9th International Symposium on Minimal Residual Cancer

September 25 -27, 2013

Pullman Paris Bercy, France

- Organizers

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CTC molecular characterization: Are we ready to move forward with clinical testing ?

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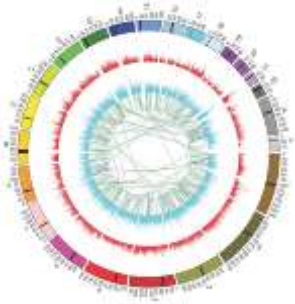
Breast cancer: Diagnostics / Treatment

2000 → 2013

Tumor Heterogeneity/ evolution

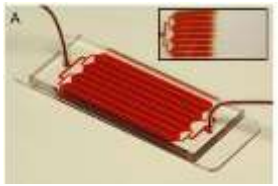


Targeted Drugs

One Drug per Pathway: Trastuzumab → Multiple Drugs per Pathway: PI3K/AKT/mTOR inh

Liquid Biopsy

CellSearch®



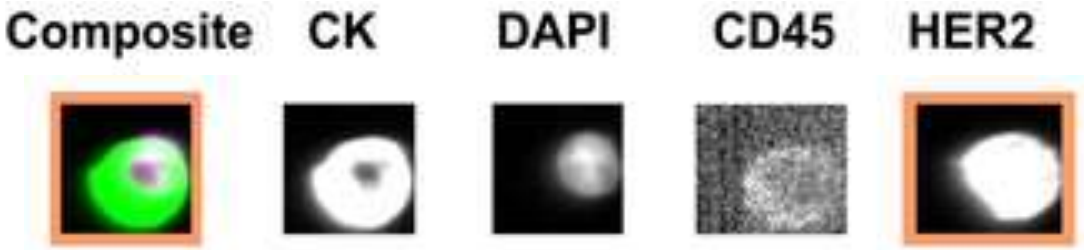
Microfluidics

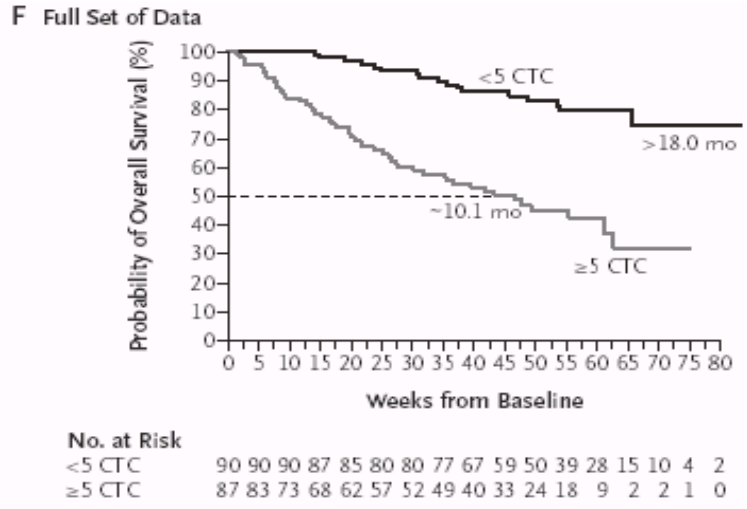
Circulating DNA

Where we stand?

- Active research for CTC molecular characterization
- >400 registered clinical trials using CTCs
- Almost all studies in metastatic disease
- Liquid biopsy: not yet in the routine clinical practice

CellSearch® (FDA-cleared)





Cristofanilli et al. NEJM 2004

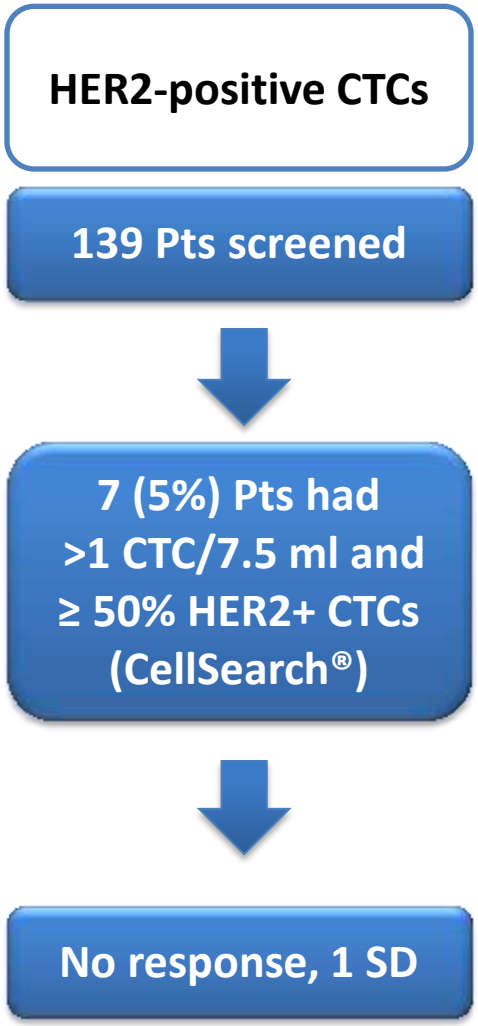
CTC enumeration for treatment decision

Ongoing trials

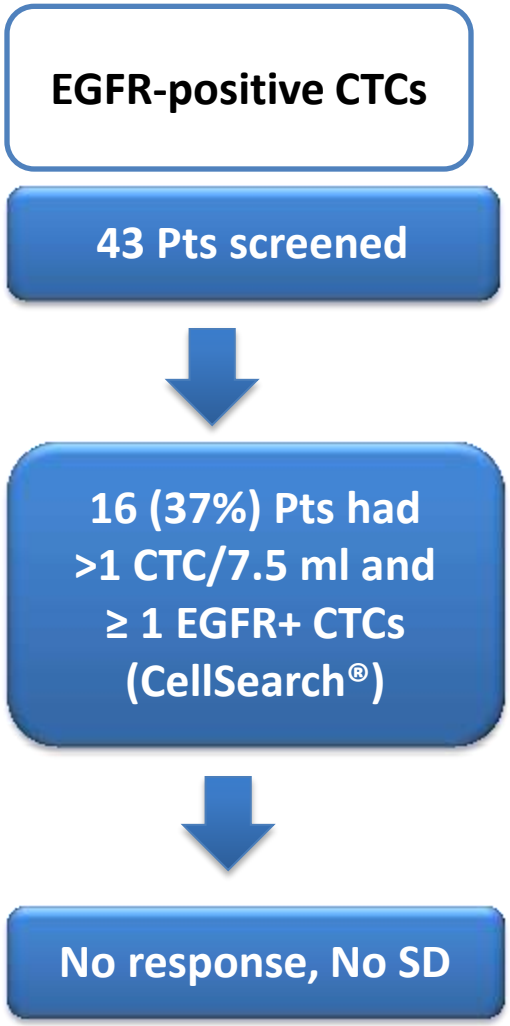
Trial	Question	Setting	Screen	Rx
SWOG 0500 (phase 3)	Does an early treatment change based on elevated CTC counts after 1 cycle of chemo lead to improved OS?	MBC starting 1st line chemo	610	120
CirCE 01 (phase 3)		MBC starting 3rd line chemo	600	304
STIC (phase 3)	CTC count vs clinician choice to decide whether to administer chemo vs hormono?	MBC ER+/HER2- starting 1st line	>994	994

CTC characterization using one marker (e.g HER2) for treatment decision

Lapatinib monotherapy in HER2-neg MBC



Pestrin et al BCRT 2012



Stebbing et al Plos One 2013

Ongoing Trials

Trial	Question	Setting	Screen	Rx
Detect III (phase 3)	Can addition of lapatinib to standard treatment increase PFS?	M+ HER2- BC, 1 HER2+ CTC/7.5ml, 1st-3rd line	1428	228
CirCEX1 (phase 2)	Response rate with TDM1?	M+ HER2- BC, before 2nd line, HER2+ CTCs by FISH	400	
COMETI P2 (phase 2)	CTC endocrine therapy index?	M+ ER+/HER2-, starting a new ET	200	

CTC characterization (beyond a single marker):

A better tool (compared to cell lines & mouse models) to study Tx response / resistance?

