AZIENDA OSPEDALIERO UNIVERSITARIA
Santa Maria della Misericordia di Udine

17.45 CTC e analisi integrate: implicazioni nella scelta clinica

D. Cesselli

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CONTENTS

nature INSIGHT

TUMOUR HETEROGENEITY

Tumour heterogeneity in the clinic

Philippe L. Bedard[1,2], Aaron R. Hansen[1,2], Mark J. Ratain[3] & Lillian L. Siu[1,2]

Recent therapeutic advances in oncology have been driven by the identification of tumour genotype variations between patients, called interpatient heterogeneity, that predict the response of patients to targeted treatments. Subpopulations of cancer cells with unique genomes in the same patient may exist across different geographical regions of a tumour or evolve over time, called intratumour heterogeneity. Sequencing technologies can be used to characterize intratumour heterogeneity at diagnosis, monitor clonal dynamics during treatment and identify the emergence of clinical resistance during disease progression. Genetic interpatient and intratumour heterogeneity can pose challenges for the design of clinical trials that use these data.

Selection and adaptation during metastatic cancer progression

Christoph A. Klein[1,2]

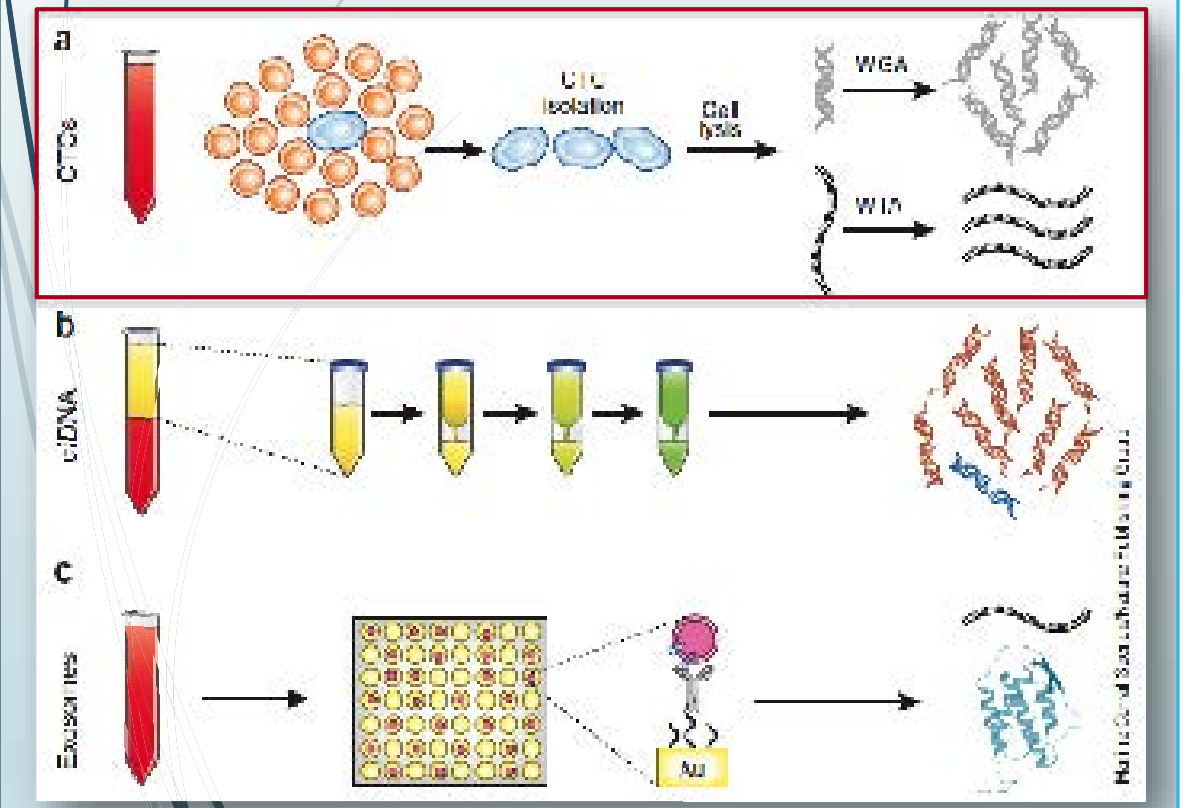
Cancer is often regarded as a process of asexual evolution driven by genomic and genetic instability. Mutation, selection and adaptation are by convention thought to occur primarily within, and to a lesser degree outside, the primary tumour. However, disseminated cancer cells that remain after 'curative' surgery exhibit extreme genomic heterogeneity before the manifestation of metastasis. This heterogeneity is later reduced by selected clonal expansion, suggesting that the disseminated cells had yet to acquire key traits of fully malignant cells. Abrogation of the cells' progression outside the primary tumour implies new challenges and opportunities for diagnosis and adjuvant therapies.

NATURE BIOTECHNOLOGY VOLUME 32 NUMBER 5 MAY 2014

Tumor signatures in the blood

Michael R Speicher & Klaus Pantel

The goal of characterizing solid-tumor genomes with nothing more than a blood sample is now within reach.



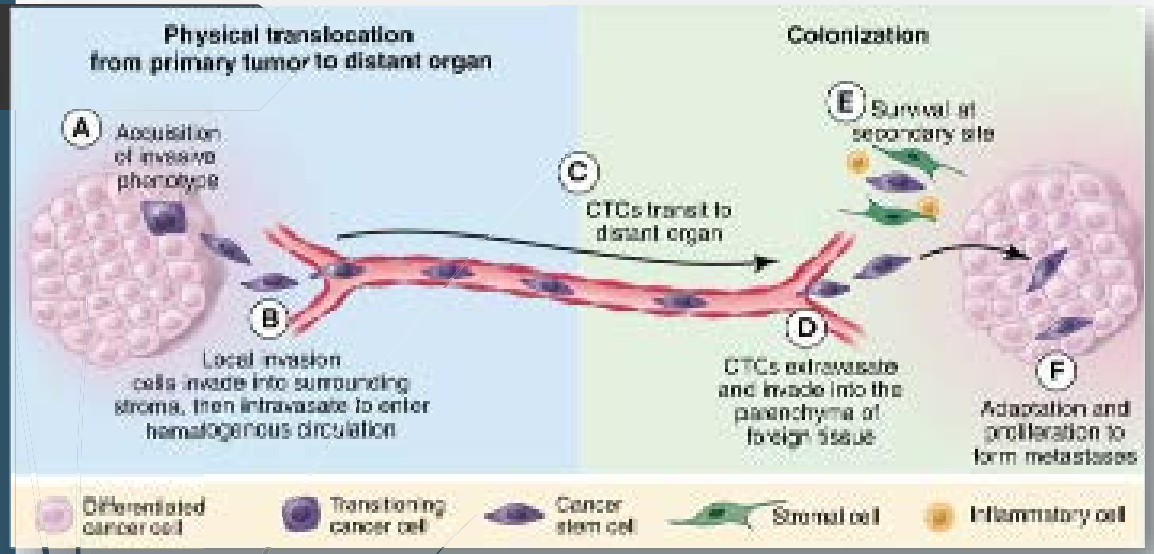
MINIMALLY INVASIVE APPROACHES TO MONITOR TUMOR GENOME EVOLUTION.

(a) CIRCULATING TUMOR CELLS (CTCs; blue) are isolated from total blood cells by cell separation systems. Cell lysis yields pure tumor DNA (gray) and RNA (black); for analysis of tumor-specific mutations, these are subjected to whole-genome or whole-transcriptome amplification, respectively (WGA, WTA). Proteins in CTCs can be analyzed by immunohistochemistry and, if viable, CTCs can be subjected to functional analyses.

(b) CELL-FREE DNA (cfDNA) is prepared from plasma by several centrifugation and filtration steps. The resulting DNA is a mixture of DNA fragments released from nonmalignant cells (brown) and from tumor cells (ctDNA; blue). Depending on the tumor stage, and especially during early disease, ctDNA may represent a minority of all cfDNA. cfDNA is analyzed by next-generation sequencing, which can reveal somatic copy number changes, single-nucleotide variants and rearrangements.

(c) EXOSOMES can be efficiently captured from blood using the nPLEX assay and their molecular constituents analyzed; for example, proteins can be quantified by nPLEX, and RNA can be quantified or sequenced.

CELLULE TUMORALI CIRCOLANTI



RARE: 1-10 CELLULE / 7.5 ML DI SANGUE

UTILIZZATE COME BIOMARCATORI IN >300 TRIAL CLINICI CHE SONO STATI REGISTRATI IN CLINICALTRIALS.GOV

SCIENCE 2011

- LE CTC SONO NOTE DA TEMPO (PRIMA RELAZIONE PIÙ DI 150 ANNI FA).
- SEMBREREBBERO RAPPRESENTARE UN EVENTO PRECOCE NELLA TUMORIGENESI.
- LE CELLULE INFILTRANTI SUBISCONO UNA TRANSIZIONE EPITELIO MESENCHIMALE (EMT) PER POTER ACCEDERE ALLA CIRCOLAZIONE SISTEMICA; ALCUNE MUOIONO, ALCUNE SOPRAVVIVONO E PERMANGONO COME CELLULE DORMIENTI IN ORGANI PERIFERICI.
- MODELLI METASTATICI HANNO DIMOSTRATO CHE CTC POSSONO RAGGIUNGERE SANGUE PERIFERICO OGNI POCHE ORE E CHE LA MAGGIOR PARTE DI ESSE SCOMPARE IN POCHI MINUTI.
- ESSE RAPPRESENTANO UN PRIMO PASSO DEL (ALTAMENTE INEFFICIENTE) PROCESSO METASTATICO.

STUDIARE LE CTC: COSA E PERCHE'

Table 1. Potential applications of circulating tumour cell (CTC) analysis.

Enumeration of CTCs	Molecular characterisation of CTCs
• Guide prognosis • Assist in measuring response to anticancer therapy — predictive and/or pharmacodynamic biomarker • May lead to more accurate prognosis when added to existing staging classifications • Select patients for adjuvant chemotherapy • Detect recurrent disease • Aid diagnostic process	• Surrogate for biological activity of underlying tumour — 'real-time biopsy' • Elucidate prognostic and predictive molecular features • Detection of treatment-resistant profiles —ease of serial sampling • Improve understanding of mechanisms of biological processes • Discover and identify new targets for therapeutic manipulation

IDENTIFICAZIONE E QUANTIFICAZIONE CTC → **DIAGNOSI, PROGNOSI, RISPOSTA ALLA TERAPIA**

MODIFICAZIONE NEI LIVELLI DI CTC → **PROGNOSI, PREDIZIONE DI RISPOSTA ALLA TERAPIA**

VALUTAZIONE FENOTIPO E GENOTIPO DELLE CTC → **DIAGNOSI, PROGNOSI, SUGGERIRE TERAPIE, SUGGERIRE SITO METASTATICO**

Ther Adv Med Oncol (2010) 2(6) 351–365

Biopsia liquida: monitoraggio in tempo reale

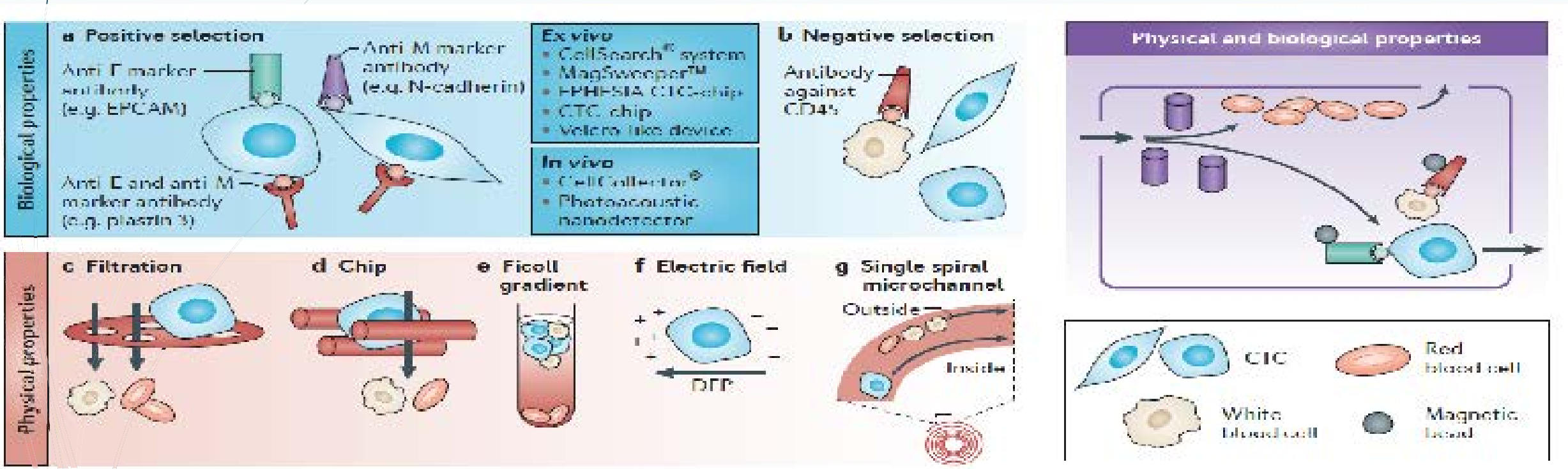
- **MONITORAGGIO DELLA "QUANTITA' DI TUMORE"**
- **MONITORAGGIO IN "TEMPO REALE" DEI CAMBIAMENTI DEL GENOMA DEL TUMORE:**
  - Pertinenza della terapia o sviluppo di resistenze
  - Valutazione precoce della efficacia di una terapia risparmiando al paziente la tossicità inutile di un farmaco che non fornisce alcun beneficio.
  - Evidenza di eventuali bersagli molecolari che potrebbero essere bersagli adatti per un nuovo trattamento.
- **POSSIBILITÀ DI USARE QUESTI TEST NELLA DIAGNOSI DEL CANCRO.**
- **COMPRENSIONE DELLO SVILUPPO DELLA MALATTIA METASTATICA**
- **IDENTIFICAZIONE DI VIE DI SEGNALE COINVOLTE NELLA INVASIVITÀ CELLULARE E COMPETENZA METASTATICA.**

