Selecting Pathology Specimens for Solid Tumor Next-Generation Sequencing

Presenter:
Sounak Gupta, M.B.B.S., Ph.D.
Assistant Professor of Pathology
Department of Laboratory Medicine and Pathology
Mayo Clinic, Rochester, Minnesota
Disclosure

• None

Molecular Testing of Tissue

• Preanalytic step - tissue selection
• Tissue metrics and requirements
  • Differ based on test platform
  • Evolve over time
• Examples
• Other tissue considerations
Tissue Considerations

- Source of tissue for Molecular testing
- Acquisition and processing of tissue
  - Fixatives
- Amount of tissue
- Amount of tumor tissue
- Percent tumor nuclei (tumor percent)

Tissue Considerations

- Sources of tissue for Molecular testing
  - Surgery
  - Biopsy
  - Cytology
  - Autopsy
  - Blood draw
- Processed any number of ways
  - Fresh
  - Frozen
  - Fixed
    - Cores or unstained slides
Tissue Requirements

- Each test has 2 requirements!
  - Adequate amount of tumor & cellularity
  - Adequate percentage of tumor nuclei

Example: LNGPR/ Lung Cancer-Targeted Gene Panel with Rearrangement by Next Generation Sequencing
  - Lung cancer panel to identify mutations at hotspots and rearrangements
  - 30 ng of total DNA
    - ~0.4 x 0.4 cm or ~5000 cells
    - >20% tumor nuclei
Test Requirements

<table>
<thead>
<tr>
<th>Test</th>
<th>Tumor %</th>
<th>FFPE (x 5 micron)</th>
<th>Cytology</th>
</tr>
</thead>
<tbody>
<tr>
<td>LNGPR RASFP MELP CAPN</td>
<td>20%</td>
<td>Preferred - 144mm², approx. 4 mm x 4 mm x 10 slides</td>
<td>Preferred - 5,000 cells, up to 3 slides</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minimum - 36mm², approx. 3 mm x 1 mm x 10 slides</td>
<td>Minimum - 3,000 cells, up to 3 slides</td>
</tr>
<tr>
<td>NONCP</td>
<td>30%</td>
<td>Preferred - 360mm², approx. 6 mm x 6 mm x 10 slides</td>
<td>Not accepted</td>
</tr>
<tr>
<td>ALK FISH</td>
<td>NA</td>
<td>100 cells</td>
<td></td>
</tr>
</tbody>
</table>

Quantity of DNA

- Normal human diploid cell = 6 pg of DNA
- 1 ng of DNA = 1000 pg (~167 cells)

<table>
<thead>
<tr>
<th># of Cells</th>
<th>Amount of DNA (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5000</td>
<td>30</td>
</tr>
<tr>
<td>500</td>
<td>3</td>
</tr>
</tbody>
</table>

- No extraction method is 100% efficient
- Cellularity/amount of tumor matters
Tissue Testing: Macrodisssection

Optimal amount of tissue available

Suboptimal amount of tissue, even with good % of tumor nuclei the tissue in the block may be exhausted before the necessary number of sections can be obtained

- Macrodisssection can help optimize the tissue used for testing

Amount of Tissue

- Amount of tissue needed, depends on test
- Quality and quantity of DNA
- Evolving with technology
**Amount of Tumor/Cellularity**

- Problem of small tissue/low cellularity
- Not enough tumor DNA
  - Higher chance of failing assay quality standards
  - Limited amount of template DNA has increased concerns with aberrant PCR products and allele drop out
- Limited tumor sampling (heterogeneity of tumors)

---

**Tissue Cellularity - Resection**

- Excellent cellularity
- High tumor percentage
- Acceptable for molecular and cytogenetic methods

![Tumor cells](image-url)
Tissue Cellularity – Small Biopsy

- High cellularity
- High tumor percentage
- Acceptable for molecular and cytogenetic methods

- Low cellularity
- Low tumor percentage
- Inadequate for molecular and cytogenetic methods

