Application of Cytologic Techniques to Circulating Tumor Cell Specimens

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MOVING INFORMATION 106TH ANNUAL MEETING USCAP

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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
- CTC 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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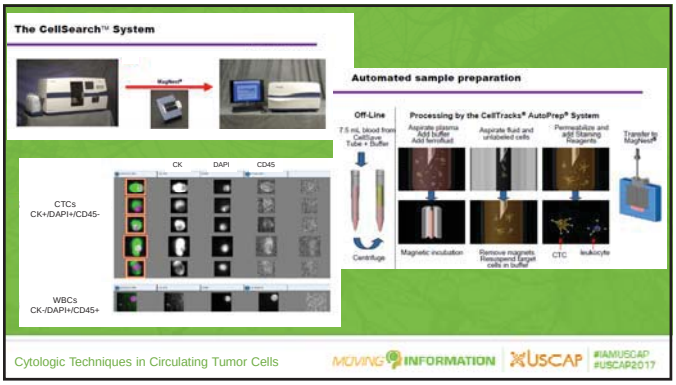
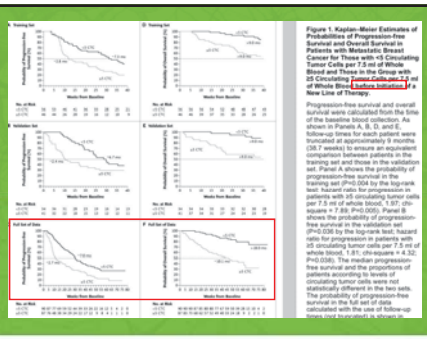
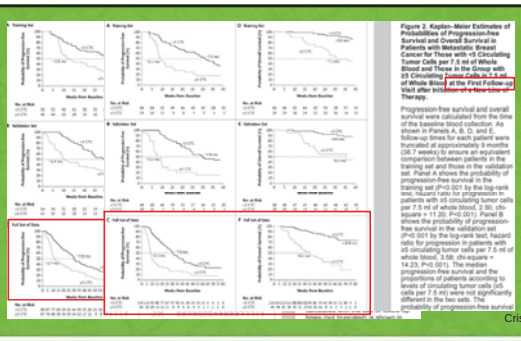




CellSearch™

Cristofanilli, *NEJM*, 2004



CellSearch™

Cristofanilli, *NEJM*, 2004

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)

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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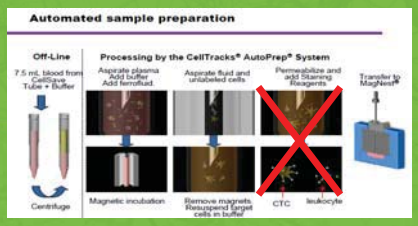
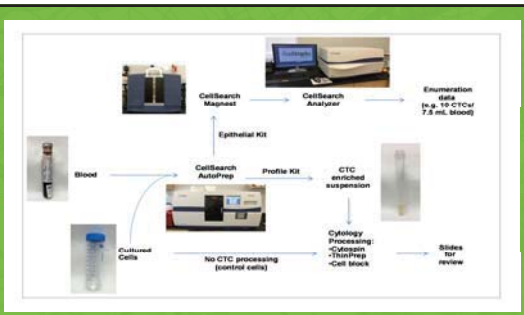
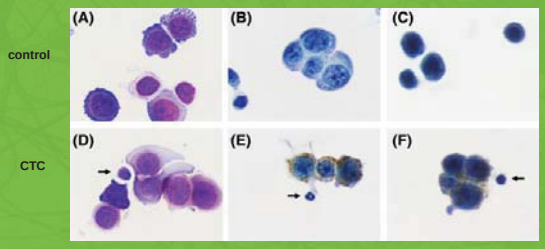
modified from Lowe, Cancer Cytopathol, 2015

CellSearch™ Profile Kit





Lowe, Cancer Cytopathol, 2015.



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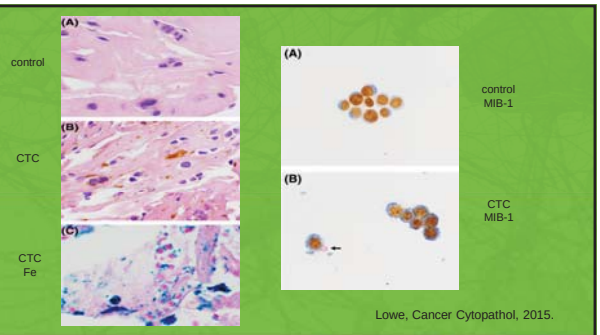

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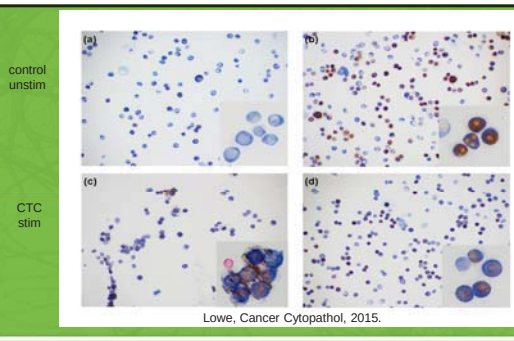
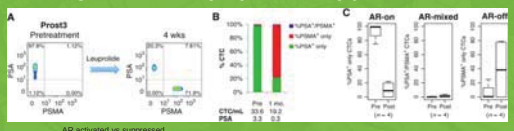


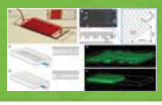
Lowe, Cancer Cytopathol, 2015.

