State of the Art in Tumor Profiling

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Disclosures

• **Intellectual Property**
  – Submission of a patent covering the SNaPshot genotyping methods

• **Consultant**
  – Bio Reference Laboratories, Inc.
State of the Art in Tumor Profiling

Learning Objectives

• Describe the main goals of clinical molecular testing

• Understand some of the challenges facing the clinical implementation of tumor molecular profiling

• Identify examples of key tumor genetic changes, for which molecular testing is being performed, to assist in therapeutic decision making
Clinical Molecular Testing

Diagnosis * Prognosis * Treatment

Molecular Diagnostics / MGH Pathology

**FISH**
- ALK
- ROS1
- EGFR
- MET
- PDGFRA
- FGFR1
- HER2 (non-breast)
- Ewing's Sarcoma (EWSR1)
- Myxoid Liposarcoma (CHOP)
- Synovial Sarcoma (SYT)
- Alveolar Rhabdomyosarcoma (FKHR)
- 1p19q

**Genotyping**
- SNaPshot Cancer Genotyping
- HER2 / ERBB2 (Exon 20 insertion/deletion)
- KRAS (codons G12, G13, Q61)
- BRAF (codon V600)
- MLH1 Promoter Methylation
- MGMT Promoter Methylation
- KRAS on Pancreatic Cyst Fluid

**Chimerism**
- Pre-transplant STR Genotyping
- Post-transplant Chimeris

**Array CGH (MICRY)**
- Proband
- Family (specify relationship to the Probands in Notes section)

Specify ICD-9 code below or in notes section:
- Multiple Congenital anomalies, NOS (759.7)
- Hypotonia, congenital (779.89)
- Dysmorphic Features (744.89)
- Delayed Milestones (783.42)
- Failure to Thrive (783.41)
- Macrocephaly (742.4)
- Microcephaly (742.1)

- CHD, unspecified (746.9)
- Cleft palate, unspecified (749.00)
- Cleft lip, unspecified (749.10)
- Skeletal anomalies, OS (756.0)
- PDD< NOS (299.90)
- Autism (299.00)

- **Hemochromatosis (HFE)**
Moving toward a genotype-based approach to guide cancer care
Specific genetic changes & sensitivity to targeted therapies

**BCR-ABL Translocation**: Imatinib
- 95% CML

**HER2 Amplification**: Trastuzumab
- 20-30% IDC

**EGFR Mutation**: Erlotinib/Gefitinib
- 20% Lung adenocarcinomas

**BRAF V600E**: Vemurafenib
- 50-60% Melanoma

**ALK Translocation**: Crizotinib
- 3-5% Lung adenocarcinoma
Oncogenic disruption of the EGFR RAS/MAPK pathway

Oncogenic Mutations

Growth factor-independent signaling

Targeted Therapies

EGFR inhibitors

PI3K inhibitors

PTEN

Akt/PKB

mTOR

MEK inhibitors

RAF inhibitors

BRAF inhibitors

MAPK-independent signaling pathways

Uncontrolled cell proliferation
Establishing a Clinical Genotyping Platform

Challenges and practical considerations

Clinical Test
- Performed in a CLIA lab
- Archived FFPE tissue
- Analytical sensitivity
- Turnaround time (2 weeks)
- Report in medical record

Actionable Targets
- Clinches diagnosis
- Yields prognosis
- Predicts response/resistance
- Stratify patients for trials
- Adaptability for new targets

Logistics
- Clinical patient coordinator
- Accessioning
- Tracking
- Automation
- Scalable to test all tumors

Other
- Economics
- Insurance and billing
- Translational research
- Bioinformatics
Goal: To provide oncologists with real-time high-throughput tumor genotyping for:

1. Clinical decision making
2. Accelerated development of new cancer therapies
New challenges in Molecular Pathology

**Which test is best?**

The problem:  
- multiple tumor types  
- many mutations  
- a growing number of targeted therapies

Possible approach:

- Tumor A → Test 1
  - Test 2
  - Test 3
  - ...no more tissue?
- Tumor B → Test X
  - Test Y
  - Test Z
  - ...no more tissue?
- Tumor C → Test α
  - Test β
  - Test γ
  - ...no more tissue?

