Evaluating tumor exome sequencing in the oncology clinic: Lessons from the BASIC3 study

A Clinical Sequencing Exploratory Research (CSER) project
Supported by NHGRI/NCI 1U01HG006485

ISMB2014
Post-genomic medical decision making in cancer

Will Parsons, MD PhD
July 14, 2014
Objective – precision oncology

To bring genomic sequencing technologies from the laboratory into the pediatric oncology clinic, providing real-time genetic information that will guide the care of each patient and family.
Challenges (partial list)

1. Limited understanding of the biologically and clinically-relevant genetic alterations
2. Limited number of preclinical models
3. Limited number of available drugs
4. Inexperience with clinical application of genomic technologies
5. Challenges of clinical trial design
   - Small numbers of patients with each tumor subtype
   - Biopsies of refractory tumors often not performed
BASIC$^3$

**Baylor Advancing Sequencing Into Childhood Cancer**

<table>
<thead>
<tr>
<th>PATIENTS</th>
<th>SEQUENCING</th>
<th>CARE</th>
<th>RETURN OF RESULTS</th>
<th>FOLLOW-UP</th>
</tr>
</thead>
<tbody>
<tr>
<td>280 children - newly diagnosed CNS and non-CNS solid tumors</td>
<td>Blood - Tumor</td>
<td>Germline - Somatic</td>
<td>EMR - GCs</td>
<td>Relapse - No relapse</td>
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<tr>
<td>SAMPLES</td>
<td>CLIA-certified whole exome sequencing</td>
<td>MUTATION REPORTS</td>
<td>MDs - Family</td>
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</tbody>
</table>

**Study objectives:**

- To integrate information from **CLIA-certified germline and tumor exome sequencing** into the care of newly diagnosed solid and brain tumor patients at Texas Children’s Cancer Center
- To perform parallel evaluation of the impact of tumor and germline exomes on families and physicians
BASIC³ inclusion criteria

• **Children** less than 18 years old having surgery for CNS or non-CNS solid tumors at Texas Children’s Hospital
  
  – Study enrollment within 60 days of final pathology report

• **Parents** of these children

• **Primary oncologists** of these children
BASIC³

Baylor Advancing Sequencing Into Childhood Cancer

PATIENTS

280 children - newly diagnosed CNS and non-CNS solid tumors

SAMPLES

Blood

Tumor

SEQUENCING

CLIA-certified whole exome sequencing

Germline

Somatic

MUTATION REPORTS

RETURN OF RESULTS

EMR

GCs

MDs

Family

FOLLOW-UP

Relapse

No relapse

PROJECT 1 (CLINICAL)

Provide tumor exome results to clinicians and families

Provide germline exome results to clinicians and families

Identify and consent patients

Measure impact on cancer surveillance and genetic testing of family members

Measure frequency of incidental findings

Measure impact on treatment decisions at relapse (2 yrs)
BASIC³

Baylor Advancing Sequencing Into Childhood Cancer

PATIENTS

- 280 children - newly diagnosed CNS and non-CNS solid tumors

SAMPLES

- Blood
- Tumor

SEQUENCING

- CLIA-certified whole exome sequencing
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- Somatic

MUTATION REPORTS

RETURN OF RESULTS

- EMR
- GCs
- MDs → Family

FOLLOW-UP

- Relapse
- No relapse

PROJECT 2 (SEQUENCING/REPORTING)

- Provide annotated tumor exome results
- Provide annotated germline exome results

Novel reporting platform (iPAD disclosure app)
BASIC³
Baylor Advancing Sequencing Into Childhood Cancer

PATIENTS
280 children - newly diagnosed CNS and non-CNS solid tumors

SAMPLES
Blood
Tumor

SEQUENCING
CLIA-certified whole exome sequencing

Germline
Somatic

MUTATION REPORTS

RETURN OF RESULTS
EMR
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FOLLOW-UP
Relapse
No relapse

Study physician-parent communication of exome results (audio-recording of disclosure visits)

Describe oncologist preferences and values for sharing and use of exome data (through longitudinal interviews)

Describe family preferences and values for receipt and use of exome data (through surveys & longitudinal interviews)

Development of ethical framework to guide shared decision-making for parents and pediatric specialists

PROJECT 3 (ELSI)
Enrollment and sample status

- 150 patients, 223 parents, and 16 oncologists

Yes (83%)

No (17%)
- Overwhelmed (10%)
- Privacy risks (3%)
- Genetic anxiety (2.5%)
- Blood draw (2%)
Tumor Diagnoses of 100 subjects

Non-CNS (n=68)