Herringbone-chip (HB-chip)



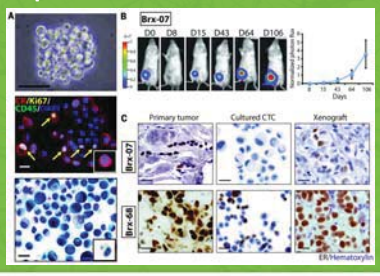
AR activated vs suppressed

Miyamoto, *Cancer Discov*, 2012.



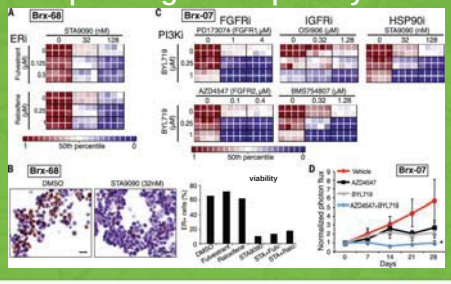
Stott et al., *PNAS*, 2010.

CTC-iChip: CTC Culture



Yu, *Science*, 2014

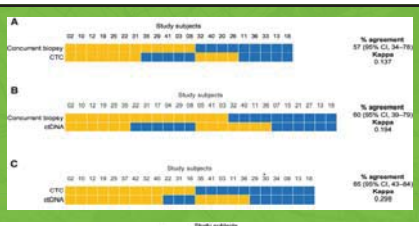
CTC-iChip: Drug Susceptibility



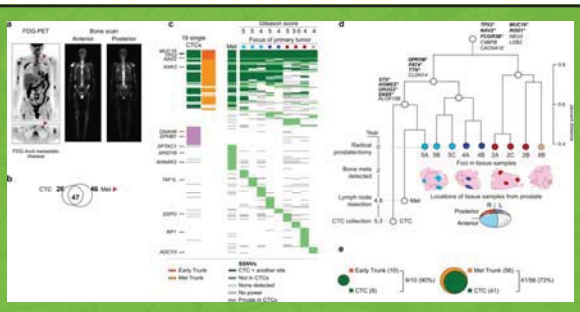
Yu, *Science*, 2014

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

HB-chip



Sundaresan, Clin Cancer Res 2016.



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: White cells (per mm³)	Red cells (per mm³)	Glucose, mg/dL	Protein, mg/dL	Cytology	Radiographic findings on the first MRI	CSF CTCs on the first lumbar puncture (cells/mL)	Definitive LM based on prespecified criteria*
1	Breast	4	30	38	106	+	+	96.4	+
2	Breast	21	10	28	137	+	+	8.53	+
3	Breast	15	129	94	40	–*	Suspicious	1.87	+
4	Breast	11	34	20	105	+	+	>150	+
5	Breast	98	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	–	–	0.27	–*
9	NSCLC	6	4	43	53	+	Suspicious	1.87	+
10	NSCLC	16	1	25	94	+	+	38.67	+
11	NSCLC	1	144	79	44	–*	Suspicious	0.13	+
12	NSCLC	1	1	88	28	+	+	51.07	+
13	NSCLC	43	168	28	143	+	+	21.87	+
14	Ovarian	16	2	80	19	+	+	18.67	+
15	Ovarian	16	0	41	232	–	+	0.62	+
16	Renal cell	2	3	24	124	–*	+	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 1 month of the first lumbar puncture.
† CSF cytology became positive within 1 month of initial lumbar puncture.
‡ 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.

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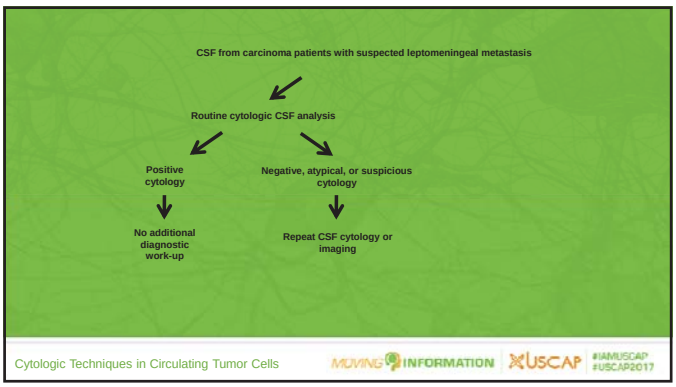

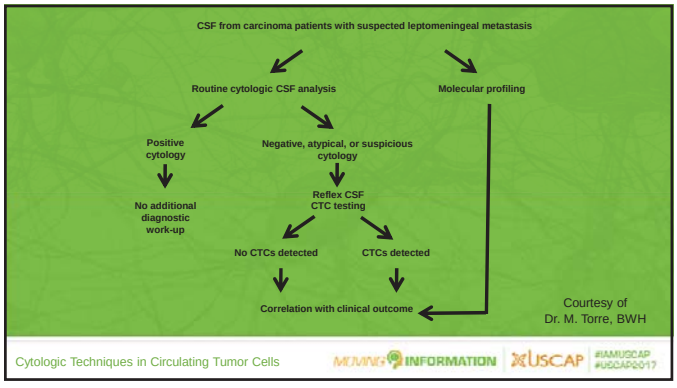

Summary/Future

- 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.

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