THE SCIENCE OF MOLECULAR DIAGNOSTICS

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Financial Disclosures

I am a stockholder and employee of Foundation Medicine Inc.
Molecularly Targeted Therapy Is Evolving

Trastuzumab in HER2 positive breast cancer

![Graph showing progression-free survival between chemotherapy alone and chemotherapy plus trastuzumab.](image)


Gefitinib in *EGFR*-mutated NSCLC

![CT scans showing change in lung tissue at baseline and 6 weeks.](image)


Vemurafenib in *BRAF* V600E mutated melanoma

![PET scans showing change at baseline and 15 days.](image)


Cabozantinib in *RET*-rearranged NSCLC

![CT scans showing change in lung tissue at baseline and 4 weeks.](image)

Drilon et al., 2013, *Cancer Discov.*
Matching the correct targeted therapy to the correct patient is diagnostically challenging as the number of “clinically relevant” genomic alterations increases.
Diagnostic Challenge

The four types of clinically relevant genomic alterations each are associated with different testing approaches:

**Base Substitutions**
e.g. *BRAF*, *EGFR*

**Insertions/deletions**
e.g. *EGFR*, *ERBB2 (HER2)*

**Focal Amplification & Homozygous Deletion**
e.g. *HER2*, *MET*

**Gene Fusion**
e.g. *ALK*, *RET*

Capillary sequencing, Mass Spectrometry

Capillary sequencing, gel size shift assays

IHC (overexpression), Fluorescence In Situ Hybridization (FISH)

RT-PCR, FISH

Multiple different diagnostic tests may exhaust precious biopsy material
The Number Of Clinically Relevant Cancer Genes Across Solid Tumors Is High

Clinically relevant genes in non-small cell lung cancer (NSCLC)

**Base Substitution:** ALK, AKT1, AKT2, AKT3, ATM, **BRAF**, BRCA1, BRCA2, CDKN2A, **EGFR, ERBB2**, FGFR1, FGFR2, GNA11, GNAS, KRAS, **MAP2K1, MAP2K2, MET**, NF1, NOTCH1, NRAS, PIK3CA, PTCH, PTEN, STK11, TSC1, TSC2

**Short Insertion/Deletion:** ATM, BRCA1, BRCA2, **EGFR, ERBB2**, MET, NF1, NOTCH1, PTCH, PTEN, **STK11**, TSC1, TSC2

**Focal Amplification:** AKT1, AKT2, AKT3, CDK4, CCND1, CCND2, CCNE1, **EGFR, ERBB2**, FGFR1, FGFR2, KRAS, MDM2, **MET**

**Homozygous Deletion:** BRCA1, BRCA2, NF1, NOTCH1, PTCH, **PTEN, STK11**, TSC1, TSC2

**Gene Fusion:** ALK, RET, ROS1, **NTRK1**, FGFR1

--- NCCN Guidelines; -- ≥1 NSCLC patient response; -- Targeted therapy trial
Low tumor purity in many clinical specimens requires diagnostic tests with high accuracy.

Mutant allele frequencies

- N=107 clinically relevant somatic mutations in FFPE non-small cell lung cancer specimens
- *Purity = relative proportion of extracted DNA originating from tumor cells

Capillary sequencing would have missed over half the mutations in this study as 20% allele frequency is the lower limit of detection.

<table>
<thead>
<tr>
<th>Fraction of mutations</th>
<th>Number of Mutations</th>
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<tbody>
<tr>
<td>&lt;5%</td>
<td>11%</td>
</tr>
<tr>
<td>&lt;10%</td>
<td>32%</td>
</tr>
<tr>
<td>&lt;20%</td>
<td>55%</td>
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<tr>
<td>&lt;25%</td>
<td>67%</td>
</tr>
<tr>
<td>&lt;50%</td>
<td>93%</td>
</tr>
<tr>
<td>&lt;100%</td>
<td>100%</td>
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</table>

Courtesy: Mike Berger, AACR 2014
Many clinical cancer specimens are small needle biopsies, FNAs and cell blocks.

Sample preparation needs to be optimized to maximize accuracy and isolate sufficient material for diagnostic testing from tiny specimens.