CNS (n=32)

Tumor available for WES

59/68 (87%)

22/32 (69%)
**BASIC³**

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- Germline
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**RETURN OF RESULTS**

**FOLLOW-UP**
- Relapse
- No relapse

**PROJECT 1 (CLINICAL)**
- Identify and consent patients
- Provide germline exome results to clinicians and families
- Provide tumor exome results to clinicians and families
- Measure impact on treatment decisions at relapse (2 yrs)
- Measure frequency of incidental findings
- Measure impact on cancer surveillance and genetic testing of family members
Clinical whole exome sequencing

• Using 1 ug of DNA isolated from frozen tissue

• Illumina Sequencing Platform
  – Capture exome on Roche-Nimblegen VCRome 2.1 from BCM HGSC
  – Paired tumor/blood samples on one HiSeq lane

• Analysis using platform developed by BCM-HGSC informatics team

<table>
<thead>
<tr>
<th>WES sample</th>
<th>Total Gb per sample</th>
<th>Unique aligned Gb</th>
<th>Mean coverage</th>
<th>Bases 20X coverage</th>
<th>Bases 40X coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germline</td>
<td>18.8</td>
<td>17.5</td>
<td>272</td>
<td>97.5%</td>
<td>95.7%</td>
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<tr>
<td>Tumor</td>
<td>18.8</td>
<td>17.6</td>
<td>276</td>
<td>97.3%</td>
<td>95.1%</td>
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</tbody>
</table>
BCM Mercury pipeline

~10Gbp of Reads
Reads pile-up over exons
Align to Reference Genome

Production & Data QA/QC

Call Variants and Estimate Quality

Variant call format (.vcf)

Annotate Variants

Cassandra

dbSNP
HGMD
TGP
Identification of somatic mutations

"tumor.vcf"

"normal.vcf"

Somatic caller (T-N)

COSMIC:
- Var_ID
- Var_Gene
- Prim_Site
- Source
- PMID

Somatic Mutation Reporting

Annotation

Rare Variants

OMIM, 1000G, dbSNP, HGMD

Germline Mutation Reporting
**Molecular Pathology variant review**

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Total variants</th>
<th>Reported variants</th>
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</thead>
<tbody>
<tr>
<td>Neuroblastoma</td>
<td>94</td>
<td>4</td>
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<tr>
<td>Medulloblastoma</td>
<td>125</td>
<td>4</td>
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<tr>
<td>Neuroblastoma</td>
<td>144</td>
<td>20</td>
</tr>
<tr>
<td>Wilms tumor</td>
<td>118</td>
<td>14</td>
</tr>
<tr>
<td>Anaplastic ependymoma</td>
<td>75</td>
<td>2</td>
</tr>
</tbody>
</table>

“soma.vcf”: >20 quality metrics >50 genotype + annotation features

Apply Filters

- Total Cov T ≥ 50X
- Q20 Var Cov T ≥ 6X
- Exon non-synon SNV + Splicing
  + Remove misaligned artifacts

Confirmation: Sanger seq

Interpret and Rank

Report
Clinical utility of tumor WES

- **Category I mutations**
  - Established clinical utility for the tumor type tested

- **Category II mutations**
  - Potential clinical utility
  - Genes that are members of cancer pathways, gene families, or functional groups; targets of approved or investigational therapeutic agents

- **Category III mutations**
  - In consensus cancer genes (not included in I and II)

- **Category IV mutations**
  - All other mutations

**Examples:**
- ALK - neuroblastoma
- MET - neuroblastoma
- PHF6 - neuroblastoma
- XIAP - neuroblastoma

A. Roy & F. Monzon
Tumor exome results

• Recurrently-mutated genes
  – \textit{CTNNB1} (8/80; 10%)
  – \textit{TP53} (4/80, 5%)
  – \textit{BRAF} (3/80, 4%)
  – \textit{KIT, KRAS, ARID1A, TSC2} (each 2/80, 2%)

• Genes mutated in only one tumor:
  – \textit{ALK, NRAS, MET, JAK2, JAK3, FGFR3, NTRK2, FBXW7, TSC2, SMARCA4, DDX3X, NF2, DICER1, NOTCH3, BRCA1, BRCA2, WT1}...