Informed Therapeutic Decisions

Other options:

- multiple therapeutic options pose a new need → multiplex testing

Tumor A → Test A
Tumor B → Test B
Tumor C → Test C

Informed Therapeutic Decisions
### SNpShot Genotyping Assay

**15 genes - 70 assays - >160 described mutations**

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<th>Gene</th>
<th>Mutation</th>
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</table>

**Multiplexed sizing assay:**

- EGFR exon 19
- EGFR exon 20
- ERBB2 exon 20
EGFR/HER2 Inhibitors
- Erlotinib
- Lapatinib
- Dacomitinib

BRAF Inhibitors
- Vemurafenib
- GSK-2118436

PI3K Inhibitors
- INK-1117
- GDC-0032
- BYL-719
- BKM-120

AKT Inhibitors
- MK-2206
- GSK-2110183
- AZD-5363
- ARQ-092

mTOR Inhibitors
- Rapamycin
- Temsirolimus
- Everolimus

- Cell proliferation
- Inhibition of apoptosis
- Angiogenesis
- Metastasis

RTK

PI3K

AKT

TSC1/2

mTOR

S6K

MEK Inhibitors
- AZD-6244
- MEK-162
- GSK-1120212

ERK

Myc
c-Jun
C-Fos
ELK-1

Ras-GTP

Raf

MEK

Sec-1
Shc
Grb2
SNaPshot Overview

Multiplex PCR → Single Base Extension Reaction → Capillary electrophoresis

Loci of interest

Electrophoretic Output

Increasing molecular weight

Relative fluorescence
SNaPshot Overview

Single Base Extension Reaction

1

G

C

2

A

T

3

C

G

Electrophoretic Output

mut
MGH SNaPshot Testing Volume

>4,500 clinical SNaPshot tests performed to date

>200 clinical SNaPshot tests per month
3 years of mutational profiling → LUNG cancer

- "Wild Type" 40%
- KRAS 24%
- EGFR 14%
- PIK3CA 4%
- TP53 6%
- NRAS 1%
- CTNNB1 2%
- BRAF 2%
- ALK rearrangement 4%
- ROS rearrangement 2%
- IDH1/AKT1/APC/PTEN/MEK1 <1%

1910 tumor specimens - 60% mutant
Overlap of Mutations - NSCLC

- **KRAS**: 129 isolated (134 total)
  - (1) **IDH1**

- **EGFR**: 63 isolated (73 total)
  - (2) **HER2**
  - 12
  - 5
  - 1

- **PIK3CA**: 15 (22 total)
  - 1
  - 2
  - 1
  - 5
  - 6

- **TP53**: (25 total)
  - 1
  - 4

- **B-cat**: (11 total)

- **ALK**: 27

Sequist et al., Annals of Oncology, 2011
Mechanisms of acquired drug resistance - NSCLC

37 patients w/ EGFR-mutant tumors → responded to EGFR inhibitors and later relapsed

Sequist et al., Science Translational Medicine, 2011
Presentation

• 61 y.o. Male never-smoker

• CT chest showed large right upper lobe mass and numerous bilateral pulmonary nodules (<5 mm) suspicious for metastasis

• Biopsy identified NSCLC, adenosquamous histology
Case 1 – NSCLC

Molecular Testing:

*EGFR* gene mutation: **EGFR L858R**

Therapeutic implication: confers sensitivity to EGFR TKI
Case 1 – NSCLC

Pre-Treatment  Therapeutic Response  Acquired Resistance

1/30/08 (RUL 6.8x4.5 cm)  3/31/08 (RUL 2.6x1.8 cm)  2/25/09 (RML 2 cm)
Case 1 – NSCLC

