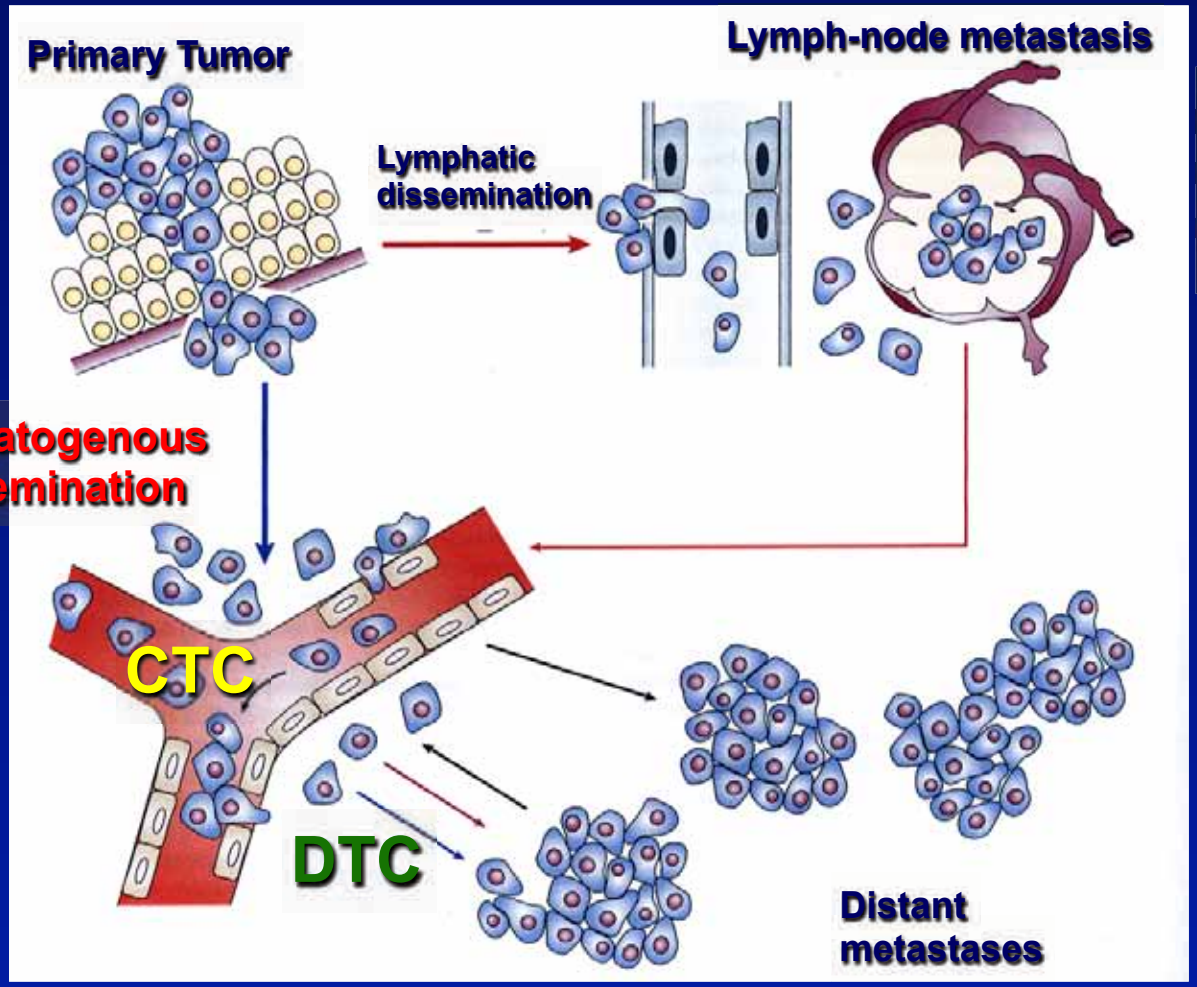
Universitätsklinikum Hamburg-Eppendorf

Institute for Tumor Biology

Professor Klaus Pantel

CTC-DTC Research: State of the Art & Perspectives

Tumor Cell Dissemination
Key Step in Tumor Progression



Izbicki/Pantel *et al.*, NEJM, 1997
De Boer *et al.*, NEJM, 2009

Universitätsklinikum Hamburg-Eppendorf

Pantel & Brakenhoff, Nat Rev Cancer, 2004; Pantel et al., Nat Rev Cancer, 2008

Detection of CTC in the peripheral blood

September 2013:

