>
<td>No iCat recommendation made</td>
<td>58 (58 [48-68])</td>
</tr>
<tr>
<td>iCat recommendation made</td>
<td>31 (31 [23-41])</td>
</tr>
<tr>
<td>Technical failure/inadequate specimen</td>
<td>11 (11 [5-17])</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
<tr>
<td>Tier of iCat recommendation</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1 (3 [0-9])</td>
</tr>
<tr>
<td>2</td>
<td>2 (6 [0-14])</td>
</tr>
<tr>
<td>3</td>
<td>8 (26 [11-41])</td>
</tr>
<tr>
<td>4</td>
<td>16 (52 [34-70])</td>
</tr>
<tr>
<td>5</td>
<td>4 (13 [1-25])</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
</tr>
<tr>
<td>Type of actionable alteration</td>
<td></td>
</tr>
<tr>
<td>Focal copy number alteration</td>
<td>20 (51 [35-67])</td>
</tr>
<tr>
<td>Mutation</td>
<td></td>
</tr>
<tr>
<td>Known or suspected deleterious</td>
<td>10 (26 [12-40])</td>
</tr>
<tr>
<td>Variant of uncertain significance</td>
<td>6 (15 [4-26])</td>
</tr>
<tr>
<td>Expressed target</td>
<td>2 (5 [0-12])</td>
</tr>
<tr>
<td>Translocation</td>
<td>1 (3 [0-8])</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
</tr>
</tbody>
</table>

*Outcome of specimen profiling reported for all patients. Tiers reported for 31 patients receiving an iCat recommendation as the highest tier per patient. Type of actionable alteration on which the iCat recommendation was based reported per iCat recommendation.
the pan-PI3K inhibitor BKM120, via a compassionate access protocol. Patient 18 also had a p.T727K PIK3CA mutation identified in the diagnostic specimen only and a p.V550L FGFR4 mutation identified in all tested specimens. Patient 81, a child with recurrent neuroblastoma with a p.R1275Q ALK mutation and MYCN high copy number gain identified in a recurrent specimen, had a tier 1 iCat recommendation and received crizotinib monotherapy. None of these patients had an objective response.

### Molecular Profiling in Pediatric Tumors

The relationship of Individualized Cancer Therapy (iCat) Recommendations and Additional Profiling Results in the 43 Patients in Whom Genomic Alterations Had Potential Clinical Significance.

#### Table 1: Known or likely deleterious mutation

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alteration</th>
<th>Diagnosis</th>
<th>Tier</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK</td>
<td>p.F1174L (c.3520T&gt;C)</td>
<td>NBL</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>p.R1275Q (c.3824G&gt;A)</td>
<td>NBL</td>
<td>1</td>
</tr>
<tr>
<td>BRAF</td>
<td>p.V600E (c.1799T&gt;A)</td>
<td>DFSP</td>
<td>2</td>
</tr>
<tr>
<td>FGFR4</td>
<td>p.V550L (c.1648G&gt;T)</td>
<td>ERMS</td>
<td>3</td>
</tr>
<tr>
<td>HRAS</td>
<td>p.Q61K (c.181C&gt;A)</td>
<td>ERMS</td>
<td>5</td>
</tr>
<tr>
<td>NRAS</td>
<td>p.Q61K (c.181C&gt;A)</td>
<td>ERMS</td>
<td>2</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>p.E545K (c.1633G&gt;A)</td>
<td>ERMS</td>
<td>4</td>
</tr>
<tr>
<td>ATM</td>
<td>p.G2023R (c.6067G&gt;A)</td>
<td>ERMS</td>
<td>5</td>
</tr>
<tr>
<td>CTNNB1</td>
<td>p.S45F (c.134C&gt;T)</td>
<td>ACC</td>
<td>4</td>
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</table>

#### Table 2: Copy number alterations (MYC/MYCN)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alteration</th>
<th>Diagnosis</th>
<th>Tier</th>
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</thead>
<tbody>
<tr>
<td>MYCN</td>
<td>High CN gain</td>
<td>NBL (n = 2)</td>
<td>3</td>
</tr>
<tr>
<td>MYC</td>
<td>High CN gain</td>
<td>OST</td>
<td>4</td>
</tr>
<tr>
<td>MYCN</td>
<td>High CN gain</td>
<td>US</td>
<td>4</td>
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#### Table 3: Variant of uncertain significance

