The application of genomics to identify diagnostic biomarkers, drivers and therapeutic targets for pediatric cancers

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TRACO
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Outline

- Success and Challenges of Treating Pediatric Cancers
- Genomics
- Next-generation Sequencing
- Application of next-generation sequencing:
  - Diagnosis
  - Identification of molecular target
- Precision Therapy
Childhood cancer

Childhood cancer: The beginning of a modern medical success story

![Diagram showing survival rates for leukemia, lymphoma, and Wilms tumors over two time periods: 1960s and 1990s.](image)

Survival rates have significantly improved over time.

Courtesy: John Maris
Mortality rates

However, in the past 16 years, no improvement in mortality rates despite increased intensity of treatment.
Pediatric cancers

Metastatic, Recurrent, & Refractory Disease Remains Incurable

- **Neuroblastoma**
  - Event-Free Survival (%)
  - Time (years)
  - N=2621; p<0.0001
  - High

- **Ewing's Sarcoma**
  - Overall Survival
  - Event-Free Survival
  - Time (months)
  - Stage 4

- **Metastatic (Spread) Rhabdomyosarcoma**
  - Probability
  - Time (years)
  - Survival
  - 27%
  - 34%
  - 26%

- **Osteosarcoma - Stage 4**
  - Overall survival
  - Event-free survival
  - Years after diagnosis
Gene expression

The dramatic consequences of gene expression in biology

Same genome →
Different expression pattern
Different proteome
Different tissues
Different physiology

Anise swallowtail, *Papilio zelicaon*
Gene expression

...but the complexity and diversity

Same genome or DNA →
- Different expression pattern
- Different proteome
- Different tissues
- Different physiology
Gene expression

Biology is driven by the simultaneous expression of large numbers of genes acting in concert

80% of the Genome is Functional

~25-30,000 Genes
>150,000 Alt Splice

~1000 miRNA
>10,000 ncRNA

3,000,000,000

Proteomics

Phenotype
Cancer Diagnosis & Response to Treatment

Genomics

Transcription
Translation

mRNA
Protein
Human genome
Genomic research

Genomic Research – identification of biomarker, driver, and target
Gene measurement

Challenge: how to measure/detect genes and their products in a massively parallel way?

- High-throughput technologies
- Computational power
First generation tools

1st generation genomic tool: microarrays

Mechanical

Electronic Piezo

Printing microarrays

Lithographic masks and de-protection through illumination

Digital micromirror device (DMD)
Microarrays

Microarrays – technologies of hybridization

1) Targets are isolated and labeled
   - Healthy
   - Cancerous

2) Labeled targets are combined with array

3) Array is washed after hybridization*

4) Hybridized array is scanned
Next-generation sequencing

Next-Generation Sequencing

1. Fragmentation
2. Size Selection
3. Adaptors Ligation
4. Amplification and Sequencing

Ref. Genome

Genomic DNA or RNA
Fragment
DNA Fragments of Similar Sizes
Genomic DNA Library
Align (Map) Reads to Ref. Genome
Genome Sequence
Massively Parallel Sequencing

- Each spot = one Sanger sequencing
- Hundred of millions spot in a flow cell
Genomic Alterations

Genomic alterations detected by DNA sequencing
Genomic Alterations

Genomic Alterations Detected by RNA Transcriptome Sequencing

- Digital Gene Expression
- Expressed Mutations
- Alternative Splicing Events
- Expressed Fusion Transcripts
- RNA editing
- Novel Transcripts
- Non-coding RNAs
Properties

Properties of the next-generation sequencing technologies

- No need to prepare clones for DNA fragments
- No need of prior knowledge for probe design
- Able to detect balanced genome structure changes
- Parallel sequencing at basepair resolution—massive-throughput (up to 100s Gb/run)
- Cheaper (per nucleotide) and faster per genome
Cancer Genomes

Next Generation Sequencing Allows for Comprehensive Analysis of Cancer Genomes on the Same Platform

- DNA
  - Whole Genome
  - Genome Partition (e.g. Whole Exome)
  - Methylation (e.g. MBD)
  - ChIP-seq

- RNA
  - Messenger RNA
  - Non-coding RNA
    - microRNA
    - Other

Next Generation Sequencing

- Copy Number
- Gene Rearangement
- Entire/Novel Methylome
- Damaging Mutations

- Gene Expression
- Chimeric Genes
- Splice Variants
- Novel Transcripts
- Damaging Mutations

Biomarkers: Diagnostic Prognostic
Biology: Drivers
Therapeutic Targets: Mutations
Clinical Vignette

