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Circulating Tumor Cells: Isolation, enrichment & clinical value

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Disclosure slide

I have received consultancy fees from Janssen Diagnostics,

I will discuss investigational use of trastuzumab.

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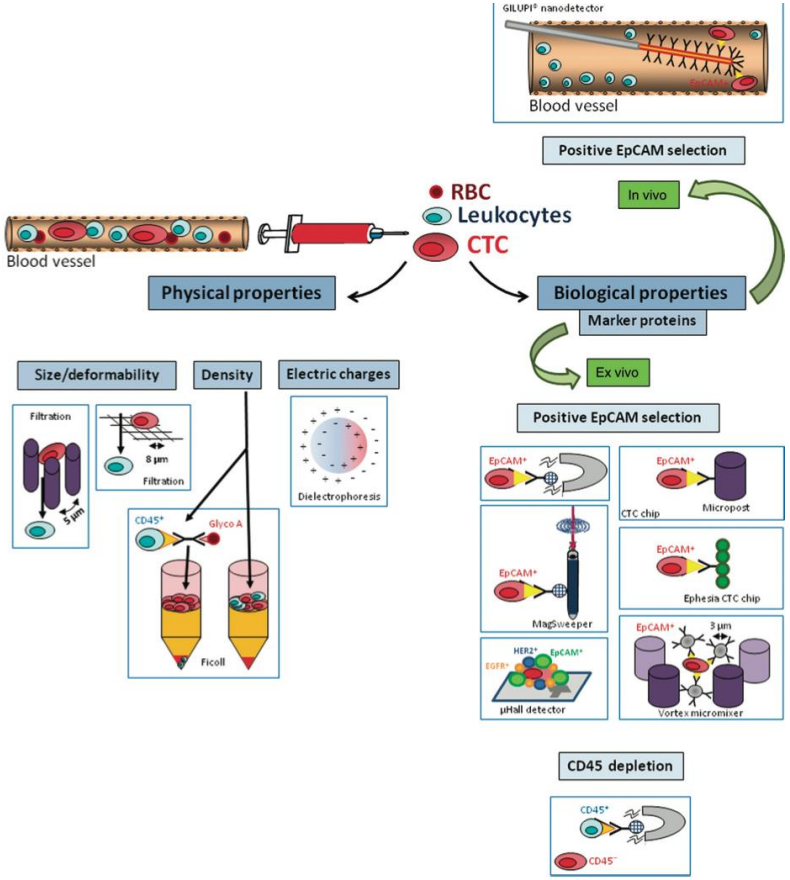
Outline

- Introduction on CTCs
- Are there any data supporting the **clinical utility** of CTCs in metastatic breast cancer?
- Could CTC elimination be used as an **early signal of drug activity** in non-metastatic breast cancer?
- What is the best tissue source for **molecular characterization**: primary tumor, metastatic biopsy, ctDNA or CTC?

