Going Beyond the Standard of Care: Translation of Personalized Medicine into the Clinical Oncology Setting

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Objectives

• Discuss the current role of somatic genetic testing in clinical practice
• Explain the purpose and value of a molecular tumor board in terms of treatment recommendations
• Identify future challenges to the implementation of genetic-guided therapy into standard oncology clinical practice
Guidelines are backward looking

With cancer, things change too rapidly for doctors to be able to rely on yesterday’s guidelines for long.

Vincent T. DeVita, Jr, MD
The Death of Cancer
Tumor vs. Patient Genome

**Tumor Genome**
- **Acquired genetic variation**
- Predicts tumor response
  - HER2: trastuzumab
  - BCR-ABL: imatinib
  - BRAF V600E: vemurafenib
  - ALK+: crizotinib

**Patient Genome**
- **Inherited genetic variation**
- Predicts drug exposure
  - Enzymes
  - Transporters
- Predicts toxicity
  - Drug targets

Targeting the Tumor Genome

- Genetic alterations in molecular pathways are involved in tumor development, survival, and progression/metastases
- We have the technology! We can profile it!
- Targeted anticancer drugs are available commercially or in clinical trials

Genetic Alterations in Lung Cancer

Current Treatment for NSCLC
Targeting Therapy in Lung Cancer

**BRAF**
- Mutations seen in up to 7% of NSCLC with more than half being the V600E mutation which is associated with more aggressive disease
- **Dabrafenib** and **trametinib**, or **vemurafenib**

**MET**
- Exon 14 skipping seen in 3-4% of NSCLC
- Amplification in 2-4% untreated patients and 5-20% in EGFR-mutated tumors as acquired resistance
- **Crizotinib** or **Cabozantinib**

**RET**
- RET fusions seen in about 1% of NSCLC, but may be closer to 6-19% in select never-smokers
- **Cabozantinib**, **vandetinib**, **lenvatinib** or **ponatinib**

**ERBB2**
- Mutations seen in 2-4% of NSCLC with the majority being exon 20 insertion mutations
- **Trastuzumab**, **afatinib**, or investigational **neratinib**

Goal of Precision Medicine

• Determine the optimal treatment or **sequence** of treatments for a patient
  – Which therapy will yield the best response?
  – How do we optimize the response?
  – How do we minimize toxicity?
In which patients do we typically do tumor genetic testing?

- **Prognostic questions**
  - Hematology, especially CLL, AML, and MDS

- **Predictive questions**
  - Patients with standard of care targeted therapy
    - EGFR in metastatic NSCLC at diagnosis
  - Patients in whom there may not be standard of care options
    - Sarcoma, merkel cell, or cancer of unknown primary
  - Patients with advanced disease who may have limited options
  - Clinical trial enrollment
    - Basket trials
Tumor Genome Analysis Workflow

1. **Tumor Genome Testing**
   - What is the goal of the test?
   - What test should be ordered?
   - What tissue is available?

2. **Identification of Variants**

3. **Clinical Interpretation**

4. **Patient – Oncologist Decision**
<table>
<thead>
<tr>
<th>Test</th>
<th>Genes Analyzed</th>
<th>Tissue Analyzed</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foundation One®</td>
<td><strong>315</strong> genes and introns of 28 genes involved in rearrangements</td>
<td>Tumor tissue</td>
<td>• Reports mutation burden (per MB) and MSI status</td>
</tr>
<tr>
<td>Foundation One® Heme</td>
<td><strong>406</strong> genes (DNA), selected introns of 31 genes to assess rearrangements and 265 genes (RNA) to detect additional fusions</td>
<td>Tumor Tissue (may be blood or bone marrow for heme malignancies)</td>
<td>• Reports mutation burden (per MB) and MSI status</td>
</tr>
<tr>
<td>Genoptix® NexCourse Complete</td>
<td><strong>236</strong> genes including select copy number alterations and rearrangements</td>
<td>Tumor Tissue (may be blood or bone marrow for heme malignancies)</td>
<td>• Does not include BCR-ABL</td>
</tr>
<tr>
<td>Foundation One® ACT</td>
<td>Complete exons of <strong>27</strong> genes, introns of 6 genes involved in rearrangements and select exons of <strong>34</strong> genes</td>
<td>Blood for cell free DNA analysis</td>
<td>• Correlation between volume of disease and concordance with cell-free DNA</td>
</tr>
<tr>
<td>Guardant360®</td>
<td><strong>73</strong> cancer related genes including 6 select rearrangements</td>
<td>Blood for cell free DNA analysis</td>
<td>• Correlation between volume of disease and concordance with cell-free DNA</td>
</tr>
</tbody>
</table>
Moffitt in-house