<table>
<thead>
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<th>Gene</th>
<th>Alteration</th>
<th>Diagnosis</th>
<th>Tier</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGFR2</td>
<td>p.C382R (c.1144T&gt;C)</td>
<td>Sialoblastoma</td>
<td>4</td>
</tr>
<tr>
<td>PTCH1</td>
<td>p.R1113C (c.3337C&gt;T)</td>
<td>ARMS</td>
<td>4</td>
</tr>
<tr>
<td>MET</td>
<td>p.T1010I (c.3030C&gt;T)</td>
<td>CCS</td>
<td>4</td>
</tr>
<tr>
<td>MAPK1</td>
<td>p.D251N (c.751G&gt;A)</td>
<td>OST</td>
<td>5</td>
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</table>

#### Table 4: Other

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alteration</th>
<th>Diagnosis</th>
<th>Tier</th>
</tr>
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<tbody>
<tr>
<td>SMARCBI</td>
<td>2 Copy deletion</td>
<td>ES</td>
<td>4</td>
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<tr>
<td>Met</td>
<td>Expression</td>
<td>RCC</td>
<td>3</td>
</tr>
<tr>
<td>CDKN2A/B</td>
<td>Loss p16 expression</td>
<td>US</td>
<td>4</td>
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</tbody>
</table>

Molecular bases of iCat recommendations are shown. For patients with more than 1 iCat recommendation, only 1 molecular basis for the iCat recommendation is displayed. (A complete list of molecular alterations leading to an iCat recommendation is provided in eTable 4 in Supplement 2.) Two patients who had both an iCat recommendation and an actionable alteration without matching therapy are shown as iCat recommendation only. ACC indicates adrenocortical carcinoma; ARMS, alveolar rhabdomyosarcoma; CCS, clear-cell sarcoma; CMN, congenital mesoblastic nephroma; DFSP, dermatofibrosarcoma protuberans; ERMS, embryonal rhabdomyosarcoma; ES, epithelioid sarcoma; EWS, Ewing sarcoma; MPNST, malignant peripheral nerve sheath tumor; NBL, neuroblastoma; OST, osteosarcoma; RCC, renal cell carcinoma; SS, synovial sarcoma; US, undifferentiated sarcoma.
The primary oncologists of all 31 patients who received an iCat recommendation were surveyed regarding whether treatment according to an iCat recommendation was delivered and if not, why not. Among the 19 responses, reasons for not delivering iCat recommended therapy included disease too advanced or patient deceased (n = 3 [16%]); no active disease, disease well controlled with use of other therapy, or attempting third-line therapy first (n = 8 [42%]); and clinical status not appropriate for iCat therapy with reason not specified (n = 4 [21%]). Four of the responses (21%) indicated that iCat recommended therapy could not be obtained because a trial was not available or the patient did not meet trial eligibility. The lack of a suitable clinical trial occurred in these 4 patients who received an iCat recommendation despite the fact that the expert panel made an iCat recommendation only if they were of the opinion that matched targeted therapy was available via a clinical trial or as a US Food and Drug Administration (FDA)-approved drug with an age-appropriate dose and formulation.

Additional Profiling Results With Potential Clinical Impact
Six patients had an actionable alteration but there was no matching targeted therapy available via a clinical trial or as an FDA-approved therapy with an age-appropriate dose and formulation (eTable 5 in Supplement 2) and so an iCat recommendation was not made.

Eleven of 89 patients had alterations identified by testing the tumor specimen that, if present in the germline, would indicate the possibility of a cancer predisposition syndrome (eTable 6 in Supplement 2). These results were returned to the primary oncologist with a suggestion for referral to a genetic counselor.

Profiling identified translocations with diagnostic implications in 3 patients (Table 3). Patient 20 had a diagnosis of Ewing sarcoma, but RNA sequencing of the diagnostic specimen, confirmed with targeted NGS of the diagnostic specimen, demonstrated an EWSR1-CREBI fusion, raising the possibility of primary pulmonary myxoid sarcoma, a diagnosis that would better fit this patient’s clinical presentation. Patient 64 had a diagnosis of stage III malignant melanoma, but RNA sequencing of the first recurrence, confirmed with targeted NGS of the fourth recurrence, revealed an EWSR1-ATF1 fusion previously reported in cutaneous clear-cell sarcoma.11 In a third patient, patient 80, a novel translocation between exon 2 of EML4 (NM_019063.3) and exon 13 of NTRK3 (NM_001012338.1) was suggested by aCGH of the diagnostic specimen and confirmed with reverse transcription polymerase chain reaction from the same specimen.