CTC detection technologies



Alix-Panabières C & Pantel K Clinical Chemistry 2013

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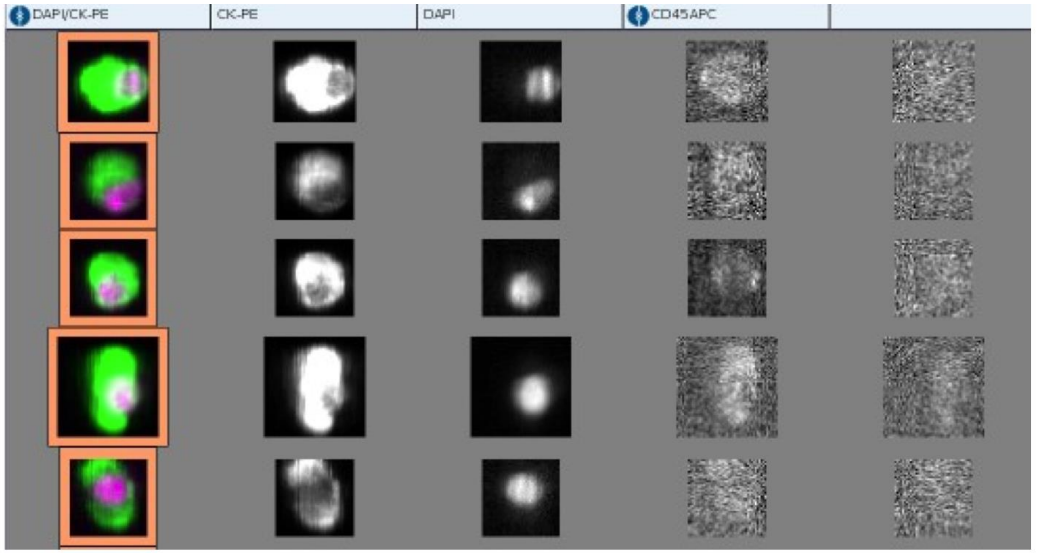
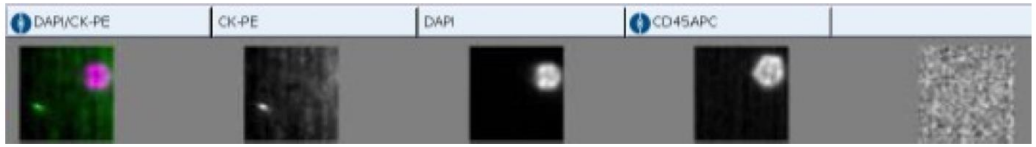
CellSearch® (FDA-cleared)

EpCAM-positive selection

Composite CK DAPI CD45 Control

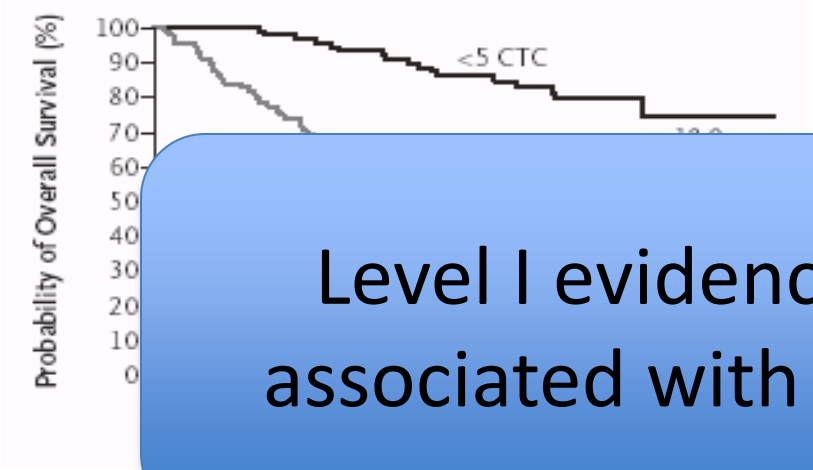


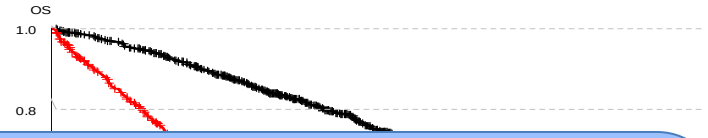


CTCs:
CK+/DAPI+/CD45-

Leukocyte:
CK-/DAPI+/CD45+

CTC detection: poor outcome in metastatic breast cancer





Level I evidence that CTC detection is associated with worse prognosis in MBC

N= 177 pts, 49% (≥ 5CTCs)
HR = 4.26
p<0.0001

Cristofanilli M et al. NEJM 2004

N= 1.944 pts, 47% (≥ 5CTCs)
HR = 2.77
p<0.0001

Bidard FC et al. Lancet Oncology 2014

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Are there any data supporting the **clinical utility** of CTCs in metastatic breast cancer?

