

# Application of Cytologic Techniques to Circulating Tumor Cell Specimens

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106TH ANNUAL MEETING

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Cytologic Techniques in Circulating Tumor Cells

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## Disclosure of Relevant Financial Relationships

No conflicts of interest  
to disclose.

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## Outline

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

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## Background

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

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## 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

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## Background

- CTCs may arise from the primary tumor or metastases
- CTC are rare, even in advanced disease
  - ~1 in 10<sup>6</sup> to 10<sup>9</sup> 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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### The CellSearch™ System

**Automated sample preparation**

**Off-Line Processing by the CellTracks™ AutoPrep™ System**

7.5 ml blood from patient → Centrifuge → Aspirate plasma → Add lysis buffer → Aspirate buff and unlabeled cells → Fluorescence and cell staining reagents → Transfer to magnet

**Processing by the CellTracks™ AutoPrep™ System**

Magnetic isolation → Sensitive magnets → CTC isolation

**CTCs** CK+DAPI+CD45-

**WBCs** CK-DAPI+CD45+

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**Figure 1. Kaplan-Meier Estimates of Probabilities of Progression-Free Survival and Overall Survival in Patients with Metastatic Breast Cancer for Those with +8 Circulating Tumor Cells per 7.5 ml of Whole Blood and Those in the Group with 0-7 Circulating Tumor Cells per 7.5 ml of Whole Blood before initiation of a New Line of Therapy.**

Progression-free survival and overall survival were calculated from the time of the baseline blood collection. As shown in Panels A, B, D, and E, follow-up times for each patient were truncated at approximately 9 months (39.7 weeks) to ensure an equivalent comparison between patients in the training set and those in the validation set. Panel A shows the probability of progression-free survival in the training set (IP=0.024 by the log-rank test; hazard ratio for progression in patients with +8 circulating tumor cells per 7.5 ml of whole blood, 1.07; chi-square = 7.83; P=0.02). Panel B shows the probability of progression-free survival in the validation set (IP=0.026 by the log-rank test; hazard ratio for progression in patients with +8 circulating tumor cells per 7.5 ml of whole blood, 1.81; chi-square = 4.32; P=0.03). The median progression-free survival and the proportion of patients according to events of circulating tumor cells were not statistically different in the two sets. The probability of progression-free survival in the full set of data, calculated with the use of follow-up times and truncated as above...

CellSearch™

Cristofanilli, NEJM, 2004

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**Figure 2. Kaplan-Meier Estimates of Probabilities of Progression-Free Survival and Overall Survival in Patients with Metastatic Breast Cancer for Those with +8 Circulating Tumor Cells per 7.5 ml of Whole Blood and Those in the Group with 0-7 Circulating Tumor Cells per 7.5 ml of Whole Blood at the First Follow-up Test after Initiation of a New Line of Therapy.**

Progression-free survival and overall survival were calculated from the time of the baseline blood collection. As shown in Panels A, B, D, and E, follow-up times for each patient were truncated at approximately 9 months (39.7 weeks) to ensure an equivalent comparison between patients in the training set and those in the validation set. Panel A shows the probability of progression-free survival in the training set (IP=0.011 by the log-rank test; hazard ratio for progression in patients with +8 circulating tumor cells per 7.5 ml of whole blood, 0.50; chi-square = 11.20; P=0.001). Panel B shows the probability of progression-free survival in the validation set (IP=0.011 by the log-rank test; hazard ratio for progression in patients with +8 circulating tumor cells per 7.5 ml of whole blood, 3.53; chi-square = 4.23; P=0.04). The median progression-free survival and the proportion of patients according to events of circulating tumor cells per 7.5 ml were not significantly different in the two sets. The probability of progression-free survival...