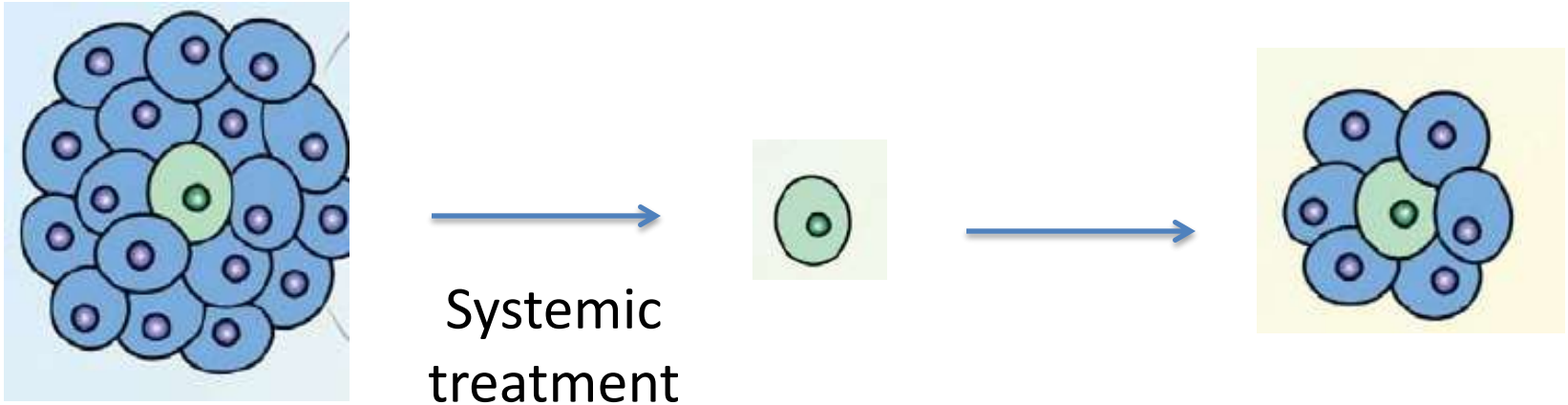
Example 1. Can characterization of EMT on CTCs helps understand and target treatment resistance?

Prevailing model of systemic treatment resistance in breast cancer

Tumor

Resistant cells
EMT / CSC-like phenotype

Relapse

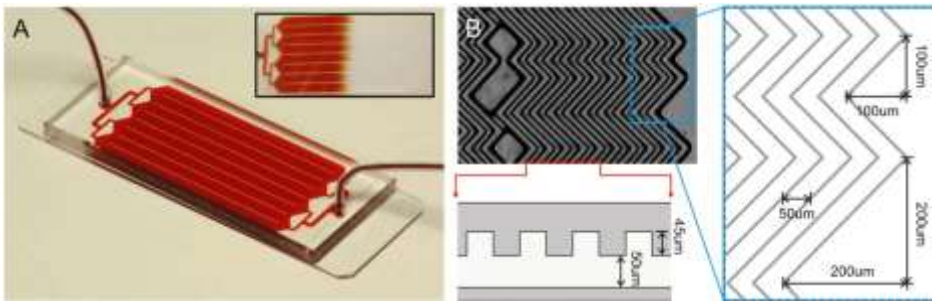


Systemic treatment

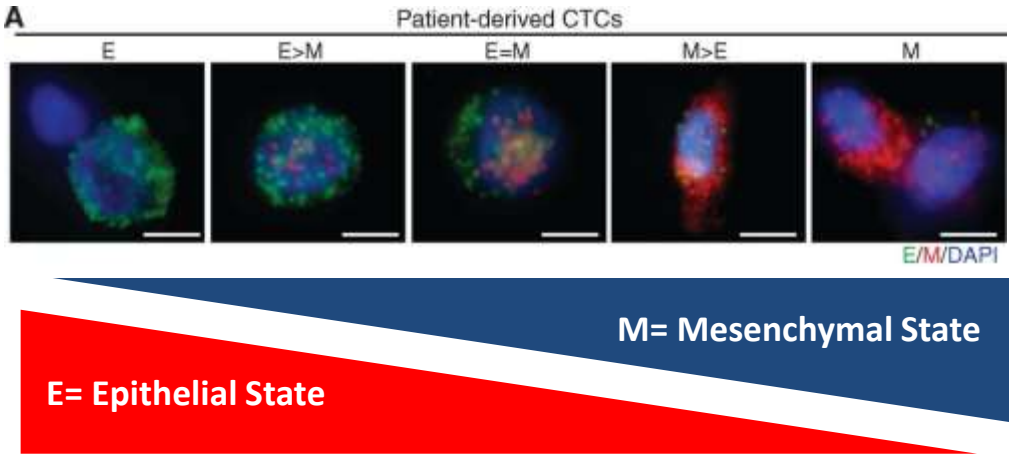
REPORTS

Circulating Breast Tumor Cells Exhibit Dynamic Changes in Epithelial and Mesenchymal Composition