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S0500

624 Registered
595 Eligible

29 ineligible or no screening CTC result

CTCs drawn at baseline prior to 1st-line chemotherapy

CTC < 5 | CTC ≥ 5

276 (46%)

319 (54%)

Arm A

Monitor for PFS & OS

CTCs drawn 3 weeks after 1st dose of chemotherapy

33 without 2nd CTC test (death, progression, or refused)

CTC < 5 | CTC ≥ 5

Arm B 163 (57%)

Maintain 1st-line chemotherapy until progression

Randomized 1º endpoint OS 123 (43%)

Arm C1

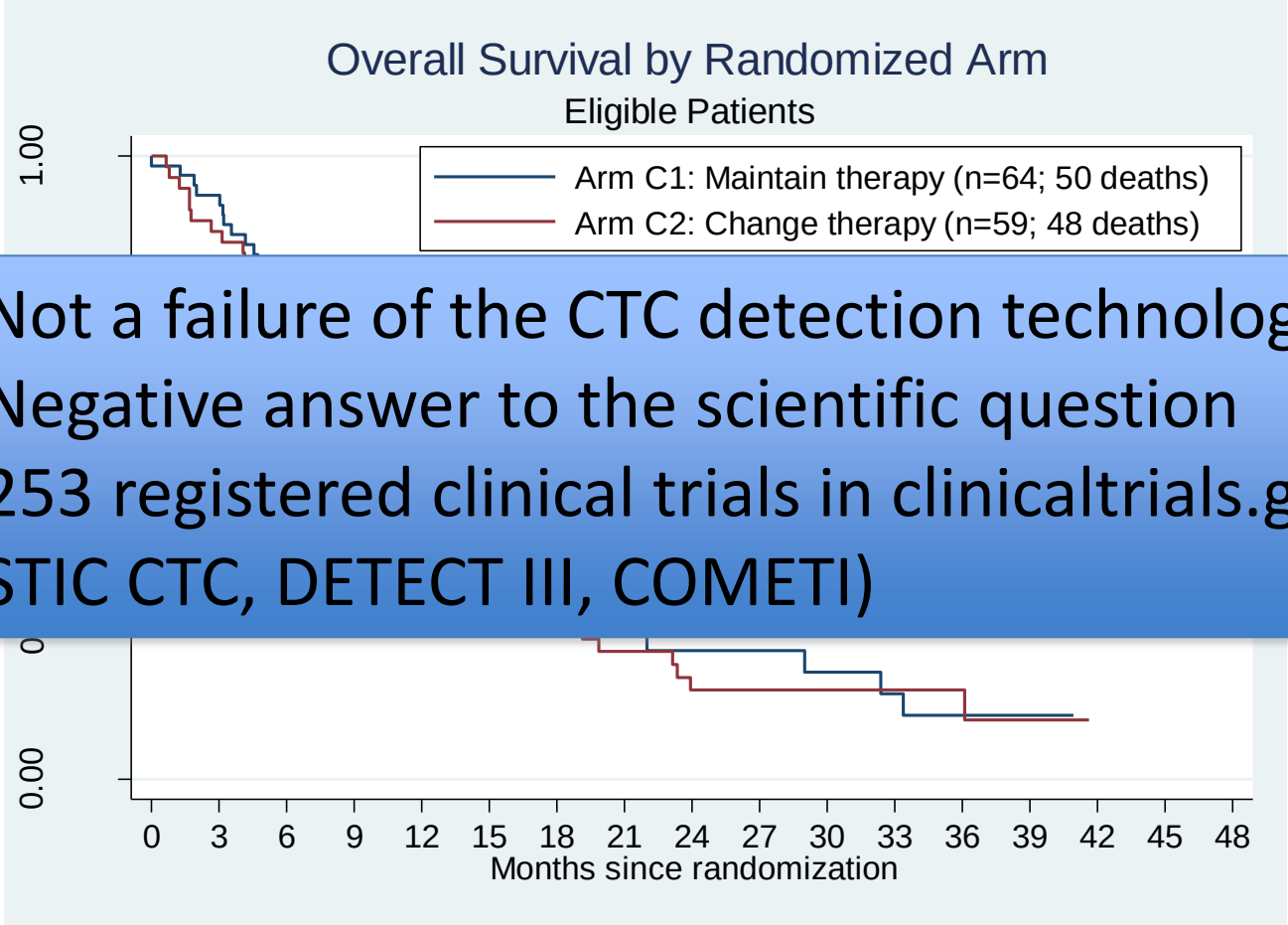
Maintain 1st-line chemotherapy

Arm C2

Switch to alternate therapy

Smerage J et al. SABCS 2013

S0500 did not meet primary endpoint



- Not a failure of the CTC detection technology
- Negative answer to the scientific question
- 253 registered clinical trials in clinicaltrials.gov (e.g. STIC CTC, DETECT III, COMETI)

Smerage J et al. SABCS 2013

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Could CTC elimination be used as an **early signal of drug activity** in non-metastatic breast cancer?