>400 registered clinical trials with CTC as biomarkers

> 13,000 publications in PubMed

Advantages over DTC detection:

- Less invasive than BM sampling
- Pool of DTC from multiple distant sites

ISMRC 2013, Paris: Scientific Sessions

- **Metastasis biology**
- **Novel CTC assays**
- CTC in clinical studies
- Molecular characterization of CTC
- CTC and other circulating markers

Metastasis Biology

Cancer Cell
Perspective

Tumor Cell Dissemination: Emerging Biological Insights from Animal Models and Cancer Patients

Yibin Kang[1,*] and Klaus Pantel[2,*]
[1]Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA
[2]Department of Tumour Biology, Center of Experimental Medicine, University Cancer Center Hamburg, University Medical Centre Hamburg-Eppendorf, Martinistr. 52, D-20246 Hamburg, Germany
*Correspondence: ykang@princeton.edu (Y.K.), pantel@uke.de (K.P.)
http://dx.doi.org/10.1016/j.ccr.2013.04.017

Tumor cell dissemination and cancer dormancy

(Uhr & Pantel, PNAS 2011)

Experimental findings:

- **Reseeding of the primary tumor:**

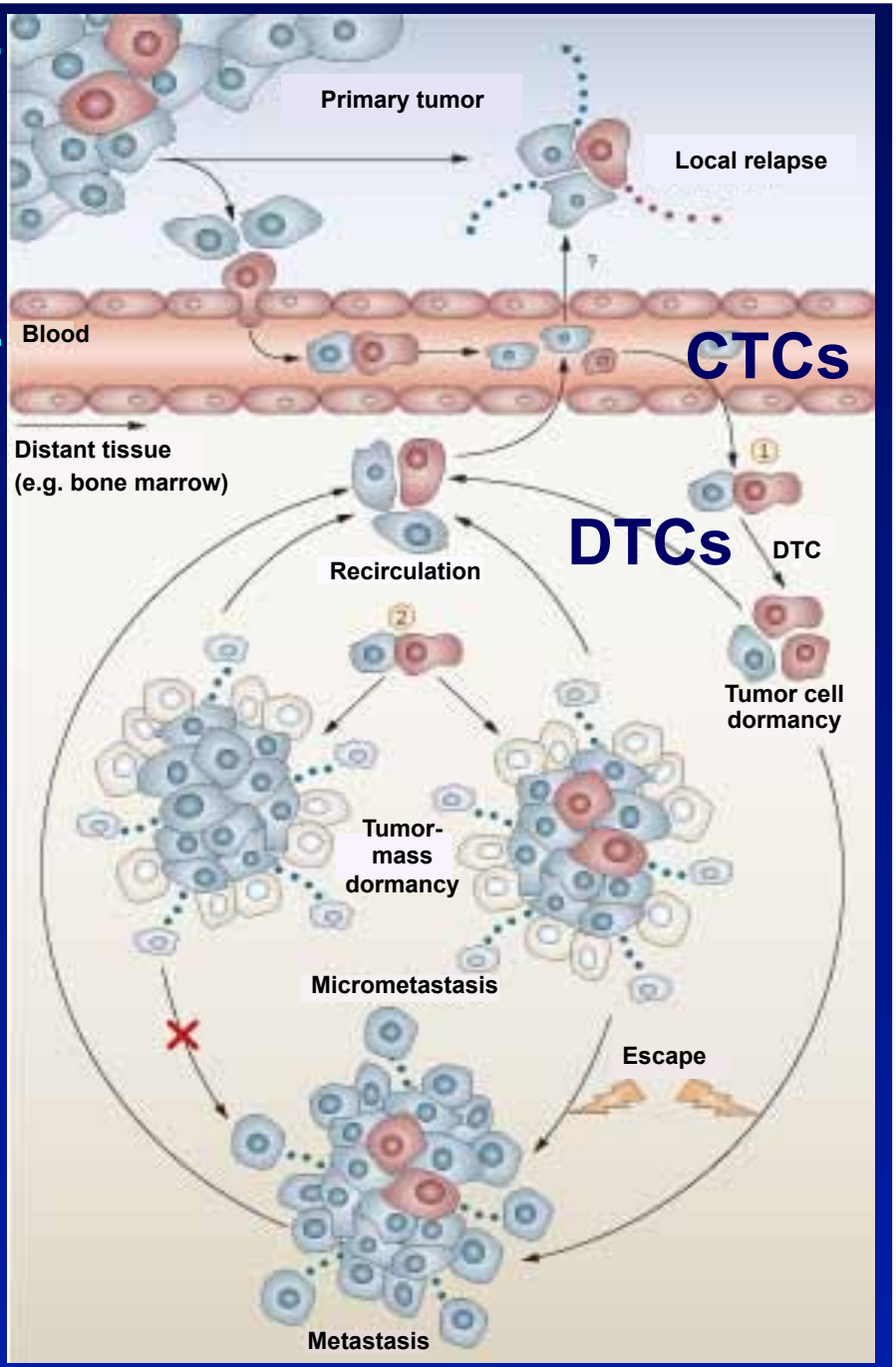
Recirculation of breast cancer cells from the bone marrow to the primary site