Therefore, 12 additional unique patients had molecular profiling results of potential clinical significance independent of the 31 patients with iCat recommendations. Thus, a total of 43 patients had results with potential clinical significance.

### Discussion
Results of the iCat study demonstrate that clinical tumor profiling to identify actionable alterations in pediatric solid tumors and make an individualized cancer therapy recommendation is feasible in the context of a multi-institution research protocol. We found potentially actionable alterations in 43% of high-risk, relapsed, or refractory pediatric solid tumors, a prevalence that may justify incorporating return of results into future genomics research projects in pediatric solid tumors. The actionable alterations identified in this study highlight the drug classes in which there is a high priority to develop early-phase clinical trials with integrated genomic characterization for children with recurrent or refractory solid tumors (Table 4), an important study outcome given the limited number of these children available to enroll in early-phase clinical trials, and the large number of novel targeted therapies not yet studied in children. The specific genomic variants identified and the frequencies of actionable alterations are generally similar to those reported in prior studies characterizing the genomic landscapes of pediatric solid tumors.12-16 Most sequencing studies of pediatric cancers are underpowered to detect mutations occurring in less than 5% to 10% of cases,17 and many rare pediatric solid tumors have not been sequenced. Therefore, it is not surprising that some actionable alterations seen in this cohort have not previously been reported to occur in the diagnoses in which we observed them.

Barriers to the administration of matched targeted therapy in this patient population were identified. Only 3 of the 31 patients who had an iCat recommendation received matched tar-
Several questions remain to be answered before clinical tumor profiling is incorporated into routine care of children with recurrent or refractory and high-risk solid tumors. Our study did not assess which tumor profiling assays optimally balance the competing factors of minimal tissue requirement, comprehensive genomic assessment, and rapid reporting. The rapid pace of technology development, a challenge to ongoing consistency, complicates this task. This issue of evolving technology presented itself even in the context of this relatively short pilot study and is a limitation of our study. Although we report outcome data of 3 patients who received targeted therapy matched to an iCat recommendation, this study was not designed primarily to assess the impact of receipt of matched targeted therapy on outcome. Additional limitations of this study include the small patient numbers; exclusion of patients with central nervous system tumors, who make up a substantial proportion of pediatric patients with cancer with recurrent disease; and the inability to obtain profiling results in 11 of the 100 subjects due to lack of available tissue or technical failure. Continuing to perform genomic tumor profiling as part of a research protocol will enable assessment of these remaining questions through large multi-institutional studies. Additional benefits of prospective clinical genomic studies in pediatric cancers include discovery of rare recurrent variants and clarity regarding the drug classes to be prioritized for clinical trials.

### Conclusions

In children with high-risk, recurrent, or refractory solid tumors, there is the potential for tumor profiling results to have clinical implications but there are barriers to receipt of matched targeted therapy. Continuing to perform tumor profiling in the context of clinical genomics research protocols permits further investigation of barriers to receipt of matched targeted therapy and assessment of the clinical impact of a precision cancer medicine approach and makes additional sequencing data from pediatric solid tumors available to the research community.

### Table 4. Most Common Drug Classes Implicated by Molecular Characterization in Children With Solid Tumors

<table>
<thead>
<tr>
<th>Target Inhibited by Drug</th>
<th>Targeted Genes Altered in Enrolled Patients</th>
<th>Patients With Alteration, No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDK4/6</td>
<td>CDKN2A/B, CDK4, CDK6</td>
<td>11</td>
</tr>
<tr>
<td>BET bromodomain</td>
<td>MYC, MYCN</td>
<td>6</td>
</tr>
<tr>
<td>BRAF/MEK/ERK</td>
<td>HRAS, NRAS, BRAF</td>
<td>3</td>
</tr>
<tr>
<td>ALK</td>
<td>ALK</td>
<td>3</td>
</tr>
<tr>
<td>PARP</td>
<td>ATM</td>
<td>2</td>
</tr>
<tr>
<td>FGF2, FGF4</td>
<td>MDM2</td>
<td>2</td>
</tr>
<tr>
<td>NTRK</td>
<td>NTRK3</td>
<td>1</td>
</tr>
<tr>
<td>PI3K/mTOR</td>
<td>PI3KCA</td>
<td>1</td>
</tr>
</tbody>
</table>

*Alterations based on variants of uncertain significance are not included. Returned known or suspected pathogenic variants without individualized cancer therapy recommendation included if the reason for no recommendation was no drug available in pediatrics.

### ARTICLE INFORMATION

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