Tissue Cellularity – Cytology Preparation

- Excellent cellularity
- Numerous tumor cell clusters
- High tumor percentage
- Acceptable for molecular and cytogenetic methods
**Tissue Cellularity – Cell Blocks**

- Poor cellularity
- Rare tumor cell clusters
- Low tumor percentage (mostly WBCs)
- Inadequate for molecular and cytogenetic methods

**Tumor Percentage**

- Problem with low tumor percentage
- Depends on the sensitivity of the assay
- Assay method
- If tumor percentage is too low, risk of false-negative
**Tumor Percentage in Large Tissues**

- Large resection tissue
- Low tumor percentage ~<5%
- Inadequate for molecular tests
- Acceptable for FISH

**Tumor Percentage in Large Tissues**

- Large resection tissue
- Low tumor percentage ~10%
- Inadequate for some molecular tests
- Acceptable for FISH
Tumor Percentage in Large Tissues

- Large resection tissue
- High tumor percentage ~90%
- Acceptable for molecular and cytogenetic tests

Tumor cells
Squamous epithelium
Benign tissue

Tumor Percentage in Small Tissues

- Biopsy
- Low tumor percentage ~<5% overall
- Macrodissected ~70% but very small amount of tissue
- Inadequate for some molecular tests
- Acceptable for FISH

The biopsy is a good size and cellular
But mostly benign liver tissue
Only a small piece is tumor
**Tumor Percentage in Small Tissues**

- Small biopsy
- High tumor percentage ~90%
- Acceptable for molecular and cytogenetic tests

**Tumor Percentage in Cytology Preparations**

- High cellularity
- High tumor percentage ~90%
- Acceptable for molecular and cytogenetic tests
Tumor Percentage in Cytology Preparations

• High cellularity
• Low tumor percentage <5%
• Inadequate for molecular tests that require 10% or more tumor nuclei
• Acceptable for FISH

Tissue Fixatives

• Formalin
• Acids
• Heavy metals
• Decalcification
**Tissue Fixatives**

- **Fixatives**
  - Formaldehyde as 10% neutral-buffered formalin
    - Results in fragmentation (~200-300 base pairs)
  - Negatively impact quality
    - Excessive time between surgery to fixation
    - Inadequate or excessive fixation (<6 or >48 hours)

---

### Tissue Fixatives

<table>
<thead>
<tr>
<th>Fixative</th>
<th>Components</th>
<th>Acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formalin</td>
<td>Formaldehyde</td>
<td>Yes</td>
</tr>
<tr>
<td>Zinc buffered formalin</td>
<td>Formaldehyde and zinc</td>
<td>Yes</td>
</tr>
<tr>
<td>Ethanol</td>
<td>70-100% ethanol</td>
<td>Yes (cytology)</td>
</tr>
<tr>
<td>Decalcification</td>
<td>Various formulations (acids vs EDTA) and decalcification times</td>
<td>No</td>
</tr>
<tr>
<td>Bouins</td>
<td>Picric acid, glacial acetic acid and formaldehyde</td>
<td>No</td>
</tr>
<tr>
<td>B5</td>
<td>Mercuric chloride and sodium acetate</td>
<td>No</td>
</tr>
</tbody>
</table>
Tissue Considerations: Inhibitors

• Number of different substances
  • For example melanin, calcium, others
• Different mechanisms of action
  • Inhibit the PCR reaction
  • Complete with the substrate

Tissue Artifacts

• Melanin
• Variable success of PCR-based assays, worth a try
Tissue Artifacts

- Crush artifact
- Difficult to determine percentage for molecular assays
- Difficult to score FISH assays
- Inadequate

Tissue Artifacts

- Necrosis
- Inadequate
Tissue Artifacts

- Cautery
- Difficult to determine percentage for molecular assays
- Difficult to score FISH assays
- Inadequate

Tissue Fixatives

- Declassification
Summary

- Factors that increase likelihood of success
  - Adequate amount of tumor (size and cellularity)
  - Adequate tumor percentage
  - Minimal fixative and artifact tissues
  - Selecting appropriate tissue can be complication – contact MLI with questions

References


Thank You