(J. Massague's group, Kim et al, Cell 2009)

- **Escape from dormancy:**

VCAM1 promotes osteoclast differentiation & activation & attracts osteoclast progenitors

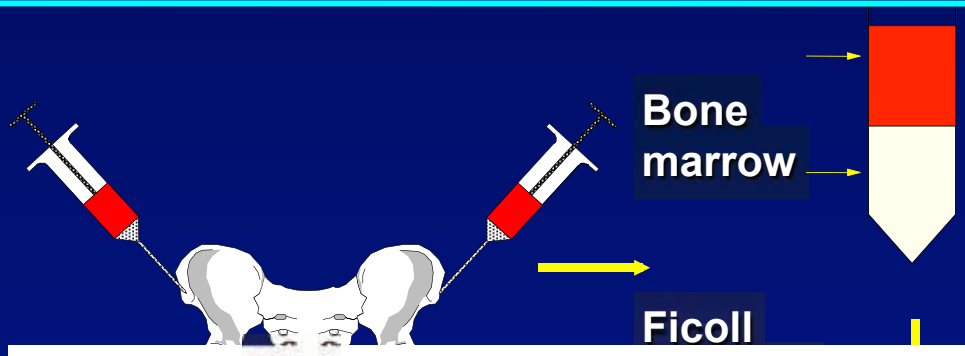
(Y. Kang's group, Lu/Pantel et al Cancer Cell 2011)



Cancer micrometastases

Klaus Pantel, Catherine Alix-Panabières and Sabine Riethdorf

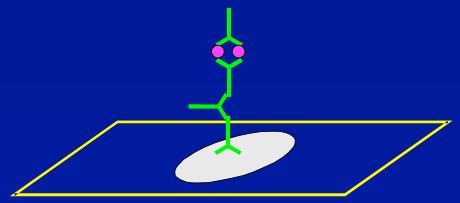
Detection of DTC in bone marrow



Breast Cancer: 199/552 (36%)
(Braun, Pantel *et al.* NEJM, 2000 & 2005)
Prostate Cancer: 86/193 (44.6%)
(Koellermann/Pantel *et al.* JCO 2008)
Nonmalignant disease: 2/191 (1%)

- DTC detection correlates with metastatic AND locoregional relapse
- Most DTC are Ki67- and have CD44+/CD24- phenotype
- DTC detection might be useful for stratification of bone-directed anti-cancer therapies (e.g., bisphosphonates, RANKL Abs)
- Bisphosponate treatment reduces DTC counts and prevents metastatic & locoregional relapse

Immunocytochemistry:
Cytokeratin staining
with mAB A45-B/B3



2 x 10^6 MNC per patient

Universitätsklinikum Hamburg-Eppendorf

Cancer Dormancy: Research questions

- Do all cancer patients have dormant tumor cells?
- Can host factors induce or break dormancy? Stress? Inflammation?
- Are there preferred reservoirs of dormant cells (e.g., bone marrow) ?
- Does the immune system play a role in dormancy?
- What is the effect of current therapies on dormant cells or dormancy?
- What signaling pathways or events reactivate dormant cells?
- Do dormant cells have properties of cancer stem cells?
- How does genetic background affect dormancy?