**Molecular Testing**
- Pre-Treatment ‘08
- Resistant ‘09

**Clinical Trial**
- MET+EGFR inhibitor therapy
- Response to therapy: tumor mass reduced by ~30%

*MET* gene amplification

Engelman et al., Science, 2007
Bean et al., PNAS, 2007
Case 2 – NSCLC

Genotype supports 2 independent primary tumors

→ mutational profiling of bilateral tumor masses → two distinct genotypes
→ support the clinical suspicion that this was not metastatic disease but rather two synchronous lower stage primary tumors
→ better prognosis and direct impact patient management (aggressive surgical therapy + adjuvant chemotherapy)

Tumor #1 - Right upper lobe
Adenocarcinoma (bronchioloalveolar)
KRAS G12C (c.34G>T)

Tumor #2 - Left upper lobe
Adenocarcinoma (acinar subtype)
BRAF V600E (c.1799T>A)
<table>
<thead>
<tr>
<th>Genotype</th>
<th>YR</th>
<th>Location</th>
<th>Clinical questions/staging</th>
<th>Informative?</th>
<th>Answer</th>
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<td>Metastases</td>
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12/13 staging dilemmas solved! 92%!
**ALK Rearrangements in NSCLC**

**Rapid integration of FISH**

**PF-02341066**: Potent & selective ATP competitive oral inhibitor of MET and ALK kinases and their oncogenic variants

---

**Identification of the transforming EML4–ALK fusion gene in non-small-cell lung cancer**

Manabu Soda, Young Lim Choi, Munehiro Enomoto, Shuji Takada, Yoshihiro Yamashita, Shunpei Ishikawa, Shin-ichiro Fujiwara, Hideki Watanabe, Kentaro Kurashina, Hisashi Hatano, Masashi Bando, Soji Ohno, Yuichi Ishikawa, Hiroyuki Aburatani, Toshiro Nishi, Yasunori Sohara, Yukihiko Sugiyama & Hiroyuki Mano

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**Genomic Alterations of Anaplastic Lymphoma Kinase May Sensitize Tumors to Anaplastic Lymphoma Kinase Inhibitors**


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**t(2;5) ALK gene breakpoint region**

Telomere  | 2p23 region | Centromere

| ~250 kb | ~300 kb |

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*Image details and diagrams*
Case 3 – NSCLC

Dramatic Improvement Following Targeted ALK Inhibition

Pre-Treatment

After 2 cycles PF02341066

50 yr-old Female
Tumor Responses to Crizotinib by Patient

Best Percent Change in Tumor Size
($n=105$ evaluable patients)

- – 30% reduction
- PD
- SD
- PR
- CR

Camidge R et al. Poster 366 presented at the 35th ESMO, 2010
PF02341066 activity in cells exhibiting ALK fusion in broad screen (McDermott et al., Canc Res, 2008)

Discovery of EML4-ALK fusions in NSCLC (Soda et al., Nature)

MGH clinical study of PF02341066 in ALK-positive NSCLC starts

FDA Approval: ALK-positive NSCLC

Timeline for approval
## Genotype-based clinical trial enrollment of advanced disease NSCLC patients

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<tr>
<th>Mutant gene and targeted agents</th>
<th>Mutation-positive patients with metastatic disease</th>
<th>Clinical trial enrollment</th>
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<td><strong>ALK</strong></td>
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<td>crizotinib ((ALK_{\text{inhibitor}}))</td>
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<td>Hsp-90(_{\text{inhibitor}})</td>
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<td><strong>BRAF</strong></td>
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<td>MEK(_{\text{inhibitor}})</td>
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<td><strong>EGFR</strong></td>
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<td><strong>KRAS</strong></td>
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<td>MEK(_{\text{inhibitor}})+ chemotherapy</td>
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<td>MEK(<em>{\text{inhibitor}})+ PI3K(</em>{\text{inhibitor}})</td>
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<td>Hsp-90(_{\text{inhibitor}})</td>
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<td><strong>Total</strong></td>
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<tr>
<td>260 mut+</td>
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<td>90 (35%)</td>
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</table>

(expanded) Sequist et al., Annals of Oncology, 2011
Tumor genetic profiling in the clinic