**Genoptix®**

**Guardant360®**

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**Clinically Relevant Results**

- FDA Approved Therapies, Prognostic Indicators, or Other Course of Action (in patient's tumor type)

**Interpretation:** A BRAF V600E mutation was detected with an allele frequency of 0.06. This activating mutation is seen in around 9% of colorectal cancer and is associated with poor prognosis. (Birnbaum et al., 2010; Jan 30, 2010; J Clin Oncol; 28(1):1:1-1:10).

**RESULTS**

- Other variants: see “All identified variants described in literature” section

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**FoundationOne® / Heme / ACT**

**Genetic Alterations**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genetic Alteration</th>
<th>Mutation Effect</th>
<th>Allele Frequency</th>
<th>Pathologist</th>
<th>Variant Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM</td>
<td>c.7758A&gt;T, p.G2586S</td>
<td>Missense</td>
<td>7%</td>
<td></td>
<td>UNCERTAIN</td>
</tr>
</tbody>
</table>

**Variant Assessment:**

The effect of the alteration on the ATM protein function is not known. Common alterations in ATM are inactivating mutations.

**Clinical Summary:**

- Nonsynonymous single nucleotide variant was detected in ATM, resulting in a predicted TM50 protein change. This variant is described in the TCGA datasets of a colorectal adenoma: c.1:1,186C>T (p.G2586S). It has also been identified as a somatic change in colorectal and lung tumors in cancer patients. A recent study has shown that coexpression of ATM and TP53 mutations is a strong predictor of colorectal cancer patients who are likely to respond to specific therapies.

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**Clinical Relevance of Detected Alterations**

- **EGFR (c.2881A>G)**
  - Alteration: c.2881A>G
  - Role in Cancer: Overexpression of EGFR is associated with increased cell proliferation and survival. EGFR inhibitors, such as gefitinib and erlotinib, are used to target this pathway.

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**INTERPRETATION:**

- **ROH1** exhibits a tumor suppressor function in the colorectal tumor microenvironment. The presence of specific ROH1 mutations is associated with a poor outcome in colorectal cancer patients. The use of ROH1 inhibitors in clinical trials is promising for the treatment of colorectal cancer.

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**Guardant360®**

- **NTRK1**
  - Alteration: c.1679_1698del
  - Role in Cancer: NTRK1 is a receptor tyrosine kinase that is commonly activated in cancer cells. NTRK1 inhibitors, such as larotrectinib, are used to target this pathway.

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**Moffitt in-house**

- **ALK (c.1494_1503del)**
  - Alteration: c.1494_1503del
  - Role in Cancer: ALK is a tyrosine kinase receptor that is often activated in lung cancer. ALK inhibitors, such as crizotinib, are used to target this pathway.
Cell Free DNA (cfDNA) Assays

- Tissue biopsies are not always feasible
- Enables serial monitoring over time to assess for resistance mutations and changes in frequency
- May better represent tumor heterogeneity
- Value of cell free DNA (cfDNA) and serial sampling
  - Plasma derived assays
    - Best concordance when higher number of metastatic sites, lower albumin, higher number of prior therapies
    - Site of disease also showed correlation
  - Cerebral Spinal Fluid (CSF)
    - Somatic alterations found in 63% of CNS metastases from solid tumors and 50% of primary brain tumors

Mutation Landscape Changes over Time

- 40 yo non-smoking female diagnosed with Stage IV NSCLC, adenocarcinoma

EGFR exon 19 del

4/2015 Started erlotinib

EGFR T790M

9/2015: D/C erlotinib
Started osimertinib

EGFR C797S

12/2015: D/C osimertinib
Started carboplatin/pemetrexed/bevacizumab

KRAS A146V and D119N

3/2016: High mutation burden, PDL1 positive
Started Pembrolizumab
**EGFR C797S and Resistance**

- We are familiar with resistance mutations:
  - Erlotinib $\rightarrow$ T790M
  - Osimertinib $\rightarrow$ C797S

- **EGFR C797S** – acquired resistance mutation
  - Covalent binding site for 2\textsuperscript{nd} and 3\textsuperscript{rd} generation EGFR-inhibitors like afatinib and osimertinib

\[\text{C797S} \text{ mutation in } \text{CIS} \text{ with } \text{T790M}\]

- Resistant to EGFR-inhibitors, use alternate therapy

\[\text{C797S} \text{ mutation in } \text{TRANS} \text{ with } \text{T790M}\]

- Combination of first- and third-line EGFR inhibitors

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Clin Cancer Res. 2015;21:3924-33
Tumor Genome Analysis Workflow

• What is the goal of the test?
• What test should be ordered?
• What tissue is available?