Young et al., Cancer Cytopathology, 2013.
Number Of Targeted Therapeutics Rising

Knowing which tests to order for a tumor type increasingly challenging
Many NGS assays have been developed for Solid Tumors

Interrogate 50 or more cancer genes in a single assay

Many can perform well on small amounts of tissue routine FFPE samples including needle biopsies and fine needle aspirates

14 day median turn around time feasible for most assays which fits in to routine clinical practice
Analytic Validation

**Base Substitutions**
(MAF 5-100%)

Sensitivity: >99.9%  PPV: >99%

**Insertions/Deletions**
(1-40bp, MAF 10-100%)

Sensitivity: 98%  PPV: >99%

**Copy Number Alterations**
(zero or ≥8 copies)

Sensitivity: >95%  PPV: >99%

**Gene Fusions**

Sensitivity: >95% (>99% for ALK fusion)  PPV: >99%

Frampton et al, *Nature Biotechnology* 2013
Real world clinical specimens are typically lower purity and MUCH harder to profile

High purity (100%) breast cancer
Profile using best academic sample prep

High purity (100%) breast
Profile using highly optimized sample prep

Low purity (20%) breast cancer
Profile using highly optimized sample prep
CGP detects clinically relevant alterations in 65% of patients with pan-negative NSCLC tested at MSKCC

**MSKCC testing:** EGFR, ERBB2, KRAS, NRAS, BRAF, MAP2K1, PIK3CA, AKT1, ALK, ROS1 & RET

- 70% of patients underwent repeat biopsy to complete MSKCC testing
- 65% of patients harbored a clinically relevant alteration by CGP
  - 26% of patients: targeted therapy in NCCN guidelines
  - 39% of patients: Approved therapy or active MSKCC clinical trial

MSKCC and FMI published results in CCR as they were so surprising

*Clinical Cancer Research Dec 2014:* Broad, hybrid capture-based next-generation sequencing identifies actionable genomic alterations in "driver-negative" lung adenocarcinomas. 158/1078-0432.CCR-14-2683
Case Presentation 1: NSCLC Patient

- Middle aged female never smoker
- Stage IV-B lung adenocarcinoma
- Sequenom negative
- ROS1 (FISH) & KIF5B-RET (PCR) testing negative
- Pemetrexed (Alimta), cisplatin and bevacizumab (Avastin) started x 6 cycles then pemetrexed and bevacizumab maintenance every 3 weeks
- 18 months after diagnosis progression of disease with new bony metastasis
Case Presentation 1: NSCLC Patient

<table>
<thead>
<tr>
<th>Genomic Alterations Detected</th>
<th>FDA Approved Therapies (on patient's tumor type)</th>
<th>FDA Approved Therapies (on another tumor type)</th>
<th>Potential Clinical Trials</th>
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<tr>
<td>RET TRIM33-RET fusion</td>
<td>None</td>
<td>Sorafenib</td>
<td>Yes, see clinical trials section</td>
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<tr>
<td>CDKN2A/B loss</td>
<td>None</td>
<td>None</td>
<td>Yes, see clinical trials section</td>
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<tr>
<td>CTNNB1 S33C</td>
<td>None</td>
<td>None</td>
<td>Yes, see clinical trials section</td>
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</table>

Baseline CT scan showing paramediastinal and pleural-based nodularities in left upper lobe.

Repeat CT scan after 28 days of anti-RET therapy: disappearance of paramediastinal and near complete resolution of pleural disease.
HER2/ERBB2 Alterations Across 27 Tumor Types
Nearly half of HER2/ERBB2 alterations missed by FISH/IHC tests.
Case Presentation 2: Treatment path for breast cancer patient destined for hospice care

58 y/o with stage I, T1a (2 mm) invasive ductal breast carcinoma

<table>
<thead>
<tr>
<th>Date</th>
<th>Procedure</th>
<th>Pathology</th>
<th>ER (IHC)</th>
<th>HER2 (FISH)</th>
<th>Therapy</th>
<th>Progression/Side Effects</th>
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<tbody>
<tr>
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<td>DCIS</td>
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<td></td>
<td>Excision</td>
<td>Carcinoma</td>
<td>+</td>
<td>+</td>
<td>Endocrine</td>
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<td>Carcinoma</td>
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<td>Regimen 4</td>
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<td>Regimen 5</td>
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</table>

*Biopsy taken in 2012 specifically for comprehensive genomic profiling
Clinical report referenced publications that demonstrate both identified ERBB2 mutations are potentially sensitive to HER2 TKIs.