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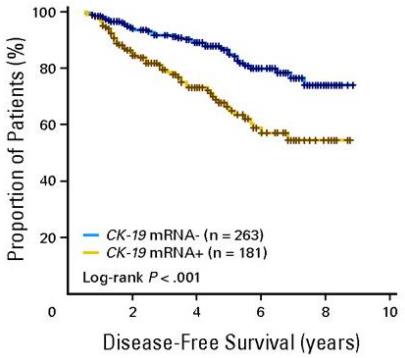
Endpoints in non-metastatic breast cancer: can we do better?

Endpoint	Pros	Cons
Overall Survival	Clinically relevant	Large trials, long f-up
Disease-free Survival	Clinically relevant	Large trials, long f-up
Pathological Complete Response	Small trials, short f-up	Prognostic, surrogacy not yet proven*
CTC elimination	Small trials, short f-up	Prognostic, surrogacy not proven

*Cortazar P et al. Lancet 2014

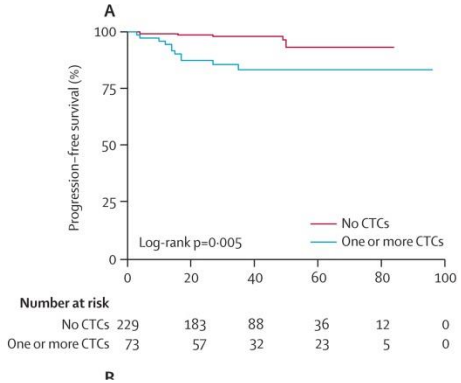
CTCs detection: poor outcome in early breast cancer

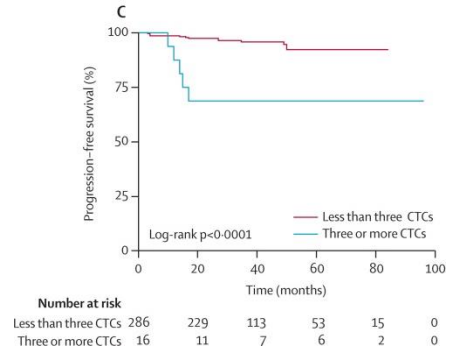
444 pts, detection rate 40% (CK19mRNA)



Ignatiadis M et al. JCO 2007

2847 pts, detection rate 20% (CellSearch®)





Pierga JY et al. CCR 2008
Bidard FC et al. Annals of Oncology 2010
Rack B et al. Recent Results Cancer Res 2012
Lucci A et al. Lancet Oncology 2012
Franken B et al. BCR 2012

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Can we use the CellSearch technology in early breast cancer (low CTC counts), in an international, multilab clinical trial?

