Advancing Precision Cancer Medicine: Novel Markers, Tests, Trials, and Biology

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The Ohio State University Comprehensive Cancer Center
James Cancer Hospital
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Disclosure Information

I have the following financial relationships to disclose:
Stockholder in: Johnson and Johnson
Advisory Board: AbbVie, Incyte
Honoraria: IDT DNA technologies

I will not discuss off label use in my presentation.
Precision Cancer Care

1) What is the right drug for our patient?
2) How can we improve that therapy?
Outline and Goals

- Precision cancer medicine
  - Gene fusions -> Targeted therapies (FGFR)
  - Microsatellite instability -> Immunotherapy
- Data sharing networks
Next Generation Sequencing Technology Enables Rapid Assessment of Cancer Genomes
How do we apply and bring genomic sequencing strategies and bioinformatics to patient care?
Personalized Oncology Through Integrative High-Throughput Sequencing: A Pilot Study

Sameek Roychowdhury,1,2* Matthew K. Iyer,1,3* Dan R. Robinson,1,4* Robert J. Lonigro,1,3
Yi-Mi Wu,1,4 Xuhong Cao,1,4,5 Shanker Kalyana-Sundaram,1,4,6 Lee Sam,1,3 O. Alejandro Balbin,1,3
Michael J. Quist,1,4 Terrence Barrette,1,4 Jessica Everett,7 Javed Siddiqui,1,4 Lakshmi P. Kunju,1,4
Nora Navone,8 John C. Araujo,8 Patricia Troncoso,8 Christopher J. Logothetis,8 Jeffrey W. Innis,9
David C. Smith,2,10 Christopher D. Lao,2,10 Scott Y. Kim,11 J. Scott Roberts,11,12
Stephen B. Gruber,2,10 Kenneth J. Pienta,1,2,10,13 Moshe Talpaz,2,10 Arul M. Chinnaiyan1,3,4,5,13†
March 2012: 34 year old woman with newly diagnosed metastatic cholangiocarcinoma.

Started therapy in a clinical trial with continuous infusion 5-FU, fixed dose rate gemcitabine, and cisplatin.

May 2012: Liver biopsy
Fibroblast Growth Factor Receptor (FGFR): A New Target for Therapy

Wu et al, Cancer Discovery, 2013

Identification of Targetable FGFR Gene Fusions in Diverse Cancers

Yi-Mi Wu\textsuperscript{1,2}, Fengyun Su\textsuperscript{1,2}, Shanker Kalyana-Sundaram\textsuperscript{1,2}, Nick Khazanov\textsuperscript{10}, Bushra Ateeq\textsuperscript{1,2}, Xuhong Cao\textsuperscript{1,7}, Robert J. Lonigro\textsuperscript{1,8}, Pankaj Vats\textsuperscript{1,2}, Rui Wang\textsuperscript{1,2}, Su-Fang Lin\textsuperscript{11}, Ann-Joy Cheng\textsuperscript{12}, Lakshmi P. Kunju\textsuperscript{1,2}, Javed Siddiqui\textsuperscript{1,2}, Scott A. Tomlins\textsuperscript{1,2}, Peter Wyngaard\textsuperscript{10}, Seth Sadis\textsuperscript{10}, Sameek Roychowdhury\textsuperscript{1,4}, Maha H. Hussain\textsuperscript{3}, Felix Y. Feng\textsuperscript{1,4,8}, Mark M. Zalupski\textsuperscript{1,3,5}, Moshe Talpaz\textsuperscript{2}, Kenneth J. Pienta\textsuperscript{1,3,6,8}, Daniel R. Rhodes\textsuperscript{1,2,5,10}, Dan R. Robinson\textsuperscript{1,2}, and Arul M. Chinnaiyan\textsuperscript{1,2,6,7,8,9}

Wu et al, Cancer Discovery, 2013
FGFR: Multiple cancer types
Genomics is Changing Clinical Trials

One size fits all

Genomics

Roychowdhury and Chinnaiyan,
Ann Rev Genomics and Human Genetics, 2014
FGFR: New questions

- Who else has the marker?
- How do we leverage big data for Patients?
- How do we diagnose it across different cancer types?
- What novel therapies can we offer them?
Our Team Approach

- Genomics
- Computational Biology
- Molecular Diagnostics
- Basket Clinical Trials

- Novel Targets/Resistance
- Autopsy and Heterogeneity
- Rare Cancers
- Immunology and Genomics