- Patient was started on HER2 targeted therapies with chemotherapy.
Case Presentation 2: Treatment path for breast cancer patient destined for hospice care

Pre-therapy: extensive active disease

Post-therapy: good response with lower/less activity

Treating physician quote

“This individual case is so impressive in the clinical evolution (from almost hospice to objective remission) that even an improvement of 3 months duration is important”
**EGFR** alterations were identified in 151 cases across 13 different tumor types

6.8% total cases

**EGFR alterations by site of origin**

![Bar chart showing EGFR alterations by site of origin](image)

**Distribution of **EGFR** mutations on **EGFR** protein**

![Diagram showing distribution of EGFR mutations](image)

6/32 “pan negative” NSCLCs harbored EGFR alterations missed by other diagnostic assays
53 year old female diagnosed with metastatic Inflammatory Breast Cancer involving liver and bone in June, 2010

*EGFR* Exon 21 L858R Point Mutation identified

Present in 10% of *lung* adenocarcinomas

Associated with unprecedented sensitivity to *EGFR-TKIs* such as gefitinib (*Iressa*) and erlotinib (*Tarceva*)

Diagnosis confirmed as Breast Cancer (not a lung metastasis)
Case Presentation 3: Identification of *EGFR* mutation in an inflammatory breast cancer patient

- September, 2012
  - *EGFR* mutation identified would never have been tested for in a patient with breast cancer
  - Genomic profile led to therapy with FDA-approved agent (off label)
  - Erlotinib commenced resulting in symptomatic and radiographic improvement: response ongoing greater than 12 months

- November, 2012
Diagnostic challenges of the liquid Biopsy
Circulating Tumor DNA (ctDNA) Assays Are Another Liquid Biopsy Option

- The FFPE Biopsy and Comprehensive Genomic Profiling will remain the gold standard for the foreseeable future and should be used in almost all clinical settings
- Considerable challenges in using ctDNA to inform targeted treatment options in the majority of patients with cancer
ctDNA Assays are Not Appropriate for Many Patients with Cancer

Up to 40% of patients will be denied standard of care treatment using ctDNA assays alone as they shed no detectable ctDNA into bloodstream.
Low Levels of ctDNA Challenge the Accuracy of Diagnostic Assays

For every fifteen patients this ctDNA assay recommends targeted c-MET therapy (e.g. Crizotinib), approximately fourteen patients will likely not have a druggable c-MET alteration.
A commercially available ctDNA assay failed to identify any alterations from the tissue biopsy. Instead, the assay identified a false positive EGFR alteration.
Circulating Tumor Cells (CTCs) have Huge Diagnostic Potential

→ **Metastatic capability:** at least a portion of the CTCs should represent the “threatening” tumor cells

→ **Representation of heterogeneity:** CTCs may provide sampling of all subpopulations in single biopsy

→ **Clinical care benefits:** low cost, no path lab required, longitudinal monitoring/serial biopsy
CTC Isolation Is An Area Of High Activity

> 50 companies using a variety of methods to capture CTCs
The Major Challenge of CTC Assay Development

Pancreatic cancer patient CK\(^+\)/CD45\(^-\) cells

Most scientists would classify these cells as CTCs by the staining.

Genomics in this patient revealed no aneuploidy or KRAS mutation.
Most scientists would classify these cells as CTCs by the staining. Genomics in this patient revealed no aneuploidy or KRAS mutation. Results were recapitulated in 8 additional patients with pancreatic cancer. Conclusion is that these are, in fact, normal cells with not a single CTC present.
Summary

• THERE IS NO ROOM FOR ERROR or OMISSION in the care of patients with cancer

• Methodologies developed for high purity research specimens need radical optimization to generate the accuracy required for clinical care

• Patients are being denied standard of care, on label therapies due to inaccurate diagnostics

• Stringent analytic validation for high accuracy to detect all classes of druggable alterations should be mandatory to maximize patient care