VALIDITÀ CLINICA E UTILITÀ CLINICA

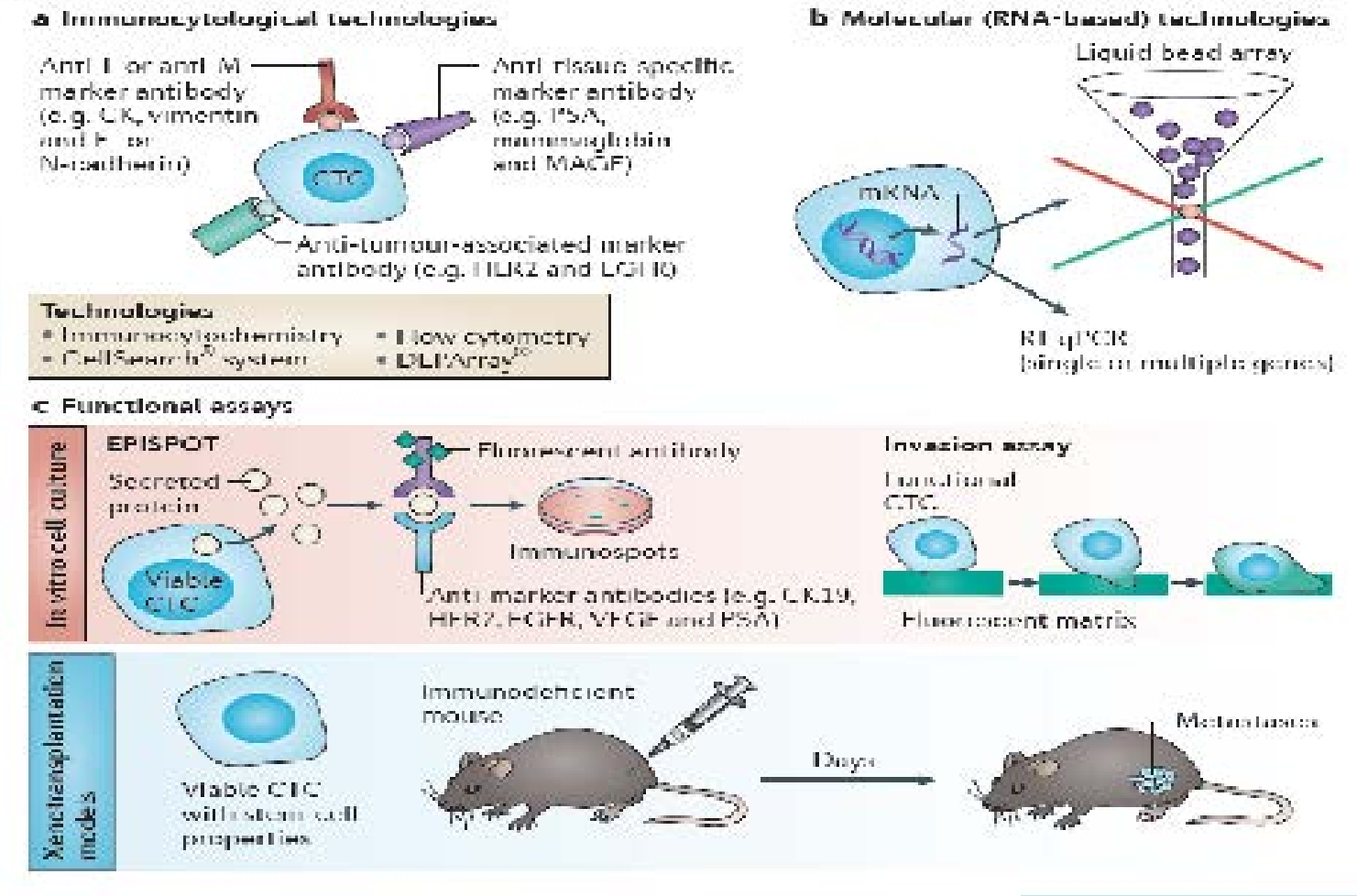
- **VALIDITA' CLINICA**: capacità del test di predire PROGNOSI e RISPOSTA ALLA TERAPIA

- **UTILITA' CLINICA**: capacità del test di modificare la prognosi del paziente guidando una scelta terapeutica

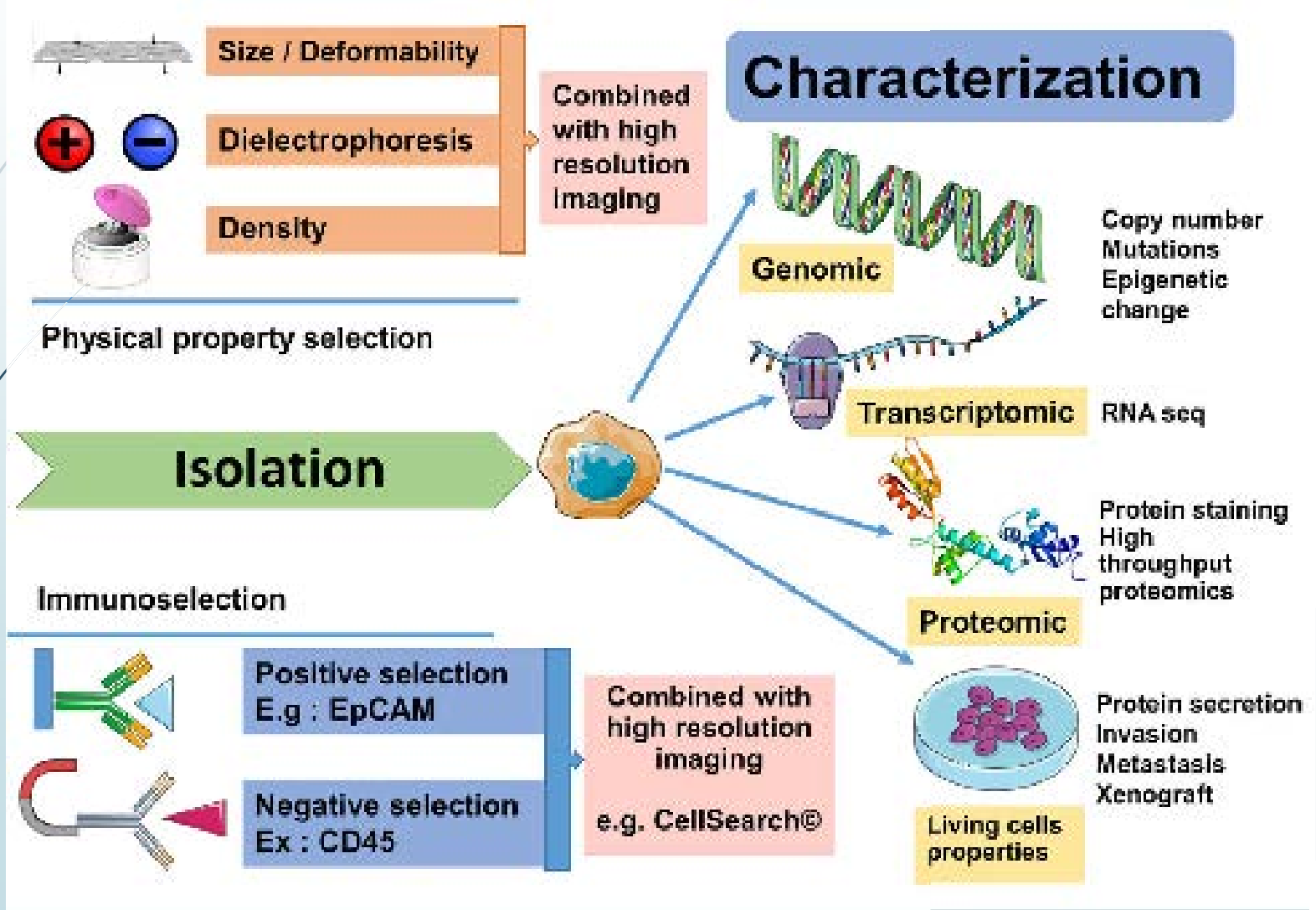
Come identificare le CTC?
1. arricchire



Come identificare le CTC?
2. selezionare



Come identificare le CTC?
3. caratterizzare



Int J Clin Oncol (2017) 22:421–430

POINT MUTATION

Heterogeneity of *PIK3CA* mutational status at the single cell level in circulating tumor cells from metastatic breast cancer patients

Marta Pestrin[a,1], Francesca Salvianti[b,1], Francesca Galardi[a], Francesca De Luca[a], Natalie Turner[a], Luca Malorni[a], Mario Pazzagli[b], Angelo Di Leo[a], Pamela Pinzani[b,]*

[a]Sandro Pitigliani Medical Oncology Department, Hospital of Prato, Istituto Toscano Tumori, Prato, Italy
[b]Department of Clinical, Experimental and Biomedical Sciences, University of Florence, Florence, Italy

MOLECULAR ONCOLOGY 9 (2015) 749–757

ERBB2 AMPLIFICATION

FISH-based determination of HER2 status in circulating tumor cells isolated with the microfluidic CEE™ platform

Julie Ann Mayer, Tam Pham, Karina L. Wong, Jayne Scoggin, Edgar V. Sales, Trisky Clarin, Tony J. Pircher, Stephen D. Mikolajczyk, Philip D. Cotter, Farideh Z. Bischoff*

Biocept Inc., San Diego, CA

Cancer Genetics 204 (2011) 589–595

DNA METHYLATION

Breast Cancer Metastasis Suppressor-1 Promoter Methylation in Primary Breast Tumors and Corresponding Circulating Tumor Cells

Maria Chimonidou[1], Galatea Kallergi[2], Vassilis Georgoulias[2], Danny R. Welch[3], and Evi S. Lianidou[1]

Mol Cancer Res; 11(10) October 2013

COPY NUMBER VARIATION

Identification of genomic signatures in circulating tumor cells from breast cancer

Nisha Kanwar[1,2], Pingzhao Hu[3], Philippe Bedard[4], Mark Clemons[5], David McCready[6] and Susan J. Done[1,2,7,8]

[1]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada
[2]The Campbell Family Institute for Breast Cancer Research at the Princess Margaret Cancer Centre, Toronto, Canada
[3]Department of Biochemistry and Medical Genetics, George and Fay Yee Centre for Healthcare Innovation, University of Manitoba, Winnipeg, Canada
[4]Division of Medical Oncology and Hematology, Princess Margaret Cancer Centre, Toronto, Canada
[5]Division of Medical Oncology, The Ottawa Hospital Cancer Centre, Ottawa, Canada
[6]Division of General Surgery and Surgical Oncology, Princess Margaret Cancer Centre, University of Toronto, Toronto, Canada
[7]Department of Medical Biophysics, University of Toronto, Toronto, Canada
[8]Laboratory Medicine Program, University Health Network, Toronto, Canada

Int. J. Cancer: 137, 332–344 (2015)

TRANSCRIPTOMIC

Annals of Oncology 26: 510–516, 2015
Published online 3 December 2014

Gene expression profiles in circulating tumor cells to predict prognosis in metastatic breast cancer patients

B. Mostert[1], A. M. Sieuwerts[1,2], J. Kraan[1], J. Bolt-de Vries[1], P. van der Spoel[1], A. van Galen[1], D. J. Peeters[3], L. Y. Dirix[3], C. M. Seynaeve[1], A. Jager[1], F. E. de Jongh[4], P. Hamberg[5], J. M. L. Stouthard[6,†], D. F. S. Kehrer[7], M. P. Look[1], M. Smid[1], J. W. Gratama[1], J. A. Foekens[1], J. W. M. Martens[1,2] & S. Sleijfer[1,2*]

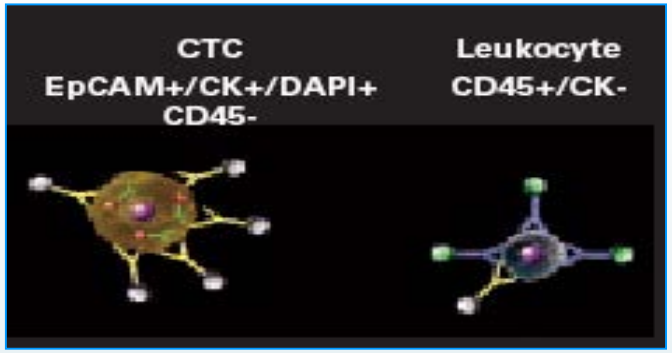
Table 1 Advantages and disadvantages of the main enrichment and detection techniques

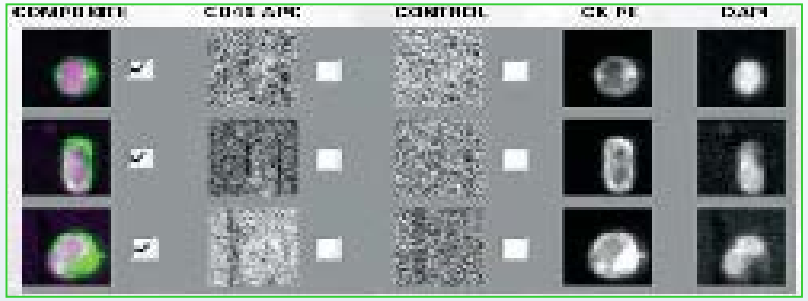
Technique	Advantages	Disadvantages	References
CTC-filtering devices by size (ISET)	Capture and analysis platform; multiplexed imaging and genetic analysis, easy and rapid, feasible for EpCAM-negative CTCs	Low specificity (lose smaller CTCs and retain larger leukocytes)	(3-5)
Density gradient centrifugation (Ficoll-hypaque or OncoQuick)	Easy and inexpensive; feasible for EpCAM-negative CTCs	Low specificity; cross-contamination of different layers (OncoQuick can resolve this issue)	(6,7)
CellSearch® system	FDA cleared; visual confirmation of CTCs, clinical relevance, automated, quantitative; highly reproducible	EpCAM-positivity dependent; no additional gene expression tests could be added for analysis of CTCs; subjective picture evaluation; costly instrumentation	(8,9)
CTC-chip	High detection rate; visual confirmation of CTCs; potential to harvest CTCs for further molecular and genetic analyses	EpCAM-positivity dependent; subjective CTC analysis; further investigation on assay specificity	(10,11)
Immunocytochemistry (ICC)	Quantification and morphological analysis of CTCs; facilitate classical cytopathological review	Time consuming; subjective evaluation	(12)
Protein assays (EPISPOT)	Detects only viable cells, limited number of markers	Clinical relevance not demonstrated, proteins must be actively secreted; no further identification and isolation of CTCs	(13,14)
Immunofluorescence-based technologies (DyLight)	Multimarker image analysis	Application in cell lines	(15)
RT-PCR (CTCscope)	High sensitivity; detects only viable cells	No morphological analysis; visualization and enumeration of CTCs is not possible	(16)
Multiplex RT-PCR (AdnaTest)	High sensitivity; detects only viable cells, saves sample and time, reduces cost; isolation and detection of stem cell and EMT markers	No morphological analysis; EpCAM and MUC1 positivity dependent assay, no quantification	(17,18)

ISET, isolation by size of epithelial tumor cells; CTCs, circulating tumor cells; EPISPOT, Epithelial ImmunoSPOT; EMT, epithelial mesenchymal transition; EpCAM, epithelial cell adhesion molecule.