Breast Cancer

130 cases

HER2 amplification ~ 20-30% IDC

PIK3CA mutant Breast Cancer
- favorable prognosis – improved OS
- decreased response to treatment with HER2 inhibitors (trastuzumab, lapatinib)
- ? PI3K/mTOR inhibitors (actively pursued, encouraging data)

KRAS / BRAF mutant Breast Cancer
- ? MEK inhibitors ? / ? BRAF inhibitors ?
65yo Female with:
2005: ER+ breast cancer (localized)
2009: Disease recurrence (bone and liver):
    - Started endocrine therapy
    - Progressed on various endocrine therapies
2011: SNaPshot revealed:
    - PIK3CA E542K mutation
    - Enrolled on PI3K-alpha inhibitor trial
Case 2 – Breast Cancer

Therapeutic Application

Marked PET response with *PIK3CA*-directed Rx

Pre-Therapy

Post-Therapy
PIK3CA Mutation Distribution Across Tumor Types

Percentage of each tumor type demonstrating PIK3CA mutation

- Total: 7%
- Lung: 4%
- Colorectal: 16%
- Brain: 7%
- Breast: 33%
- Pancreas: 2%
- Other: 5%

Legend:
- # cases
- # mutant cases
- # PIK3CA mutant cases
BRAF V600E mutations are common in a subset of pediatric brain tumors

Activating mutations in PIK3CA in a rare and very aggressive skin malignancy

identify new targets for rare/poorly studied malignancies
enable rapid translation to patient care (genotype-driven clinical trials)

BRAF V600E Mutations Are Common in Pleomorphic Xanthoastrocytoma: Diagnostic and Therapeutic Implications
Dias-Santagata et al., PLoS ONE, 2011

Activation of PI3K Signaling in Merkel Cell Carcinoma
Nardi et al., Clin Cancer Res, 2012
Future Goals

Moving toward a more comprehensive tumor genetic fingerprint

Future sequencing strategies include:

- **Sanger Sequencing**
  - One exon
  - >160 hotspot mutations
  - 15 cancer genes

- **SNaPshot**
  - "Wild type"

- **Next Generation Sequencing**
  - All exons
  - Hundreds of cancer genes
Tumor Genetic Changes Drive Cancer Development

- **Copy number**
- **Indels**
- **Point mutations**
- **Rearrangements**
- **Gene expression**
- **Proteomics**
- **Gene expression**
- **Proteomics**
- **Epigenetics**
- **Non-coding RNAs**
- **FISH**

NexGen
The Next Generation of Clinical Cancer Genotyping

**Clinical** targeted sequencing of FFPE DNA

*Goals*

- 300-800 genes (5 Mb)
- 200-500X coverage
- 5 Gb data per tumor-normal pair
- 3-4 week turnaround time
- $500-700 raw reagent cost
- Tumor vs. normal
- SNV, indel, copy number
Clinical Application of Tumor Genotyping

Challenges

- **Biological/Clinical**
  - Tumor Heterogeneity
  - Tumor Evolution

- **Practical/Logistical**
  - Data Management – How to interpret data and deliver results quickly
  - Maximum Sensitivity – Resistance mechanisms
  - Rapid Evolution of Knowledge and Clinical Indications
  - Cost and Reimbursement
Prospective, multiplexed genotyping can be efficiently incorporated into clinical practice to aid clinical decision-making and to accelerate stratified clinical trial enrollment.

Broad-based tumor genotyping can identify new prognostic/therapeutic markers not previously identified in a specific cancer type.

Emerging tumor genotyping technologies including Next Generation Sequencing will be an important tool in expanding our efforts to:

- identify novel therapeutic targets
- understand differential response to treatment
- discover additional mechanisms of acquired resistance
MGH Clinical Translational Collaborators
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Jose Baselga (Phase I/Director)
David Louis (Director)

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Rebecca Heist (Lung Cancer)
Ignatius Ou (Lung Cancer)
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Thank you to the PATIENTS and their Families!