• What type of variants will be assessed?
• Lower limit of quantitation, number of reads, etc

• How is actionability determined?
• Priority given to multiple actionable variants?
• How to handle variants of unknown or almost known significance?
• Germline variants?
Clinical Actionability

- Genetic alteration predicts response to a particular therapy
  - Benefit or resistance to a particular therapy
  - FDA approved therapy in the patient’s tumor or another type of tumor
  - Clinical trial for the particular alteration or reasonable based on molecular biology

- Genetic alteration provides diagnostic or prognostic information

- Clinically relevant germline alteration that informs disease risk or pharmacokinetic or pharmacodynamics
### Supporting Data
- Comparative trial with biomarker selection/stratification (patient’s tumor type or different tumor type)
- Retrospective cohort or case-control trials
- Biomarker association with response less robust (secondary endpoint)
- Case study or case series
- Preclinical data only (in vitro or in vivo models)

### Clinical Actionability
- FDA approved therapy in patient’s tumor type
- FDA approved therapy in different tumor type
- Clinical trial based on specific mutation
- Clinical trial based on application of pathway biology
- Prognostic information
- Not clinically actionable at this time
Variants of Almost Known Significance

- Variation found in clinically significant gene in area of known tyrosine kinase binding or other known relevant area
  - Specific alteration itself is unknown
  - Example: EGFR N771Y
    - Located in the EGFR tyrosine kinase domain in exon 20 but has not been previously reported in COSMIC or other sources

- Value of functional based assays
- Importance of data sharing, especially regarding relevant clinical outcomes
Tumor Genome Analysis Workflow

• What is the goal of the test?
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• How is actionability determined?
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• Germline variants?

• Ability to qualify and travel for a clinical trial?
• Ability to acquire off label therapy?
• Other patient factors to consider?
Translating Recommendations into Clinical Decision Making

• Researching and presenting available data to facilitate the decision making process
• Considering the interaction of all the mutations together
  – Cyclin D pathway alteration + RB1 loss
• Consideration of each patient’s unique characteristics
  – Desire for a clinical trial and ability to travel
  – Availability and ability to qualify for a clinical trial
  – Sequencing of treatment options
  – Insurance coverage and ability to afford off label therapy
  – Patient preference on treatment options
  – Where patient is in his/her treatment course
Personalized Medicine Clinical Service (PMCS) and Clinical Genomics Action Committee (CGAC)

Tumor Genome Analysis Workflow

- Tumor genetic testing ordered and results returned
- Personalized Medicine Clinical Service discussion and review
- Expedited consult communicated to ordering clinician
- Referral to Clinical Genomics Action Committee
- Consult report generated and documented in EHR
- Discussion with oncologist and patient
- Assistance with acquisition of off-label therapy if needed

Clinical Genomics Action Committee (CGAC)

- Breast
- Thoracic
- Leukemia
- Bioinformatics
- Myeloma
- Genetic Couns
- Medical Gen.
- PCM Fellow
- Heme Pathology
- GU
- Pharmacy
- Hem/onc fellow
- Mol Pathology
- Sarcoma

