Towards Precision Medicine:
A Framework for Clinical Genome Sequencing in Cancer

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Relevant Disclosures:

A.M.C. serves on the Scientific Advisory Board or as a consultant to Life Technologies, Paradigm, Hologic, and MolecularMD.
The Application of High-Throughput Sequencing for Precision Cancer Therapy

**MI-ONCOSEQ**: The Michigan Oncology Sequencing Program

*Roychowdhury et al, November 2011*
Why we need a comprehensive sequencing approach to personalize cancer therapy?

• Tumors generally have a private, “combination” of mutations or rare driver mutations (1-5%) (e.g., RAF fusions or AKT mutations in prostate cancer, ALK fusions in lung cancer).

• Targeted sequencing assays can miss new “drivers”

• To molecularly enrich Phase 1 and 2 clinical trials of targeted therapies

• Characterize mechanisms of resistance with repeat tumor biopsy and assessment

• Opportunity for new discoveries
MI-ONCOSEQ: The Michigan Oncology Sequencing Center

Informed Consent
Genetic Counseling
Tumor Biopsy
Buccal swab or Blood
Sequencing
Analysis
1) Actionable Results?
2) Incidental Results?
Precision Medicine Tumor Board
Genetic Counselor
Disclosure of Results
Individualized Therapy Based on Molecular Enrichment (Any Histology)

Molecular Eligibility?

- PIK3CA mut
- CDK6 amp
- FGFR1 amp
- AR amp
- TCL1A
- PI3KR2 mut
- PTEN del

Tumor Genomic Analysis

- PI3K inhibitor
- CDK inhibitor
- FGFR inhibitor
- Anti-androgen
- AKT inhibitor
- PI3K inhibitor

1) Molecular enrichment and eligibility
2) Endpoints: Response Rate
3) Comparison of primary responders versus non-responders
4) Pre-treatment and Post-resistance biopsy: mechanisms of resistance
Clinically Relevant Timeframe?

Tissue Biopsy to Sequencing Results

- **Tumor Biopsy**: Day 1
- **Pathology**: Day 2
- **Sample Prep**: Day 3
- **Sequencing**: Day 4-17
- **Analysis**: Day 17-26
- **Sequence Results**: Day 27-30

MI-ONCOSEQ
# Multi-Disciplinary Precision Medicine Tumor Board

## Diagram

- **Clinical Oncology**
- **Genomics and Informatics**
- **Pathology**
- **Clinical Genetics**
- **Patient Advocate**

## Table

<table>
<thead>
<tr>
<th>Class</th>
<th>Subtype</th>
<th>Example 1 (Patient with colorectal cancer)</th>
<th>Example 2 (Hypothetical)</th>
</tr>
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<tbody>
<tr>
<td><strong>Class 1</strong></td>
<td><strong>Approved Agents</strong></td>
<td><strong>KRAS wildtype in metastatic colorectal cancer and cetuximab</strong></td>
<td><strong>BRAF mutation in melanoma and vemurafenib</strong></td>
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<tr>
<td></td>
<td>A: FDA-approved indication</td>
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<td>B: Off Label</td>
<td><strong>KRAS wildtype and erlotinib</strong></td>
<td><strong>BRAF mutation in cholangiocarcinoma and vemurafenib</strong></td>
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<tr>
<td><strong>Class 2</strong></td>
<td><strong>Investigational Agents</strong></td>
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<td>A. Phase 2, 3 clinical trials</td>
<td><strong>NRAS mutation and downstream of RAS signaling such as MEK inhibitors</strong></td>
<td><strong>BRAF mutation in cholangiocarcinoma and RAF inhibitor</strong></td>
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<td>B. Phase 1 clinical trials</td>
<td><strong>CDK8 amplification and CDK inhibitors</strong></td>
<td><strong>PIK3CA mutation in breast cancer and PI3K inhibitor</strong></td>
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<td><strong>Class 3</strong></td>
<td><strong>Pre-clinical</strong></td>
<td><strong>TP53 mutation and deletion</strong></td>
<td><strong>AKT mutation in prostate cancer and AKT inhibitor</strong></td>
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<td>A. In vivo or in vitro</td>
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<td>B. Potential Research</td>
<td><strong>AURKA kinase domain mutation</strong></td>
<td><strong>Novel kinase domain mutation</strong></td>
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<td><strong>Class 4</strong></td>
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<td><strong>CCR3 mutation</strong></td>
<td><strong>CCR3 deletion</strong></td>
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<td>Unknown Significance in cancer</td>
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MI-ONCOSEQ
Case Examples
Case 1:  
Discovery of a pathognomonic gene fusion for a rare cancer