Uhr & Pantel PNAS 2011; Kang & Pantel, Cancer Cell 2013

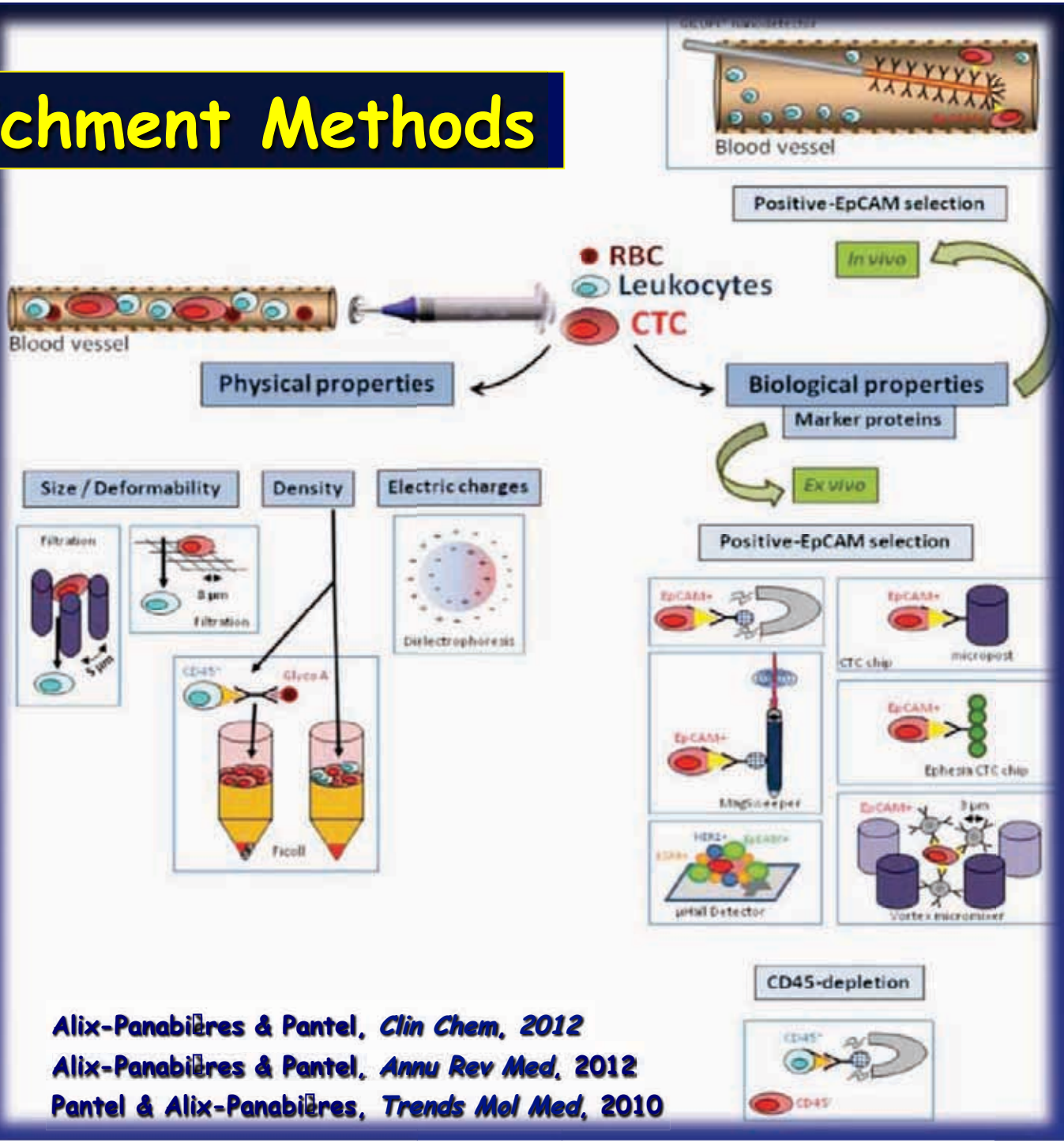
Novel CTC Assays

CTC Enrichment Methods

2013: > 50 different CTC assays !

The technical challenge:

Finding one tumor cell in 10^6 – 10^8 normal blood cells



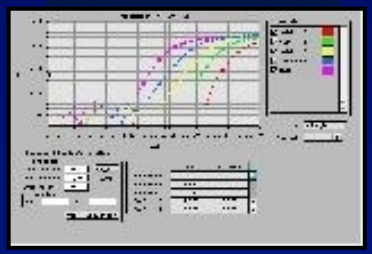
Alix-Panabières & Pantel, *Clin Chem*, 2012