Use of Diagnostic Assay

- 4.5 year old female 2nd opinion from POB, NCI from Germany with questionable Diagnosis
- 6-week history of weight loss, reduced appetite, fever, abdominal pain
- On examination left sided abdominal mass
Wilms tumor

MRI: 9 x 8 x 9 cm mass in upper pole left kidney, tumor in Left renal vein and inferior vena cava

Initial diagnosis: Wilm’s tumor
Cancer diagnosis

Diagnosis of cancers using gene expression profiles

- Wilm’s tumor
- Neuroblastoma

- Patient was switched to high risk neuroblastoma treatment included stem cell transplant
- Doing well 1 yr after diagnosis
Diagnosis

Diagnosis of fusion positive pediatric tumors using whole genome sequencing

Rhabdomyosarcoma

\[ t(2;13) \text{ PAX3-FOXO1} \]

Ewing’s Sarcoma

\[ t(11;22) \text{ EWS-FLI1} \]

Shern et al., Cancer Discovery 2014, 4(2):216-31

Brohl et al., PLOS Genetics 2014, 10(7):e1004629
Rearrangement

Novel in-frame *PAX3-INO80D* fusion with massive 2q rearrangement in RMS, Expression fusion gene verified by RNaseq
Pediatric cancer mutations

Pediatric cancers have a low number of somatic and actionable mutations at initial diagnosis

- Is there an enrichment for actionable mutations at relapse?
- Is the mutational load similarly low at relapse?
- Is there clinical utility of comprehensive genomics of cancers at relapse?
- Feasibility of performing genome guided precision therapy trials in pediatrics?

Somatic mutation frequency (per Mb)
MultiDimensional ClinOomics for Precision Therapy of Children and Adolescent Young Adults with Relapsed and Refractory Cancer: A Report from the Center for Cancer Research

Wendy Chang¹,²,³, Andrew S. Brohl¹,⁴, Rajesh Patidar¹, Sivasish Sindiri¹, Jack F. Shern¹,², Jun S. Wei¹, Young K. Song¹, Marielle E. Yohe¹,², Berkley Gryder¹, Shile Zhang¹, Kathleen A. Calzone⁵, Nityashree Shivaprasad¹, Xinyu Wen¹, Thomas C. Badgett¹,⁶, Markku Miettinen⁷, Kip R. Hartman⁸,⁹, James C. League-Pascual²,⁸, Toby N. Trahair¹⁰, Brigitte C. Widemann², Melinda S. Merchant², Rosandra N. Kaplan², Jimmy C. Lin¹, and Javed Khan¹

Clin Cancer Res. May 2016

Protocol Number: 10-C-0086
Title: “Comprehensive Omics Analysis of Pediatric Solid Tumors and Establishment of a Repository for Related Biological Studies” or Omics protocol
Study Design

• Pilot study to determine the utility and feasibility of performing comprehensive genomic analyses to identify clinically actionable mutations in pediatric and young adult patients with metastatic, refractory or relapsed solid tumors
• 59 patients enrolled to the pediatric oncology branch, Center for Cancer Research (CCR), NCI (2010-2014)
• Age 7 months-25 years
• 20 diagnostic categories (non-CNS, solid tumors)
• Comprehensive multi-omics exome germline & tumor, RNAseq tumor & Illumina Omni SNP arrays of tumor
Mutations

Definitions: Actionable

• **Actionable germline mutation:** loss of function mutation or known hotspot activating mutation of a cancer consensus gene or pathogenic or likely pathogenic mutation of an American College of Medical Genetics (ACMG) Gene

• **Actionable somatic mutation:** genomic alterations that changes the patient’s diagnosis, or may be targeted with FDA approved drugs or in the context of existing clinical trials according to the NCI-adult MATCH-Criteria
Multi-omics integrated landscape

- RNAseq
  - Diagnostic, Driver, Actionable
- DNAseq and RNAseq
  - Somatic: Driver, Actionable
- DNA copy number & RNAseq
  - Somatic: Driver, Actionable
- DNAseq
  - Germ line: Disease causing, Actionable
Fusion genes

Presence or absence of fusion genes and/or expression profiles confirms diagnosis or leads to revision of diagnosis

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Clinical Diagnosis</th>
<th>Age at Enrolment</th>
<th>Prior chemotherapy</th>
<th>Fusion Genes</th>
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Somatic mutations

Somatic mutation burden at relapse increases 2-3X. Not all somatic SNVs are expressed in mRNA.
## Match criteria

