Application of Cytologic Techniques to Circulating Tumor Cell Specimens

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Cytologic Techniques in Circulating Tumor Cells

Disclosure of Relevant Financial Relationships
No conflicts of interest to disclose.

Outline
• Background
• Circulating tumor cell (CTC) enumeration
• At BWH
• CTC characterization
• Application to non-blood specimens
• Summary/Future

Background
• Metastasis
  • Hallmark of malignancy
  • Primary cause of death in solid tumors
• Mechanism: invasion, survival in circulation, seeding, and establishment

Background
• “Liquid biopsy” – not clearly defined, tumor sampling via blood
• Encompasses
  • Circulating tumor derived nucleic acid
  • Tumor derived extracellular vesicles (exosomes, microvesicles, oncosomes)
  • Circulating tumor cells – intact cells, may be viable or dying
Background

- CTCs may arise from the primary tumor or metastases
- CTCs are rare, even in advanced disease
  - ~1 in 10^6 to 10^9 cells in blood
- First noted in 1869 by Ashworth
- Recent technologies allow reliable identification/isolation
  - Biophysical (size, deformability, density, charge, etc.) or antigenic differences from WBCs leveraged to enrich for and detect CTCs

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CTC Enumeration

- Prognostic
  - At diagnosis and during treatment
  - For localized and metastatic disease
  - By numerous different technologies (only CellSearch is FDA cleared)
  - In many tumor types (carcinoma, also others)

The CellSearch® System

CK+/DAPI+/CD45-

CTCs

Advantages

- Automated sample preparation
- Single cell PCR
- Sensitive
- Selective
- Noninvasive
- Easy to use

CellSearch®

Cristofanilli, NEJM, 2004

CK-/DAPI+/CD45+

WBCs

Cristofanilli, NEJM, 2004

CTC Characterization

- Cytologic
- Molecular
- Functional

Application to non-blood specimens

- Tumor biopsy
- Urine
- Pleural effusion
- Peritoneal effusion
- Bone marrow

Summary/Future

- Improvements in technology
- Increased clinical utility
- Future directions

CTC Enumeration

• Not all patients have identifiable CTCs
  • Sampling (e.g., low stage disease and small sample volume)
  • CTC and identification technology mismatches

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At BWH

• CellSearch system within Cytology since 2005
  • CLIA approved space
  • CTC Clinical Lab – enumeration
  • CTC Core Lab – enumeration and isolation

Current BWH Research

• Clinical protocols for enumeration with breast, GU, and H&N groups
• Clinical protocols for isolation with the breast and lung groups
• Reference for CTC technology development
• Developing CTC characterization protocols and other applications of the technology

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CellSearch™ Profile Kit

Automated sample preparation

Off-line

Preparation by the CellTracks™ AutoPap® System

Chamber preparation

Automated process

Manual process

CTC

control

unstim

stim

CTC

control

MIB-1

CTC

Fe

CTC stim w/ incubation

control

MIB-1

CTC

stim

Lowe, Cancer Cytopathol, 2015.
Herringbone-chip (HB-chip)

Miyamoto, Cancer Discov, 2012.

Stott et al., PNAS, 2010.

Cytologic Techniques in Circulating Tumor Cells

CTC-iChip: CTC Culture

Yu, Science, 2014

CTC-iChip: Drug Susceptibility

Yu, Science, 2014

Concurrent tissue biopsy, CTC, and ctDNA analyses for T790M in EGFR mut patients on TKI

HB-chip


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Cytologic Techniques in Circulating Tumor Cells

Table 1: CSF and MRI findings in patients with positive CDM-CTCs

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Gender</th>
<th>Race</th>
<th>Type of CTC</th>
<th>CDM-CTC</th>
<th>CSF cytology</th>
<th>MRI findings</th>
<th>Correlation with clinical outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55</td>
<td>M</td>
<td>White</td>
<td>1.2</td>
<td>1.5</td>
<td>Negative</td>
<td>Negative</td>
<td>No additional diagnostic workup</td>
</tr>
<tr>
<td>2</td>
<td>62</td>
<td>F</td>
<td>Black</td>
<td>1.3</td>
<td>1.2</td>
<td>Positive</td>
<td>Positive</td>
<td>Repeat CSF cytology or imaging</td>
</tr>
</tbody>
</table>

Abbreviations: CTC = circulating tumor cell; CDM = CTC enumeration; MRI = magnetic resonance imaging.

Table 2: CSF cytology analysis

<table>
<thead>
<tr>
<th>Patient</th>
<th>CSF Cytology</th>
<th>Additional diagnostic workup</th>
<th>Correlation with clinical outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative</td>
<td>No additional diagnostic workup</td>
<td>No additional diagnostic workup</td>
</tr>
<tr>
<td>2</td>
<td>Positive</td>
<td>Repeat CSF cytology or imaging</td>
<td>Repeat CSF cytology or imaging</td>
</tr>
</tbody>
</table>

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Summary/Future

- Multiple CTC platforms exist
- CTC enumeration is prognostic
- CTC characterization of patient samples is imminent
- Non-blood CTC applications hold promise
- CTC specimens are cytology!
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No claims can be processed after that date!

After September 30, 2017 you will NOT be able to obtain any CME or SAMs credits for attending this meeting.