CTC Isolation

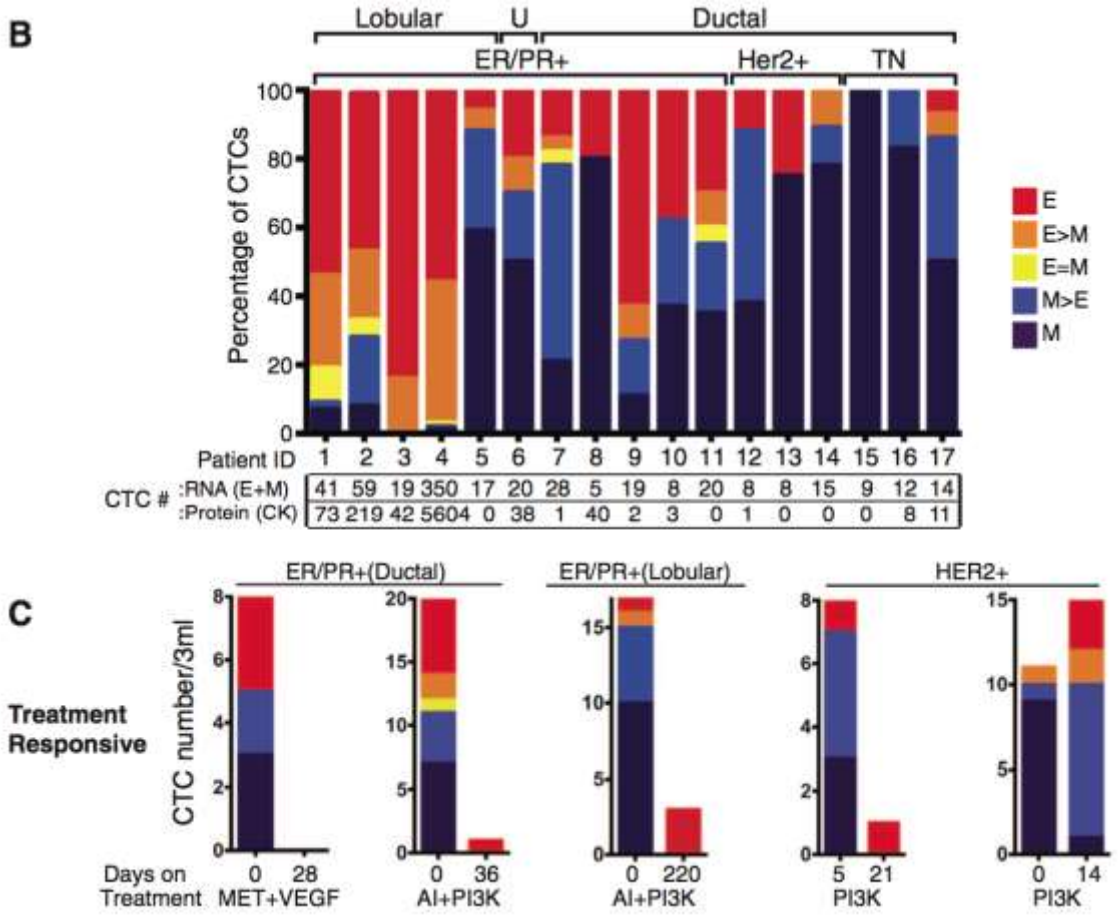


RNA ISH for a panel of Epithelial and Mesenchymal Markers



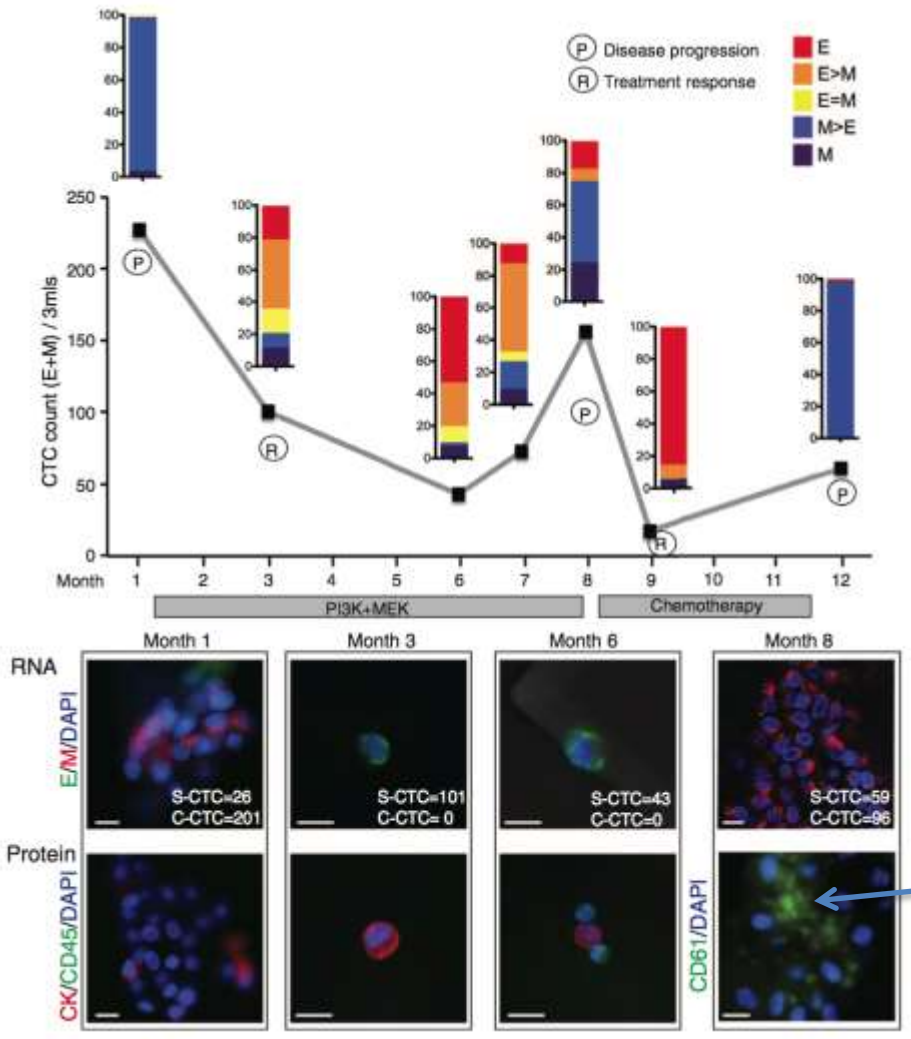
M Yu et al. Science 2013

Contrary to the prevailing model, the E/M ratio on CTCs increases after treatment



M Yu et al. Science 2013

Increase in Mesencymal CTCs is associated with CTC clusters



platelets

M Yu et al. Science 2013

Conclusion

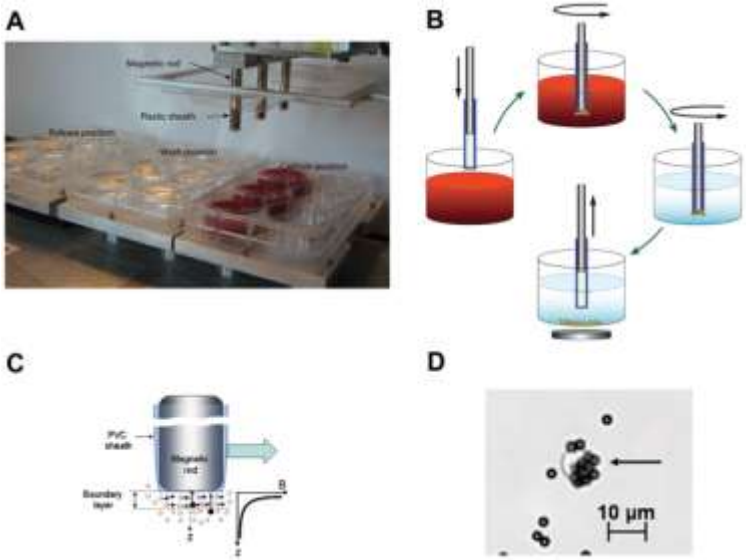
The demonstration of dynamic changes in Epithelial and Mesenchymal composition of CTCs

- sheds light into the mechanisms of treatment resistance
- suggests new treatment targets and,
- can serve as a surrogate efficacy marker in trials using agents that target ‘stemness’ and EMT

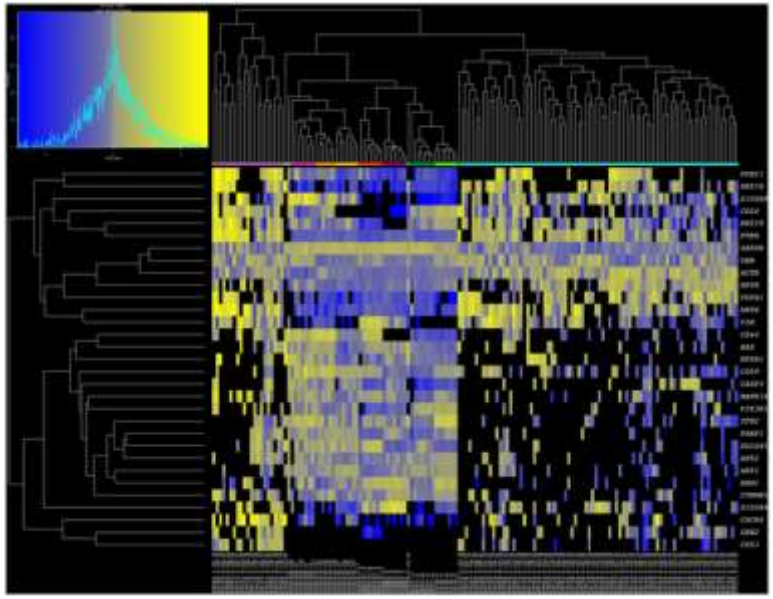
Example 2. Can the study of CTCs gene expression improve drug development?