Moffitt Cancer Center
### CGAC Clinical Database

#### Mutation Analysis
- Patient Summary
- Add Patient
- Patient List
- By Gene and Protein Change

#### Reports
- Report by Gene
- Report by Cancer Type
- Patient-Mutation Report

#### Review List
- Review List

#### Help
- Glossary

#### Other Tools by CIC
- MutationID
- ExpressionID
- GeneID

#### List of Findings for patient

<table>
<thead>
<tr>
<th>Gene</th>
<th>Location</th>
<th>Mutation</th>
<th>Significant</th>
<th>CNA</th>
<th>MAF</th>
<th>In EVS</th>
<th>Protein Domain</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP300</td>
<td>22q13.2</td>
<td>R695P</td>
<td>NO</td>
<td></td>
<td></td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPS3</td>
<td>17p13.1</td>
<td>R337C</td>
<td>YES</td>
<td>No</td>
<td>P53_tetramer</td>
<td></td>
<td>Ex</td>
<td>Detail</td>
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<tr>
<td>NUP93</td>
<td>16q13</td>
<td>A72V</td>
<td>NO</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RB1</td>
<td>13q14.2</td>
<td>L331F?</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDAC7</td>
<td>12q13.1</td>
<td>R166H</td>
<td>NO</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LRRK2</td>
<td>12q12</td>
<td>Q923H</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
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<tr>
<td>KRAS</td>
<td>12p12.1</td>
<td>C180N</td>
<td>NO</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLX1</td>
<td>7q22.1</td>
<td>S1134C</td>
<td>NO</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP3K1</td>
<td>5q11.2</td>
<td>A19S</td>
<td>NO</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOTCH2</td>
<td>1p13-p11</td>
<td>P615T?</td>
<td>YES</td>
<td>No</td>
<td>EGF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMGL3</td>
<td>T23M</td>
<td></td>
<td>NO</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Add Gene and Mutation

**Gene:**

**Mutation (Change):**

**Significant:**

**CNA:**
CGAC Database

3. Mutation Frequency in TCC Samples

Tumor Samples vs. Normal Samples

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Proteo</th>
<th>Sample with Mutation</th>
<th>Total Sample</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrium</td>
<td>G2023R</td>
<td>1</td>
<td>200</td>
<td>0.5</td>
</tr>
<tr>
<td>Esophagus</td>
<td>G2023R</td>
<td>1</td>
<td>41</td>
<td>2.7273</td>
</tr>
<tr>
<td>HEME-CLL</td>
<td>G2023R</td>
<td>2</td>
<td>94</td>
<td>2.12766</td>
</tr>
<tr>
<td>Kidney</td>
<td>G2023R</td>
<td>1</td>
<td>243</td>
<td>0.41152</td>
</tr>
<tr>
<td>Lung</td>
<td>G2023R</td>
<td>1</td>
<td>603</td>
<td>0.16584</td>
</tr>
</tbody>
</table>

Mutation Frequency of ATM(G2023R)
<table>
<thead>
<tr>
<th>Category</th>
<th>Resource</th>
<th>Utility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variants of Unknown Significance</td>
<td>1000 Genomes Project (<a href="http://www.1000genomes.org/">http://www.1000genomes.org/</a>)</td>
<td>Provide a probability of the variant being germline</td>
</tr>
<tr>
<td></td>
<td>Exome Variant Server (<a href="http://evs.gs.washington.edu/EVS/">http://evs.gs.washington.edu/EVS/</a>)</td>
<td>Provide a probability of the variant being germline</td>
</tr>
<tr>
<td></td>
<td>HCI Breast Cancer Gene Prior Probabilities (<a href="http://priors.hci.utah.edu/PRIORS">http://priors.hci.utah.edu/PRIORS</a>)</td>
<td>Data on all possible single nucleotide substitutions in BRCA1/2</td>
</tr>
<tr>
<td></td>
<td>American College for Clinical Genetics (ACMG)</td>
<td>Association of a variant with an inherited disease</td>
</tr>
</tbody>
</table>

# Clinically Important Genetic Resources

<table>
<thead>
<tr>
<th>Category</th>
<th>Resource</th>
<th>Utility</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variants from across Cancer Types</strong></td>
<td>cBioPortal (<a href="http://www.cbioportal.org/">http://www.cbioportal.org/</a>)</td>
<td>The frequency of a variant across cancer types and the location of the variant in the functional domains of the gene</td>
</tr>
<tr>
<td></td>
<td>Catalogue of Somatic Mutations in Cancer (COSMIC) (<a href="http://cancer.sanger.ac.uk/cosmic">http://cancer.sanger.ac.uk/cosmic</a>)</td>
<td>The frequency of a variant across cancer types</td>
</tr>
<tr>
<td><strong>Therapeutic Association</strong></td>
<td>MyCancerGenome (<a href="http://www.mycancergenome.org/">http://www.mycancergenome.org/</a>)</td>
<td>Association of mutation with tumorigenesis, related therapeutic implications and available clinical trials</td>
</tr>
<tr>
<td></td>
<td>PharmGKB (<a href="https://www.pharmgkb.org/">https://www.pharmgkb.org/</a>)</td>
<td>Interactive tool for researchers investigating how genetic variation effects drug response</td>
</tr>
<tr>
<td></td>
<td>Personalized Cancer Therapy Knowledge Base for Precision Oncology (<a href="https://pct.mdanderson.org">https://pct.mdanderson.org</a>)</td>
<td>Knowledge base resource for the implementation of personalized cancer therapy and integrating information about tumor DNA, RNA, protein and metabolomics profiles with predicted therapy response</td>
</tr>
</tbody>
</table>

In vitro bladder cancer cell data supports this mutation induced phosphorylation of PLCγ1, FRS2 and ERK1/2. Differences were seen between different FGFR3 mutations and different cell types.