\textbf{n = 81 exomes}
Tumor exome results

Median of 8 somatic mutations **PER PATIENT**

*Note: Cat 4 mutations have not been validated*
Tumor exome results

**HIGHEST category of mutation PER PATIENT**

- **Cat. 1** (2%)
  Mutations of known clinical utility in that tumor type

- **Cat. 2** (26%)
  Mutations of potential clinical utility

- **Cat. 3** (23%)
  Mutations in other “cancer genes”

- **Cat. 4** (49%)
  Only mutations in “non-cancer genes”

n = 81 exomes
14 y/o girl with metastatic OS

Tumor exome sequencing result: frameshift mutation in TSC2 (and loss of other allele)

- Diagnosis: metastatic osteosarcoma
- Prognosis: poor
- Tumor exome result: TSC2 mutation
- Clinical consideration: use of mTOR inhibitor?
7 y/o boy with malignant liver tumor

- Diagnosis: malignant epithelial hepatic neoplasm
- Prognosis: poor
- Tumor exome result: NRAS mutation
- Clinical consideration: targeting RAS pathway?

NRAS c.181C>A, p.Q61K
14 y/o boy with medulloblastoma

Tumor exome sequencing results:

- **CTNNB1** p.D32Y
- **DDX3X** p.V345L
- **ARID1B** c.3345+1G>A
- **FOXO3** p.W233X

β-catenin: subset of tumor cells with nuclear reactivity

*Courtesy of A. Roy*
16 year old girl with GBM

Tumor exome sequencing results:

- **H3F3A** p.G34R
- **ATRX** p.Q2192X
- **TP53** p.R273H
- **BCOR** p.S1261fs
- **MED12** p.R1994W

TP53
BASIC³

Baylor Advancing Sequencing Into Childhood Cancer

**Patients**

- 280 children - newly diagnosed CNS and non-CNS solid tumors

**Samples**

- Blood
- Tumor

**Sequencing**

- CLIA-certified whole exome sequencing

**Mutation Reports**

- Germline
- Somatic

**Care**

- Return of results
  - EMR
  - GCs
  - MDs
  - Family

**Follow-up**

- Relapse
- No relapse

**Complementary Genomic Analyses**

- CGH/SNP arrays
- Whole genome sequencing
- RNA sequencing
- Tumor heterogeneity
- Treatment response/resistance
- Other research studies

**Additional**

- Additional patient blood sample
- Additional parental blood sample
- Tumor sample from first surgery
- Tumor samples from additional surgeries
RNAseq – integration with WES

14/44 (32%) Category 4 mutations expressed

Linghua Wang & David Wheeler, Baylor College of Medicine HGSC
Preclinical model development

- Same location
- Same depth
- Same cell number (vol)

**Intra-cerebral (IC)**
**Intra-cerebellar (ICb)**
**Intra-brain stem (IBs)**

- Microsurgical drill: Burr hole (0.7 mm in diameter)
- 10 μl Hamilton syringe
- Depth: 3 mm for intra-cerebrum, 3 mm for intra-cerebellum, 6 mm for intra-brain stem
- Cell suspension: 2 μl

**Integrated genomic characterization**
- Primary tumor, blood, normal, xenograft, cell line(s)
- WES, RNA-seq, CGH/SNP array, WGS

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Cases</th>
<th>Exome sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medulloblastoma</td>
<td>27</td>
<td>13</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>DIPG</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>ATRT</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Germinoma</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Ependymoblastoma</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>sPNET</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ganglioglioma</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>65</strong></td>
<td><strong>36</strong></td>
</tr>
</tbody>
</table>

Xiao-Nan Li, MD PhD
**BASIC³**

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**Provide tumor exome results to clinicians and families**

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**Measure impact on treatment decisions at relapse (2 yrs)**
Diversity of germline exome results

**TUMOR REPORT**
- All
- **All Somatic**
  - **BRAF V600E**

**GERMLINE REPORT**
- Gene
  - Other Medically Actionable
  - PCG Genes
- Other Phenotype
  - Cancer or Other Patient Phenotype
- Genes
  - *CFTR* D
- Muta&on
  - Pathogenic
  - VUS
  - Pathogenic
  - FDA Indication
  - Pathogenic
- Example
  - *DICER1* nonsense
  - Rare *WT1* missense
  - *SCN5A* mut
  - *CYP2A* mut
  - *CFTR* DF508

Opt-In
14 year old girl with GBM

- Sequencing revealed **c.1697delA frameshift mutation in** *MSH2* transmitted from her mother.
- *MSH2* mutations are associated with increased risk of multiple cancers, including glioma.
- **Cancer screening recommendations** made for patient, mother and other *MSH2* positive family members.
## Mutations related to phenotype