Alix-Panabières & Pantel, *Annu Rev Med*, 2012

Pantel & Alix-Panabières, *Trends Mol Med*, 2010

CTC Identification Methods

Real-time RT-PCR

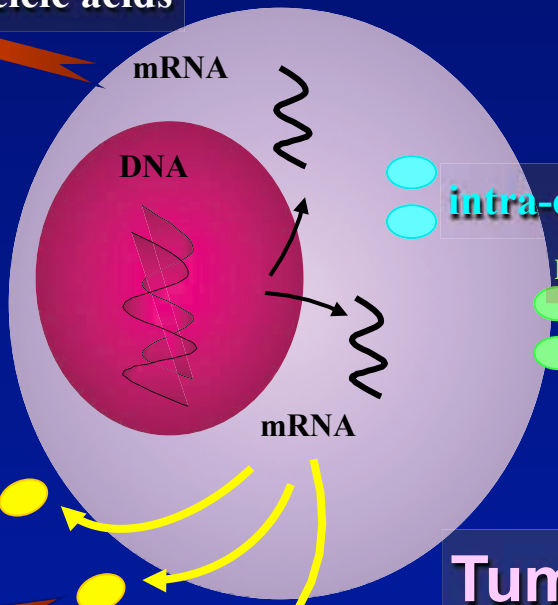


Cytokeratins as standard CTC markers

BUT differential expression of individual CKs

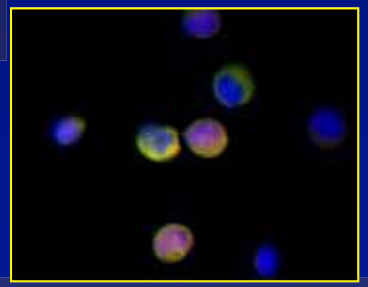
(Joosse/Pantel *et al., Clin Cancer Res* 2012)

nucleic acids



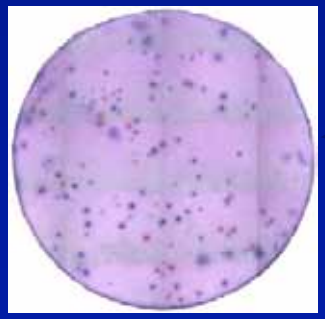
intra-cytoplasmic proteins

membrane proteins



Immunocytochemistry

Tumor cell



EPISPOT assay

secreted proteins by VIABLE cells

Alix-Panabières *et al.*, Clin Cancer Res, 2008

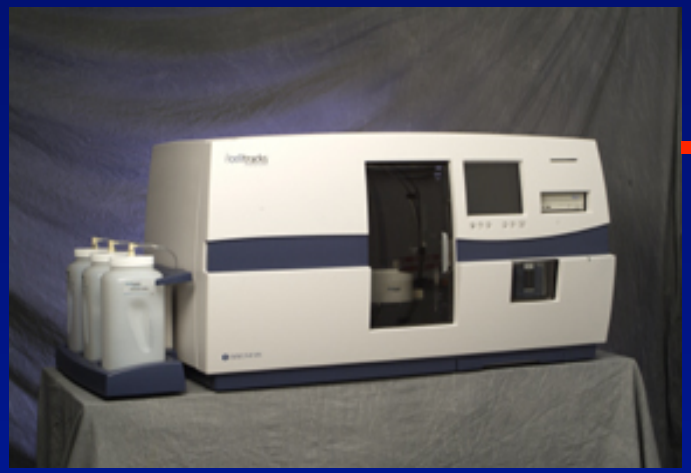
Design of robust automated systems for reproducible CTC detection

CellSearch™ System (FDA-cleared)