(from Toss A and Coll.; *Ann Transl Med* Nov 2014)

Cellsearch® System

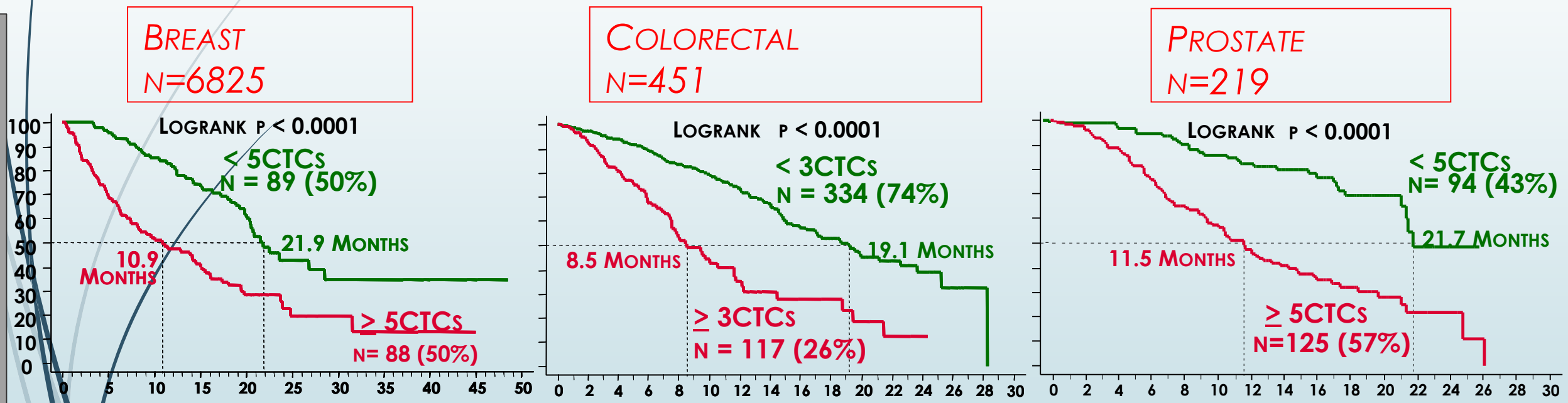




- E' IL **SOLO METODO APPROVATO FDA** PER LA QUANTIFICAZIONE DELLE CTC *(CANCRO METASTATICO DI MAMMELLA, COLON, PROSTATA)*
- E' BASATO SULL'ARRICCHIMENTO IMMUNOMAGNETICO (USO DI **ANTI-EPCAM** AB): 10^8->10^4
- LE CTC SONO DEFINITE COME CELLULE NUCLEATE (DAPI POSITIVE) POSITIVE PER LE CITOCHERATINE 8, 18, 19 E NEGATIVE PER CD45
- LE CELLULE SONO ESAMINATE DA UN MICROSCOPIO A FLUORESCENZA
- I RISULTATI SONO ESPRESSI COME **NUMERO DI CELLULE PER 7,5 ML DI SANGUE PERIFERICO**

VALUTAZIONE DELLE CTC PRIMA DELLA TERAPIA: VALORE PROGNOSTICO

METASTATIC CARCINOMAS



M CRISTOFANILLI ET AL
NEJM 2004 & JCO 2005
ZHANG ET AL. CLIN CANCER RES 2012
BIDARD ET AL, LANCET ONCOL 2014

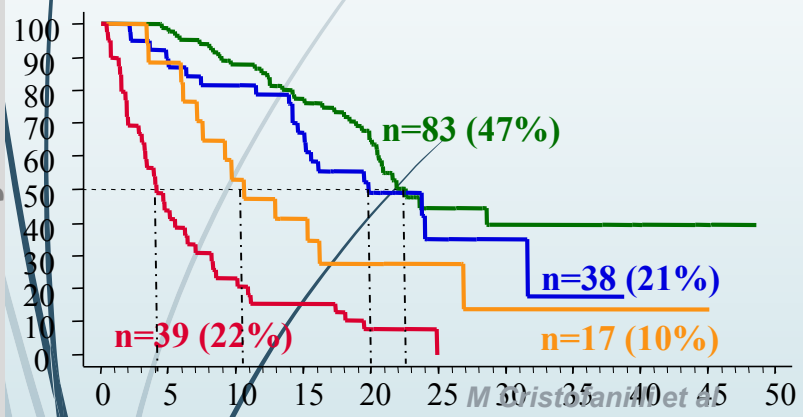
S COHEN ET AL
JCO 2008

DE BONO ET AL
CCR 2008

MT SANDRI

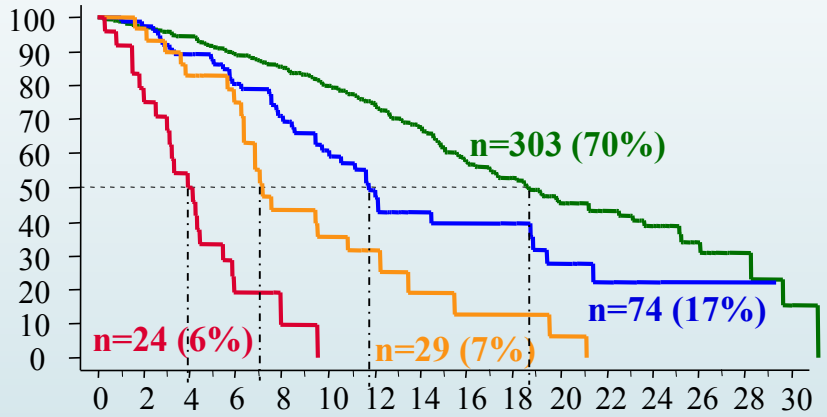
Cambiamento del numero di CTC in risposta alla terapia

Breast



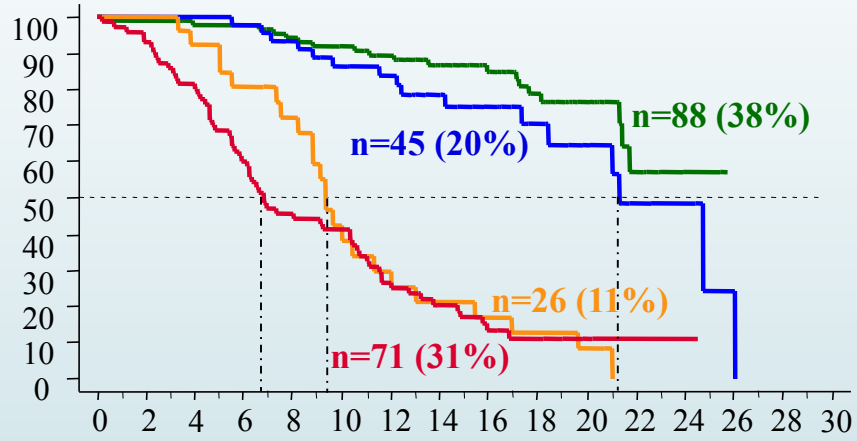
M Cristofanilli et al
NEJM 2004 & JCO 2005
Budd et al CCR 2006
Hayes et al CCR 2006

Colorectal



S Cohen et al
JCO 2008

Prostate



De Bono et al
CCR 2008

Time from Baseline (Months)

REMAIN FAVORABLE

REMAIN UNFAVORABLE

CONVERT TO UNFAVORABLE

CONVERT TO FAVORABLE

MT SANDRI

Rilevanza clinica: PROGNOSI TUMORE METASTATICO

Meta-Analysis of the Prognostic Value of Circulating Tumor Cells in Breast Cancer

Liling Zhang[1], Sabine Riethdorf[2], Gang Wu[1], Tao Wang[1], Kunyu Yang[1], Gang Peng[1], Junli Liu[1], and Klaus Pantel[2]

Abstract

Purpose: The prognostic value of circulating tumor cells (CTC) detected in breast cancer patients is currently under debate. Different time points of blood collections and various CTC assays have been used in the past decades. Here, we conducted the first comprehensive meta-analysis of published literature on the prognostic relevance of CTC, including patients with early and advanced disease.

Experimental Design: A comprehensive search for articles published between January 1990 and January 2012 was conducted; reviews of each study were conducted and data were extracted. The main outcomes analyzed were overall survival (OS) and disease-free survival (DFS) in early-stage breast cancer patients, as well as progression-free survival (PFS) and OS in metastatic breast cancer patients. Pooled hazard ratio (HR) and 95% confidence intervals (CIs) were calculated using the random and the fixed-effects models. Subgroup and sensitivity analyses were also conducted.

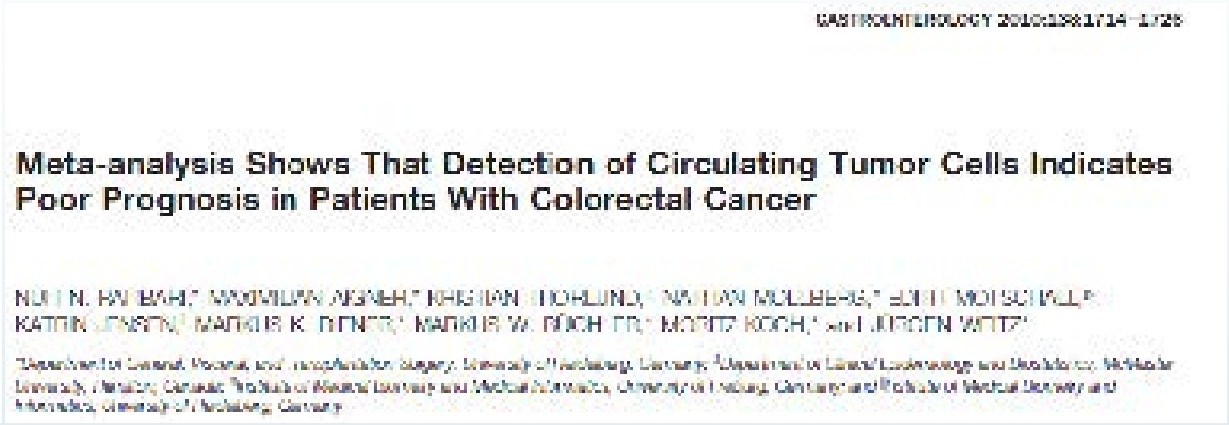
Results: Forty-nine eligible studies enrolling 6,825 patients were identified. The presence of CTC was significantly associated with shorter survival in the total population. The prognostic value of CTC was significant in both early (DFS: HR, 2.86; 95% CI, 2.19–3.75; OS: HR, 2.78; 95% CI, 2.22–3.48) and metastatic breast cancer (PFS: HR, 1.78; 95% CI, 1.52–2.09; OS: HR, 2.33; 95% CI, 2.09–2.60). Further subgroup analyses showed that our results were stable irrespective of the CTC detection method and time point of blood withdrawal.

Conclusion: Our present meta-analysis indicates that the detection of CTC is a stable prognosticator in patients with early-stage and metastatic breast cancer. Further studies are required to explore the clinical utility of CTC in breast cancer. *Clin Cancer Res; 18(20); 5701–10.* ©2012 AACR.

Rilevanza clinica: PROGNOSI TUMORE METASTATICO

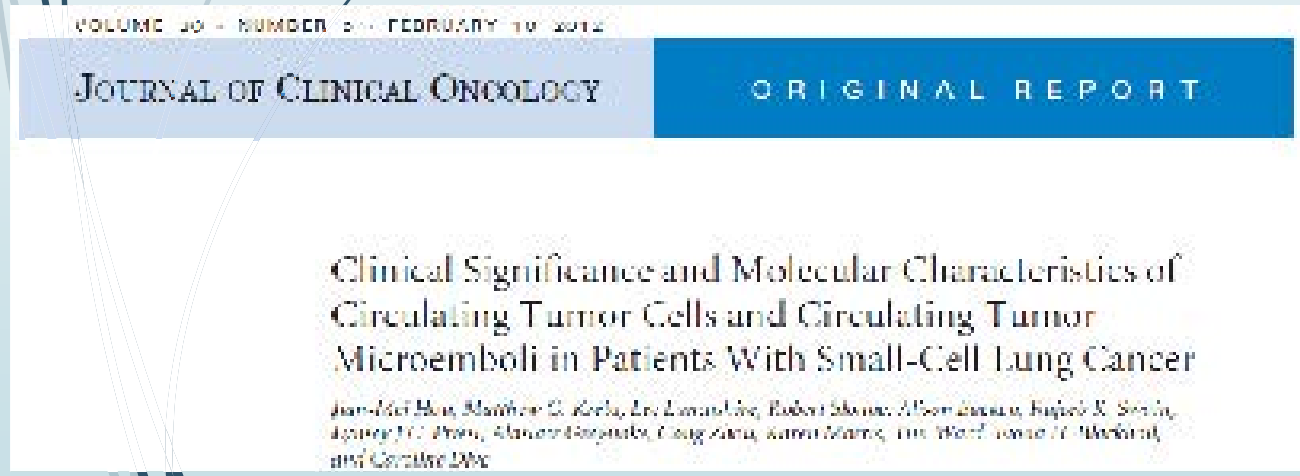
JOURNAL OF CLINICAL ONCOLOGY | ORIGINAL REPORT

Circulating Tumor Cell Biomarker Panel As an Individual-Level Surrogate for Survival in Metastatic Castration-Resistant Prostate Cancer



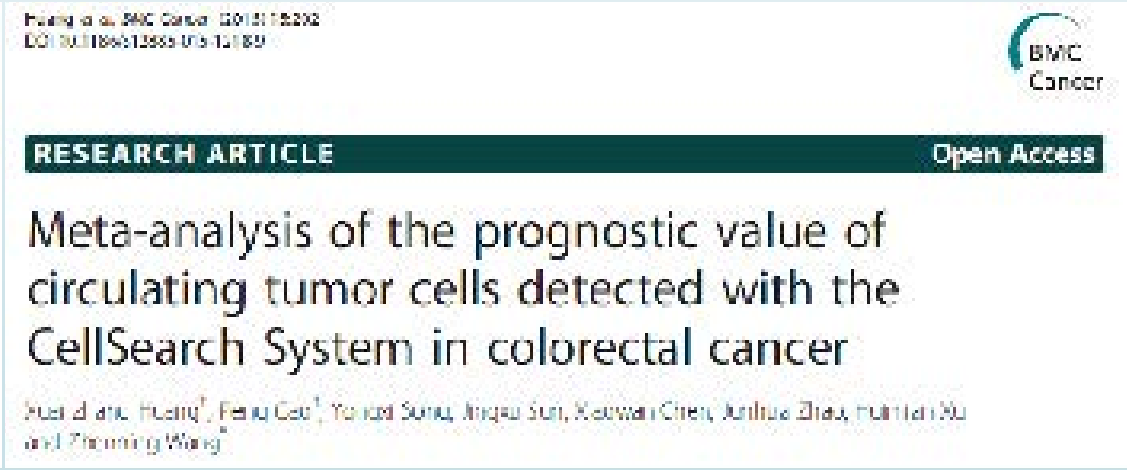
GASTROENTEROLOGY

Meta-analysis Shows That Detection of Circulating Tumor Cells Indicates Poor Prognosis in Patients With Colorectal Cancer



JOURNAL OF CLINICAL ONCOLOGY | ORIGINAL REPORT

Clinical Significance and Molecular Characteristics of Circulating Tumor Cells and Circulating Tumor Microemboli in Patients With Small-Cell Lung Cancer



BMC Cancer

RESEARCH ARTICLE | Open Access

Meta-analysis of the prognostic value of circulating tumor cells detected with the CellSearch System in colorectal cancer

Rilevanza clinica: prognosi tumore non metastatico

Table 2 CTC detection in BC treated by neoadjuvant therapy.

