ctDNA: A Clearer Picture of Cancer

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Rocky Mountain Oncology Society
Thursday, September 18, 2014
I have the following financial relationships to disclose:

- Founder of Pagerbox, Inostics, Personal Genome Diagnostics, Inc.

- Consultant for Spectrum Pharmaceuticals and Amgen.

Under separate licensing agreements between Inostics, Personal Genome Diagnostics and the Johns Hopkins University, Dr. Diaz is entitled to a share of royalty and milestone payments received by the University on sales of products related to research described in this presentation.
Human Cancer Exomes Sequenced

- Glioblastoma (35)
- Head and Neck Cancer (66)
- Non-Hodgkin Lymphoma (74)
- Lung Cancer (Non-Small Cell) (147)
- Lung Cancer (Small Cell) (163)
- Breast Cancer (33)
- Esophageal Adenocarcinoma (57)
- Esophageal Squamous Cell Carcinoma (79)
- Pancreatic Cancer (45)
- Gastric Cancer (53)
- Hepatocellular Cancer (39)
- Colorectal Cancer (66)
- Ovarian Cancer (42)
- Endometrial Cancer (49)
- Chronic Lymphocytic Leukemia (12)
- Prostate Cancer (41)
- Acute Myeloid Leukemia (8)
- Melanoma (135)
- Rhabdoid Cancer (4)
- Neuroblastoma (12)
- Glioblastoma (14)
- Medulloblastoma (8)
- Acute Lymphocytic Leukemia (11)
Cancer Mutations
September 2014

Coding Mutations
1,712,998

Genes
28,426
Clinical Application of Cancer Genetics

- Prognostic Markers
- Somatic Cancer Genome Data
- Immune Antigens
- Predictive Markers
- Dynamic Biomarkers
Access to Somatic Mutations

Tumor Tissue
• FFPE
• Frozen tissue

Blood & other bodily fluids
• Cell-free DNA
• Circulating tumor cells (CTCs)
Liquid Biopsy

**Plasma**
- Water 91%
- Proteins 7%
- Metabolites (trace)
- Cell-free DNA (trace)

**Cellular Components**
- White Blood Cells 2–3%
- Platelets 2–3%
- Red Blood Cells 90%
- Circulating tumor cells (trace)
Circulating Cell-free DNA

Bone Marrow: 80-90%
GI Tract: 5-10%
Skin: 5-10%

Pool of Cell-free DNA: 1-10%
Circulating Cell-free DNA

Bone Marrow
80-90%

GI Tract
5-10%

Skin
5-10%

Pool of Cell-free DNA

Fetus
1-10%
Circulating Cell-free DNA

Bone Marrow: 80-90%
GI Tract: 5-10%
Skin: 5-10%
Transplanted Organ: 1-10%
Circulating Cell-free DNA

Bone Marrow: 80-90%

GI Tract: 5-10%

Skin: 5-10%

Tumor: 0.01-10%
Clinical Application of Cancer Genetics

Dynamic Biomarkers
Objectives

• Biology
Mutations are highly specific

- Pre-Cancer Cell
- Cancer Cell
- Normal Cells

Mutations
No Mutations
Size distribution of DNA in maternal plasma

1,810–12,639 DNA fragments /mL @ baseline

Variation with Trauma & Surgery

DNA is in small fragments

Fan H C et al. PNAS 2008;105:16266-16271
Monitoring cell-free DNA in the circulation

Diehl et al. Nature Medicine, 2008
ctDNA clearance after resection.

$T_{1/2} = 114$ min

Diehl et al. Nature Medicine, 2008
Circulating tumor DNA

Diehl et al Nature Medicine, 2008
Survey of ctDNA
14 metastatic tumor types (n=136)

Bettegowda et al, Sci Tran Med Feb 2014
## Comparison of CTCs with ctDNA

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Cellular DNA (mutant fragments per 5 mL)</th>
<th>Plasma DNA (mutant fragments per 5 mL)</th>
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<td>Bladder cancer</td>
<td>0</td>
<td>226</td>
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<td>Colorectal cancer</td>
<td>0</td>
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<td>690</td>
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<tr>
<td>Breast cancer</td>
<td>0</td>
<td>9,900</td>
</tr>
</tbody>
</table>
Survival vs. ctDNA

metastatic CRC tumors
(n=120)

Bettegowda et al, Sci Tran Med Feb 2014
Objectives

• Biology
  – Highly Specific
  – Small Fragments ~180bp
  – Dynamic – intra and inter patient
  – Short half-life – 2 hours
  – Heterogeneous across tumor types
  – More abundant than CTCs
  – Levels correlate with survival
Objectives

• Biology

• Beyond point mutations
Methylated Tumor DNA

Methyl-BEAMing

Mitochondrial Tumor DNA

Mitochondrial mutations

Yiping He

Nick Papadopoulos

Tarted Rearrangement Detection

PARE approach for development of personalized biomarkers

Rebecca Leary

Victor Velculescu

Leary et al Science Translational Medicine, April 2011.
Whole Genome Sequencing of Plasma