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>N</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autosomal dominant</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Suspected clinically and ordered as part of care</td>
<td>4</td>
<td><em>DICER1, VHL, TP53 x 2</em></td>
</tr>
<tr>
<td>Not considered by clinical team but FH+</td>
<td>3</td>
<td>*MSH2, BRCA1, BRCA2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>*WT1 (mosaic)</td>
</tr>
<tr>
<td>Autosomal recessive; single mutation</td>
<td>4</td>
<td><em>FANCL x 2, FANCA, MUTYH</em>; Oncologist discretion to pursue work-up further</td>
</tr>
<tr>
<td>Diagnostic for other medical problems</td>
<td>3</td>
<td><em>TJP2 - Liver disease/HCC; CLCN5-renal disease; MNX1- neurologic disorders</em></td>
</tr>
</tbody>
</table>

*One TP53 described as likely deleterious in text of report*
Mutations unrelated to phenotype

• 3 patients with mutations on the ACMG list
  – *SCN5A* (long QT syndrome)
  – *DSP* (cardiomyopathy)
  – *LDLR* (familial hypercholesterolemia)
• 1 patient with *TNFRSF13B* immunodeficiency mutation
• 2 patients with mtDNA mutations including MELAS
• Referrals to appropriate TCH clinics made and evaluation initiated based on incidental finding
• Larger dataset of 2000 exomes at BCM-WGL found closer to 4.5% of actionable mutations reported
Other common germline findings

- Median of 3 VUS in genes related to phenotype
  - 98% carry at least one VUS in cancer genes
  - Generally no further follow-up of these variants at this time

- Median of 2 recessive carrier mutations
  - Majority are very rare syndromes
  - Only 2-3% carry mutation in a gene on the ACMG population screening list for recessive disorders
  - Can identify X-linked carrier status as well

- Median of 2 FDA pharmacogenetic variants
## Yield of germline/tumor WES (n=100)

<table>
<thead>
<tr>
<th>Germline</th>
<th>1</th>
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</table>

### Germline

- Diagnostic cancer susceptibility: 8
- Diagnostic non-cancer: 3
- Medically actionable: 7

### Tumor

- Category 1: 2
- Category 2: 18

40%
Conclusions

• Pipeline for clinical sequencing of patient tumor and blood samples successfully established

• Mutations of potential clinical utility identified by tumor/germline WES in ~40% of pediatric solid tumors

• Diversity of mutations identified suggests that genome-scale (or more pediatric-specific targeted) diagnostic approaches may be favored

• The most useful genomic “test” (or combination of tests, e.g WES and RNAseq) remains to be proven

• Further characterization of both temporal and intra-tumoral heterogeneity will be necessary
Looking forward

• There is significant patient/family interest in these clinical genomic “personalized” approaches

• Specialized expertise/training is necessary for informed consent and result reporting for clinical genomics

• Major interpretive challenges remain (the definition of “actionable” is a fairly loose one)

• It will be critical to understand the preferences of physicians and families for reporting of genomic data

• For any given patient the tumor or germline report may be more informative

• Clinical trials are needed to test if clinical genomics will be beneficial for pediatric oncology patients
A Clinical Sequencing Exploratory Research (CSER) project Supported by NHGRI/NCI 1U01HG006485

**BASIC³ Project 1 (clinical)**
- Sharon Plon, MD, PhD (Project PI)
- Will Parsons, MD, PhD (Project PI)
- Murali Chintagumpala, MD (co-I)
- Stacey Berg, MD (co-I)
- Susan Hilsenbeck, PhD (co-I)
- Tao Wang, PhD (co-I)

**BASIC³ Project 2 (sequencing and reporting)**
- Richard Gibbs, PhD (co-PI)
- Christine Eng, MD (co-PI)
- Yaping Yang, PhD (co-I)
- Angshumoy Roy, MD, PhD (co-I)
- Federico Monzon, MD (co-I)
- David Wheeler, PhD (Co-I)
- Donna Muzny, MS

**BASIC³ Clinical Project Team**
- TXCCC pediatric oncologists
- Robin Kerstein, MT, CCRA
- Sarah Scollon, MS, CGC
- Katie Bergstrom, MS, CGC
- Stephanie Gutierrez (Data manager)
- Ryan Zabriskie (Laboratory manager)

**TCH/BCM Pathology**
- Angshumoy Roy, MD, PhD
- Dolores López-Terrada, MD, PhD
- Adekunle Adesina, MD, PhD

**TCH Surgery and Neurosurgery**

**BCM/TCH leadership**
- David Poplack, MD
- Susan Blaney, MD
- Arthur Beaudet, MD
- James Versalovic, MD, PhD
- Jed Nuchtern, MD

**BASIC³**

**BCM Advancing Sequencing Into Childhood Cancer Care**