Trial name (if any) references	Patients number tumor type	CTC detection rate (≥1 CTC/7.5 ml)				Correlation between CTC detection and		Prognostic impact	
		Before neo. tt.	During neo. tt.	After neo. tt., before surgery	After neo. tt., after surgery	Tumor characteristics	Pathological complete response	Disease free survival	Overall survival
REMAGUS02 Pierga et al. [illegible]	N = 115 all subtypes	23%	–	17%	–	No	No	Yes	Yes
GEPARQUATTRO Riethdorf et al. [illegible]	N = 213 all subtypes	22%	–	11%	–	No	No	–	–
GEPARQUINTO Riethdorf et al. [illegible]	N = 384 all subtypes	23%	14%	11%		No	No		
[illegible] Hospital [illegible] et al. [illegible]	N = 95 all subtypes	[illegible]	–	–	–	No	No	No	–
[illegible] et al. [illegible]	N = [illegible] HER2 positive	[illegible]	[illegible]	[illegible]		No	No		
[illegible] Onstenk et al. [illegible]	N = [illegible] HER2 negative	[illegible]	[illegible]			No	No		
[illegible] et al. [illegible]	N = [illegible] HER2 positive	[illegible]	[illegible]	[illegible]	–	No	No	–	–
MD Anderson Hall et al. [illegible]	N = 57 triple negative	–	–	[illegible]	–	No	No	Yes	Yes
[illegible] Pierga et al. [illegible]	N = [illegible] HER2-positive inflammatory	[illegible]	[illegible]	[illegible]	[illegible]	No	No	Yes	Yes
[illegible] Pierga et al. [illegible]	N = [illegible] HER2 negative inflammatory	[illegible]	[illegible]	[illegible]	[illegible]	No	No	Yes	Yes
MD Anderson Mego et al. [illegible]	N = 77 all subtypes inflammatory	[illegible]	–	–	–	No	No	No	No
MD Anderson Hall et al. [illegible]	N = [illegible] all subtypes inflammatory			[illegible]		No	No	Yes	No

Studies that specifically enrolled inflammatory breast cancer patients are displayed at the bottom of the table. Neo. tt.: neoadjuvant therapy.

Bidard F-C, Michiels S, et al (2016) IMENEO: International MEta-analysis of circulating tumor cell detection in early breast cancer patients treated **by NEOadjuvant chemotherapy**. In: Abstracts of the thirty-ninth annual CTRC-AACR San Antonio Breast Cancer Symposium, San Antonio, Texas, 6-10 December 2016.

Rilevanza clinica: prognosi tumore non metastatico

Circulating tumor cells in locally advanced pancreatic adenocarcinoma: the ancillary CirCe 07 study to the LAP 07 trial

F. D. Bidard[1,2*], F. Huguet[3], G. Louvet[4], L. Mineur[5], O. Bouché[6], E. Chibaudel[7], P. Artru[5], F. Desseigne[2], J. B. Bachet[8], C. Mahjoubi[1], J. Y. Pierga[1,2] & P. Hammel[3]

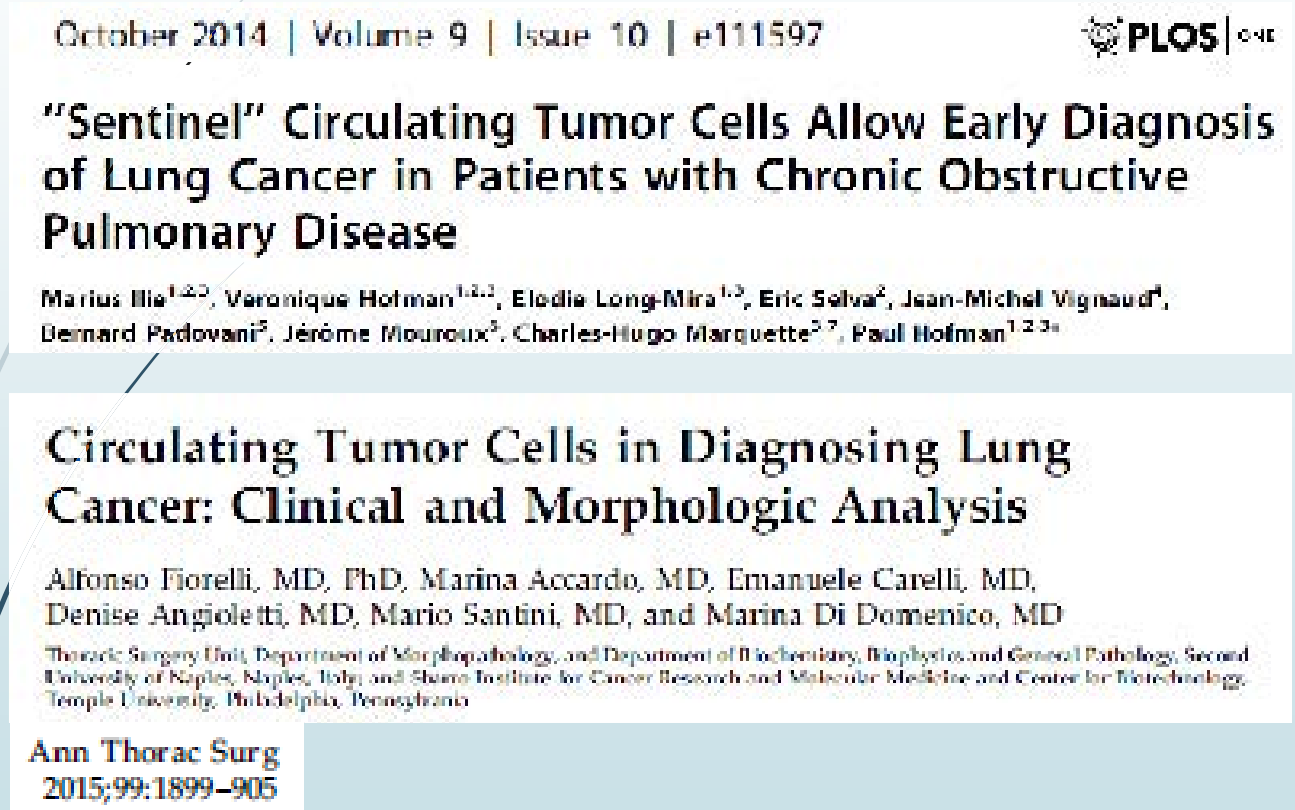
Presenza di una o piu'CTC associate a prognosi peggiore (9%)

COLON CANCER:

- Valore prognostico del numero pre-operatorio di CTC (non post-operatorio);
- Alto numero di CTC dopo adiuvante, aumentato rischio di recidiva

Rilevanza clinica: SCREENING

- Cellule presenti in basso numero e solo in una piccolo frazione di pazienti



October 2014 | Volume 9 | Issue 10 | e111597

PLOS ONE

"Sentinel" Circulating Tumor Cells Allow Early Diagnosis of Lung Cancer in Patients with Chronic Obstructive Pulmonary Disease

Marius Ilie[1,2], Veronique Hofman[1,2,3], Elodie Long-Mira[1,3], Eric Selva[2], Jean-Michel Vignaud[4], Bernard Padovani[5], Jérôme Mouroux[5], Charles-Hugo Marquette[2,7], Paul Hofman[1,2,3]*

Circulating Tumor Cells in Diagnosing Lung Cancer: Clinical and Morphologic Analysis

Alfonso Fiorelli, MD, PhD, Marina Accardo, MD, Emanuele Carelli, MD, Denise Angioletti, MD, Mario Santini, MD, and Marina Di Domenico, MD

Thoracic Surgery Unit, Department of Morphopathology, and Department of Biochemistry, Biophysics and General Pathology, Second University of Naples, Naples, Italy; and Sbarro Institute for Cancer Research and Molecular Medicine and Center for Biotechnology, Temple University, Philadelphia, Pennsylvania

Ann Thorac Surg
2015;99:1899–905

NCT02500693: Circulating Tumor Cells and Early Diagnosis of Lung Cancer in Patients With Chronic Obstructive Pulmonary Disease

NCT02608346: Circulating Tumor DNA and Follow-up of BRCA1 Mutation Carriers (CirCa 01)

UTILITA'CLINICA?

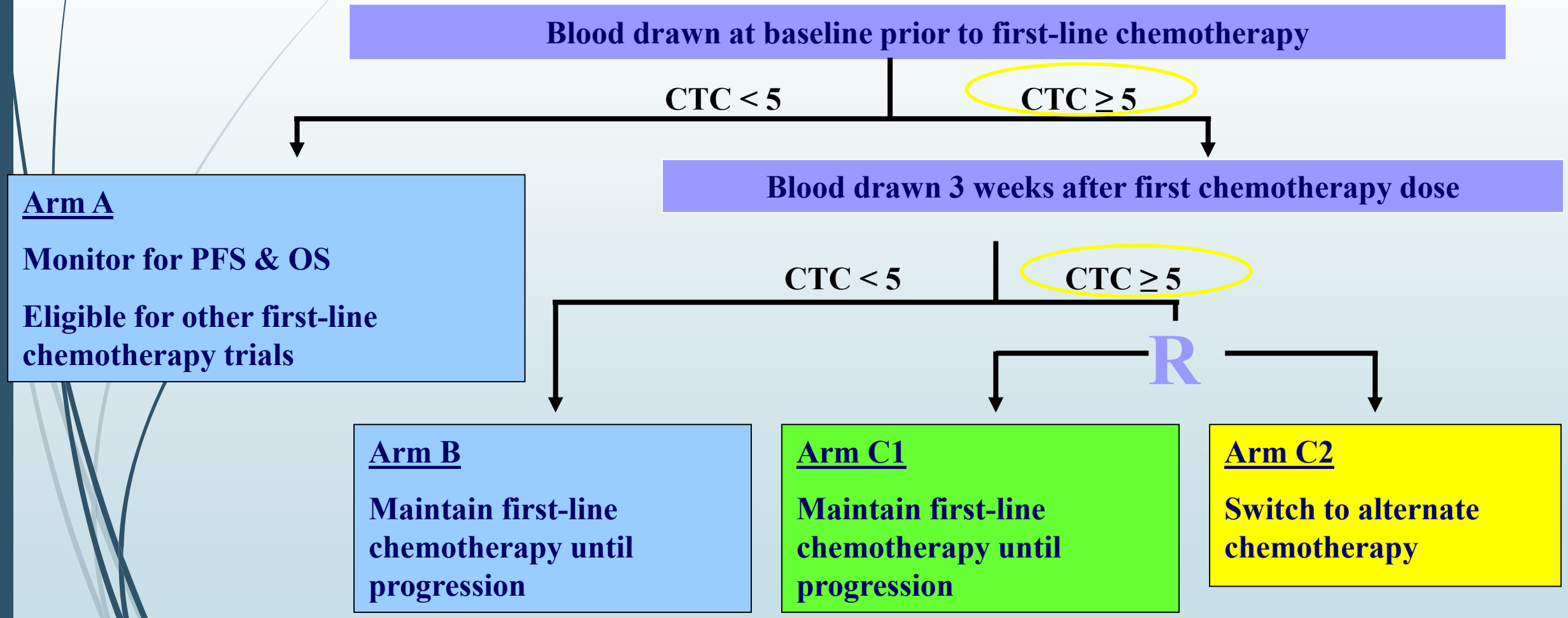
Table 1 Clinical utility of CTCs: published or ongoing phase II or III trials

Trial name and organ	Primary objective	Results (or predicted results)
Phase III		
SWOG S0500 [illegible] BREAST	Evaluate whether changing chemotherapy after one cycle of first-line chemotherapy in MBC patients with persistent CTC detection can improve the outcome	No significant increase of survival (PFS and OS) in patients randomized to the CTC-based management arm
[illegible] NCT01349842 BREAST	Evaluate whether CTCs can guide chemotherapy from the third line of chemotherapy for MBC patients	Ongoing
DETECT III [illegible] BREAST	Evaluate the efficacy of lapatinib in HER2-negative MBC patients and HER2-positive CTCs	Ongoing
STIC CTC NCT01710605 BREAST	Assess the value of baseline CTCs [illegible] hormone therapy vs chemotherapy [illegible] hormone receptor positive MBC patients. Randomization between treatment [illegible] by the clinician or based on CTC level (hormone therapy if <5 CTCs, chemotherapy if ≥5 CTCs)	Ongoing
[illegible] NCT01640405 COLON	Assess the value of first-line triplet chemotherapy (FOLFOXIRI-bevacizumab) vs doublet chemotherapy (FOLFOX-bevacizumab) in metastatic colorectal cancer patients with [illegible] CTC counts ([illegible] CTCs)	Ongoing
Phase II		
[illegible] [illegible] BREAST	Evaluate lapatinib in HER2-negative MBC and HER2-positive CTCs	[illegible] patients screened had HER2-positive CTCs, no objective response (OR) was observed
Stebbing et al. [illegible] BREAST	Evaluate lapatinib in eliminating EGFR-positive CTCs in women with MBC	[illegible] patients screened had EGFR-[illegible] [illegible] evaluable patients had a decrease in CTC counts, especially for EGFR-positive CTCs
Agelaki et al. [illegible] BREAST	Evaluate lapatinib in eliminating cytokeratin-positive CTCs in women with MBC	[illegible] patients treated by lapatinib: [illegible] had a CTC response
Georgoulias et al. [illegible] BREAST	Evaluate trastuzumab vs placebo added to chemotherapy in HER2-negative primary breast cancer patients with HER2(−)CTCs before and after adjuvant chemotherapy	[illegible] of the [illegible] analyzed patients had HER2 CTC(+) and were randomized. Median PFS was longer in the trastuzumab arm than in the placebo arm
[illegible] et al. [illegible] BREAST	Evaluate trastuzumab [illegible] in HER2-negative MBC and HER2-positive CTCs	[illegible] patients treated: [illegible] cases of early progression [illegible] weeks, [illegible] response ([illegible] weeks), [illegible] disease ([illegible] weeks)
TREAT CTC [illegible] BREAST	Evaluate trastuzumab in HER2-negative patients with primary breast cancer who, after completing neo/adjuvant chemotherapy and surgery, have detectable CTCs in peripheral blood	Ongoing
CirCe T-DM1 NCT01975142 BREAST	Evaluate trastuzumab emtansine (T-DM1) in HER2-negative MBC patients who have HER2-amplified CTCs	Ongoing
NCT01185509 BREAST	Evaluate the efficacy and safety of trastuzumab and vinorelbine in MBC with HER2-negative primary tumors and HER2(+)CTCs	Study was stopped: not enough confirmed responses to continue treatment

MBC metastatic breast cancer, *CTCs* circulating tumor cells

Int J Clin Oncol (2017) 22:421–430

CTC count and therapy: SWOG 0500



Randomized phase III clinical trial

Circulating Tumor Cells and Response to Chemotherapy in Metastatic Breast Cancer: SWOG S0500

Jeffrey B. Smerage, William E. Barlow, Gabriel N. Hortobagyi, Eric P. Winer, Brian Leyland-Jones, Gordan Srkalovic, Sheela Tejwani, Anne F. Schott, Mark A. O'Rourke, Danika L. Lew, Gerald V. Doyle, Julie R. Gralow, Robert B. Livingston, and Daniel F. Hayes

A B S T R A C T

Purpose
Increased circulating tumor cells (CTCs; five or more CTCs per 7.5 mL of whole blood) are associated with poor prognosis in metastatic breast cancer (MBC). A randomized trial of patients with persistent increase in CTCs tested whether changing chemotherapy after one cycle of first-line chemotherapy would improve the primary outcome of overall survival (OS).

