Development and clinical application of an integrative genomic approach to personalized cancer therapy

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May 17, 2016
Personalized cancer therapy (PCT)

• Goal
  – recommend personalized therapeutics, clinical trials for each cancer patient based on her/his genetic and genomic profiles

• Experiments
  – Tumor: WES, genotyping, RNA-Seq
  – Blood: WES, genotyping
  – Adjacent normal: RNA-Seq when available
Our cohort of patients who received genomics reports

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (N=46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis of most recent primary (median and range, years)</td>
<td>48 (12-69)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>26 (56.5%)</td>
</tr>
<tr>
<td>Men</td>
<td>20 (43.5%)</td>
</tr>
<tr>
<td>Cancer type</td>
<td></td>
</tr>
<tr>
<td>Colorectal</td>
<td>18 (39.1%)</td>
</tr>
<tr>
<td>Other (single-primary)</td>
<td>7 (15.2%)</td>
</tr>
<tr>
<td>Breast</td>
<td>6 (13.0%)</td>
</tr>
<tr>
<td>Multiple primaries</td>
<td>6 (13.0%)</td>
</tr>
<tr>
<td>Medullary thyroid carcinoma</td>
<td>5 (10.9%)</td>
</tr>
<tr>
<td>Unknown primary</td>
<td>4 (8.7%)</td>
</tr>
<tr>
<td>Had metastatic disease at diagnosis</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>21 (45.7%)</td>
</tr>
<tr>
<td>No</td>
<td>23 (50.0%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (4.3%)</td>
</tr>
<tr>
<td>Sequenced tumor specimen type</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>22 (47.8%)</td>
</tr>
<tr>
<td>Metastatic</td>
<td>13 (28.2%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (8.7%)</td>
</tr>
<tr>
<td>Primary and metastatic</td>
<td>3 (6.5%)</td>
</tr>
<tr>
<td>Lymph node</td>
<td>2 (4.3%)</td>
</tr>
<tr>
<td>Primary and lymph node</td>
<td>1 (2.2%)</td>
</tr>
<tr>
<td>Local recurrence</td>
<td>1 (2.2%)</td>
</tr>
</tbody>
</table>
**Patient**
- tumor tissue
- blood (normal)
- adjacent normal tissue (if available)

**Assays**
- whole-exome sequencing (Illumina HiSeq 2500)
- targeted panel (Ion PGM Hotspot v2)
- SNP genotyping (OmniExpress Exome array)
- RNA-Seq (HiSeq 2500)

**Bioinformatics**
- variant calling (somatic and germline)
- copy number variation
- gene fusions
- RNA abundance changes

**In-house knowledge base**
- technical (QC)
- biological / medical
- mirrors of public and private databases

**Work flow**

**Deliver final findings to patient & treating oncologist**

**Internal meeting to review draft summary documents**

**Treatment suggestions**
- Functional annotation and impact prediction of all variants
- Integrated pathway analysis (TCGA-based): gain or loss of pathway activity
- Literature and drug knowledge mining
- Germline variant analysis

**Integrated pathway analysis (TCGA-based): gain or loss of pathway activity**
Selection of genomic assays

- gDNA < 1.5µg for either normal or tumor specimen
  - Only targeted panel assay was run
- gDNA 1.5-2.5µg for both normal and tumor
  - Both targeted panel and WES were run
- gDNA 2.0-2.5µg
  - WES libraries were attempted up to two times
- gDNA > 2.5µg
  - All assays (targeted panel, WES, and SNP microarray) were run
What do the genomic findings look like when presented to patient and treating physician?

