Cancer Update: Metastasis

Circulating Tumor Cells

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Why Study Metastasis?

90% of all cancer-related deaths are due to metastatic disease
Tumor Metastasis:
What we know so far

Hematologic spread of carcinoma results in incurable metastasis

The basic characteristics and mechanism of travel of tumor cells in the bloodstream are not yet understood
Case History

- Patient CL, 58 year-old lady, palpates breast lump on self examination
- Mammogram reveals a spiculated 3cm mass in right breast
- Lumpectomy and sentinel node biopsy performed
- Invasive ductal carcinoma, pT2N1a, ER/PR +ve
- Hormone Therapy + Chemotherapy
Case History cont’d

- Surveillance: Scans
- 3 years s/p resection: Patient reports incapacitating back pain
- Imaging studies show multiple lytic lesions in spine
- Repeat chemotherapy
- 6 months later: Patient reports weight loss and lethargy
- Imaging studies show lesions in bilateral lungs and single lesion in left lobe of liver
- Palliative care
- Patient succumbs to disease
Can we capture metastasis in action?
The Metastatic Process

Wirtz D et al Cancer Volume 11 July 2011
Do all tumors shed tumor cells?

- Yes! It is estimated that approximately 1 million circulating tumor cells are shed per gram of tumor tissue
- Don’t know whether this is active or passive shedding of cells
- Within 24 hours, < 0.1% of those cells are still viable
- This means that < 0.1% survive to produce metastases
- This means that circulating tumor cells are rare
What are circulating tumor cells?

- Ashworth TR. A case of cancer in which cells similar to those in the tumour were seen in the blood after death. *Aust Med J*, 1869
- Cells believed to have detached from a solid tumor and entered the peripheral blood stream
How do we capture CTCs?

- CellSearch System®: Capture, analysis and enumeration of circulating tumor cells
- Can detect as few as 1 CTC per 7.5mL blood
- Approved for use in metastatic breast, prostate and colorectal cancer
- Suggested use is to evaluate prognosis and assist in therapeutic management of cancer patients with metastatic disease
Isolation and Enumeration of CTCs

- Sampled enriched with magnetic beads containing epithelial-cell adhesion molecule ("EpCAM") antibody which will isolate epithelial cells
- Cells labeled with fluorescent nuclei acid dye (DAPI) to visualize nucleus for morphologic evaluation
- In order to distinguish epithelial cells from leukocytes:
  - Fluorescently labeled antibodies that bind cytoplasmic filaments present in epithelial cells = CK
  - Antibodies specific for leukocytes = CD45
- Enumeration is based on fluorescence-based microscopy system which creates computer-generated reconstruction of cellular images
CTC Identification

Tumor (carcinoma) cells = CK +ve
Leukocytes = CD45 +ve
Epithelial cell nucleus > leukocyte nucleus

Circulating Tumor Cells are thus defined as:
EpCAM isolated
CK+ve/CD45-ve cells
Intact nucleus larger than the surrounding leukocytes
Review of Images

Epithelial cells = Red/WBC = Green

Review of Images
Intermediate Phenotypes = CTCs?

Marrinucci D et al *Phys Biol* 2012
CTC Identification Problems

- CK/CD45+ve cells: hybrid epithelial and leukocyte phenotype?
- CK/CD45-ve cells: neither epithelial nor hematopoietic?
- Spotty CK staining
- Irregular nuclei
- Incomplete cells/cell fragments
- Poorly differentiated carcinomas lose their cytokeratin profile
The Metastatic Phenotype
Epithelial Mesenchymal Transformation (“EMT”)

Chaffer CL et al Science Vol 331 25 March 2011
CTC Adhesion and Extravasation
Location of Metastatic Sites

Wirtz D et al Cancer Vol 11 July 2011
Clinical Significance of CTCs

- Can CTC assays be used as a “fluid biopsy” before radiologically detectable disease is identified?
- Is there any correlation between number of CTCs and clinical outcome?
What would serial CTC assays have shown?
The Clinical Significance of CTCs
CellSearch® Use in Metastatic Breast Ca Clinical Trial

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>177</th>
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<tbody>
<tr>
<td>CTC testing interval</td>
<td>4 weeks</td>
</tr>
<tr>
<td>CTC Cutoff</td>
<td>≥ 5 cells per 7.5mL blood</td>
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<tr>
<td>Duration of Testing</td>
<td>6 months</td>
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<td>PFS with baseline count &lt; 5</td>
<td>7.0 months</td>
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<tr>
<td>PFS with baseline count ≥ 5</td>
<td>2.7 months</td>
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<tr>
<td>OS with baseline count &lt; 5</td>
<td>21.9 months</td>
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<tr>
<td>OS with baseline count ≥ 5</td>
<td>10.9 months</td>
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## The Clinical Significance of CTCs

**CellSearch® Use in Metastatic Colorectal Ca Clinical Trial**

<table>
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<td>CTC Cutoff</td>
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<td>Duration of Testing</td>
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<tr>
<td>PFS with baseline count ≥ 3</td>
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<tr>
<td>OS with baseline count &lt; 3</td>
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<td>OS with baseline count ≥ 3</td>
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The Clinical Significance of CTCs
CellSearch® Use in Metastatic Prostate Ca Clinical Trial

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<td>21.7 months</td>
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<tr>
<td>OS with baseline count ≥ 5</td>
<td>11.5 months</td>
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Cohen SJ et al J Clin Oncol 2008
Clinical Significance of CTCs

- In all 3 studies, number of circulating tumor cells before treatment was an independent predictor of progression-free survival and overall survival
### Clinical Significance of CTCs
#### Evolving Technology (GEDI)

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<tr>
<th></th>
<th>N</th>
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<th>≥5</th>
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<td>Prostate</td>
<td>20</td>
<td>90%</td>
<td>80%</td>
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<tr>
<td>Breast</td>
<td>30</td>
<td>80%</td>
<td>70%</td>
<td>60%</td>
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<td>Pancreas</td>
<td>18</td>
<td>61%</td>
<td>44%</td>
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<td>Normal</td>
<td>15</td>
<td>7%</td>
<td>0%</td>
<td>0%</td>
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What we know so far about CTCs

- Cells believed to be of tumor origin identified in the peripheral blood
- They are very rare (3-5 CTCs/ 7.5mL blood)
- Difficult to classify: CK/CD45 +/-
- Associated with an unfavorable prognosis
Remaining Challenges

- If CTCs do not label with epithelial or hematopoietic markers, how should we classify them?
- How do we classify cells that have undergone EMT?
- How many parameters can we use to define CTCs?
- Why are there so few CTCs in the blood?
- Why are some CTCs found in normal controls?
- Why are CTCs not identified in 100% of patients with known metastatic disease
Remaining Challenges

- Do CTCs reflect tumor burden or tumor biology?
- Can we use CTCs as a “metasta-meter”?
  - Do they always produce a metastasis, ie are all CTCs malignant?
  - Do all CTC’s produce metastatic disease?
- What is the best use of CTC enumeration?
  - Monitor remaining disease?
  - A prognostic marker: predictor of metastasis
  - Monitor response during treatment?
Future Directions

- Ongoing clinical trials:
  - SWOG S0500: What is the value of immediate change in chemotherapy based on CTC enumeration versus waiting for clinical evidence of progression?
  - Other types of cancer: urothelial, NSCLC, melanoma
Thank You