Pre-analytical variables affecting Fine Needle Aspiration Biopsies (FNAs) and their influence on clinical diagnosis

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Disclosure

Dr. Clark is entitled to a share of equity as a founder of BioMarker Strategies, LLC. He currently is an officer for the company. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.
Objectives

- To understand the biospecimen challenges and opportunities presented by Fine Needle Aspiration Biopsies (FNAs) of solid tumors.

- To understand how FNAs can facilitate development and application of predictive markers for cancer therapy.
Personalized Cancer Treatment: A JH case study

• 36 yr old female w/ history of breast carcinoma in 2004
• Mother died at young age of breast cancer
• Patient carries a germline BRCA1 mutation
• Now presents with RUL lung mass, mediastinal adenopathy. R/O Lung primary.
Pre-carinal Lymph Node Transbronchial FNA

• Specimen handled by pulmonologist, processed using traditional methods

• Dx: Poorly-differentiated adenocarcinoma.
Clinician’s response...

- The key issue in this case is to differentiate between breast and lung. If breast cancer, then predictive markers of response (i.e., ER/PR/HER2) are needed to decide if endocrine therapy or trastuzumab might be an option. If lung, molecular markers looking for k-ras and EGFR mutations might be useful as predictors of response to EGFR TKIs like erlotinib.

- Let's hope (if indeed breast) that she now has HER2-positive disease or (if lung) that it has EGFR mutation ...
Performing an FNA

- 25 gauge needle
- It’s NOT an aspiration biopsy.
- **Excursion of the needle through the lesion,** NOT aspiration, is key to obtaining material.
- Ultrasound-guided

(Ljung) [http://www.papsociety.org/fna.html](http://www.papsociety.org/fna.html)
FNA Sample Processing: highly variable

- Direct Smears
  - Air-dried, methanol-fixed
  - EtOH-fixed
- Cytospins
- ThinPrep® (Hologic)
- PrepStain™ (BD)
- Cell block (FFPE)

Cytopreparation (Gary Gill) http://www.cytopathology.org:80/website/download.asp?id=867
19th Century Processing vs. 21st Century Technology

• Tissue fixation with formaldehyde was discovered by Ferdinand Blum in 1863.

• Paraffin wax embedding was described by Edward Klebs in 1869.

• FNA processing methods were developed in the 1960’s-1980’s.

Edward Klebs (1834-1913)

Biomarker information is lost during routine tissue processing

- Surgical Excision
- Manual Fixation
- Tissue Processing
- Manual Embedding
- Manual Sectioning
- Contamination

Lost Information

Current basis for MDx
Biospecimen Challenges of FNAs for Molecular Diagnostics

- Relatively small sample size.
  - 200,000-10^6 cells
  - Representative of whole?
- Heterogeneous and variable cellular composition.
- Minimal, but unique pre-analytical variability.
Biospecimen Advantages of FNA sampling:

• Avoids pre-analytical ischemia.
• Less invasive and more cost-effective than surgical excisional biopsies.
• Can obtain live cells.
There is a strong, but unmet, clinical need for MDx on FNAs

- Surgery is not indicated for most patients with advanced, metastatic disease.
- Neo-adjuvant treatment regimens will require pre-operative molecular characterization.
  - Tumor may be gone or altered after treatment.
- Approx. 50% of these cancer patients are NOT surgical candidates
- Tumor should be sampled via FNA

Sample Size: Biomolecules in FNA samples

- **Tumor cells**
  - $3 \times 10^4 - 1 \times 10^6$
  - $>92\%$ tumor cells (47\%-98\%)

- **DNA**
  - 10 ug

- **RNA**
  - $>1$ ug (0.5-12 ug)
  - Breast FNA: 3.6 ug (2.8 ug in core biopsy)

- **Protein**
  - 200 ug

Successful Examples of Cytology MdxF

- **DNA-based biomarkers**

- **DNA Methylation**

- **Proteomics**

- **Quantitative RT-PCR/Microarrays**

- **Immunocytochemistry**

Summary of JH EGFR and KRAS Testing -2008

5% FNAs at Brigham; Smouse, et al. Cancer 2009; 117:67-72
FNAs: Heterogeneous Cellular Composition

Breast FNA: 80% tumor; 15% lymphs; 5% stroma

Core Bx: 50%/20%/30%

Symmans et al, Cancer, 97:12, 2003
Impact of Specimen Heterogeneity on MDx: *PIK3CA* DNA Sequence

- **E545**: Control
- **E545**: Heterogeneous False Negative
- **E545K**: Enriched
- **E545**: Negative
Model systems for FNA Biospecimen Research: FNAs on murine xenografted human tumors

Molecular assays:
⇒ mRNA levels to determine response to anti-EGFR therapy
⇒ Methylation-specific PCR to monitor treatments that modify chromatin structure

Slight Reduction in FNA RNA Integrity Numbers (RIN)

- RNA Integrity Numbers (RIN) lower in FNAs relative to frozen, excised tumor.
- FNA RINs decrease slightly over time at 37°C.
- Still fit-for-purpose.
- Higher than FFPE (RIN=2.5).
- Mechanism?
- Solutions?
Static vs. Dynamic Biomarkers
The **live cell** opportunity: Ex vivo biomarkers provide **functional** pathway information.

- **Biopsy Sample**
- **Dispersion + Enrichment**
- **Growth factor Stimulation**
- **Drug**
- **Control**

**Ex vivo biomarkers:** mRNA phosphoproteins

**Predictive Biomarkers**

**Drug Inhibition**
Ex vivo Biomarkers

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<th>Tissue</th>
<th>Stimulus</th>
<th>Inhibitor</th>
<th>Ex vivo Biomarker</th>
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<tr>
<td>AML</td>
<td>G-CSF</td>
<td>None</td>
<td>p-ERK; p-STAT3</td>
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<td>EGF</td>
<td>Erlotinib</td>
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<td>Cyclin B1 mRNA</td>
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Question: This tumor is sensitive to which of the following drugs?

a. Iressa  
b. Tarceva  
c. Cetuximab  
d. None of the above
Susceptibility testing for tumors?

FNA for diagnosis + molecular characterization for drug selection.

Monitor patient for responsiveness:
Repeat FNAs of tumor

Modify therapy in response to drug resistance.
Conclusions

• FNAs are clinically-important biospecimens.
• Molecular Diagnostic testing is possible on FNAs.
• FNAs present unique biospecimen challenges and opportunities.
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