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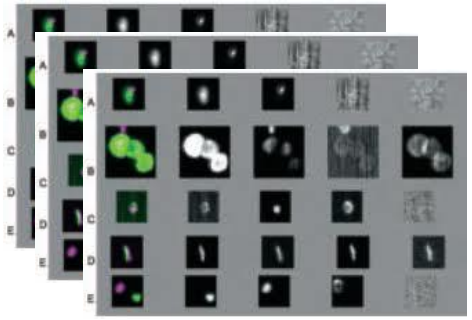
Inter-reader variability for CTCs

8 Independent Veridex readers

22 Independent academic readers

Gallery of 272 images



Each image : CTC yes vs no

% Agreement Veridex consensus vs each academic reader

Ignatiadis M. et al BCR 2014

Conclusions of the inter-reader variability study

- Overall very good agreement between academic readers and Veridex consensus (VC) for CTC detection
- Lower agreement for images from patients with M0 disease, <5CTCs
- Continuous training, adherence to guidelines and independent image review is suggested in this setting

Ignatiadis M. et al BCR 2014

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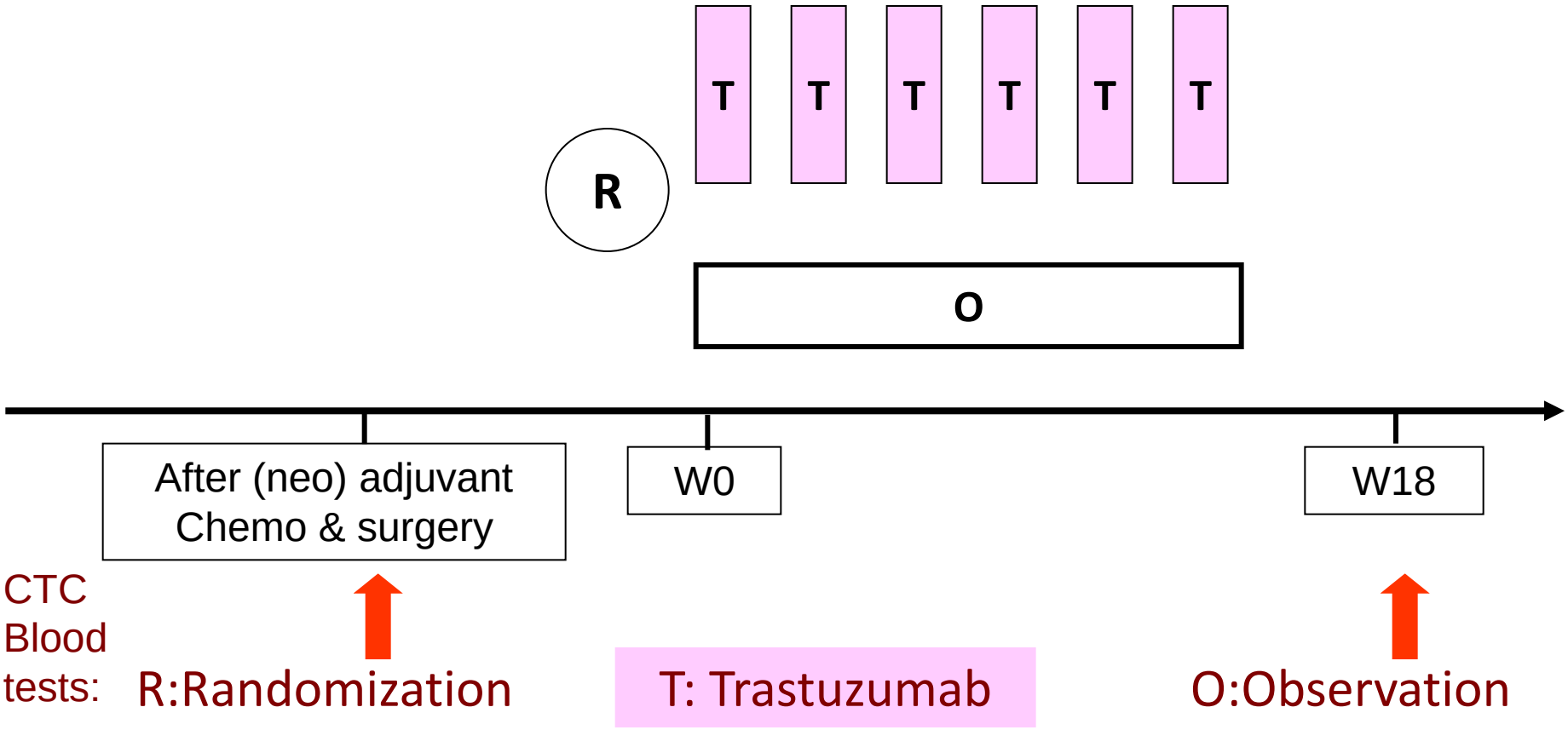
BIG Breast International Group