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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)

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## 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



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## 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

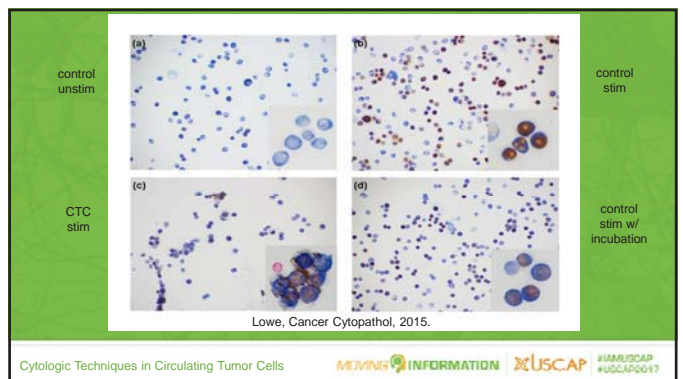
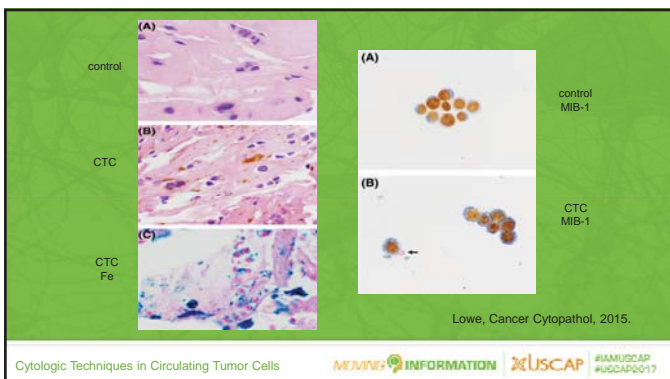
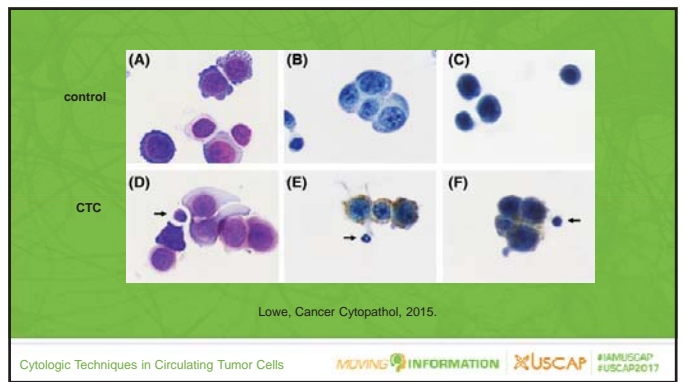
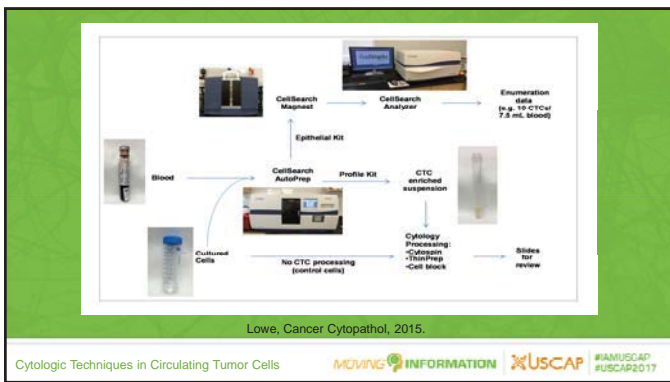
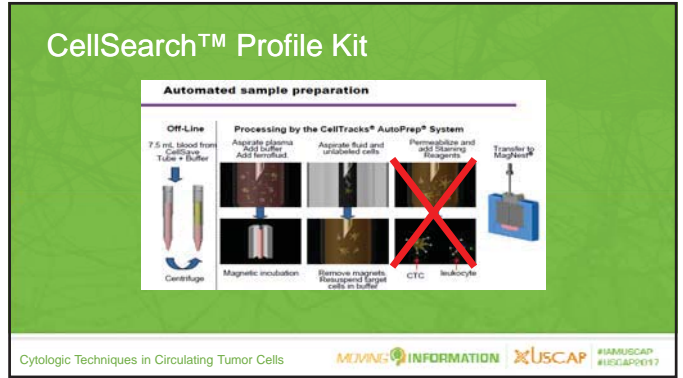
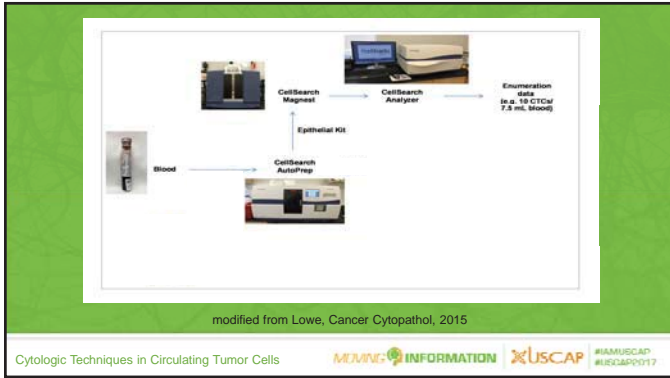
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### Herringbone-chip (HB-chip)