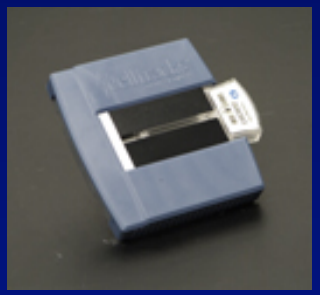
cellsave Preservative Tube

CellSearch™
Epithelial Cell Kit

celltracks™ autoprep system

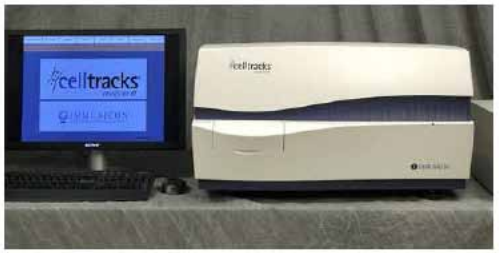


MagNest™



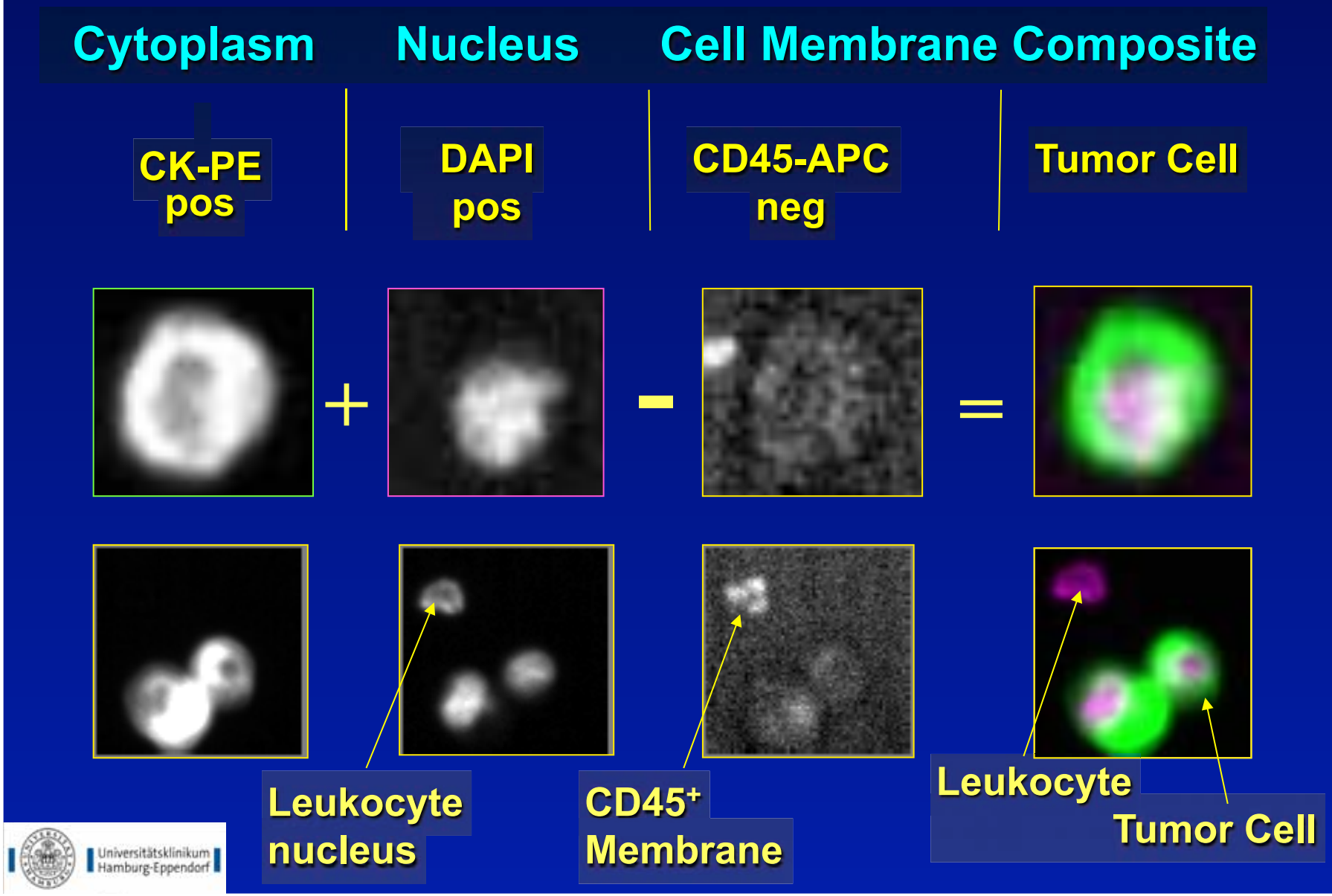
Enrichment of CTC with anti-EpCAM ferro fluids

Cristofanilli et al., NEJM, 2004
Riethdorf et al., CCR, 2007 & 2010
DeBono et al, CCR, 2008
Cohen et al, JCO, 2008
Krebs et al, JCO, 2012