### Molecular Analysis Summary: Tumor Classification

### Analysis Summary: Predictive

### Clinical Trial Connection

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Phase</th>
<th>Title</th>
<th>Target</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT01750918</td>
<td>Phase I/II</td>
<td>BRAF/MEK/EGFR Inhibitor Combination Study in Colorectal Cancer</td>
<td>BRAF, MEK, EGFR</td>
<td>US GSK Clinical Trials Call Center 877-379-3718</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><a href="mailto:GSKClinicalSupportHD@gsk.com">GSKClinicalSupportHD@gsk.com</a></td>
</tr>
<tr>
<td>NCT01347866</td>
<td>Phase I</td>
<td>Clinical Study Of PI3K/mTOR Inhibitors In Combination With An Oral MEK Inhibitor Or Irinotecan In Patients With Advanced Cancer</td>
<td>MEK, PI3K/mTOR</td>
<td>Pfizer CT. gov Call Center 1-800-718-1021</td>
</tr>
<tr>
<td>NCT01927341</td>
<td>Phase Ib/II</td>
<td>Phase Ib/II Study of Efficacy and Safety of MEK162 and Panitumumab, in Adult mCRC Patients With Mutant or Wild-type RAS Tumors</td>
<td>MEK</td>
<td>Novartis Pharmaceuticals 1-888-669-6682</td>
</tr>
<tr>
<td>NCT02079740</td>
<td>Phase I/II</td>
<td>Trametinib and Navitoclax in Treating Patients With Advanced or Metastatic Solid Tumors</td>
<td>MEK, BCL2</td>
<td>Principal Investigator: Ryan Corcoran Dana-Farber Cancer Institute 617-726-8599 <a href="mailto:rbcorcoran@partners.org">rbcorcoran@partners.org</a></td>
</tr>
<tr>
<td>NCT01351103</td>
<td>Phase I</td>
<td>A Study of Oral LGK974 in Patients With Malignancies Dependent on Wnt Ligands</td>
<td>PORCN (Wnt Signaling pathway)</td>
<td>Novartis Pharmaceuticals 1-888-669-6682</td>
</tr>
</tbody>
</table>
Strength of integrative approach

• **Identify more cancer relevant mutations and more actionable alterations.**
  • Enable data interpretation at pathway level
  • Identify novel or rare activating mutations
  • Germline variants – pharmacogenomic biomarkers; cancer predisposing variants for prognosis and therapeutic implications.
• RNAseq – confirm SNVs/indel; prioritize/validate CNVs; cancer sub-classification; gene fusion; gene expression biomarkers without genetic level alterations.
### Comparative analysis of integrative genomic approach and cancer panels

<table>
<thead>
<tr>
<th>Genomic approach</th>
<th>Mean number of cancer-relevant somatic mutations (range)</th>
<th>Number of patients with tier 1 drug recommendations</th>
<th>Number of patients with tier 2 drug recommendations</th>
<th>Number of patients with actionable alterations</th>
<th>Mean number of actionable alterations (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion AmpliSeq Cancer Hotspot Panel v2</td>
<td>1.3 (0-4)</td>
<td>24 (52%)</td>
<td>16 (35%)</td>
<td>24 (52%)</td>
<td>0.65 (0-3)</td>
</tr>
<tr>
<td>Oncomine Comprehensive Panel</td>
<td>2.5 (0-11)</td>
<td>39 (85%)</td>
<td>24 (52%)</td>
<td>41 (89%)</td>
<td>2.4 (0-6)</td>
</tr>
<tr>
<td>FoundationOne</td>
<td>3.7 (0-22)</td>
<td>39 (85%)</td>
<td>24 (52%)</td>
<td>41 (89%)</td>
<td>2.6 (0-7)</td>
</tr>
<tr>
<td>This study</td>
<td>17.3 (1-79)</td>
<td>40 (87%)</td>
<td>26 (57%)</td>
<td>42 (91%)</td>
<td>4.9 (0-14)</td>
</tr>
</tbody>
</table>

Of 4.9 actionable alterations, 1.5 were somatic mutations, 0.6 were CNAs, 2.2 were germline variants, 0.7 were gene expression alterations
Actionable alterations by tumor type

**Actionable** = any alteration that has clinical implications for:

- **Tier 1 therapeutics**
  - FDA-approved for this cancer
- **Tier 2 therapeutics**
  - any therapeutics (including experimental) whose molecular basis of action is relevant given the patient’s dysregulated pathways

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Tier 1</th>
<th>Tier 2</th>
<th>Tier 1&amp;2</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>breast</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CRC</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MTC</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>other</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

N = 7               18              5               16
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  - RNAseq – confirm SNVs/indel; prioritize/validate CNVs; cancer sub-classification; gene fusion; gene expression biomarkers without genetic level alterations.
Enable data interpretation at pathway level

Colon cancer

Breast cancer (ER+/PR+/Her2-)

Squamous cell carcinoma (skin)
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A case study

• Diagnosed with cancer of unknown primary at age 55
• Genomic analysis of a metastatic liver tumor, which was classified as poorly differentiated adenocarcinoma with signet ring features
• No known somatic mutations with available targeted therapeutic agents
• A novel EGFR D587H somatic mutation
  – Close to hotspots located at P596 and G598
EGFR mutation frequencies from TCGA