Patients and Methods
Patients with MBC who did not have increased CTCs at baseline remained on initial therapy until progression (arm A). Patients with initially increased CTCs that decreased after 21 days of therapy remained on initial therapy (arm B). Patients with persistently increased CTCs after 21 days of therapy were randomly assigned to continue initial therapy (arm C1) or change to an alternative chemotherapy (arm C2).

Results
Of 595 eligible and evaluable patients, 276 (46%) did not have increased CTCs (arm A). Of those with initially increased CTCs, 31 (10%) were not retested, 165 were assigned to arm B, and 123 were randomly assigned to arm C1 or C2. No difference in median OS was observed between arm C1 and C2 (10.7 and 12.5 months, respectively; $P = .98$). CTCs were strongly prognostic. Median OS for arms A, B, and C (C1 and C2 combined) were 35 months, 23 months, and 13 months, respectively ($P < .001$).

Conclusion
This study confirms the prognostic significance of CTCs in patients with MBC receiving first-line chemotherapy. For patients with persistently increased CTCs after 21 days of first-line chemotherapy, early switching to an alternate cytotoxic therapy was not effective in prolonging OS. For this population, there is a need for more effective treatment than standard chemotherapy.

J Clin Oncol 32.

SE QUANTIFICARE NON BASTA?

- CELLSEARCH QUANTIFICA SEMPLICEMENTE LE CTC MA NON LE CARATTERIZZA ULTERIORMENTE
- NUOVI METODI DI ISOLAMENTO DI CTC
- NUOVI STRUMENTI PERMETTONO DI ISOLARE LE CELLULE ED OTTENERE ULTERIORI INFORMAZIONI.

M30 Neoepitope Expression in Epithelial Cancer: Quantification of Apoptosis in Circulating Tumor Cells by CellSearch Analysis

Elisabetta Rossi, Umberto Basso, Romina Celadin, et al.

Clin Cancer Res 2010;16:5233-5243. Published OnlineFirst October 26, 2010.

Translational Relevance

The absolute number of circulating tumor cells (CTC) has proved to be a robust predictor of poor prognosis in metastatic breast, colorectal, and prostate cancer. Moreover, in the absence of tumor biopsies CTC provide a "surrogate" index for monitoring response to treatment. However, the CTC biological significance is as yet undefined: why did the median overall survival not further decrease when >5 CTC (poor-prognosis threshold, very few CTC indeed) were detected in 7.5 mL of blood?

By exploiting a M30 integrated CTC assay, we show here that CTC are a heterogeneous cell population, which includes both apoptotic and viable cells: exceedingly high numbers of live CTC were associated with radiologic recurrence of disease, and also when a switch under the threshold of poor prognosis was observed during the therapy. Our data offer a rationale to the option that a CTC subpopulation not expressing M30 may be associated with decreased chances of survival.

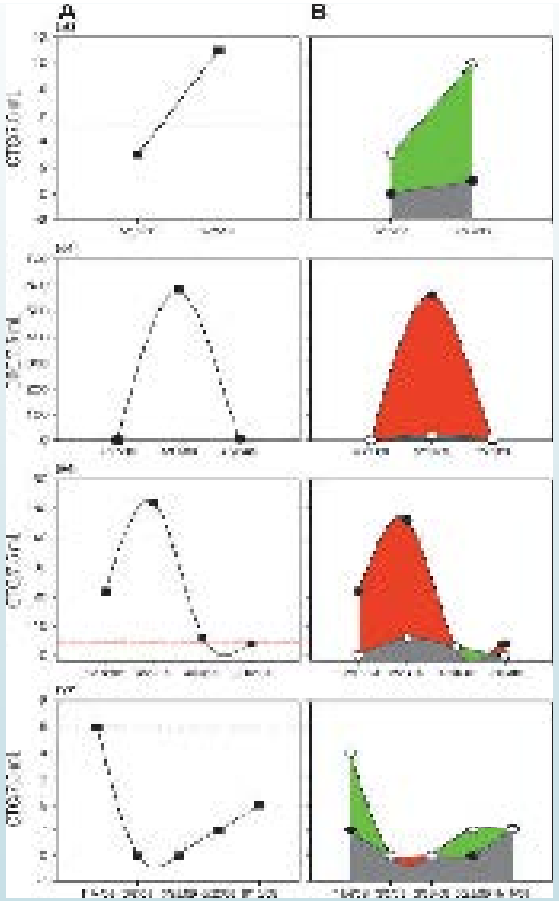


Fig. 4. Changes in the number of CTC in breast cancer patients. A, longitudinal graphs of CTC count over the indicated follow-up period for patients 50, 84, 94, and 97. Patients 94 and 97 were diagnosed with metastatic cancer at enrolling in this study; red lines, threshold of poor prognosis (≥ 5 CTC/7.5 mL for MUC; ref. 5). B, area under the blood concentration-time curve over the follow-up period of M30-negative (–, red area) and M30-positive (+, green area) CTC; gray area, overlay of the M30-positive and M30-negative longitudinal graphs. Extra-five CTC over the follow-up (positive ΔAUC) were detected in patients 84 and 94, which were PD by imaging; on the contrary, extra-apoptotic CTC over the follow-up period (negative ΔAUC) were detected in patients 50 and 97, which were SD/PR by imaging.

Monoclonal antibody (M30) targeting a neoepitope disclosed by caspase cleavage at cytokeratin 18 (CK18) in early apoptosis.

Reports on HER2 status changes

Comparison of HER2 status between primary tumor and disseminated tumor cells in primary breast cancer patients

Conclusions. HER2 positive DTC can be detected in patients with HER2 negative primary tumors. Therefore, the antigenic profile of DTC may be considered for treatment decision since these patients might actually benefit from trastuzumab. However, the HER2 overexpression on DTC is heterogeneous in individual patients which may reduce the efficacy of an immunotherapy based strategy directed against HER2-antigen only.

5-38% of patients with HER2-negative Primary breast cancer had HER2 Overexpression in CTCs

Research a
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Tanja Fehm[1],
Diethelm Wal

ients with initially negative or
elevated serum HER2 levels
the time of development of
ly a small number of patients
s are of clinical relevance
do not have access to HER2-

HER-2 gene amplification can be acquired as breast cancer progresses

Songdong Meng[a], Debasish Tripathy[b], Sanjay Shete[c], Raheela Ashfaq[d], Barbar
Aminollah Khan[g], David Euhus[h], Cynthia Osborne[h], Eugene Frenkel[b], Susan I
Ellen Vitetta[a], Larry Morrison[i], Dorothee Herlyn[k], Leon W. M. M. Terstappen[l],
Thomas Tucker[a], Nancy Lane[a], Jianqiang Wang[a], and Jonathan Uhr[a,m,n]

Nine of 24 breast cancer patients whose primary tumor was *HER-2*-negative each acquired *HER-2* gene amplification in their CTCs during cancer progression, i.e., 37.5% (95% confidence interval of 18.8–59.4%). Four of the 9 patients were treated with Herceptin-containing therapy. One had a complete response and 2 had a partial response.

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JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Detection of Circulating Tumor Cells Harboring a Unique *ALK* Rearrangement in *ALK*-Positive Non–Small-Cell Lung Cancer

Emma Pailler, Julien Adam, Amélie Barthélémy, Marianne Oulhen, Nathalie Auger, Alexander Valent, Isabelle Borget, David Planchard, Melissa Taylor, Fabrice André, Jean Charles Soria, Philippe Vielh, Benjamin Besse, and Françoise Farace

See accompanying editorial on page 2236

All authors: Institut de Cancérologie Gustave Roussy, Villejuif, France.

Published online ahead of print at www.jco.org on May 13, 2013.

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Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/13/3118ie-2273w/$20.00

DOI: 10.1200/JCO.2012.44.5832

A B S T R A C T

Purpose
The diagnostic test for *ALK* rearrangement in non–small-cell lung cancer (NSCLC) for crizotinib treatment is currently done on tumor biopsies or fine-needle aspirations. We evaluated whether *ALK* rearrangement diagnosis could be performed by using circulating tumor cells (CTCs).

Patients and Methods
The presence of an *ALK* rearrangement was examined in CTCs of 18 *ALK*-positive and 14 *ALK*-negative patients by using a filtration enrichment technique and filter-adapted fluorescent in situ hybridization (FA-FISH), a FISH method optimized for filters. *ALK*-rearrangement patterns were determined in CTCs and compared with those present in tumor biopsies. *ALK*-rearranged CTCs and tumor specimens were characterized for epithelial (cytokeratins, E-cadherin) and mesenchymal (vimentin, N-cadherin) marker expression. *ALK*-rearranged CTCs were monitored in five patients treated with crizotinib.

Results
All *ALK*-positive patients had four or more *ALK*-rearranged CTCs per 1 mL of blood (median, nine CTCs per 1 mL; range, four to 34 CTCs per 1 mL). No or only one *ALK*-rearranged CTC (median, one per 1 mL; range, zero to one per 1 mL) was detected in *ALK*-negative patients. *ALK*-rearranged CTCs harbored a unique (3′5′) split pattern, and heterogeneous patterns (3′5′, only 3′) of splits were present in tumors. *ALK*-rearranged CTCs expressed a mesenchymal phenotype contrasting with heterogeneous epithelial and mesenchymal marker expressions in tumors. Variations in *ALK*-rearranged CTC levels were detected in patients being treated with crizotinib.

Conclusion
ALK rearrangement can be detected in CTCs of patients with *ALK*-positive NSCLC by using a filtration technique and FA-FISH, enabling both diagnostic testing and monitoring of crizotinib treatment. Our results suggest that CTCs harboring a unique *ALK* rearrangement and mesenchymal phenotype may arise from clonal selection of tumor cells that have acquired the potential to drive metastatic progression of *ALK*-positive NSCLC.

J Clin Oncol 31:2273-2281. © 2013 by American Society of Clinical Oncology

mRNA and microRNA Expression Profiles in Circulating Tumor Cells and Primary Tumors of Metastatic Breast Cancer Patients

Anieta M. Sieuwerts[1], Bianca Mostert[2], Joan Bolt-de Vries[1], Dieter Peeters[6], Felix E. de Jongh[4], Jacqueline M.L. Stouthard[5], Luc Y. Dirix[5], Peter A. van Dam[5], Anne Van Galen[1], Vanja de Weerd[1], Jaco Kraan[2], Petra van der Spoel[2], Raquel Ramirez-Moreno[7], Carolien H.M. van Deurzen[3], Marcel Smid[1], Jack X. Yu[8], John Jiang[8], Yixin Wang[8], Jan W. Gratama[2], Stefan Sleijfer[2], John A. Foekens[1], and John W.M. Martens[1]

Abstract

Purpose: Molecular characterization of circulating tumor cells (CTC) holds great promise. Unfortunately, routinely isolated CTC fractions currently still contain contaminating leukocytes, which makes CTC-specific molecular characterization extremely challenging. In this study, we determined mRNA and microRNA (miRNA) expression of potentially CTC-specific genes that are considered to be clinically relevant in breast cancer.

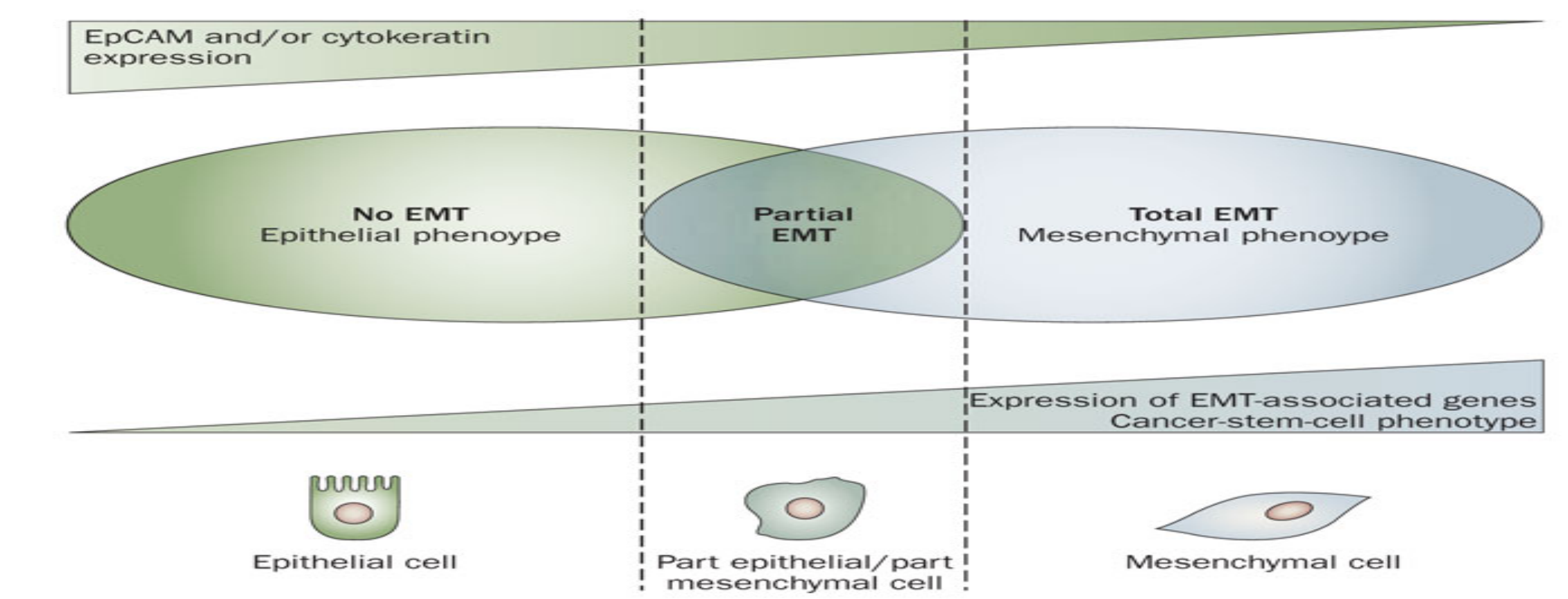
Experimental Design: CTCs were isolated with the epithelial cell adhesion molecule–based CellSearch Profile Kit. Selected genes were measured by real-time reverse transcriptase PCR in CTCs of 50 metastatic breast cancer patients collected before starting first-line systemic therapy in blood from 53 healthy blood donors (HBD) and in primary tumors of 8 of the patients. The molecular profiles were associated with CTC counts and clinical parameters and compared with the profiles generated from the corresponding primary tumors.

Results: We identified 55 mRNAs and 10 miRNAs more abundantly expressed in samples from 32 patients with at least 5 CTCs in 7.5 mL of blood compared with samples from 9 patients without detectable CTCs and HBDs. Clustering analysis resulted in 4 different patient clusters characterized by 5 distinct gene clusters. Twice the number of patients from cluster 2 to 4 had developed both visceral and nonvisceral metastases. Comparing transcript levels in CTCs with those measured in corresponding primary tumors showed clinically relevant discrepancies in estrogen receptor and HER2 levels.