AR activated vs suppressed

Miyamoto, *Cancer Discov.*, 2012.

Stott et al., *PNAS*, 2010.

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### CTC-iChip: CTC Culture

Yu, *Science*, 2014

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### CTC-iChip: Drug Susceptibility

Yu, *Science*, 2014

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

HB-chip

Sundaresan, *Clin Cancer Res* 2016.

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MagSweeper

Lohr, *Nat Biotech*, 2014.

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Table 2 CSF and MRI findings in patients with positive CSF CTCs

Patient no.	Type of cancer	CSF results on first lumbar puncture				Cytology	Radiographic findings on the first MRI	CSF CTCs on the first lumbar puncture (cells/mL)	Definitive LM based on prespecified criteria*
		White cells (per mm <sup>3</sup> )	Red cells (per mm <sup>3</sup> )	Glucose, mg/dL	Protein, mg/dL				
1	Breast	4	30	38	106	+	+	96.4	+
2	Breast	21	10	28	137	+	+	8.53	+
3	Breast	15	129	84	40	- <sup>b</sup>	Suspicious	1.87	+
4	Breast	11	34	20	105	+	+	>150	+
5	Breast	88	388	28	156	-	+	125.6	+
6	Breast	66	1,900	33	230	+	+	106.8	+
7	Breast	8	3	16	33	+	-	>150	+
8	Breast	2	1	64	31	-	Suspicious	0.27	- <sup>c</sup>
9	NSCLC	6	4	43	53	+	+	1.87	+
10	NSCLC	16	1	28	64	+	+	38.97	+
11	NSCLC	1	144	79	44	- <sup>b</sup>	Suspicious	0.13	+
12	NSCLC	1	1	88	28	+	+	51.07	+
13	NSCLC	43	168	28	143	+	+	21.87	+
14	Ovarian	18	2	80	19	+	+	18.87	+
15	Ovarian	16	0	41	232	-	+	0.62	+
16	Renal cell	2	3	24	124	- <sup>b</sup>	+	5.33	+

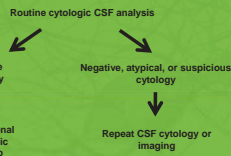
Abbreviations: CTC = circulating tumor cell; LM = leptomeningeal metastasis; NSCLC = non-small cell lung cancer.  
 \* This definition takes into consideration results of repeat CSF or MRI within 3 months of the first lumbar puncture.  
<sup>b</sup> CSF cytology became positive within 3 months of initial lumbar puncture.  
<sup>c</sup> This patient was considered to have a false-positive CSF CTC result, but she developed MRI signs of LM 6 months after the tap (see text for explanation).  
 Nayak, Neurology, 2013.

Table 2 CSF and MRI findings in patients with positive CSF CTCs

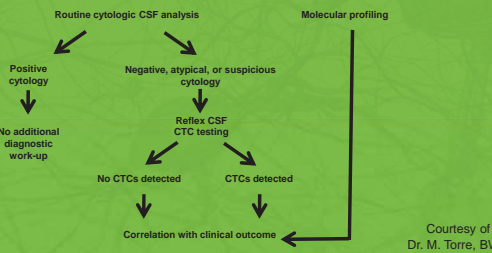
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 Nayak, Neurology, 2013.

### CSF from carcinoma patients with suspected leptomeningeal metastasis



### CSF from carcinoma patients with suspected leptomeningeal metastasis



Courtesy of Dr. M. Torre, BWH

## 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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