### NCI-Adult MATCH
Criteria for Matching Mutation to Drug

<table>
<thead>
<tr>
<th>Level</th>
<th>Match Criteria</th>
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<tbody>
<tr>
<td><strong>Level 1</strong></td>
<td>Gene variant approved for selection of an approved drug (BRAF V600E and vemurafenib). The variant will be Level 1 in all tissues open to treatment with the approved drug.</td>
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<tr>
<td><strong>Level 2a</strong></td>
<td>Gene variant is an eligibility criteria for an ongoing clinical trial for that treatment.</td>
</tr>
<tr>
<td><strong>Level 2b</strong></td>
<td>Gene variant has been identified in an N of 1 responses (TSC1 and everolimus) for that treatment</td>
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</table>
| **Level 3** | Preclinical inferential data (*in vivo* and *in vitro* models) that provide biological evidence sufficient to support the use of a variant for treatment selection, e.g.  
  - Models with variants respond to treatment and models without variant do not respond to treatment  
  - Gain of function mutations demonstrated in pre-clinical model, e.g. D769H variant of ERBB2 results in increased tyrosine kinase-specific activity and up regulates pathway signaling (does not require treatment evidence)  
  - Loss of function genes, tumor suppressor or pathway inhibitor (e.g. NF1) any variant that produces a stop codon including frameshift or demonstrated loss of function in pre-clinical model (does not require treatment evidence) |
**Tumor mutations**

Approximately 50% of Pediatric and Adolescent Young Adults with Cancers have Actionable Tumor Mutations

<table>
<thead>
<tr>
<th>Sample</th>
<th>Diagnosis</th>
<th>Code</th>
<th>Stage</th>
<th>Modality</th>
<th>AA Change</th>
<th>Level</th>
<th>Drug</th>
<th>Clinical Trail</th>
<th>Pediatric</th>
<th>PDA</th>
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</table>

**NCI-Adult MATCH Criteria for Matching Mutation to Drug**

- **Level 1**: Gene variant approved for selection of an approved drug (BRAF V600E and vemurafenib). The variant will be Level 1 in all tissues open to treatment with the approved drug.
- **Level 2a**: Gene variant is an eligibility criterion for an ongoing clinical trial for that treatment.
- **Level 2b**: Gene variant has been identified in an N of 1 responses (TSC1 and exorinhib) for that treatment.
- **Level 3**: Preclinical inferential data (in vitro and in vivo models) that provide biological evidence sufficient to support the use of a variant for treatment selection.
  - Models with variants respond to treatment and models without variant do not respond to treatment
  - Gain of function mutations demonstrated in pre-clinical model, e.g. D769H variant of ERBB2 results in increased tyrosine kinase-specific activity and up-regulates pathway signaling (does not require treatment evidence)
  - Loss of function genes, tumor suppressor or pathway inhibitor (e.g. NF1) any variant that produces, a step, a code in frameshift, or demonstrates loss of function in pre-clinical model (does not require treatment evidence)
Germline mutations

~10% of Pediatric and Adolescent Young Adults with Cancers have Actionable Germline Mutations some Therapeutically

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<tr>
<th>Sample</th>
<th>Diagnosis</th>
<th>Gene</th>
<th>Mutation</th>
<th>Disease</th>
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<th>Notes</th>
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<td>MM</td>
<td>ATM</td>
<td>p.Y380fs</td>
<td>Ataxia-Telangiectasia and Cancer Predisposition Syndrome</td>
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<td>BRCA1</td>
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<td>R175H</td>
<td>Li-Fraumeni Syndrome</td>
<td>Yes</td>
<td>Patient Tumor has LOH of Wild-Type TP53 on Other Allele</td>
<td>No</td>
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<tr>
<td>NCI0226</td>
<td>ACC</td>
<td>TP53</td>
<td>A159K</td>
<td>Li-Fraumeni Syndrome</td>
<td>Yes</td>
<td>Tumor has LOH of Wild-Type TP53 on Other Allele, Novel, 2 Base Non-Frameshift Substitution, c.358_359delGCinsTT</td>
<td>No</td>
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<tr>
<td>MM</td>
<td>TSC1</td>
<td>TSC1</td>
<td>p.S828R</td>
<td>Tuberous Sclerosis Type 1, Lymphangioleiomyomatosis, Focal Cortical Dysplasia, and Everolimus Sensitivity</td>
<td>No</td>
<td>Nonsynonymous SNV, Autosomal Dominant, Patient also has a Germline TSC2 Mutation</td>
<td>No</td>
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<tr>
<td>MM</td>
<td>TSC2</td>
<td>TSC2</td>
<td>p.T246A</td>
<td>Tuberous Sclerosis Type 2, and Lymphangioleiomyomatosis</td>
<td>Yes</td>
<td>Nonsynonymous SNV, Autosomal Dominant, Patient also has a Germline TSC1 Mutation</td>
<td>No</td>
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</tbody>
</table>
Summary