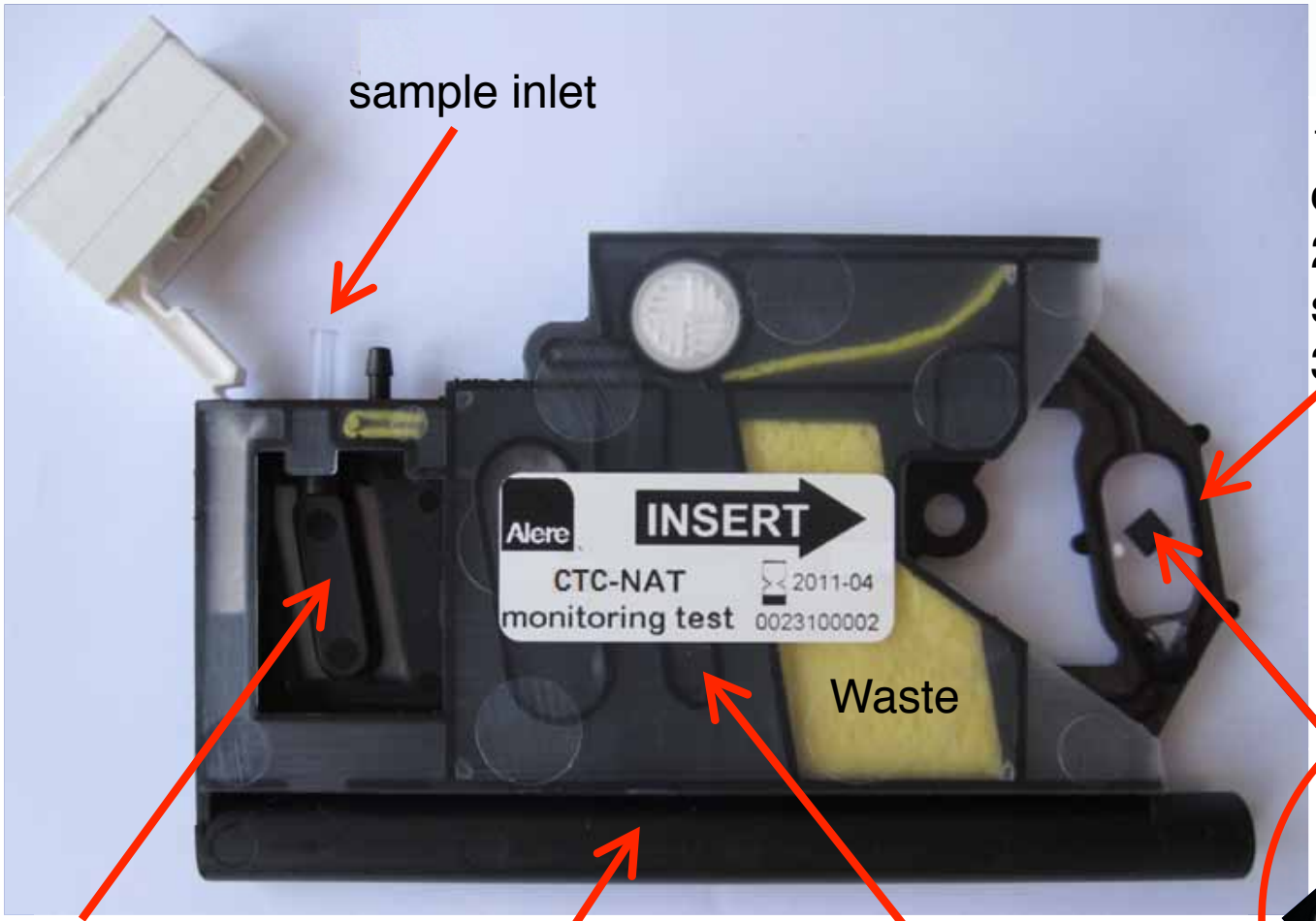
CellTracks® Analyzer II
w/ Linux operating system