- 44 y.o. woman had surgery and post-op radiation for anaplastic meningeal malignant Solitary Fibrous Tumor (SFT)/hemangiopericytoma in 2002
- Presented to MI-ONCOSEQ program, CT-guided liver biopsy was performed

referred by Dr. Scott Schuetze
Discovery of the NAB2-STAT6 gene fusion

Robinson et al, Nature Genetics 2013
Recurrent NAB2-STAT6 fusions in SFT/hemangiopericytoma

52/52 cases of SFT from MSKCC (C. Antonescu)
U.Mich (L. Kunju)

Robinson et al, *Nature Genetics* 2013
A novel mechanism of oncogenesis: Conversion of a transcriptional repressor into an activator by gene fusion

Robinson et al, Nature Genetics 2013
Case 2: Discovery of an actionable gene fusion in a pediatric leukemia

- 9 yr old girl diagnosed with Pre-B ALL in 2008
- 83% blasts in bone marrow
- Initially treated with COG high risk ALL protocol AALL0232
- CNS relapse
- No match for BMT so underwent COG AALL 0433 and whole brain radiation
- Improved but relapsed again during a Make-A-Wish trip
- BMT and COG AAML 0531 protocol
- Developed left sided chest pain approximately mid May 2012 and was found to have a left-sided pleural effusion.
- Pleural fluid and bone marrow were collected on 6/6/12 for the study.

Referred by Dr. Rajen Mody
Discovery of an actionable ETV6-ABL1 fusion in a pediatric oncology patient
CLIA Validation of ETV6-ABL Fusion by Cytogenetics

Normal Metaphase

PO_3003

Green: ETV6 probe; Red: ABL probe
The fusion of TEL and ABL in human acute lymphoblastic leukaemia is a rare event.

Department of Paediatrics II, University of Ulm, Germany.

Abstract
We have recently identified a common ALL patient which harboured a chromosomal fusion between the TEL gene on chromosome 12 and the ABL gene on chromosome 9. We designed an RT-PCR assay to screen 186 adult ALL and 30 childhood ALL patients for this novel translocation. We were unable to identify any additional cases with a TEL/ABL fusion product.

Molecular cytogenetic and clinical findings in ETV6/ABL1-positive leukemia.

Centre for Medical Genetics, Ghent University Hospital, Ghent, Belgium.

Abstract
Rearrangements of 12p, resulting from deletions or translocations, are common findings in hematologic malignancies. In many cases, these rearrangements target the ETV6 gene (previously called TEL) located at 12p13. Various partner genes have been implicated in the formation of fusion genes with ETV6. These include PDGFRB, JAK2, NTRK3, ABL2, and ABL1, each of which encodes for proteins with tyrosine kinase activity. To date, ETV6/ABL1 transcripts have been detected in only four patients with a leukemic disorder. Here, we describe one adult with chronic myeloid leukemia and a child with T-cell acute lymphocytic leukemia with ETV6/ABL1. Molecular cytogenetic analysis confirmed that formation of an ETV6/ABL1 fusion in these patients required at least three chromosomal breaks and showed that each of these translocations is the result of a complex chromosomal rearrangement. Molecular analysis showed the presence of two fusion transcripts in both patients as the result of alternative splicing, questioning the suggested role of these transcripts in the lineage specificity. Clinical findings of these patients were compared to those of previously reported cases, and the possible clinical and biological similarities between ETV6/ABL1 and other fusion genes leading to increased tyrosine kinase activity are discussed.
Cells from ETV6-ABL1 patient are sensitive to imatinib in vitro

Patient was enrolled in an Imatinib + chemo trial and is currently in remission (> 1yr)
Cases 4, 5, 6, 7: Identification of Targetable FGFR Gene Fusions in Diverse Cancers

Wu et al, Cancer Discovery 2013
Cholangiocarcinoma

FGFR2-BICC1 gene fusion

Case 1: MO_1636

- Patient: 34-yr old female
- Cancer type: Cholangiocarcinoma
- SNVs: 8 mutations (ARID1A, FBXW7)
- Gene fusions: 8 fusions (FGFR2-BICC1)

FGFR2-BICC1 fusion (1663 a.a.)

Gene copy number ratios by chromosome

Case 2: MO_1039

- Patient: 61-yr old male
- Cancer type: Cholangiocarcinoma
- SNVs: 27 mutations (TP53)
- Gene fusions: 5 fusions (FGFR2-BICC1)

FGFR2-BICC1 fusion (1663 a.a.)