Conclusions: Our study shows that molecular profiling of low numbers of CTCs in a high background of leukocytes is feasible and shows promise for further studies on the clinical relevance of molecular characterization of CTCs. *Clin Cancer Res; 17(11); 3600–18. ©2011 AACR.*

CTC RICONOSCIUTE SULLA BASE DELL'ESPRESSIONE DI EPCAM:

E LE CELLULE IN *TRANSIZIONE EPITELIO-MESENCHIMALE?*



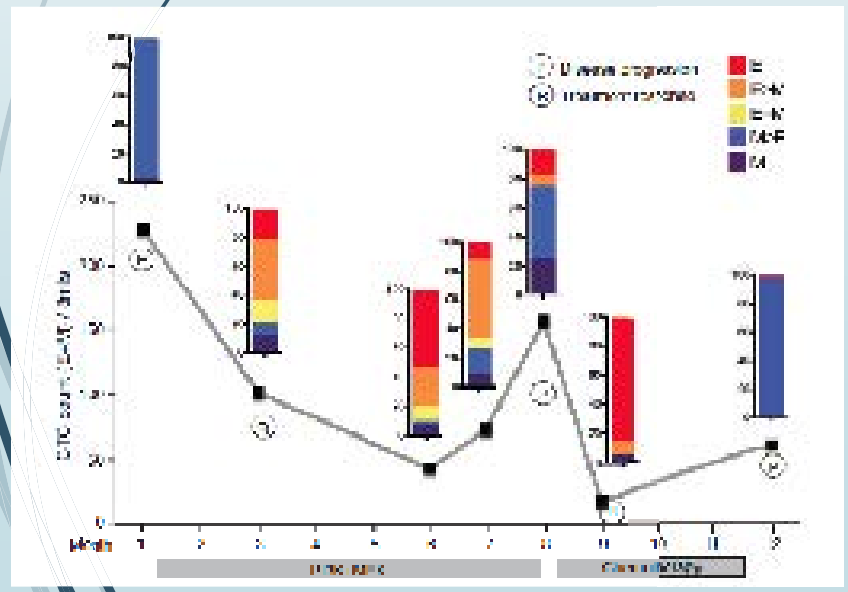
EPCAM-negative CTC?

Mego, M., Mani S. A. & Cristofanilli, M. (2010), *Nat. Rev. Clin. Oncol.*

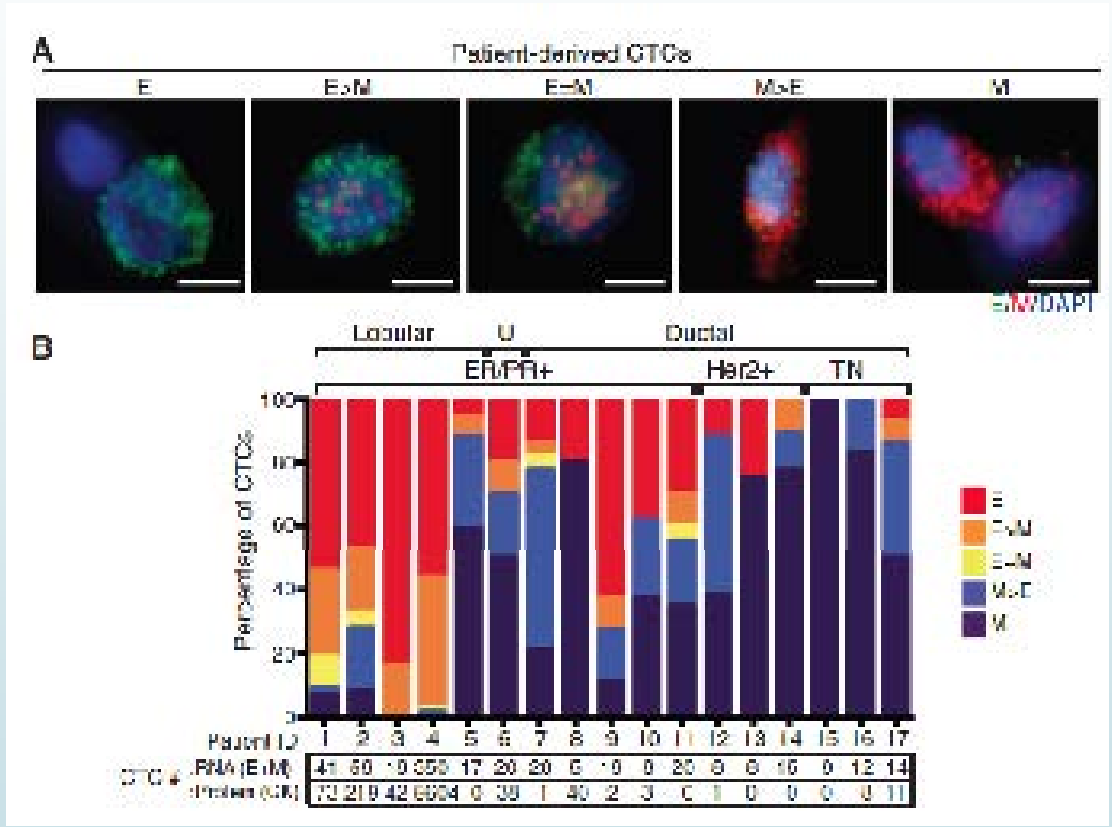
REPORTS

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

Min Yu,[1,4]* Aditya Bardia,[1,3]* Ben S. Wittner,[1] Shannon L. Stott,[1,2] Malgorzata E. Smas,[1] David T. Ting,[1] Steven J. Isakoff,[1,3] Jordan C. Ciciliano,[1] Marissa N. Wells,[1] Ajay M. Shah,[2] Kyle F. Concannon,[1] Maria C. Donaldson,[1] Lecia V. Sequist,[1,3] Elena Brachtel,[1,4] Dennis Sgroi,[1,4] Jose Baselga,[1,3] Sridhar Ramaswamy,[1,3] Mehmet Toner,[2,5] Daniel A. Haber,[1,3,6]† Shyamala Maheswaran[1,5]†



Science **339**, 580 (2013)



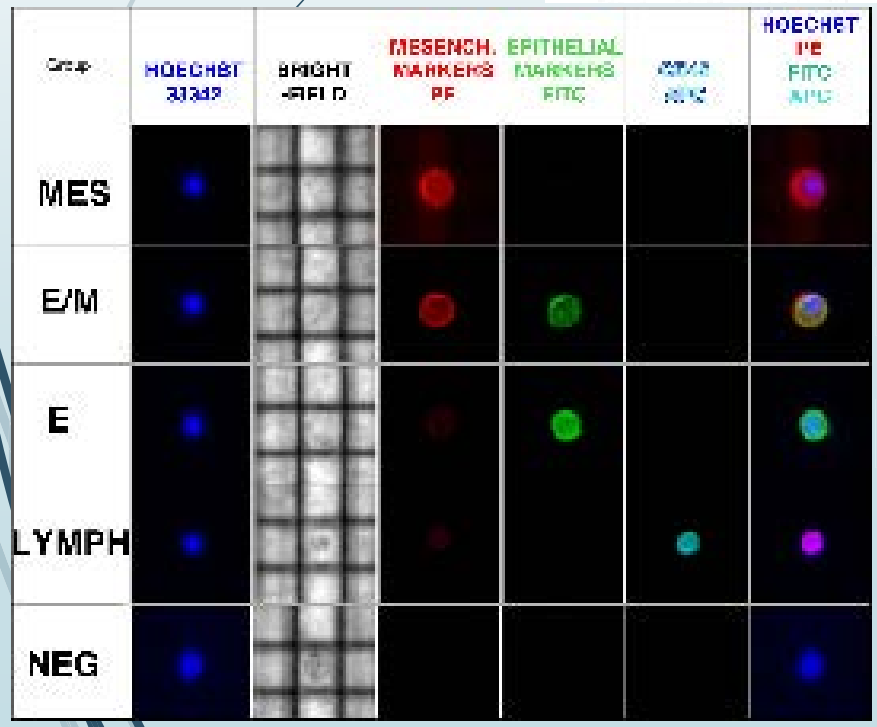
M. Bulfoni

Breast Cancer Research

RESEARCH ARTICLE Open Access

In patients with metastatic breast cancer the identification of circulating tumor cells in epithelial-to-mesenchymal transition is associated with a poor prognosis

Michela Bulfoni, Lorenzo Gerratana, Fabio Del Ben, Stefania Marzinotto, Marisa Sorrentino, Matteo Turetta, Giacinto Scoles, Barbara Toffoletto, Miriam Isola, Carlo Alberto Beltrami, Cara Di Loreto, Antonio Paolo Beltrami, Fabio Puglisi and Daniela Cesselli



→ MESENCHYMAL CELL: MES

→ EPITHELIAL CTC IN EMT: CTC-EMT

→ EPITHELIAL CTC: CTC-E

→ LYMPHOCYTES

→ NEG CELLS

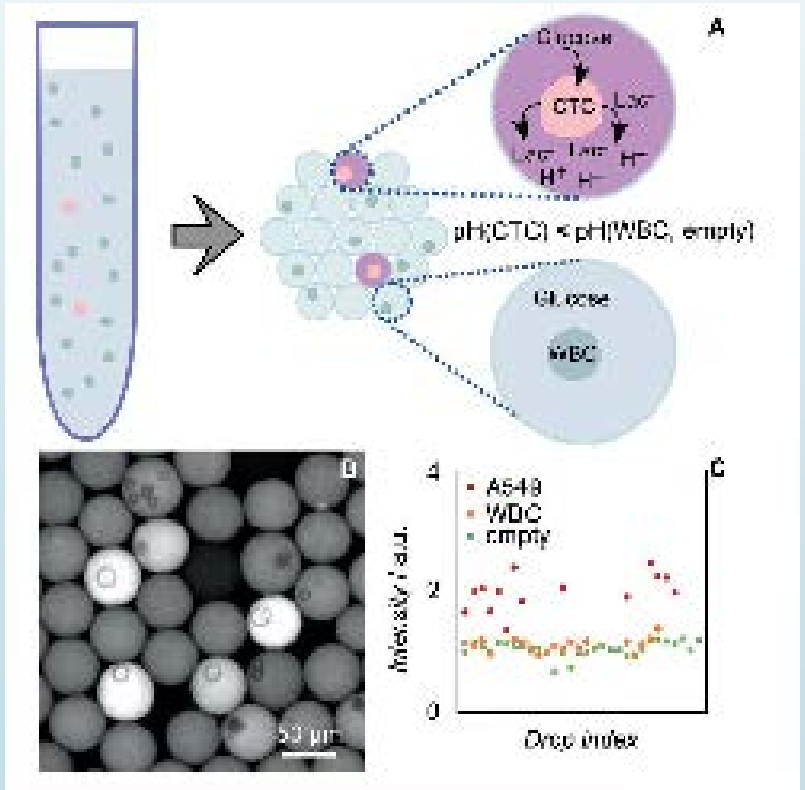
GDCh Communications Angewandte Chemie

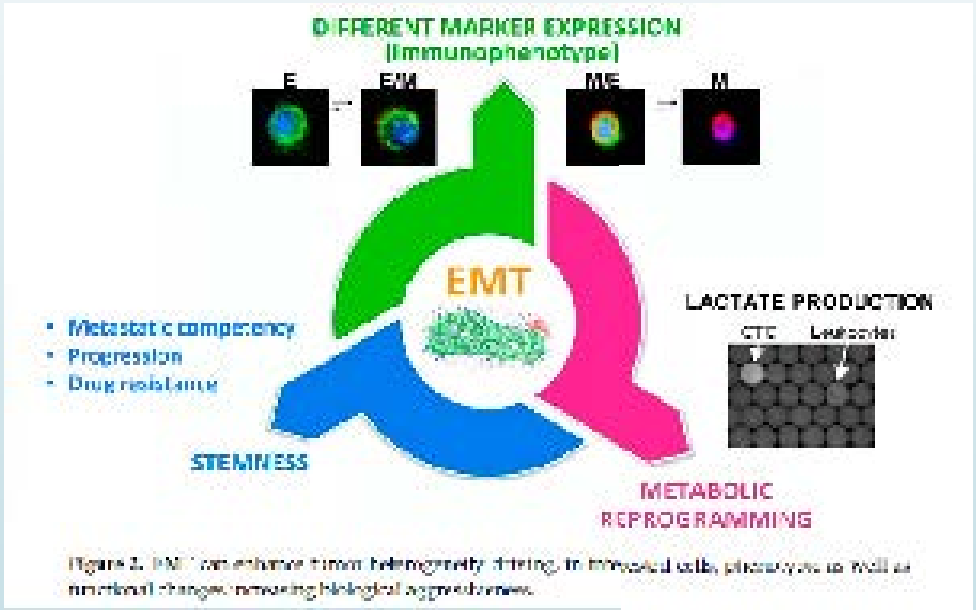
Microfluidics Hot Paper

International Edition: DOI: 10.1002/anie.201602328
German Edition: DOI: 10.1002/ange.201602328

A Method for Detecting Circulating Tumor Cells Based on the Measurement of Single-Cell Metabolism in Droplet-Based Microfluidics

*Fabio Del Ben', * Matteo Turetta', Giorgia Celetti, Agata Piruska, Michela Bulfoni, Daniela Cesselli, Wilhelm T. S. Huck,* and Giacinto Scoles**





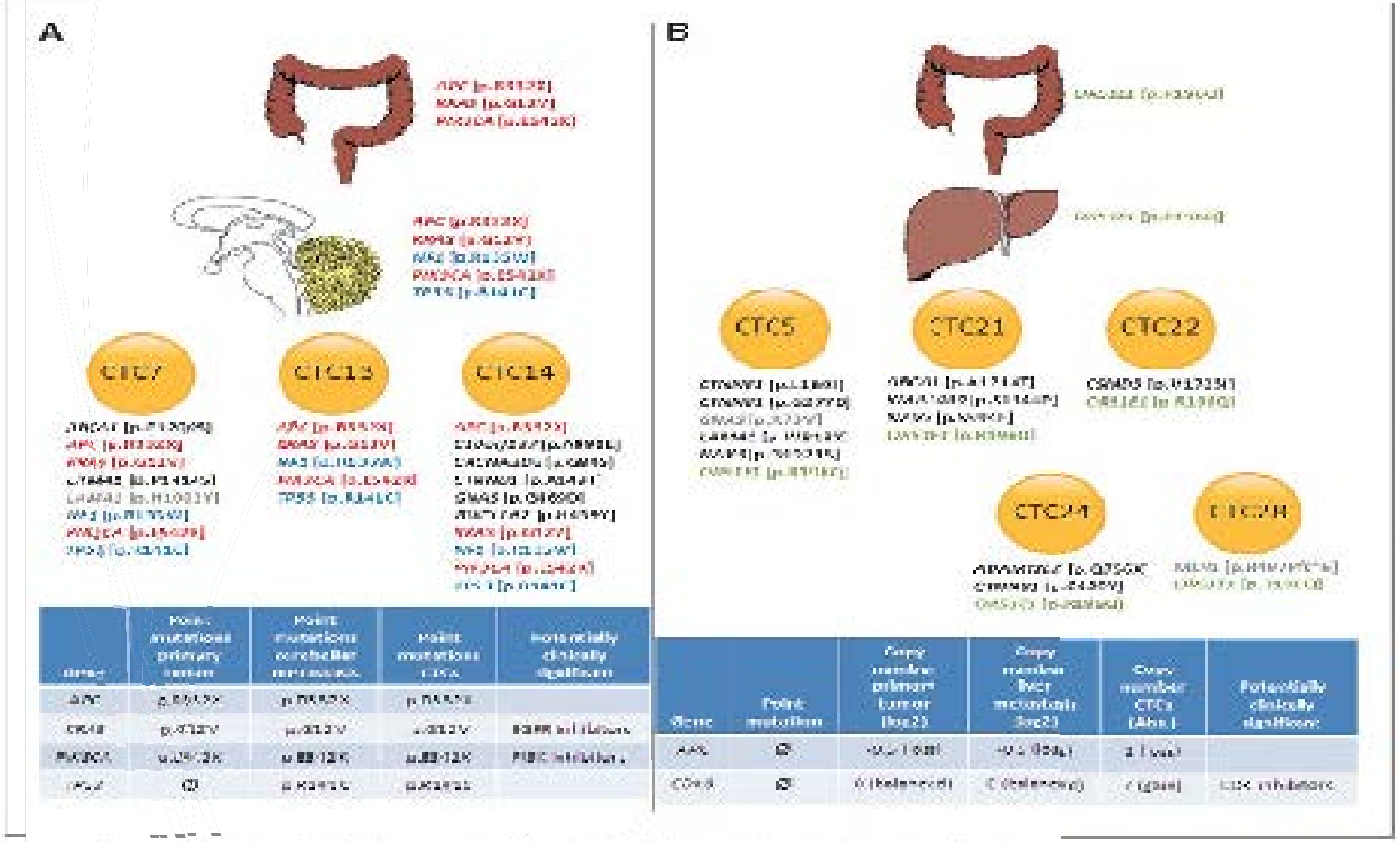
Bulfoni M et al.; Int. J. Mol. Sci. 2016, 17, 1775 x

Complex Tumor Genomes Inferred from Single Circulating Tumor Cells by Array-CGH and Next-Generation Sequencing

Ellen Heitzer, Martina Auer, Christin Gasch, et al.