Leary et al Science Translational Medicine, Nov 2012.
<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Fold Change in Read Density</th>
<th>Tumor fraction</th>
<th>PA-score</th>
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<tbody>
<tr>
<td>0</td>
<td>0%</td>
<td>0%</td>
<td>0.3</td>
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<td>0.3%</td>
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<tr>
<td>21</td>
<td>43.8%</td>
<td>32.6</td>
<td>32.6</td>
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</tbody>
</table>

Unmatched normal plasma (N1)

Primary Tumor (CRC14-PT)

Plasma 0 months (CRC14-0)

Plasma 4 months (CRC14-4)

Plasma 62 months (CRC14)
Objectives

• Biology

• Beyond point mutations

• Clinical Applications
Clinical Application of Cancer Genetics

Monitoring Response
Monitoring Response

(a) Chemotherapy 1
Surgery 1
Chemotherapy 2
Surgery 2
PIK3CA

(b) Chemotherapy 1
Surgery 1
Chemotherapy 2
Surgery 2
Chemotherapy 3
Surgery 3
TP53

Mutant DNA fragments per sample (n)
A phase II trial of low-dose multiagent chemotherapy with GTX-C in subjects with metastatic pancreatic cancer

- 1st line pancreatic cancer
- Chemo and radiation naïve population
- GTX-C (capecitabine 500 mg bid on days 1–14, and the combination of gemcitabine 500 mg/m² (10 mg/m²/min), taxotere 20 mg/m² and cisplatin 20 mg/m² on days 4 and 11)
- Primary endpoint was 6-month PFS rate
  - active if the 6-month PFS rate was >75%
  - inactive if < 50%.
Radiographic Response
BEST RESPONSE

Target Lesions Change from Baseline SLD (%)

-100 -50 0

Clinical PD (n=1; 4%)
PD (new lesions) (n=2; 7%)
SD (n=11; 39%) PR (n=14; 50%) DCR (n=25; 89%)
Patient 001

ctDNA levels

Initiation of GTXC

ctDNA Spike

Partial Response
Clinical Application of Cancer Genetics

Tracking Resistance
Tracking Resistance

Tumor

Targeted Therapy

Response

Recurrence

Resistant to Therapy

Tracking Resistance

EGFR blockade in Colorectal Cancer

**Primary resistant** – Exon 12 or 13 KRAS mutation

**Response Rate** – 17%

**Progression Free Survival** – 12.3 weeks
Tracking Resistance

Monitoring the emergence of resistant mutations in KRAS WT patients treated with EGFR blockade

Tracking Resistance

Interrogated all exons of KRAS, NRAS, BRAF, PIK3CA and EGFR

96% of cases had at least 1 mutation KRAS or NRAS

<table>
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<th>Sample ID</th>
<th>Pre-Treatment</th>
<th>Post-Treatment</th>
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<td></td>
<td>KRAS 12</td>
<td>KRAS 13</td>
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<td>AMG 011</td>
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<td>AMG 022</td>
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<td>AMG 028</td>
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<td>AMG 034</td>
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<td>AMG 040</td>
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<td>AMG 046</td>
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<td>AMG 109</td>
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<td>AMG 114</td>
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<td>AMG 121</td>
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<td>AMG 132</td>
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<td>AMG 140</td>
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<td>AMG 148</td>
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<td>AMG 155</td>
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<td>AMG 161</td>
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<td>AMG 167</td>
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<td>AMG 180</td>
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<td>BARD 101 PLS</td>
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<tr>
<td>CRC 191 PLS</td>
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</table>

Red: Single mutation
Orange: Multiple mutations

INSIGHTS

1. SECONDARY RESISTANCE TO EGFR BLOCKADE

2. METASTATIC HETEROGENEITY

3. FEASIBILITY: (167 tumors amongst 24 patients) X 7 time points = 1,169 biopsies to recreate the time course data

Tracking Resistance

EGFR BLOCKADE

KRAS Mutant

NRAS Mutant

KRAS Mutant

EGFR Mutant

KRAS WT

NRAS WT

EGFR WT
Clinical Application of Cancer Genetics

Minimal Residual Disease
Molecular Analysis
CEA measured 6-8 weeks following curative resection of mCRC

CT scan negative
After Surgery
Day 42

CT scan positive
After Surgery
Day 244

13.4 %
0.015 %
0.11 %
0.66 %

Percent Mutant DNA
CEA measured 6-8 weeks following curative resection of mCRC
ctDNA measured 6-8 weeks following curative resection of mCRC
250* patients with Stage II colon cancer

Tumor Tissue

Massively parallel sequencing of TP53, APC, KRAS, BRAF, PIK3CA, FBXW7 and SMAD4 mutations

Mutations identified in tumors

4-10 weeks post-op blood samples

3-monthly follow-up blood draw (N = 175)

- ctDNA quantification with Safe-SeqS
  - CEA analysis

- Use of adjuvant chemo at clinician’s discretion
  - 3-monthly clinical review
  - 6-monthly restaging CT

Recurrence and survival data

J. Tie and Peter Gibbs
ctDNA measured 6-8 weeks following curative resection of stage II CRC
Clinical Application of Cancer Genetics

Early Detection
Early Detection

Bettegowda et al, Sci Tran Med Feb 2014
Early Detection

Bettegowda et al, Sci Tran Med Feb 2014
Clinical Application of Cancer Genetics

Early Detection – Other Fluids
Early Detection

Early detection of ovarian and endometrial cancer

- 69,000/year diagnosed
- 8,000/year deaths from Endometria Cancer
- 15,000/year from Ovarian Cancer
- No recommended screening test exists
Ovarian cancer

Sloughed-off cancer cells and cellular fragments drain into the endocervical canal.

