Overcoming Operational Challenges of Personalized Cancer Therapy

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- 423 patients consented
  - Out of how many new metastatic patients?

- 195 “targetable” mutations
  - 23 (5%) had standard of care altered outside trials
  - 13 (3%) had clinical benefit from their therapy

- Important research topic
  - Didn’t change standard of care for 95% of patients
  - No evaluation of the psychological/QoL consequences of this unactionable extra knowledge

Clinical Testing: Real Clinical Utility or Irrational Exuberance?

- Multidisciplinary decision team
- Distinguish between clinical validity based on level of evidence and “actionable” interest based on hypothesis
  - Outside of a clinical trial
- Avoid or manage conflicts
  - Academic (research intent as clinical service)
  - Competition (between institutions or with industry)
NGS Clinical Test Volume Grows Rapidly

- DNA sequencing with 46-gene panel
- 3354 CMS46 reports 2012-2013
- 3035 unique patients at MDACC
BREAST CANCER

Mutation frequency (%)

- TP53
- PIK3CA
- AKT1
- ATM
- MET
- ABL1
- NRAS
- EGFR
- SMAD4
- HRAS
- APC
- STK11
- PTEN
- ERBB2
- MLH1
- BRAF
- KRAS
- FGFR2
- ERBB4
- FGFR3
- PTPN11
- MPL
Clinical NGS requires high coverage depth

- the trade-off in generating so many parallel sequences using PCR /DNA polymerase is loss of accuracy.

- NGS platforms have approximately 10-fold higher error rates (1 in 1000 bases) versus Sanger sequencing (1 in 10,000 bases).

- For clinical accuracy, each template requires 100’s of sequence reads to account for sequencing errors, non-neoplastic DNA “contamination”, and artifacts from formalin.
CLIA next-gen sequencing of solid tumors

- High coverage: multiple (~500x) reads of the same sequence to gain confidence in result
  - Critical when ratio of neoplastic to non-neoplastic cells is low
  - Allows signal to be sifted from the noise
- Examination of reads in both directions to rule out artifacts
- Confirm or rule out sequence variant using an additional method (e.g. Sanger)
Not all biopsies are equal!
Adequacy for histologic diagnosis vs. adequacy for biomarker testing

- Very difficult issue
- Not unusual to make a diagnosis of cancer from only a few cells
- These same cases might be unsuitable for molecular testing
- Focus on tissue qualification in Pathology
- Engage the interventional radiologists and the cytologists
What about an FNA sample?

Methods in Pathology

Next-generation sequencing-based multi-gene mutation profiling of solid tumors using fine needle aspiration samples: promises and challenges for routine clinical diagnostics

FNA Direct Smears or Cell Block Sections (FFPE) Are Equivalent To Excised Tumor Tissue (FFPE) For Next-Gen DNASeq In The Clinical Lab

What about Nucleic Acid Preservatives?

Prospective registry trial at MDACC (PI: Stacy Moulder).

Samples collected at time of clinical procedure following a SOP

Pass rates represent Dx, & yield & quality of RNA & gene expression microarray data

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>ER+/HER2-</th>
<th>TNBC</th>
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<tbody>
<tr>
<td></td>
<td>Pass</td>
<td>Fail</td>
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<tr>
<td>FNA</td>
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<td>7</td>
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<tr>
<td>CBX</td>
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<td>Surgical</td>
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Clinical Testing For Personalized Cancer Therapy

1. Requires a truly multidisciplinary decision and active participation

2. Seek clinical validity and clinical utility before implementation as standard of care
   • Otherwise, it is reasonable to perform within a clinical trial

3. Only perform in an accredited diagnostic laboratory
   • Procedures and requirements are different from research

4. Quality of the sample is critical to success
   • Ideally collect samples with the best quality molecules
   • Otherwise, sample qualification is essential
     • Need to demonstrate feasibility with limited samples