Gene copy number ratios by chromosome

Case 4: MO_1081

- Patient: 57-yr old male
- Cancer type: Prostate cancer
- Gene fusions: 8 fusions (SIL2R1/FITR2)

Wu et al, Cancer Discovery 2013
Metastatic breast cancer

**FGFR2-AFF3 gene fusion**

*Wu et al, Cancer Discovery 2013*
Metastatic prostate cancer

$SLC45A3$-$FGFR2$ gene fusion

Wu et al, *Cancer Discovery* 2013
FGFR-TACC fusions in Solid Tumors

- FGFR-TACC fusions in 3% of GBM (Singh et al, Sept 2012, *Science*; Parker et al Feb 2013, *JCI*)
- Proposed mechanisms of actions of fusion protein include 1) mislocalization to mitotic spindle and 2) loss of miR binding site
## FGFR Fusions in Diverse Cancers

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<th>FGFR2 Fusions</th>
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<td>FGFR2-CCDC6</td>
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<td>CCDC6</td>
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<td>FGFR2-KIAA1967</td>
<td>LUSC(n=1)</td>
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<td>SLC45A3-FGFR2</td>
<td>Prostate cancer</td>
<td>n=1</td>
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<td>SLC45A3</td>
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| FGFR3 Fusions          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| FGFR3-TACC3            | Exon16-exon4-exon10-RT-4 | n=1      | TK       | TK       | TACC3    | 783      | 1488     |          |          |          |          |          |          |
| FGFR3-TACC3            | Exon16-exon10-Oral cancer | n=1      | TK       | TK       | TACC3    | 780      | 966      |          |          |          |          |          |          |
| FGFR3-TACC3            | Exon18-exon11-Bladder cancer | n=3      | TK       | TK       | TACC3    | 760      | 951      |          |          |          |          |          |          |
| FGFR3-BAIAP2L1         | Bladder cancer  | n=1      | TK       | TK       | BAIAP2L1 | 700      | 1254     |          |          |          |          |          |          |

| FGFR1 Fusions          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| BAG4-FGFR1             | LUSC(n=1) |          | TK       | TK       | BAG4     | 128      | 741      |          |          |          |          |          |          |
| ERLIN2-FGFR1           | Breast cancer | n=1    | TK       | TK       | ERLIN2   | 138      | 1037     |          |          |          |          |          |          |

Wu et al, *Cancer Discovery* 2013
FGFR Fusions in Diverse Cancers

Glioblastoma
Cholangiocarcinoma
Breast Cancer
Prostate Cancer
Bladder Cancer
Lung Squamous Cancer
Thyroid Cancer
Oral Cancer
Head & Neck Cancer

Wu et al, Cancer Discovery 2013
FGFR Fusion Partners Mediate Receptor Oligomerization

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<tr>
<th>MYC-Tag</th>
<th>V5-Tag</th>
<th>FGFR3-BAIAP2L1</th>
<th>FGFR3-TACC3</th>
<th>FGFR2-BICC1</th>
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<th>FGFR3</th>
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Wu et al, *Cancer Discovery* 2013
FGFR Fusions Enhance Cell Proliferation

Wu et al, *Cancer Discovery* 2013
FGFR fusion positive bladder cancer xenografts may be more sensitive to FGFR Inhibitors than activating FGFR point mutants
Metastatic Cholangiocarcinoma – *FGFR2* Rearrangements and Fusions

David Craig, Winnie Liang, Ph.D., Angie Baker, Rebecca Reiman, Lori Phillips, Jackie McDonald, Jessica Aldrich, Ahmet Kurdolgu, Alexis Christoforides, Sara Nasser, Tyler Izatt, Steve Mastria, Jeffrey Trent, Daniel Von Hoff, John Carpten TGen, Phoenix, AZ

Mitesh Borad, Alan Bryce, Jan Egan, Mia Champion, Robert McWilliams, Ann McCullough, Katherine Hunt, Rafael Fonseca, A. Keith Stewart

Mayo Clinic, Scottsdale, AZ

Presented at 2013 AACR Annual Meeting
SU2C Prostate Dream Team
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Dream Team Co-Leader
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Dream Team Principal
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Dream Team Principal
Dana Farber Cancer Institute

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Johann de Bono, MD, PhD
Institute of Cancer Research/Royal Marsden Hospital

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Priya Kunju
Amy Gursky
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Chun-Liang Chen
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J.M. Mosquera

Bioethics
Scott Roberts
Scott Kim
Ray De Vries
Brian Zikmund-Fisher

Prostate SPORE
Challenges Moving Forward...

- What type of molecular aberrations should be monitored?
- What type of sequencing should be employed?
- How do we handle broad sequencing in the context of regulatory approval?
- How will we pay for drugs used off label but with a compelling molecular rationale?
- How should future clinical trials be designed?
- How do we match combinations of mutations/pathways with multiple targeted therapies?