Cell lines differ from CTCs in gene expression

CTC isolation using the MagSweeper



Single Cell Gene expression of a panel of 31 selected genes



CTC CI

Cell Lines

CTC CII

Powell AA, et al PloS One 2012

Drug development: CTCs vs cell lines

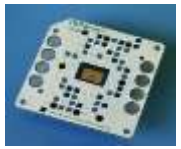
Differentially expressed transcripts	Names
Increased in CTCs	*FOXC1, KRT18, PTEN, NPTN, TGFß1, KRT8, ZEB2, and CXCR4*
Increased in cell lines	*RRM1, AKT1*, and *AKT2*

For early trials using e.g. an AKT-inhibitor,
tailor drug dose based on PIK3CA/AKT pathway activity:
CTCs or cell lines?

Example 3. Can we perform whole genome sequencing on single CTCs?

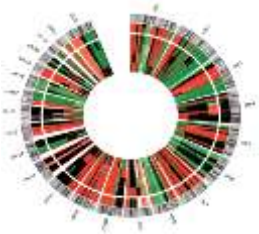
Whole genome sequencing of single cells

Spiking HCC38 cells



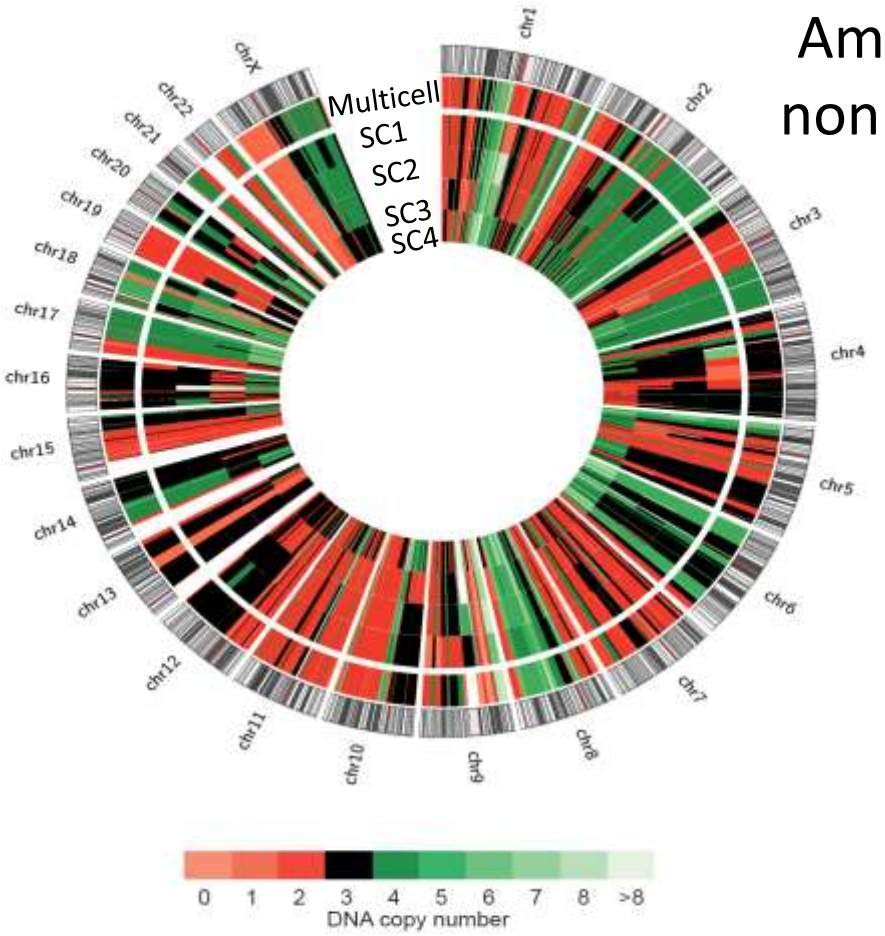
single / pool of cells

WGA Ampli1 followed by low coverage whole genome sequencing using the HiSeq 2000 ILLUMINA



Peeters et al. ISMRC 2013

Copy Number Variation single cell profiles of the HCC38 breast cancer cell line



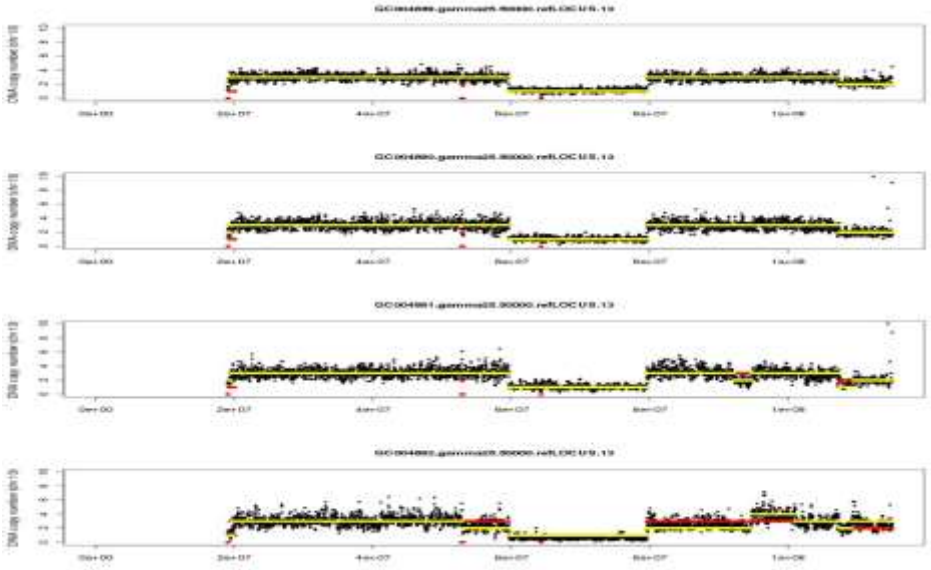
Amplified DNA from single cells vs non amplified DNA from many cells (HCC38 cell line)

Sample	Copy Number Concordance
SC1	78.8%
SC2	88.7%
SC3	76.2%
SC4	44.5%

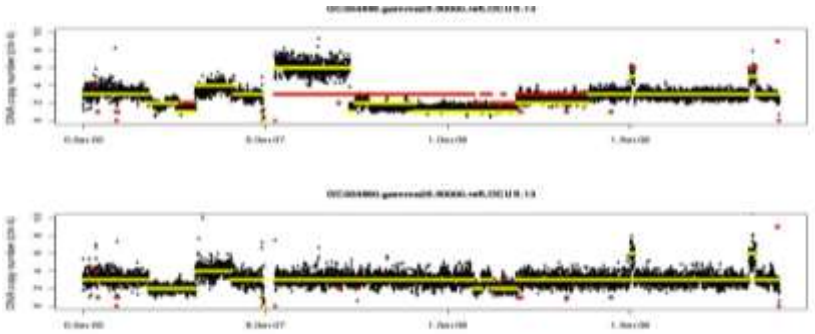
Peeters et al. ISMRC 2013

DNA Copy Number: amplified DNA from single cells vs non amplified DNA from pool of cells