Endometrial cancer

Massively parallel sequencing
Early Detection - GenePap

Endocervical Fluid Analysis for tumor DNA

1. DNA harvested from standard Pap smear fluid from women with suspected endometrial and ovarian cancer

2. Interrogated 46 different gene regions in genes commonly mutated in Ovarian and Endometrial Cancers

3. Applied SafeSeq methodology
Safe Sequencing by NGS (Safe-Seq)

Next-Generations sequencing of ctDNA

Isaac Kinde

Essential elements of Safe-SeqS.

- WT
- Mutant
- UID Assignment
- Amplification
- Redundant Sequencing

Kinde I et al. PNAS 2011;108:9530-9535
## Early Detection

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Type</th>
<th>Subtype</th>
<th>Subtype distribution (%)</th>
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<tbody>
<tr>
<td>Ovarian</td>
<td>Epithelial</td>
<td>High-grade serous</td>
<td>60</td>
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<tr>
<td></td>
<td></td>
<td>Endometrioid</td>
<td>15</td>
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<td></td>
<td></td>
<td>Clear cell</td>
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<td></td>
<td></td>
<td>Low-grade serous</td>
<td>8</td>
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<tr>
<td></td>
<td></td>
<td>Mucinous</td>
<td>2</td>
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<td>Transitional cell</td>
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<td>Other</td>
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<td>Endometrial</td>
<td>Type I: endometrioid</td>
<td>Endometrioid</td>
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<td>Type II: non-endometrioid</td>
<td>Papillary serous</td>
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<td>Clear cell</td>
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## Early Detection

<table>
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<tr>
<th>Tissue</th>
<th>Subtype</th>
<th>Common Mutations (Frequency)</th>
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<tr>
<td>Endometrial</td>
<td>Endometrioid</td>
<td>PTEN (64%)</td>
<td>PIK3CA (59%)</td>
<td>ARID1A (55%)</td>
<td>CTNNB1 (32%)</td>
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<td>MLL2 (32%)</td>
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<td>RNF43 (27%)</td>
<td>APC (23%)</td>
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<td>FGFR2 (18%)</td>
<td>KRAS (9%)</td>
<td>PIK3R1 (9%)</td>
<td>EGFR (14%)</td>
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<td>AKT1 (5%)</td>
<td>NRAS (5%)</td>
<td>TP53 (5%)</td>
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<td>Papillary</td>
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<td>serous</td>
<td>Clear Cell</td>
<td>PT53 (81.6%)</td>
<td>PIK3CA (24%)</td>
<td>FBXW7 (19.7%)</td>
<td>PPP2R1A (18.4%)</td>
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<td>TP53 (56%)</td>
<td>KRAS (40%)</td>
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### Tissue Subtype Common Mutations (Frequency)

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<th>Tissue</th>
<th>Subtype</th>
<th>Common Mutations (Frequency)</th>
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<td>ARID1A (30%)</td>
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<td>CTNNB1 (26%)</td>
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<td>PTEN (17%)</td>
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<td>PIK3CA (15%)</td>
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<td>KRAS (10%)</td>
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<td></td>
<td></td>
<td>PPP2R1A (11%)</td>
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<td></td>
<td></td>
<td>CDKN2A (12%)</td>
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<td>BRAF (8%)</td>
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<td>ARID1A (57%)</td>
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<td>PIK3CA (40%)</td>
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<td>PPP2R1A (7.1%)</td>
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<td>KRAS (4.7%)</td>
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<td>BRAF (38%)</td>
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<td>KRAS (40%)</td>
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<td>PPP2R1A (33%)</td>
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<td>CDKN2A (16%)</td>
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<td>PTEN (11%)</td>
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Early Detection - GenePap

N = 26

Endometrial tumors

N = 22

Ovarian tumors

Percent mutant alleles in liquid Pap smear specimen
Summary

Somatic mutations can be effective biomarkers largely because of specificity

Digital Genomics has improved sensitivity and throughput sufficient for real clinical application

Future applications will focus on unmet clinical needs
Summary

Targeted

- Point Mutations
  - Regions of Interest
    - Structural Changes/Exome
      - Whole Genome

Unbiased

- $$$
  - Cost of Sequencing
Thank you

B. Vogelstein  Ken Kinzler  N. Papadopoulos  V. Velculescu  Shibin Zhou