Patient → Gene(s) Marker(s) → Therapy → Response → Resistance → Rational combinations
CLIA-Cancer Genomics Laboratory

Novel Molecular Diagnostic Tests

Tumor Biopsy  Pathology  DNA & RNA Qty QC  Library Prep(s)  Sequence  Bioinformatics Analysis  Review and Report

The James
RNAseq to Detect Gene Fusions

Maher et al., PNAS 2009
RNA sequencing to detect gene fusions

Gene A

Gene B

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>➢ Unbiased (no knowledge of breakpoint/partner gene required)</td>
<td>➢ Complex (but focused) data analysis</td>
</tr>
<tr>
<td>➢ Novel fusion discovery</td>
<td>➢ Limited by genes on panel</td>
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<tr>
<td>➢ Gene expression information</td>
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Spanning Actionable RNA Kinase Fusions [OSU-SpARKFuse]

Sample Requirements and RNA Quality Control

1. Pathologist review
   - Minimum 20-50µm FFPE tissue or single frozen biopsy specimen
   - Tumor content requirement >= 25%
2. RNA Extraction and QC
   - RIne and DV200

Library Preparation and Hybridization/Capture

1. 250ng input
2. rRNA depletion, fragmentation, cDNA synthesis, library construction
3. Multiplexed hybridization and capture using biotinylated probes (IDT)

Illumina Sequencing and Quality Metrics

1. 2 x 100 paired end sequencing on MiSeq
2. QC report:
   - Alignment
   - Coverage
   - Kinase %
   - ERCC %
   - rRNA %

Fusion Calling and Report Generation

1. Fusion callers - ChimeraScan and TopHat-Fusion
   - Number of fusion spanning reads
2. Flagging Known fusions

Reeser et al, Journal of Molecular Diagnostics, 2017
## Gene List

### KINASE

| Kinase | Kinase | Kinase | Kinase | Kinase | Kinase | Kinase | Kinase | Kinase | Kinase | Kinase | Kinase | Kinase | Kinase | Kinase |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| ABL1   | ABL2   | AKT1   | AKT2   | AKT3   | ALK    | AR     | ARAF   | ATM    | AURKA  | CDK1   | CDK10  | CDK2   | CDK3   | CDK4   | CDK5   |
| AURKB  | BLK    | BRAF   | BTK    | CDK8   | CDK9   | CSF1R  | CSF2RA | CSF3R  | DDR2   | EGFR   | EPHA6  | ETV1   | ETV4   | ETV5   | ETV6   |
| CDK6   | CDK7   | CDK8   | CDK9   | CSF1R  | CSF2RA | CSF3R  | DDR2   | EGFR   | EPHA6  | ETV1   | ETV4   | ETV5   | ETV6   | ETV6   |
| EPOR   | ERBB2  | ERBB3  | ERBB4  | ERG    | ESR1   | ETV1   | ETV4   | ETV5   | ETV6   | FYN    | FYN    | FYN    | FYN    | FYN    |
| EWSR1  | FGFR1  | FGFR2  | FGFR3  | FGFR4  | FGR    | FLT1   | FLT4   | FRK    | FYN    | FYN    | FYN    | FYN    | FYN    | FYN    |
| HCK    | HRAS   | IGF1R  | IGF2R  | IL7R   | ITK    | JAK1   | JAK2   | JAK3   | KDR    | KDR    | KDR    | KDR    | KDR    | KDR    |
| KIT    | KRAS   | LCK    | MAP2K1 | MAP2K2 | MAPK1  | MAPK3  | MET    | MPL    | MPL    | MPL    | MPL    | MPL    | MPL    | MPL    |
| MTO1   | MYC    | NRAS   | NTRK1  | NTRK2  | NTRK3  | PDGFB  | PDGFRB | PDGFRB | PIK3CA | PIK3CA | PIK3CA | PIK3CA | PIK3CA | PIK3CA |
| PIK3R1 | PIK3R2 | RAF1   | RARA   | RET    | ROS1   | SRC    | STK11  | SYK    | TSC1   | TSC1   | TSC1   | TSC1   | TSC1   | TSC1   |
| TSC2   | TYK2   | YES1   |        |        |        |        |        |        |        |        |        |        |        |        |        |