Cancer Res 2013;73:2965-2975. Published OnlineFirst March 7, 2013.

same patient. This study paves the way to use CTCs as a liquid biopsy in patients with cancer, providing more effective options to monitor tumor genomes that are prone to change during progression, treatment, and relapse. *Cancer Res; 73(10); 2965–75. ©2013 AACR.*



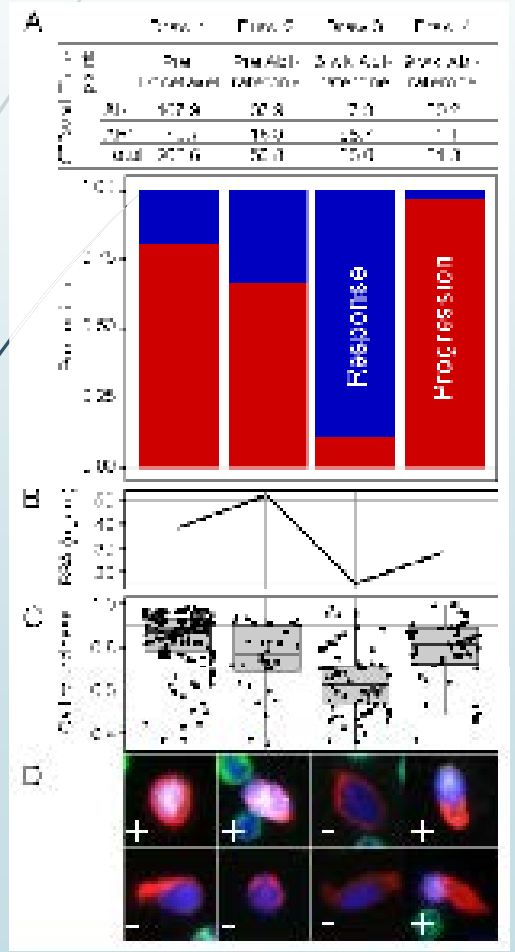
NATURE BIOTECHNOLOGY VOLUME 32 NUMBER 5 MAY 2014

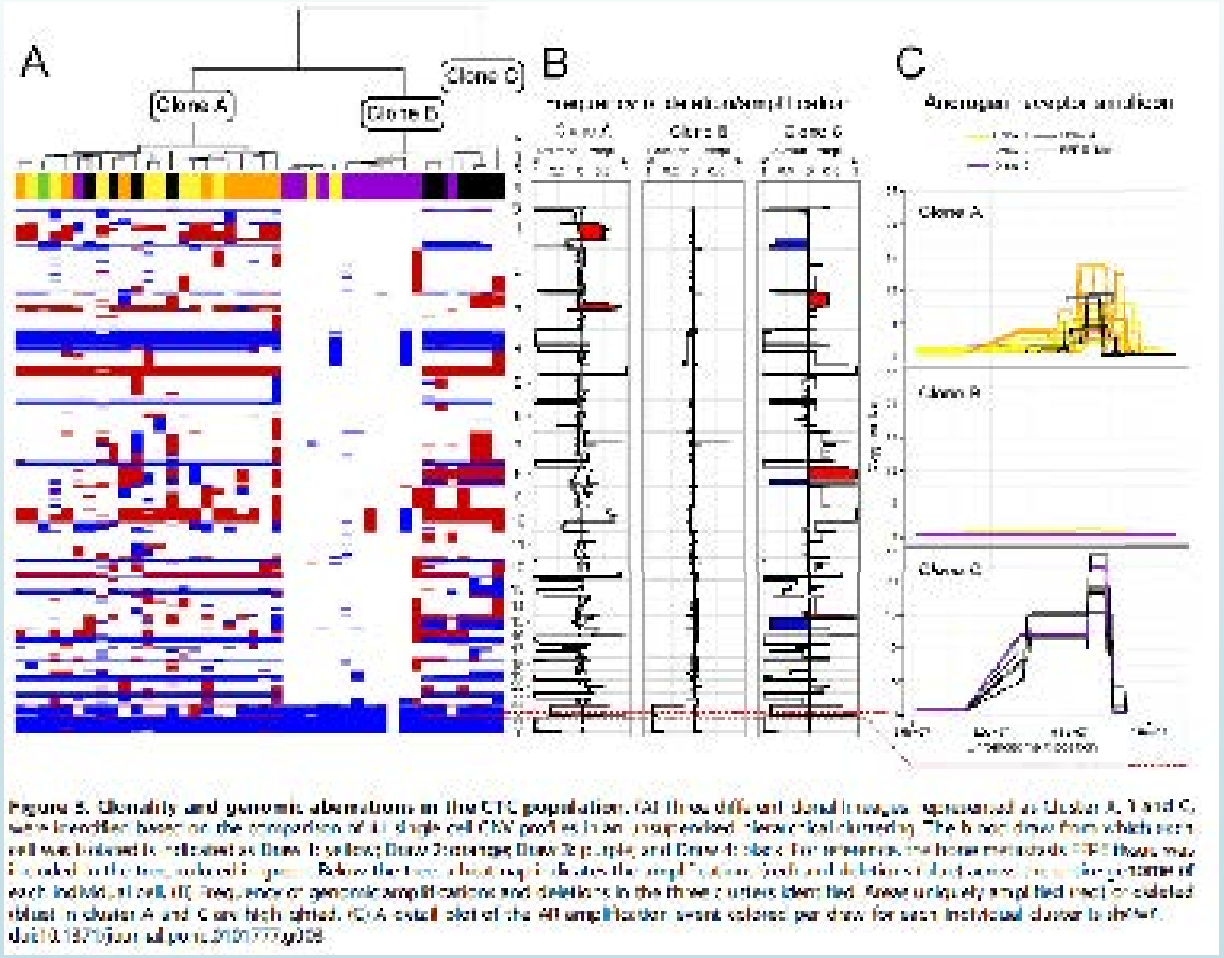
Whole-exome sequencing of circulating tumor cells provides a window into metastatic prostate cancer

OPEN ACCESS Freely available online August 2014 | Volume 9 | Issue 8 | PLOS ONE

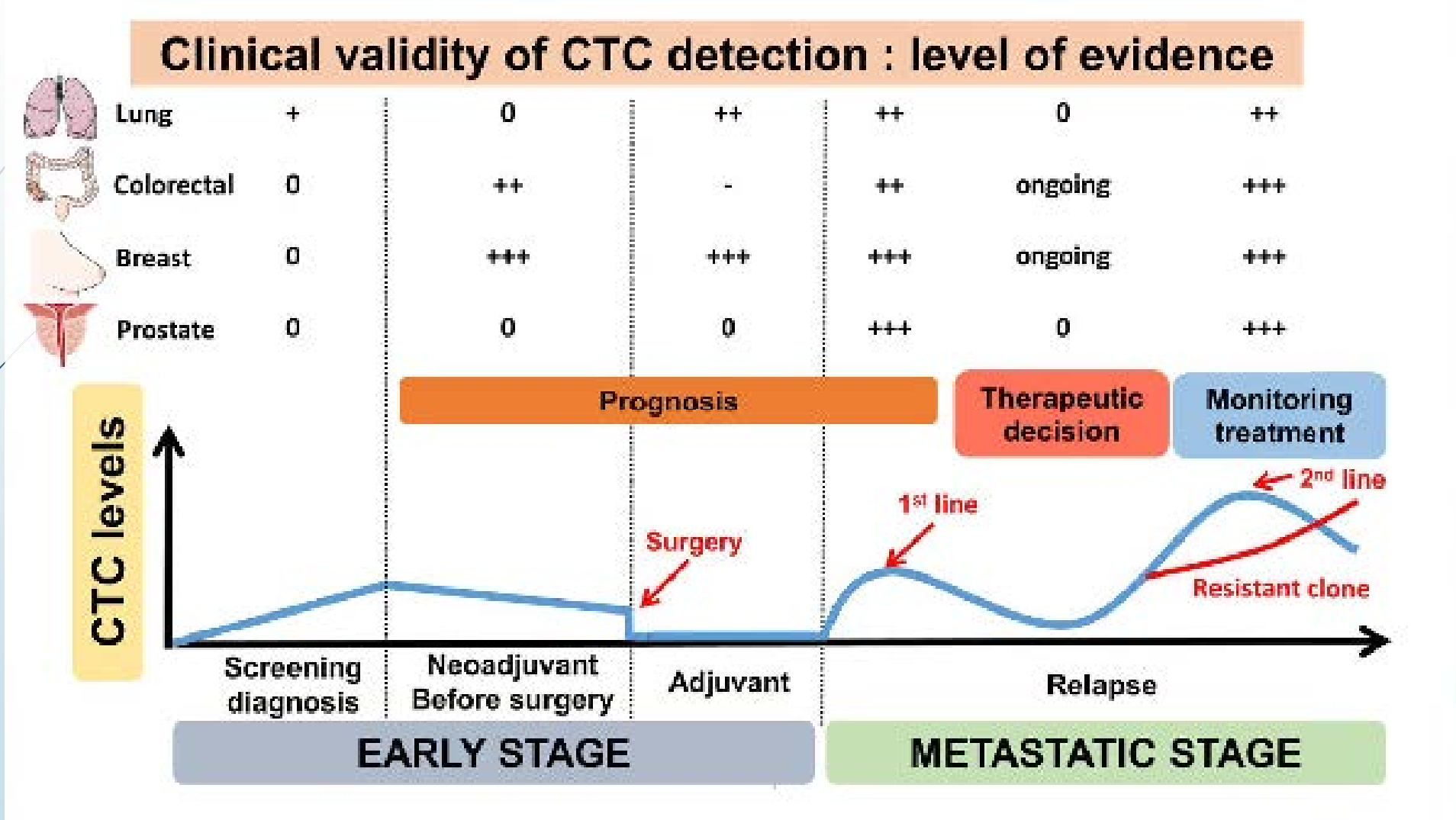
Rapid Phenotypic and Genomic Change in Response to Therapeutic Pressure in Prostate Cancer Inferred by High Content Analysis of Single Circulating Tumor Cells

Angel E. Dago[1,2], Asya Stepansky[2,3,4], Anders Carlsson[1], Madelyn Luttgen[1], Jude Kendall[2,3], Timour Baslan[2,5], Anand Kolatkar[2], Michael Wigler[6], Kelly Bethel[1,2], Mitchell E. Gross[8], James Hicks[2,7], Peter Kuhn[2,9]