- D587 is located near hotspot at G598 within domain IV
Treatment course was changed based on a rare activating EGFR mutation

- EGFR auto-phosphorylation is augmented by D587H
- D587 activates EGFR signaling
- Recommended targeted anti-EGFR therapy
- This mutation would not be called somatic if tumor-only sequencing were performed using cancer panels
Strength of integrative approach

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• Identify novel or rare activating mutations
• Germline variants – pharmacogenomic biomarkers; cancer predisposing variants for prognosis and therapeutic implications
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Germline variants infers pharmacogenomics biomarkers

- A metastatic colorectal cancer case
- Genomic profiling report
  - Predicting insensitivity to cetuximab based on NRAS Q61R
  - Germline variants in KDR and CXCR2 associated with increased benefit to bevacizumab
  - Germline variants in ERCC1, ERCC2, ERCC5, XRCC1 associated with decreased benefit to oxaliplatin
- Altered treatment course
  - Treatment with bevacizumab and 5-FU resulted in brisk response that allowed for cryoablation of remaining oligometastatic lung disease
  - Initial platinum-based regimen (oxaliplatin) had limited efficacy
- Complete remission for 16 months
cancer predisposing variants for prognosis and therapeutic implications

• A breast cancer case

• Identified BRCA1 W1712fx germline variant

• Recommendation for Cisplatin chemotherapy
Strength of integrative approach

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• Identify novel or rare activating mutations
• Germline variants – pharmacogenomic biomarkers; cancer predisposing variants for prognosis and therapeutic implications
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RNA-Seq augments the utility of genetic testing I

• More accurate molecular characterization
  – A breast cancer case
  – Discrepancy between pathology and RNA-Seq
    • Pathology: ER+/PR-/HER2-
    • RNA-Seq: Basal like
  – Only 10% tumor nuclei stained positive for ER, ER staining was weak (1+).
RNA-Seq augments the utility of genetic testing II

• Driver pathways are activated by abnormal expression in the absence of genetic alteration
  – A quadruple negative colon cancer case
  – Expression of EGFR ligands epiregulin and amphiregulin were elevated by 113 and 29 fold
  – Predicting favorable outcome in response to cetuximab treatment
Limitation of comprehensive integrative genomic approach

• Cost of WES and RNA-Seq are higher
• Longer time for data generation and interpretation
• Higher requirement for sample quantity and quality
• Lower sequencing depth
Recommendation

- A stagger approach
- Targeted panel sequencing first
- Progress to deeper characterization if actionable alteration are not identified
- Selecting WES depth based on initial tumor purity estimate from the panel
Follow up patient survey

• 10 patients consented for survey
  – 1 consented but chose not to respond
• 78% (7 out of 9) stated the genomic study findings met their expectation
• All 9 patients expressed some difficulty understanding the findings
• All 9 patients discussed results with their treating physicians
• 67% (6 out of 9) stated that findings are useful
• The course of treatments were altered for 4 patients
Summary

• An integrative approach to personalized cancer therapy (WES, tumor/match normal, RNA-Seq)
  – Identify more cancer relevant mutations and more actionable alterations
  – Enable data interpretation at pathway level
  – Identify novel or rare activating mutations
  – Germline variants for pharmacogenomic biomarkers, prognosis and therapeutic implications
  – RNAseq for cancer sub-classification and gene expression biomarkers without genetic level alterations

• Recommend a stagger approach
My team
Acknowledgements

Collaborators

• Mount Sinai School of Medicine
  – Eric Schadt
  – Johan Bjorkegren
  – Jason Kovacic
  – Robert Sebra
  – Lisong Shi
  – Giulio Pasinetti
  – Lisa Edelmann
  – Joel Dudley
  – Eliza Geer
  – Andrew Steward
  – John Martignetti
  – Janina Longtine
  – Michael Donovan
  – Ke Hao
  – George Diaz
  – Jason Bobe

• Uconn Health
  – Andrew Arnold

• Sage Bionetworks
  – Stephen Friends

• Geisinger Health System
  – David Carey
  – Uyenlinh Mirshahi
  – Michael Murray

• Columbia University
  – Hongxia Ren

Consortium

• Uk10K
• UK Biobank
• 1000 genome
• GERA
• Wellderly
• TCGA
• ExAC
• dbGap
• ......

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