Materials and Methods

**Background:** High-risk neuroblastoma patients have a survival rate below 50% despite dose-intensive chemotherapy. Treatment using molecularly targeted therapy could more effectively manage patients with less toxicity, but would be based on detection and identification of GAs that support responsiveness to such therapies. A recent study by Pugh et al. (2013) found that 16% of neuroblastoma cases demonstrated a gain of function mutation in 3769 exons of 236 cancer-related genes. Herein, we report on the identification of actionable alterations in 17 high-risk neuroblastoma patients and explore their potential impact on targeted therapy.

**Objective:** The objective of this study is to identify actionable alterations in neuroblastoma patients treated with high-risk therapy and explore their potential impact on targeted therapy.

**Methods:**

1. **Sample Collection:** Specimens were collected from 17 high-risk neuroblastoma patients treated with high-risk therapy.

2. **DNA Extraction:** DNA was extracted from the samples using a required minimum of 20% nuclei derived from tumor nuclei.

3. **NGS:** Next-generation sequencing (NGS) was performed on hybridization-captured, adaptor ligation-based libraries using DNA extracted from the samples.

4. **Analysis:** The genomic alterations detected by FoundationOne were further divided into actionable and non-actionable categories.

5. **Clinical Pharmacy and Therapeutics:** The potential impact of actionable alterations on targeted therapy was explored.

**Results/Discussion:**

- **Overview of Clinical Experience:**
  - **CDK4 amplification:** CDK4 amplification was identified in 10% of patients (3/37 cases, range 7-16 copies).
  - **FGFR1 Amplification:** FGFR1 amplification was observed in 18% of cases (3/17 cases, range 7-16 copies).

- **CDK4 amplification as actionable alteration:** CDK4 amplification was identified in 10% of patients (3/37 cases, range 7-16 copies).

- **FGFR1 activating alterations:** FGFR1 activating alterations were identified in 12% of cases (3/25 cases).

- **ALK Base Substitutions:**
  - **ALK** genes were identified in 16% of patients (2/12 cases).

- **BEND5-ALK Fusion:** BEND5-ALK fusion was identified in 9% of patients (2/23 cases).

- **Conclusions:**
  - **CDK4 amplifications occur at a frequency of 10% in neuroblastoma:** This frequency is greater than previously reported frequencies.
  - **Neuroblastoma cases harbored FGFR1 N546K in 12% of cases:** This is a novel finding.
  - **A novel BEND5-ALK fusion was identified in neuroblastoma:** This fusion is predicted to be activating and responsive to crizotinib.
  - **The existence of multiple actionable genomic alterations within neuroblastoma argues for the incorporation of comprehensive genomic profiling into prospective clinical trials with therapy matching.
We identified 361 genomic alterations in 193 pediatric patients (range 0-14, average 2.5 per patient). The most common alterations were found in: TP53, CDKN2A, ALK, and BRAF. At least one actionable alteration was identified in 109 (56%) of these pediatric patients. This approach has led to novel insights into pediatric cancers including novel fusions and non-fusion alterations in known drug targets.

Definition of Actionability:
- FDA approved targeted therapy in tumor type
- FDA approved targeted therapy in another tumor type
- Open clinical trial of therapy targeting alteration in gene
- "Hotspot" panels would not have detected 71% (176/249) of the actionable genomic alterations

Materials and Methods
Hybridization capture of 3,320 exons from 182 cancer-related genes and 37 introns of 14 genes commonly rearranged in cancer (current version of the test) or 3,769 exons from 236 cancer related genes and 37 introns of 14 genes commonly rearranged in cancer (previous version of the test) was applied to ≥ 50ng of DNA extracted from over 190 pediatric FFPE tumor specimens and clinical FFPE specimens including needle biopsies and malignant effusions. Genomic alterations (base substitutions, small indels, rearrangements, copy number alterations) were determined and then reported for these patient samples.

Comprehensive next-generation sequencing-based genomic profiling identifies actionable genomic alterations in diverse pediatric tumor types: The Foundation Medicine (FMI) experience

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Comprehensive NGS-based genomic profiling assay workflow

Introduction
Solid tumor oncology is amidst a paradigm shift with the advent and increasingly successful utilization of targeted therapies that inhibit specific genomic alterations driving an individual patient’s disease. Unfortunately, many pediatric tumors lack approved targeted therapies, and routine genomic profiling of pediatric tumors has yet to be applied in a widespread manner. More comprehensive testing platforms are required to determine the landscape of genomic alterations in pediatric solid tumors and thereby broadly target treatment options. We have developed a solid tumor next-generation sequencing (NGS)-based diagnostic test, optimized for routine clinic FFPE specimens including needle biopsies and malignant effusions, and report here on 193 pediatric patients’ tumors analyzed to date in our CLIA-certified and CAP-accredited laboratory.

Results
Initial 193 Pediatric Cases Profiled

<table>
<thead>
<tr>
<th>Number of Samples</th>
<th>Total number of genomic alterations</th>
<th>Mean number of alterations per sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>193</td>
<td>361</td>
<td>2.2 (0-14)</td>
</tr>
</tbody>
</table>

Pedicant Tumor Types Analyzed

FoundationOne Reveals Actionable Alterations Not Found by "Hotspot" Panels

Summary of Gene Alterations

Summary of Alterations by Tumor Type

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Patients</th>
<th>Samples</th>
<th>Average Alters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Sarcoma</td>
<td>13</td>
<td>41</td>
<td>3.7</td>
</tr>
<tr>
<td>Leukemia</td>
<td>11</td>
<td>40</td>
<td>3.1</td>
</tr>
<tr>
<td>Brain</td>
<td>5</td>
<td>56</td>
<td>2.4</td>
</tr>
<tr>
<td>Soft Tissue Sarcoma</td>
<td>6</td>
<td>60</td>
<td>2.2</td>
</tr>
<tr>
<td>Liver</td>
<td>13</td>
<td>43</td>
<td>1.7</td>
</tr>
<tr>
<td>Kidney</td>
<td>6</td>
<td>7</td>
<td>1.2</td>
</tr>
<tr>
<td>Lung</td>
<td>5</td>
<td>1</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Results and Conclusions

- We identified 361 genomic alterations in 193 pediatric patients (range 0-14, average 2.5 per patient).
- The most common alterations were found in: TP53, CDKN2A, ALK, and BRAF.
- At least one actionable alteration was identified in 109 (56%) of these pediatric patients.
- "Hotspot" panels would not have detected 71% (176/249) of the actionable genomic alterations.

This approach has led to novel insights into pediatric cancers including novel fusions and non-fusion alterations in known drug targets.