Chr 13



Chr 4

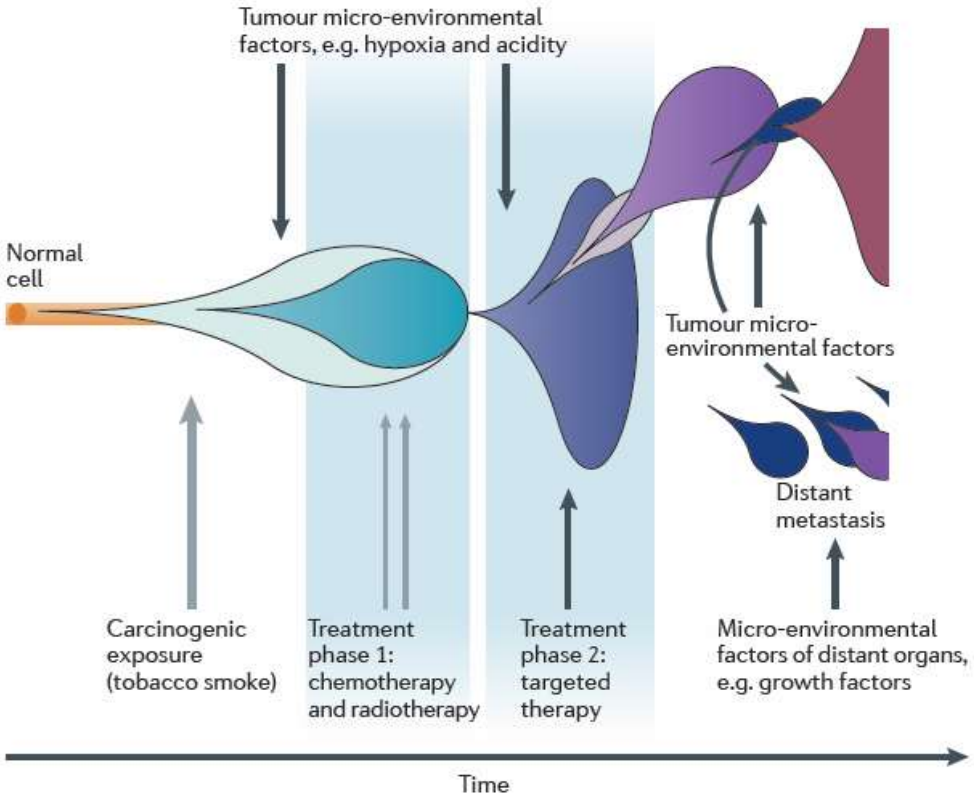


Yellow lines: amplified DNA
Red Lines: Non amplified DNA

Peeters et al. ISMRC 2013

Example 4. Is the study of CTCs suitable to capture tumor evolution?

Cancer evolution: Implications for treatment



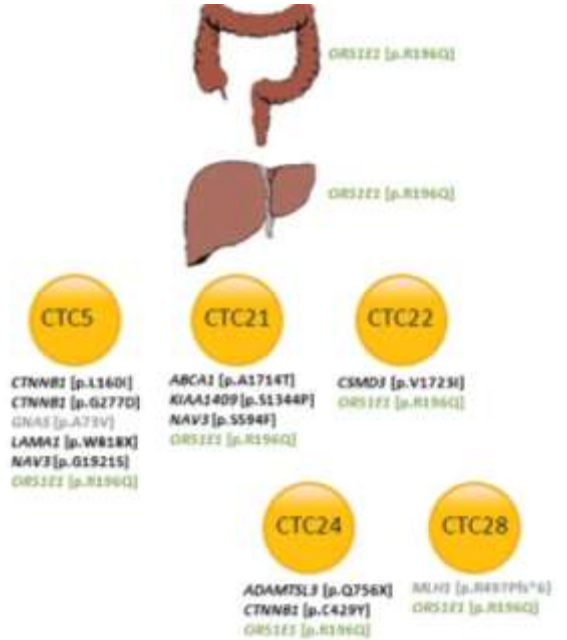
Multiple, serial biopsies are needed to capture spatial and temporal tumor heterogeneity

Yates and Campbell et al. Nat Rev Gen 2012
Gerlinger et al. NEJM 2012

CTC analysis: a “druggable” CDK8 gain not present in primary tumor

CTC isolation using micromanipulation, WGA

Array CGH, NGS panel 68 genes



Gene	Point mutation	Copy number primary tumor (log2)	Copy number liver metastasis (log2)	Copy number CTCs (Abs.)	Potentially clinically significant
APC	Ø	-0.5 (loss)	-0.5 (loss)	2 (loss)	
CDK8	Ø	0 (balanced)	0 (balanced)	7 (gain)	CDK inhibitors

Heitzer E et al. Cancer Res 2013

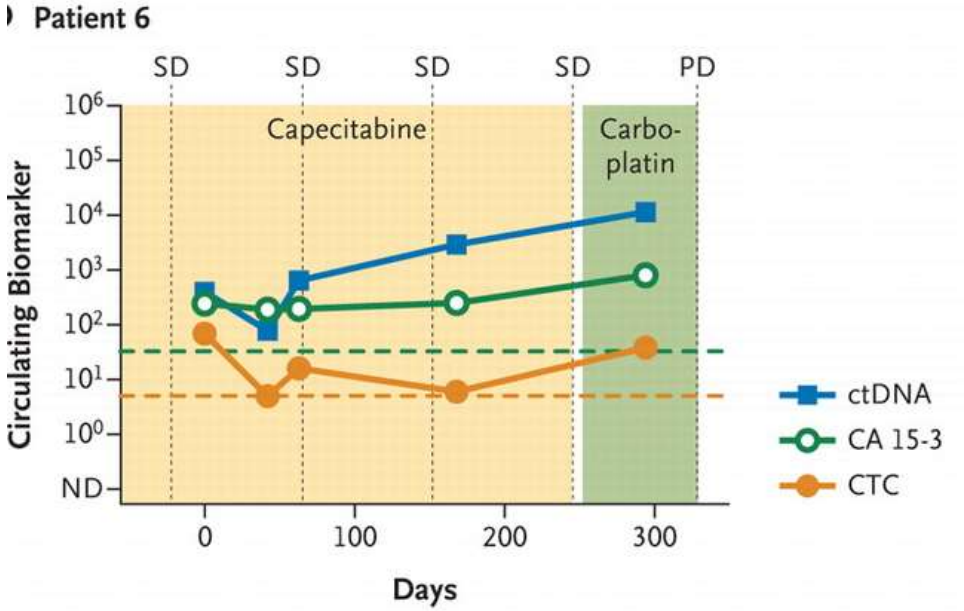
Beyond CTCs: Circulating tumor DNA?

ctDNA: Targeted approach

Primary Tumors
(low coverage whole genome or targeted gene screen for selected mutations e.g p53, PIK3CA)

PCR

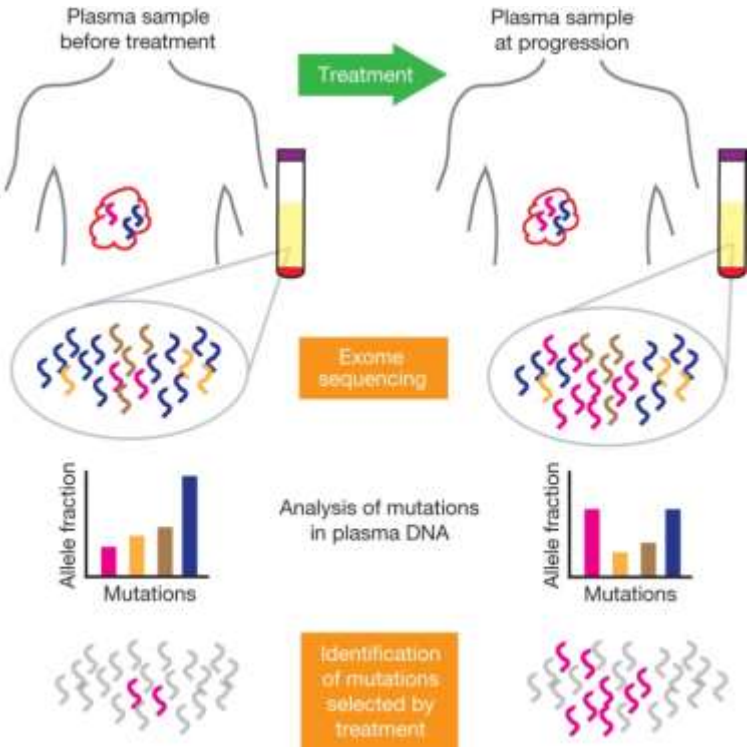
Plasma



Targeted screen: feasible in samples with mutation present even in <1% of cDNA

Diehl et al. Nat Med 2008
Dawson et al. NEJM 2013

ctDNA: Unbiased approach



Exome sequencing: feasible if mutation present in at least 10% of cDNA

Murtaza et al. Nature 2013

CTC or ctDNA?
Targeted or not?