TRastuzumab in HER2-negative Early breast cancer as secondary Adjuvant Treatment for Circulating Tumor Cells

"Treat CTC" trial

EORTC The future of cancer therapy

"Treat CTC" design



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Objectives

Primary objective

- To evaluate whether trastuzumab eliminates CTCs in patients with HER2-negative primary BC

Secondary objectives

- To evaluate feasibility, reliability, within patient reproducibility of the CTC assay
- To evaluate the safety of trastuzumab in these women
- To compare clinical outcomes between the trastuzumab and observation arms
- To perform translational research

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Status

Sample Size: Screen 2150 patients to randomize 174

Accrual: 92 sites, 6 countries (Austria, Belgium, France, Germany, Greece, UK), 7 academic labs

CTC Labs QC program: Q 6-month spiking experiments, central image review

Current status: 56 patients screened, 2 patients randomized (Belgium, Germany)

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Challenges

CTC detection rate: Low

Few CTCs detected: 1-2 CTCs per positive sample

High Discordance: 8 patients identified as CTC-positive but not confirmed after central image review

Action: EORTC organized webex TC (central labs & Janssen Diagnostics) and a consensus was reached: **4 out of 8 patients** were considered as CTC-positive

Continuous training and adherence to guidelines for image interpretation (Ignatiadis et al BCR 2014)

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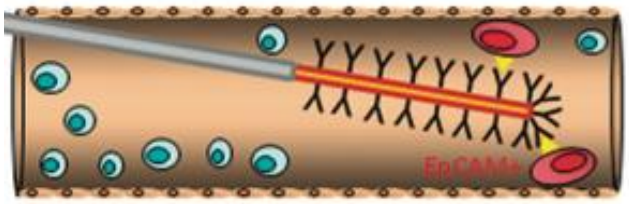
Minimal residual disease in early breast cancer: sensitive technologies are needed

CellSearch: validated in ~3000 pts **but** low detection rate, low CTC counts (1-3CTCs/7.5ml of blood)

Newer CTC detection technologies



ctDNA, digital PCR, sensitivity 0,01%

Diaz L et al. JCO 2014
Beaver JA et al. CCR 2014

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What is the best tissue source for **molecular characterization**: primary tumor, metastatic biopsy, plasma ctDNA or CTCs?

Primary tumor	Metastatic biopsy	Plasma ctDNA	CTCs
Intact cells	Intact Cells	Fragmented DNA	Intact cells (few)
Accessible, mostly used	Invasive, not always accessible	Non-invasive, accessible, easy to process	Non-invasive, accessible, laborious to isolate
DNA, RNA, protein, cell culture, xenografts	DNA, RNA, protein, cell culture, xenografts	DNA	DNA, RNA, protein, cell culture, xenografts

Two approaches for the study of ctDNA

Candidate mutation

- ✓ Mutation(s) are known or first identified in the primary tumor and then followed in plasma [1,2,3,4]
- ✓ Higher sensitivity, feasible even when low disease burden
- ✓ Resistance mechanisms must be known