- Demonstrated the importance and feasibility of performing multi-dimensional ClinOmics in the clinical setting in real time
- ~50% of children with pediatric or AYA patients with relapsed or refractory cancers have actionable somatic mutations
- ~10% have actionable germline mutations
- Importance of performing parallel germline sequencing; some therapeutically actionable (e.g. DNA repair, PTEN, TSC1, TSC2, HRAS, RET, ALK)
- Increased tumor burden in relapsed tumors; implications for immunotherapy
- Single agent pediatric MATCH like trials are planned by COG-NCI
Future Trials
Genomics Enabling Precision Therapy-The Future for Pediatric Trials

- Metastatic Disease
- Genomics-Biomarkers
- Good Signature
- Poor Signature

- Amplification
- Mutation
- Translocation
- Over Expression
- Alternative Spliced Gene

Standard Therapy
Targeted Individualized Combinatorial Therapy

KIT
ClinOmics program

CCR ClinOmics Program-CLIA

Patient referred to CCR → Patient enrolled

Obtain Tissue ← ClinOmics Protocol

Experimental Workflow

Research Data → Germline Data → Genetics Board

Somatic Data → Tumor Board

Electronic Medical Record

4 Weeks Turnaround

PI Protocol

Pediatrics & Adults

Precision Therapy
Operational goals

Clinical Genomics Platform
- Enable precision therapy trials for patients with cancer
- Panel & Exome for tumor and normal

Research Comprehensive Genomics
- RNAseq transcriptome analysis of tumor
- SNP arrays tumor, normal
- Methylation arrays tumor

Patient-derived Tumor Models
- PDX from tumor and blood
- Conditional reprogrammed cells from tumor, PDX
- Exome / RNAseq on models

Biobanking & Tissue Repository
- Tumor, germ line
- DNA, RNA
- Plasma, Serum, Urine, Circulating tumor cells, DNA, RNA
Exome vs. Panel

Exome vs. Panel (both CLIA)

- **Exome-All Protein Coding Genes**
  - Mutations in dominant clone
  - Novel driver mutations
  - Actionable secondary/incidental findings in germline in non-cancer genes

- **Panel- Cancer Genes**
  - Validates exome NGS results
  - Deeper coverage allows subclone detection
  - Copy number changes and LOH
  - Fusion gene detection
Clinomica Website

ClinOmics Website for Data Presentation

- NIH credential login
  - Secured data deposit
  - Access control
- Interactive Data viewing
  - QC
  - DNA sequencing: exome and cancer panel
  - RNAseq
QC Report: Sequencing Statistics & Genotyping

### Run Statistics

<table>
<thead>
<tr>
<th>Sample</th>
<th>QC1</th>
<th>QC2</th>
<th>QC3</th>
<th>QC4</th>
<th>QC5</th>
<th>QC6</th>
<th>QC7</th>
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### Genotyping

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</tbody>
</table>

Showing 1 to 15 of 15 entries.
QC report

QC Report: Coverage

Circos

RNA Coverage

Coverage

Hotspot Coverage
**Mutation View**

### Table Example

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<thead>
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<th>Details</th>
<th>VUS</th>
<th>chrome</th>
<th>start</th>
<th>end</th>
<th>ref</th>
<th>alt</th>
<th>gene</th>
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<th>hotspot</th>
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<th>prediction</th>
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<th>cancer1</th>
<th>hgmd1</th>
<th>reported1</th>
<th>correlation1</th>
</tr>
</thead>
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</tbody>
</table>

Status: **active** | **Mutations:** 117/1892

---

Mutation View is a software interface used for analyzing genetic mutations. It provides a detailed view of mutations, including their chromosome positions, gene changes, and associated data such as hotspot predictions and reported mutations.
Conclusions

1. Integrated analysis of the cancer genome identifies biologically relevant diagnostic, prognostic biomarkers and novel targets for therapy
2. Powerful emerging tools of next generation sequencing (including whole genome, exome, and transcriptome) will determine the complete genomic portrait of pediatric cancers at the base pair level
3. This will lead to the identification of key drivers and will enable the development of future novel therapies and precision therapy
Acknowledgements

Oncogenomics Section
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