Update on Molecular Testing of Cytology Specimens:
Beyond the Cell Block

Dara L. Aisner, M.D., Ph.D.
Assistant Professor
Co-Director, Colorado Molecular Correlates Laboratory

Disclosures
• None relevant to this talk
• Other (>12 months)
  – AstraZeneca
  – Clovis Oncology
  – Oxford Oncology

Molecular Cytopathology
• Growing recognition of the role of combining cytopathology sampling and diagnostic approach with molecular analysis
• Cytopathology has always been a pioneering force – cardinal examples:
  – HPV testing
  – FISH analysis of tumor imprints for sarcoma
  – UroVysion testing for bladder cancer

Molecular Cytopathology – Solid Tumor
• Very high growth of molecular testing in the area of solid tumor
• Applications in cytopathology are increasing in a parallel fashion
Tumor Types Commonly Tested

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Molecular Targets</th>
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<tbody>
<tr>
<td>NSCLC</td>
<td>EGFR, ALK, ROS1, BRAF, ERBB2 (HER2) Others</td>
</tr>
<tr>
<td>Thyroid</td>
<td>BRAF, RET, RAS family others</td>
</tr>
<tr>
<td>Melanoma</td>
<td>BRAF, others</td>
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<tr>
<td>GIST</td>
<td>KIT</td>
</tr>
<tr>
<td>CRC</td>
<td>KRAS, NRAS, BRAF</td>
</tr>
<tr>
<td>Many others…</td>
<td></td>
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</tbody>
</table>

The Cell Block

- In many cases, molecular testing has been validated on cell block preparations
  - Most analogous to surgical pathology specimens
- **Pros:**
  - Validation based on similarity to SP specimens
- **Cons:**
  - Variability in preparation methods
  - Variability in implementation
  - Can be paucicellular
  - FFPE damage/artifacts

Beyond the Cell Block

Based on cell block limitations PLUS

Availability of Smears / Touch Prep / LBC

Numerous groups have worked to validate molecular testing on non-cell block cytopathology preparations

<table>
<thead>
<tr>
<th></th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Cell blocks (FFPE)</td>
<td>Ease of acquisition</td>
<td>Lack of immediate assessment</td>
</tr>
<tr>
<td></td>
<td>Ease of validation</td>
<td>May have low cellularity</td>
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<tr>
<td></td>
<td>Easy to get serial sections</td>
<td>Degradation of nucleic acid due to formalin fixation</td>
</tr>
<tr>
<td></td>
<td>High quality nucleic acid</td>
<td>Partial nuclei in standard 4-5 μm sections (lower DNA yield)</td>
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<tr>
<td></td>
<td>Whole cells= whole nuclei (higher nucleic acid yield)</td>
<td></td>
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<tr>
<td>Direct smears/touch prep</td>
<td>Immediate assessment for tumor adequacy</td>
<td>More difficult to validate</td>
</tr>
<tr>
<td></td>
<td>High quality nucleic acid</td>
<td>Requires technical support and skill to prepare smears</td>
</tr>
<tr>
<td></td>
<td>Whole cells= whole nuclei (higher nucleic acid yield)</td>
<td>May need to sacrifice slide (medicolegal issues)</td>
</tr>
<tr>
<td>Liquid-based prep</td>
<td>Standardized processing with optimal preservation of nucleic acids</td>
<td>More difficult to validate</td>
</tr>
<tr>
<td></td>
<td>High quality nucleic acid</td>
<td>Lack of immediate assessment</td>
</tr>
<tr>
<td></td>
<td>Ease of use</td>
<td>Inability to assess presence/quantify tumor in tested sample</td>
</tr>
<tr>
<td></td>
<td>Whole cells= whole nuclei (higher nucleic acid yield)</td>
<td>Variable preservative capacity of liquid preparations – requires validation for every type</td>
</tr>
</tbody>
</table>
Cytology Smears / TP & Molecular Testing

• Numerous studies have demonstrated suitability of smears for various molecular platforms
• Availability depends on local/regional validation
  – If you need testing performed on a smear / TP sample:
    • Numerous laboratories have validated these approaches
    • Details vary – need to check!
• Impending regulatory changes may present obstacles to these innovative approaches

Smears/TP and Molecular Testing

Often requires de-coverslipping
Highly cellular, tumor rich specimen
- scrape whole slide
- can preserve some cells for archive
Specimen needing tumor enrichment
- Cell lift/transfer techniques
- Microdissection
Upfront knowledge
- Smear/TP slides not cover-slipped
Medicolegal considerations

FISH & Smears / TP

• TP has been a specimen acquisition method for FISH (e.g. sarcoma assays)
• Applications to smears ongoing
  • As new assays become available, validation on TP/smears increase access

FISH & Smears / TP

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Smears/TP: Other Evolutions

- Cell-transfer techniques
  - Anaplastic Lymphoma Kinase (ALK) Immunocytochemistry on Cell-Transfered Cytologic Smears of Lung Adenocarcinoma
    - Chen Zhou, M.D., Kevin L. Buck, M.D.,>You and J. James, M.D., and Henry M. Cosset, M.D.,
  - Courtesy of Marileila Varella-Garcia

- Multi-color assays

Smears/TP: Other Evolutions

- Liquid based cytology testing
  - ALK Status Testing in Non-Small-Cell Lung Carcinoma by RISH on ThinPrep Slides with Cytology Material
    - Kiyohiko Uchiyama, MD, PhD, Christopher P. Legare, MD, PhD, and G. Rafael Fausto, MD, PhD

Cytology & Next Generation Sequencing

Using "Residual" FNA Rinse and Body Fluid Specimens for Next-Generation Sequencing: An Institutional Experience

Highly Accurate Diagnosis of Cancer in Thyroid Nodules With Papanicolaou Cytology: Massively Parallel DNA Sequencing From Routinely Processed Cytological Smears

Factors Affecting the Success of Next-Generation Sequencing in Cytology Specimens

Slides used for smears:
- FF: Fully frosted
- FT: Frosted tip (non-frosted)
- PC: Positively charged

<table>
<thead>
<tr>
<th>TABLE 3. NGS Success and Failure With Direct Smears by Slide Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smears (N = 150)</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>FF</td>
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<tr>
<td>FT</td>
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<tr>
<td>FT + FF (N = 15)</td>
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<td>FF + FT (N = 15)</td>
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</table>
Patient with known EGFR mutation, now with progressing lesions
Chest wall mass; FNA on site

- **EGFR T790M** – patient eligible for 3rd line EGFR TKI
- No need to schedule IR procedure
Summary

• Cytopathology specimens are a critical component of molecular testing for patients
• Expansion of testing to cytopathology specimens requires individualized validation
  – Results in increased patient access to testing
• Cell blocks work fine – but smears/TP are better!

Thank You

• CMOCO Laboratory
• Thoracic Oncology Team
• Department of Pathology
• University of Colorado Cancer Center
• Colorado Center for Personalized Medicine
• Marileila Varella Garcia
• Wilbur Franklin
• Dan Merrick
• Sinchita Roy Chowdhury
• Our patients!