Pazopanib was shown in vitro to inhibit FGFR3 activating mutations at an IC50 of 100nM-1uM and one SqCC head and neck cancer patient with an FGFR2 P253R mutation had a response to pazopanib.

67 yo woman with metastatic papillary urothelial carcinoma s/p several chemotherapy agents found to have FGFR3 amp and S249C (58%), treated with pazopanib and had a PR > 6 months.

AZD4547 is part of the NCI-MATCH trial expanded arms

- Subprotocol W (FGFR1-3 amplifications, mutations or translocations)
Germline Challenges

• If tumor is analyzed with matched normal tissue, can subtract out alterations found in the normal tissue
  – If normal tissue not analyzed, more difficult to separate
  – Allele frequency of 50% or 100% may indicate germline alterations in some assays

• Available databases
  – Exome variant server
  – ClinVar

• ACMG recommendations regarding incidental findings for suspected germline mutations in tumor tissue
Mutation Load and Immunotherapy

• **Exciting therapy, but not everyone has a response**
  – Durable responses to anti-PD1 therapy were seen in:
    • 31-44% of melanoma
    • 19-20% of lung cancer
    • 22-25% of renal cell carcinoma
  – Potential biomarkers:
    • Density of CD8+ T cells in tumors
    • Expression of PDL1 on tumors
    • **Mutation burden and microsatellite instability:** now being reported by some molecular testing companies for individual patients

**Example:** MSI: Stable
Mutation Burden: **High**, 25 mutations per megabase

• Nat Rev Cancer. 2016;16:275-287
Mutation Load and Immunotherapy

**Number of Mutations**

- Improved **overall survival** with CTLA4-inhibitors in melanoma patients with > 100 mutations (p=0.04)
  - 64 patients treated with ipilimumab or tremelimumab
  - Neoantigen response signature developed

- Improved **mPFS** in lung cancer patients treated with pembrolizumab with high mutation burden
  - Patients with durable responses had a median of 302 mutations vs. 148 in those without a durable response (p=0.02)

**Microsatellite Instability**

- 41 patients with MMR-deficient colorectal cancer, 9 patients with other MMR-deficient cancer and 21 MMR-intact colorectal cancer patients
  - All treated with pembrolizumab

- Whole exome sequencing mean number of somatic mutations per tumor
  - MMR-deficient: 1782 mutations
  - MMR-intact: 73 mutations
  - Higher somatic tumor burden = improved mPFS

N Eng J Med. 2015;372:2509-20
Future of Somatic Genomics

• What are the optimal mutational profiling approaches?

• How do we translate these findings into clinical practice for the average oncologist?
  – Defining “clinically actionable”
  – Handling “variants of unknown significance”
  – Facilitating patient discussions
  – Ethics on germline findings

• What clinical trials should we be doing?
  – Novel trial design like “Basket Studies”
Ongoing Challenges

• Identify, interrogate and validate the correct biomarkers for targeted and immunotherapies
• Utilize novel clinical trial designs to assess outcomes across tumor types and mutations
  – Basket trials
  – Genetic-guided Registry trials
  • **Targeted Agent and Profiling Utilization Registry (TAPUR)**
    – Goal: To learn from the real world practice of prescribing targeted therapies to patients with advanced cancer whose tumor harbors a genomic variant known to be a drug target or to predict sensitivity to a drug
    – Currently open at 4 sites with many more planned, 15 arms
    – NCT02693535
Optimizing Targeted Therapy

- Translate our understanding of cancer biology crosstalk and feedback signaling into rationale drug combinations
- Modify the immune environment to improve tumor identification and destruction
- Improve biomarker identification and validation to target the right genetic drivers

Acknowledgements

• **Personalized Medicine Clinical Service**
  – Howard McLeod, PharmD
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  – Richard Lu, PhD

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• **Our Patients**