Conclusioni



Int J Clin Oncol (2017) 22:421–430

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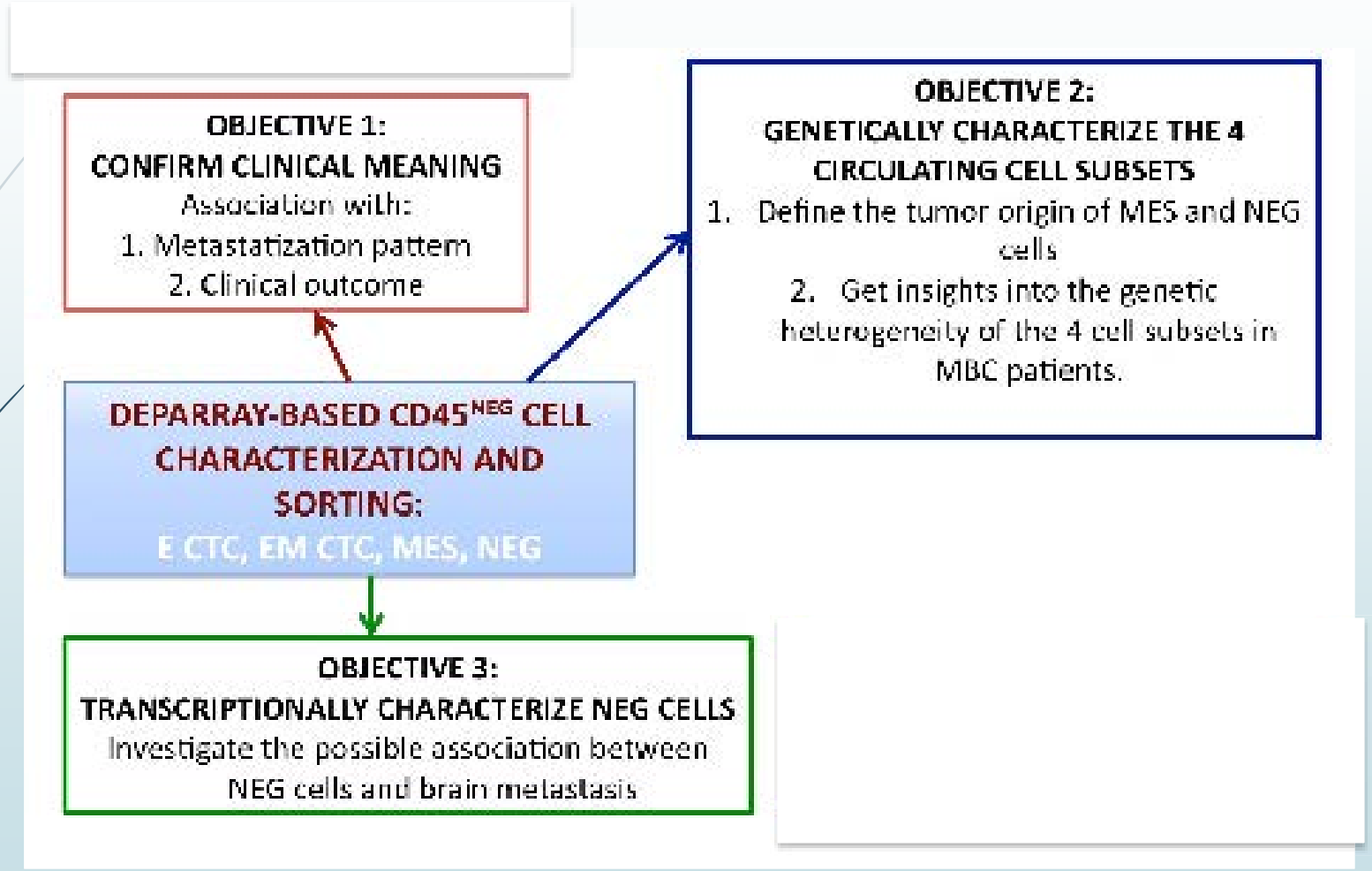
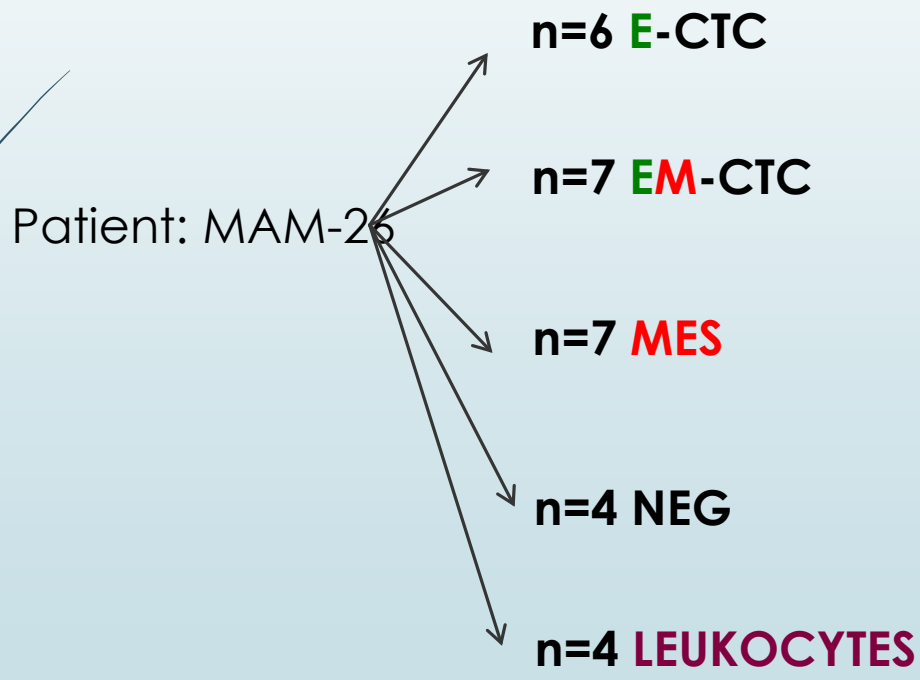
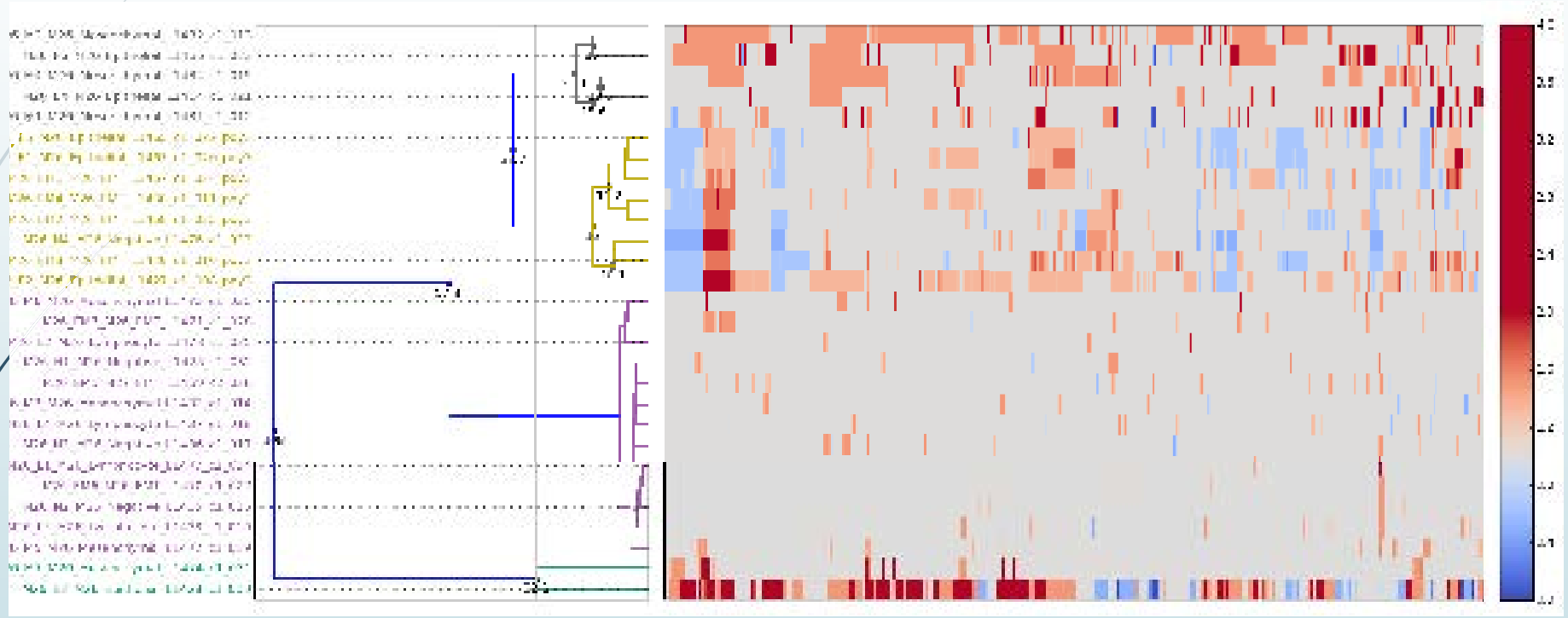

OBJECTIVE 1:
CONFIRM CLINICAL MEANING
Association with:
1. Metastatization pattern
2. Clinical outcome
OBJECTIVE 2:
GENETICALLY CHARACTERIZE THE 4 CIRCULATING CELL SUBSETS
1. Define the tumor origin of MES and NEG cells
2. Get insights into the genetic heterogeneity of the 4 cell subsets in MBC patients.
DEPARRAY-BASED CD45NEG CELL CHARACTERIZATION AND SORTING:
E CTC, EM CTC, MES, NEG
OBJECTIVE 3:
TRANSCRIPTIONALLY CHARACTERIZE NEG CELLS
Investigate the possible association between NEG cells and brain metastasis

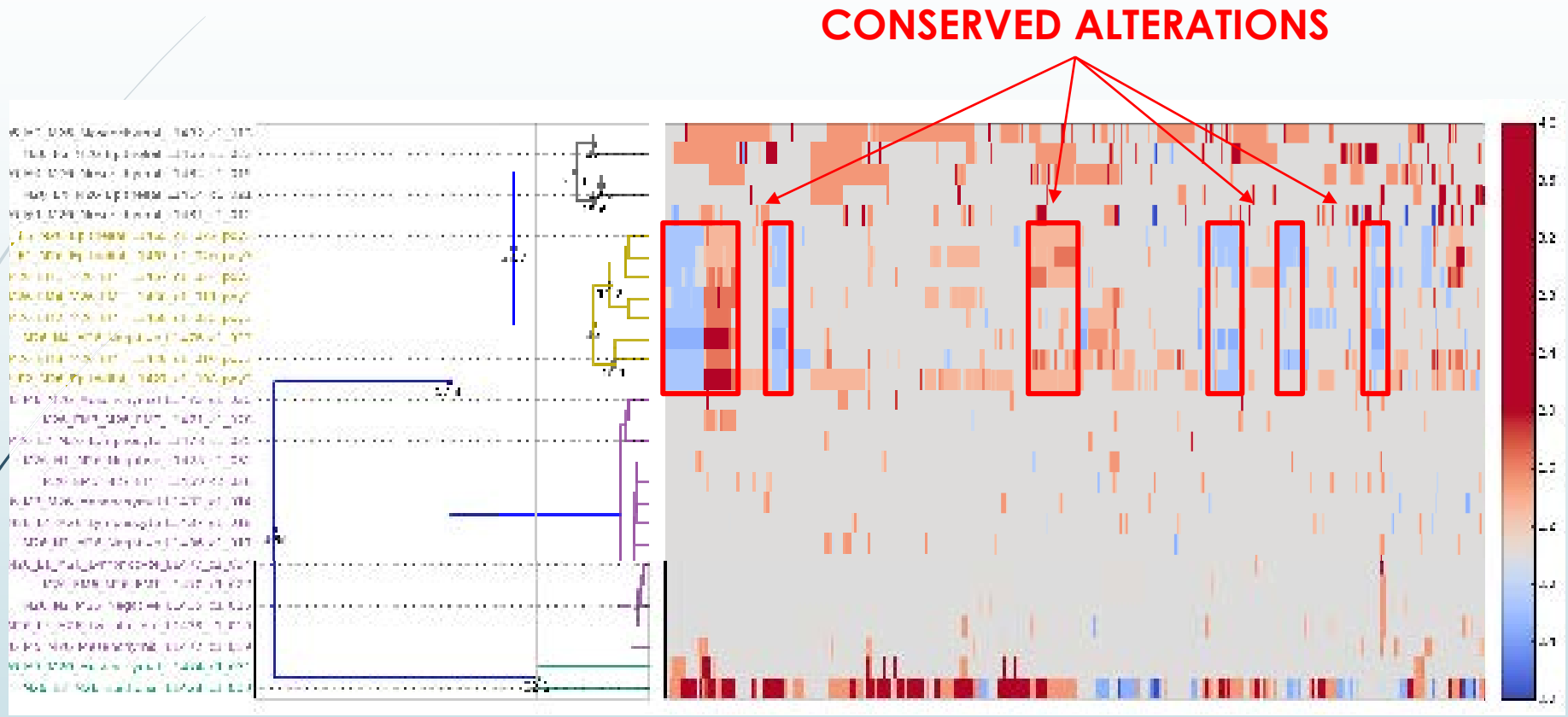
*Ampli*1™ LowPass
CNA analysis on single-CTCs obtained by DEPArray™ following enrichment by depletion



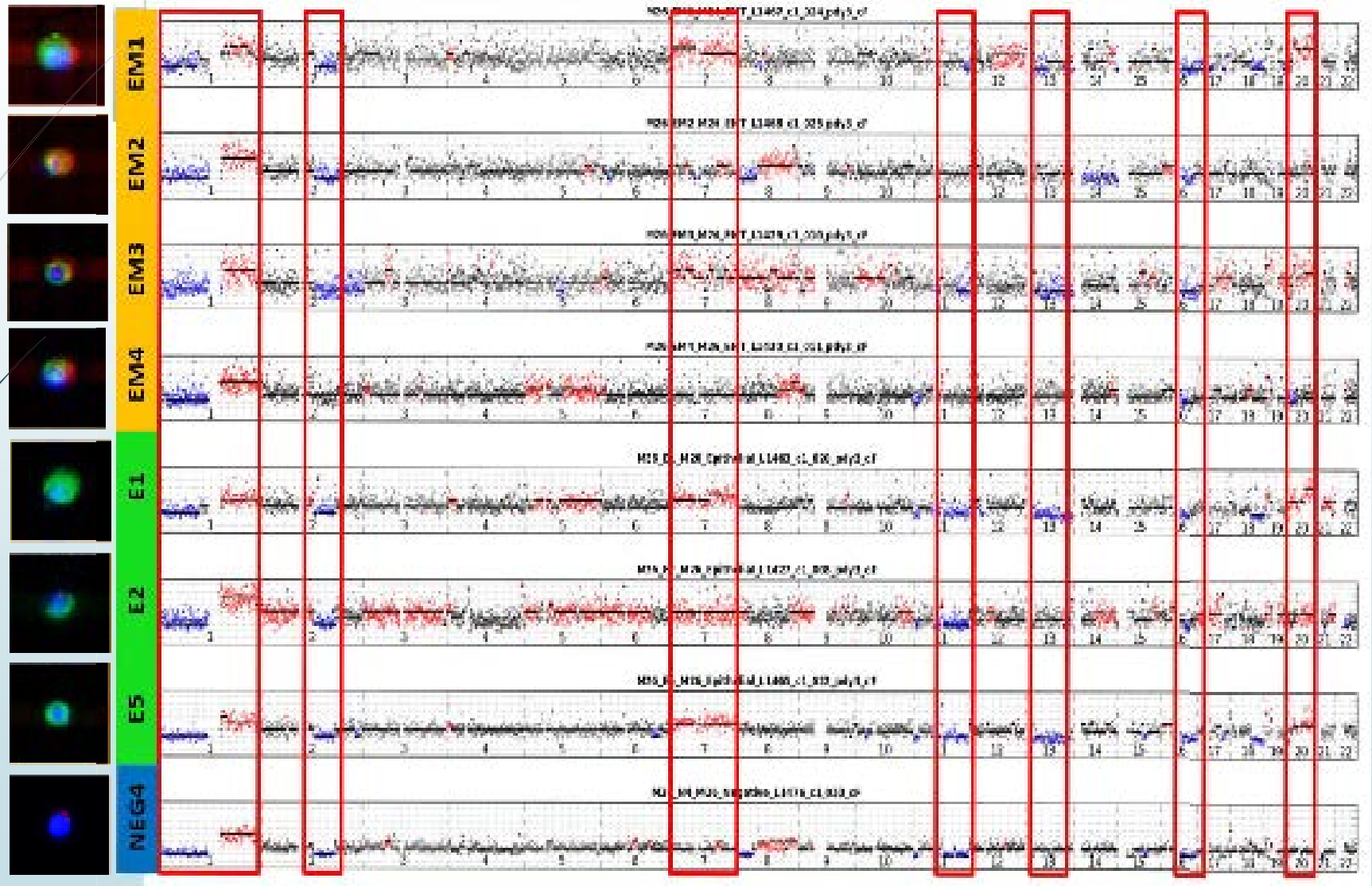
Clustering



Clustering



CNA profiles - Cluster



Conclusions

- Aberrations were found in E, and some EMT, M, and negative cells, confirming them as bona-fide CTCs
- Certain EMT, M and negative cells showed relatively flat profiles, possibly normal cells
- A clear cluster of profiles included representatives from E (n=3), EMT (n=4), and Negative (n=1) class.
- At least one other E CTC had a clearly distinct aberration profile