### HOUSE KEEPING

<table>
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<tr>
<td>ASCC3</td>
<td>CNTF</td>
<td>EPM2A</td>
<td>HEATR4</td>
<td>NOL10</td>
<td>PRPSAP1</td>
<td>RGRIP1</td>
<td>SFT2D3</td>
<td>SPDYA</td>
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### ERCC

| ERCC-00017    | ERCC-00025    | ERCC-00039    | ERCC-00057    | ERCC-00060    | ERCC-00084    | ERCC-00108    | ERCC-00109    | ERCC-00154    | ERCC-00164    |
Genomics Closes the Gap from Discovery to Patients

Microscopy
Immunohistochemistry
Karyotype
PCR
Sanger sequencing
FISH
Microarray
Immunohistochemistry

1900s 1960s 1970s 1980s 1990s 2000s 2010s

BCR-ABL (Leukemia) 1960
IMATINIB 2001
BRAF (Melanoma) 2002 VEMURAFENIB 2010
ALK (Lung) 2007 CRIZOTINIB 2010
FGFR and Trials 2013-2015
Three FGFR inhibitor trials for patients with activating FGFR gene alterations

1) **Ponatinib** for **Any** Cancer with **FGFR** gene alterations

2) **BGJ398** for **cholangiocarcinoma** with **FGFR** gene alterations

3) **INCB054828** for **Any** Cancer with **FGFR** gene alterations
Clinical response to FGFR inhibitor in patient with FGFR2 fusion-positive metastatic cholangiocarcinoma
Intratumor heterogeneity
Rapid Research Autopsy

- Informed Consent
- Transport to Morgue
- Autopsy
- Tissue Procurement
- Return to Funeral Home
- Genomics

Clinical Research Team
Hui-Zi Chen, MD, PhD
Melanie Krook, PhD
Julie Reeser, PhD
Michele Wing, PhD, FNP

Autopsy Team
Patricia Allenby, MD
Jen Sachire
Jakob Durakovic

Tissue Procurement Team
Kelly Hamilton

- Tumor Heterogeneity
- Drug Resistance
- Patient Derived Xenografts
Acquired Resistance to INCB54828

Gem/Cis  OSU-15241: INCB54828  FOLFOX

Jan. 2017  Mar. 2017 Tumor Bx  
Targeted Seq.

Apr. 2017 Death & Autopsy

FGFR2 N549H

PELOTONIA
Summary:
Patients teaching us about gene fusions

- Novel FGFR fusions
- 3 FGFR inhibitor Trials
- Acquired Resistance
- Research Autopsy
- Tumor Heterogeneity in Cholangiocarcinoma

Melanie Krook, PhD
Postdoctoral Fellow
Cancer Biology

Melanie is studying mechanisms of resistance to FGFR inhibitors and how to overcome this resistance.

Hui-Zi Chen, MD, PhD
Medical Oncology Fellow
Medical Oncology

Hui-Zi is treating patients with FGFR inhibitors on trial and leading research autopsy
Outline and Goals

- Precision cancer medicine
  - Gene fusions -> Targeted therapies (FGFR)
  - Microsatellite instability -> Immunotherapy
- Data sharing networks
Rhonda Ball

- Metastatic adenocarcinoma of unknown primary, summer of 2015.
- Radiation, chemotherapy, surgery
- Found to have MSI-H+ marker on her tumor.
- Started immunotherapy trial. Complete response.
Microsatellites are short, repeating DNA sequences

- 1-5 bp repeat, for 10-60 bp total

\[
\text{CAGGTA} \ldots \text{AGGGTTC}
\]

- Dispersed throughout the genome

- Repeat count must be preserved through repeated cell divisions
  - By DNA mismatch repair (MMR) system
DNA repair deficiency leads to hypermutation

- Cancer cells with **deficient** DNA mismatch repair (MMR) system have lots of mutations

- **Hypermutated** cancer cells have resulting **Neo-antigens** that can be recognized by the immune system

- But the immune system needs a little help…
T cells have many gas pedal(s) and brake(s): Implications for cancer immunotherapy

GAS PEDALS & CHECKPOINTS

Cell cycle progression
- Proliferation
- Survival
- Effector function
- Cytokine production
- Memory

Lesokhin et al, Science Translational Medicine 2015
PD-1 Blockade in Tumors with Mismatch-Repair Deficiency


B Radiographic Response

- Mismatch repair–proficient colorectal cancer
- Mismatch repair–deficient colorectal cancer
- Mismatch repair–deficient noncolorectal cancer

Change from Baseline in the Sum of Longest Diameters (%)

20% increase (progressive disease)

30% decrease (partial response)
One of five clinical trials that helped lead to ….