Unbiased

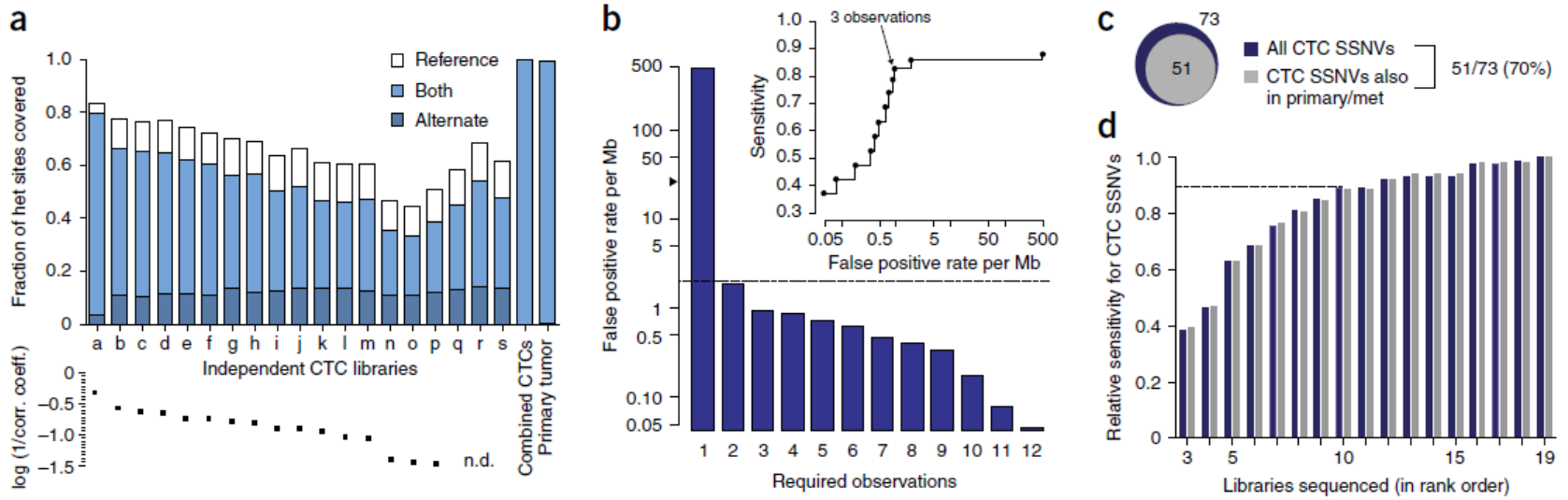
- ✓ Direct plasma ctDNA detection without prior analysis of tumor[5]
- ✓ Lower sensitivity, high disease burden required
- ✓ Can uncover new resistance mechanisms

Mc Bride et al. Gene Chromosomes Cancer 2010
Dawson et al. NEJM 2013
Forshew et al. Sci Trans Med 2012
Heitzer et al. Cancer Res 2013
Murtaza et al. Nature 2013

Whole exome sequencing of CTCs: a window in metastatic prostate cancer

Mapping of >99.995% of the standard exome is possible in CTCs

LETTERS



Lohr et al Nature Biotechnology 2014

Conclusions (I)

- There is now level I evidence that CTC detection using CellSearch is an adverse prognostic factor in metastatic breast cancer and ongoing clinical trials are testing its **clinical utility**
- The ongoing Treat CTC trial is testing **CTC elimination as an early signal** of trastuzumab activity in HER2-negative early breast cancer
- The role of **more sensitive CTC detection technologies** or **ctDNA** for monitoring minimal residual disease in the early breast cancer setting should be further explored

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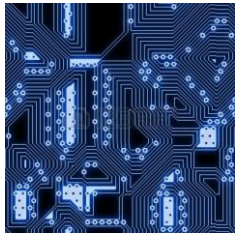
Conclusions (II)

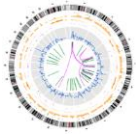
- **Plasma ctDNA** should be prospectively tested as a tool for **treatment selection and monitoring** in clinical trials of patients with metastatic breast cancer
- Technological advances have allowed **CTC** analysis as a 'liquid biopsy' to **study tumor evolution**
- **CTC analyses** offers a unique window of opportunity to **assess treatment resistance** at the cellular level

‘Liquid biopsy’ for precision medicine

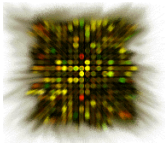
CTC chip



Mutation Profile



GEP for pathways activation



Drug Sensitivity Profile

Algorithm for best combination Tx

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FNRS

Women with breast cancer

MEDIC Foundation