Targeted approach

- ✓ Tumor or patient specific assays[1,2,3,4]
- ✓ Feasible even when low disease burden in blood
- ✓ Better suited for non enriched samples (plasma cDNA)
- ✓ Lower cost

Unbiased approach

- ✓ Whole exome[3] / genome sequencing
- ✓ Feasible only when high disease burden in blood
- ✓ Better suited for enriched samples (e.g. CTCs)
- ✓ Higher cost

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

Metastatic biopsies vs CTC vs cDNA
Who will be the winner?

Ongoing study
(N=10 metastatic breast cancer patients)

Metastatic biopsy

Plasma cDNA

Single CTCs /WBCs DNA

Normal DNA

Ion Torrent
Ion AmpliSeq™ Cancer Hotspot Panel v2:
50 genes

Illumina HISeq 2000
Exome Sequencing

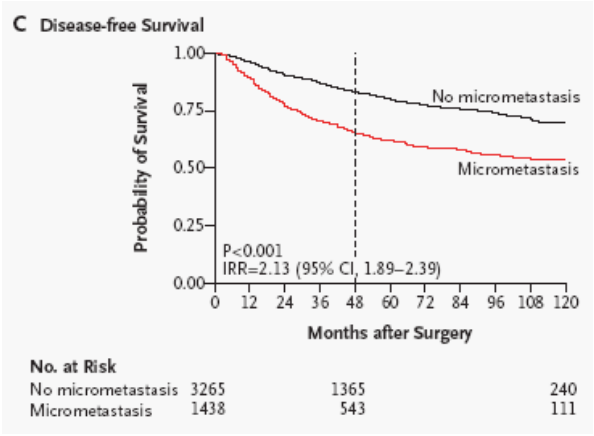
Personal opinion

- Liquid biopsy will be the preferred option by physicians / patients for monitoring treatment resistance
- Circulating tumor DNA (apoptotic cells) to monitor known mutations that confer treatment resistance / sensitivity: Promising approach but no solid data today
- CTC molecular analysis (viable cells): **a unique window** to understand treatment resistance in humans

Early breast cancer:
Can the use of “liquid biopsy” increase cure rates in breast cancer ?

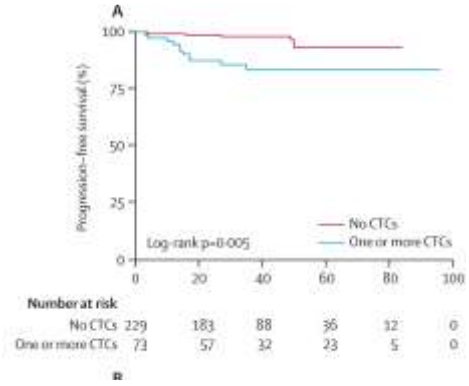
DTCs & CTCs:
poor outcome in early breast cancer

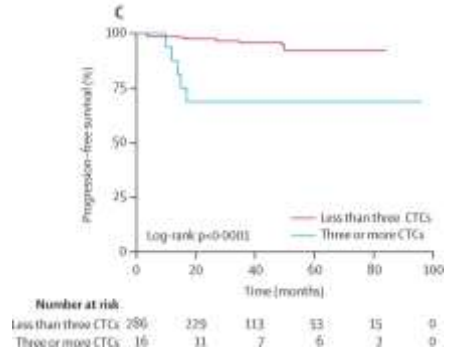
4703 patients, detection rate 30%



Braun et al. NEJM 2005

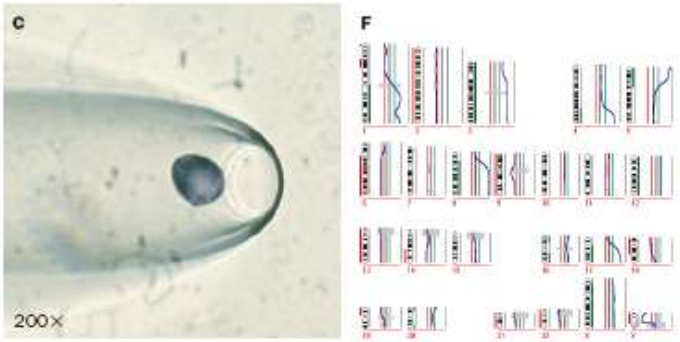
2847 patients, detection rate 20% (CellSearch®)





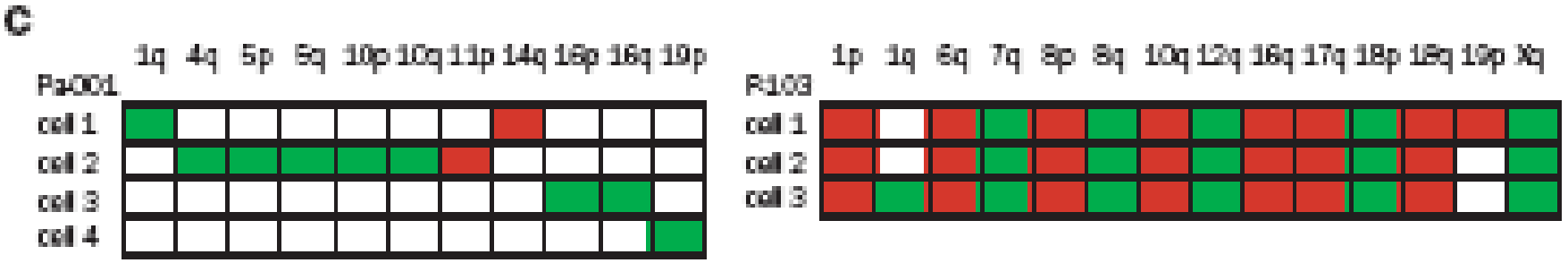
Pierga et al. CCR 2008
Bidard et al. Annals of Oncology 2010
Rack et al. Recent Results Cancer Res 2012
Lucci et al. Lancet Oncology 2012
Franken et al. BCR 2012

Bone marrow DTCs display marked heterogeneity in early breast cancer: Is there a common driver?