FDA News Release

FDA approves first cancer treatment for any solid tumor with a specific genetic feature

For Immediate Release

May 23, 2017
MSI: New questions

- Who else has the marker MSI-H?
- How do we leverage big data for Patients?
- How do we diagnose it across different cancer types?
- What novel therapies can we offer them?
Landscape of Microsatellite Instability Across 11,000+ cancers

Bonneville, Krook, et al, JCO Precision Oncology, 2017
MSIDx

Next generation sequencing to detect **MicroSatellite Instability-High** (MSI-H)

![Diagram showing comparison between normal and tumor samples with custom probes and sequencing & analysis](image-url)
Phase 2 Trial of Combination IDO-1 inhibitor and Pembrolizumab immunotherapy for any tumor with MSI-H

- **Tumor:** Pretreatment and Post-treatment Tumor Biopsies, Research Autopsy (resistance)
- **Host:** Serial blood and urine (immune cells, circulating markers)
- **Extrinsic:** Stool Microbiota

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Novel Diagnostics and Therapy

- Algorithm to Detect MSI-H
- Novel Diagnostic Test: MSIDx
- Clinical Trial Immunotherapy

- Published Landscape of MSI-H marker across 39 Cancer Types (June 2017)
- Developed concept for pan-cancer test ("MSIDx")
- UH2/UH3 funding for developing MSIDx (Sept 2017)
- New clinical trial for Immunotherapy

Russell Bonneville
Graduate Student
Computational Biology

Michele Wing, PhD, FNP
Research Scientist
Cancer Molecular Diagnostics

Julie Reeser, PhD
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Outline and Goals

- Precision cancer medicine
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What can we expect from advanced genomic testing for our patients?

- ~3% will have a germline alteration that may confer heritable risk
- ~10% will have an actionable genomic alteration that leads to new therapy
A National Cancer Center Alliance to integrate “Big Data” and Data Sharing For Cancer Research and Care

Mission:
Accelerating cancer discovery and delivering hope through collaborative learning and partnerships
Oncology Research Information Exchange Network (ORIEN)
Oncology Research Information Exchange Network (ORIEN): Investigator Initiated Trials for Marker+ patients

- MSI-H Hypermutated
- FGFR fusions
- ALK and ROS1 fusions

Analysis of Whole Exome and RNAseq

MARKER+ Candidates? → CLIA Testing → Trial Therapy → Drug Resistance
Looking Ahead to Novel Diagnostics and Targets

**OSU-IGNITE (DNA)**
- Point Mutations and InDel’s
  - Missense
  - Nonsense
  - Frameshift
- Wild-type: AGA
- V600E Mutant: AGA

**OSU-SpARKFuse (RNA)**
- Gene Expression
  - Outlier Expression
  - Signatures
  - Pathways
- Structural Rearrangements
  - Translocations
  - Gene Fusions
  - Inversions
  - EML4-ALK

**Novel Biomarkers and Diagnostics**
- **OSU** Undisclosed
  - Methylation
  - Undisclosed
- **OSU MSIDx**
  - MSI-H
  - Immunotherapy
- **OSU Microbiome**
  - ?
  - Diet?
- **OSU T cell**
  - ?
  - Combination Immunotherapy
Summary

- Patients first

**Examples:**
- Biomarkers to predict response to therapy
- Novel Diagnostic tests
- Therapies in clinical trials

- Team work
- Data Sharing Networks
- Training

The James
The Team

Genomics Diagnostics
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PELOTONIA
American Lung Association
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The James
American Cancer Society
The Ohio State University Comprehensive Cancer Center

Collaborators
Kristin Dittmar
Aharon Freud
Wei Chen
Tricia Allenby
John Hays
Thank you!

Questions?
## Molecularly Matched Therapeutic Trials at OSUCCC-James (Examples)

<table>
<thead>
<tr>
<th>Alteration</th>
<th>Trials</th>
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<tbody>
<tr>
<td>FGFR alterations</td>
<td>FGFR inhibitors (3)</td>
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<tr>
<td>RET alterations</td>
<td>RET (3)</td>
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<tr>
<td>ALK alterations</td>
<td>ALK (3+)</td>
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<tr>
<td>ROS1 alterations</td>
<td>ROS1 (2)</td>
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<tr>
<td>NTRK alterations</td>
<td>NTRK (1)</td>
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<td>MYC alterations</td>
<td>Bromodomain inhibitors (2)</td>
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<tr>
<td>BRCAness</td>
<td>PARP inhibitors (1+)</td>
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<td>MSI-H</td>
<td>PD1 inhibitors, Multiple</td>
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