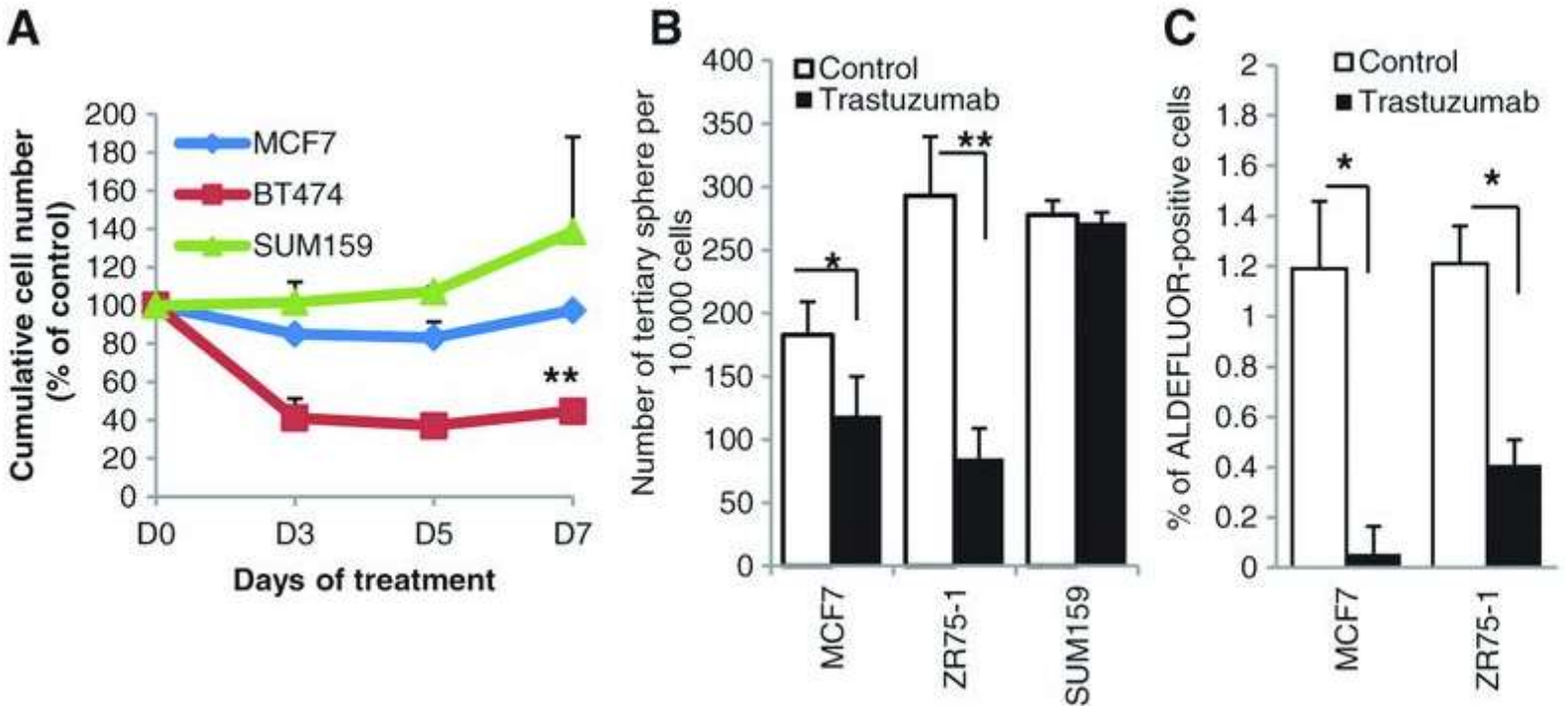
Early breast cancer (M0 DTCs)

Metastatic breast cancer (M1 DTCs)



Klein et al. Lancet 2002
Klein Nature 2013

Trastuzumab targets CSCs in luminal breast cancer cells



MCF7: HER2 non-amplified

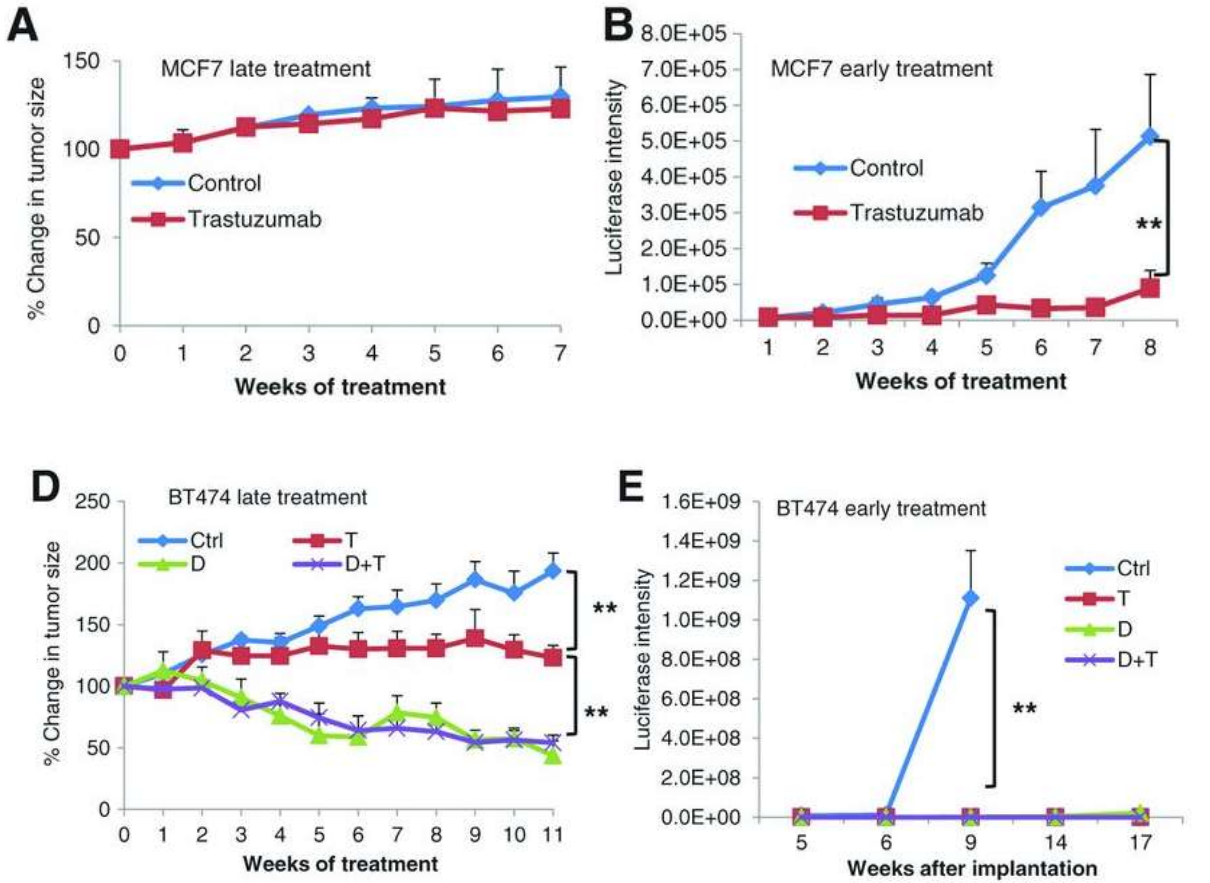
BT474: HER2 amplified

Ithimakin S et al. Cancer Res 2013

Effect of trastuzumab on mouse tumor xenograft depend on the timing of administration

HER2 non-amplified

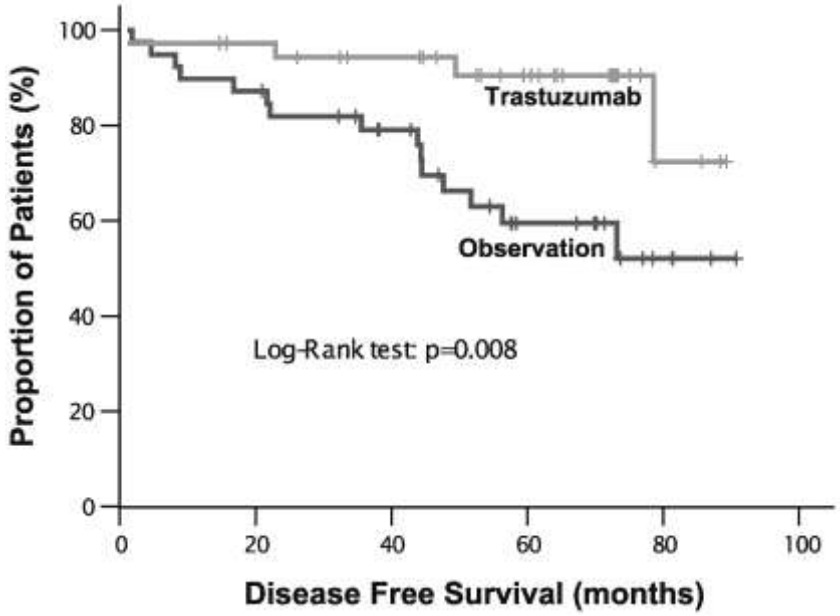
HER2 amplified



Ithimakin S et al. Cancer Res 2013

The first reported “liquid biopsy” trial

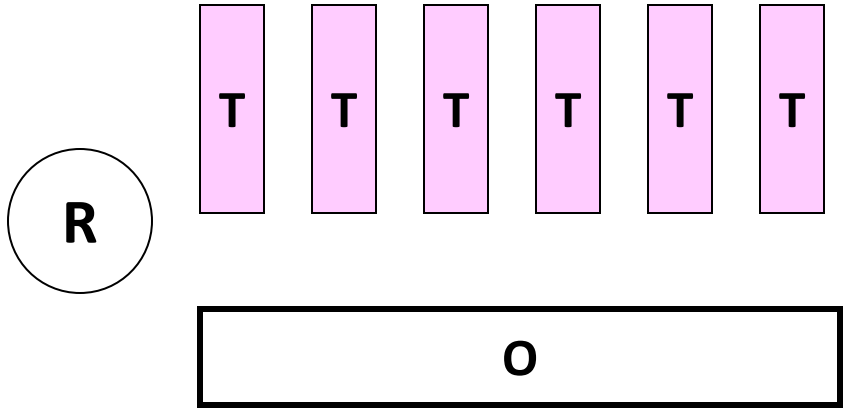
- Early Breast Cancer
- Patient selection based on CK19 mRNA
- Randomized phase 2 single center study
- Trastuzumab (x6) vs observation (N=75 pts)

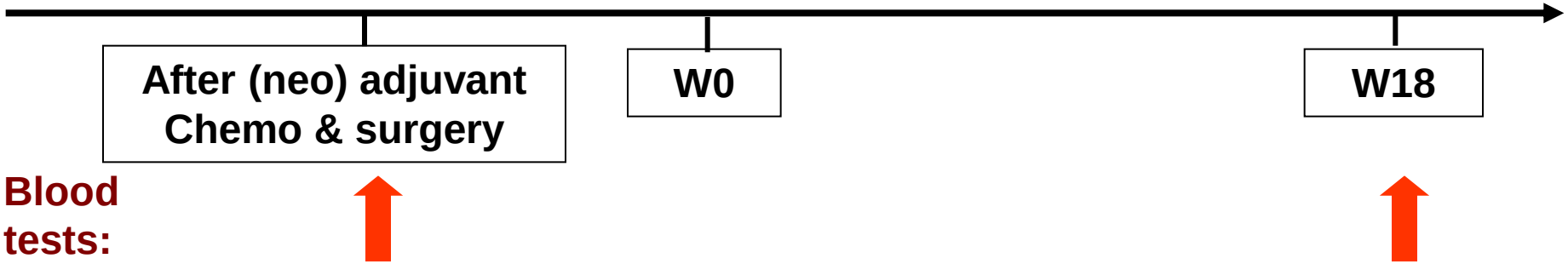


Georgoulias et al. Annals of Oncology 2012

"Treat CTC"

Screen: >2000 Pts with HER2- BC
Randomize: 174 CTC+ Pts (CellSearch®)





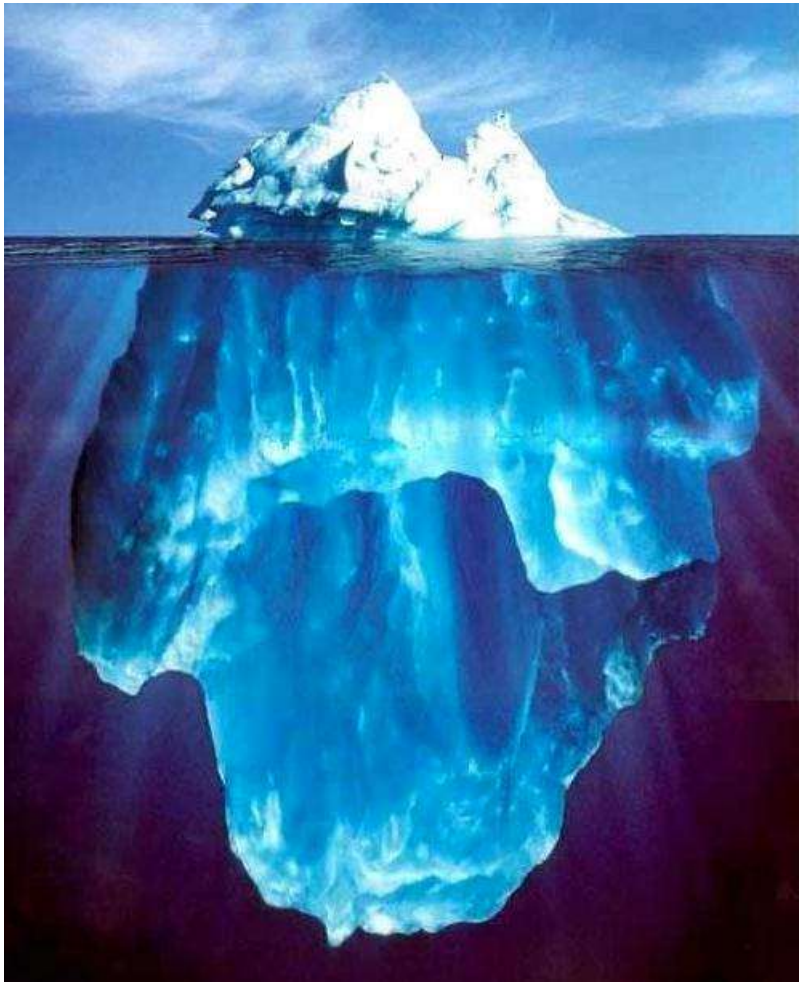
R:Randomization **T:Trastuzumab** **O:Observation**

Study started in Belgium
5 more countries by the end of the year

PI : M Ignatiadis

Co-PIs: JY Pierga, C Sotiriou, B Rack, M Piccart

CTCs molecular characterization:
A lot to be discovered in the coming years…



Acknowledgements

Institut Jules Bordet
Françoise Rothé
Marion Maetens
Ghizlane Rouas
Christine Desmedt
Christos Sotiriou
Martine Piccart

University of Crete
Dimitris Mavroudis
Vassilis Georgoulias

University of Athens
Evi Lianidou

Sint-Augustinus Hospital
Dieter Peeters
Luc Dirix

KUL Leuven
Thierry Voet Group

Sanger, UK
Peter Campbell Group

OncoDNA
Jean-Francois Laes

Bio.be
Xavier Deghorain

EORTC

BIG
Breast International Group

Les Amis de
l'Institut Bordet

the Breast Cancer Research Foundation

FNRS

Women with breast cancer

MEDIC
Foundation