CellSearch® System: Images of Tumor Cells



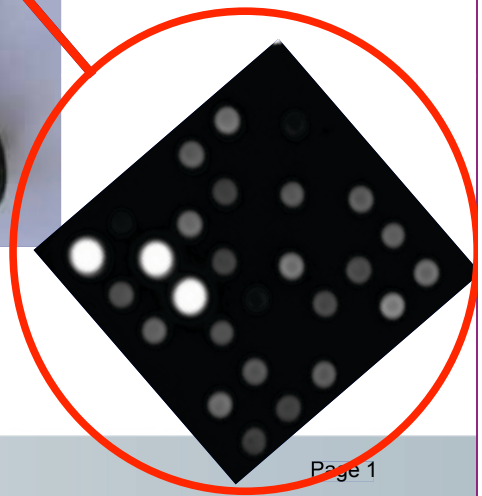
Universitätsklinikum Hamburg-Eppendorf

Automated multiplex q-RT-PCR: Lab-in-a-cartridge



Reaction chamber:
1. mRNA-capturing
2. c-DNA synthesis
3. PCR

Array:
Signal monitoring of real time PCR



Lysis chamber:
binding of Biotin-Oligos to mRNA

Processing Fluid

dry reagents
RT, TAQ, Primer, Reporter

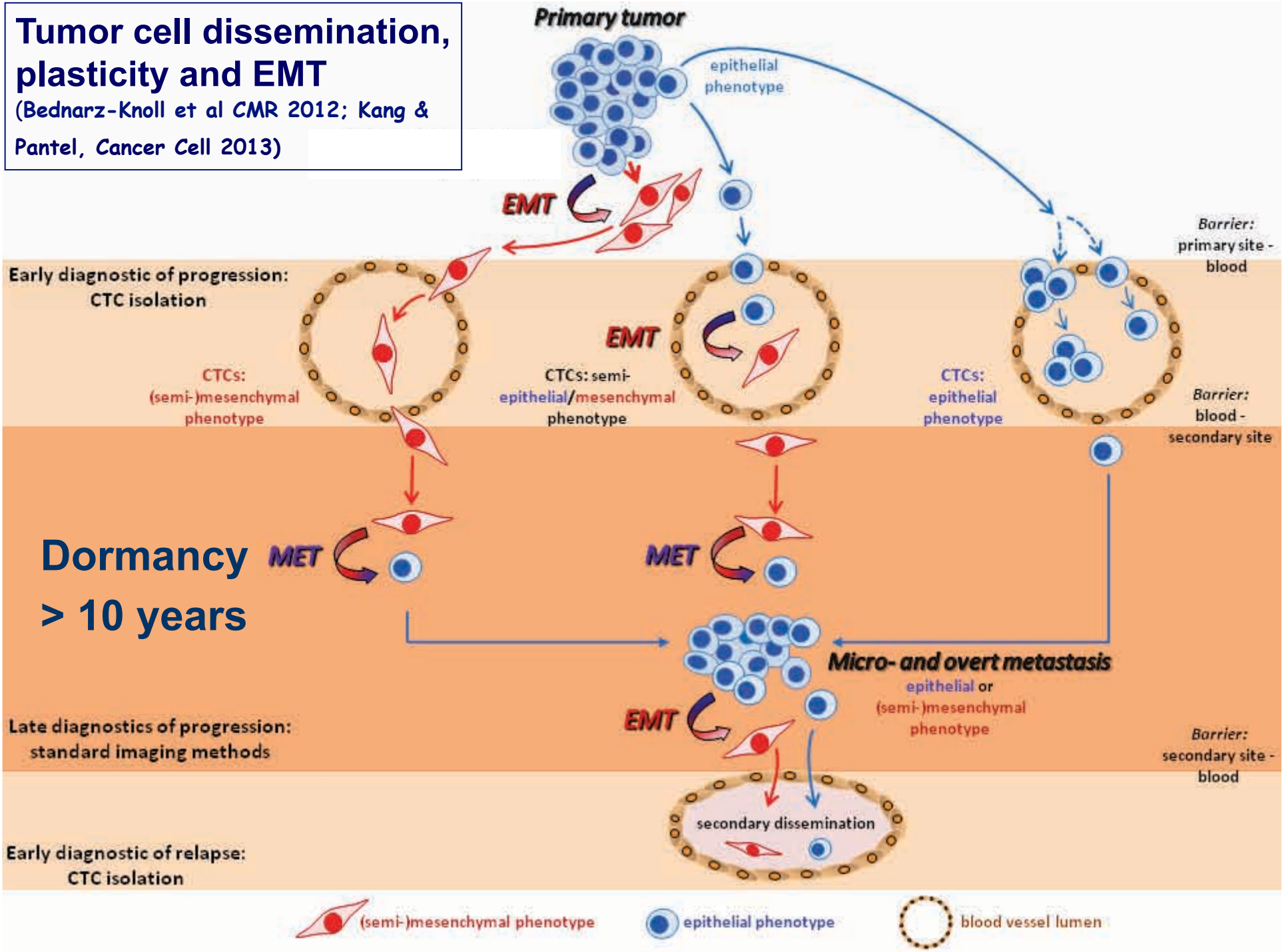
Challenge of CTC detection:

Epithelial-Mesenchymal Transition (EMT)

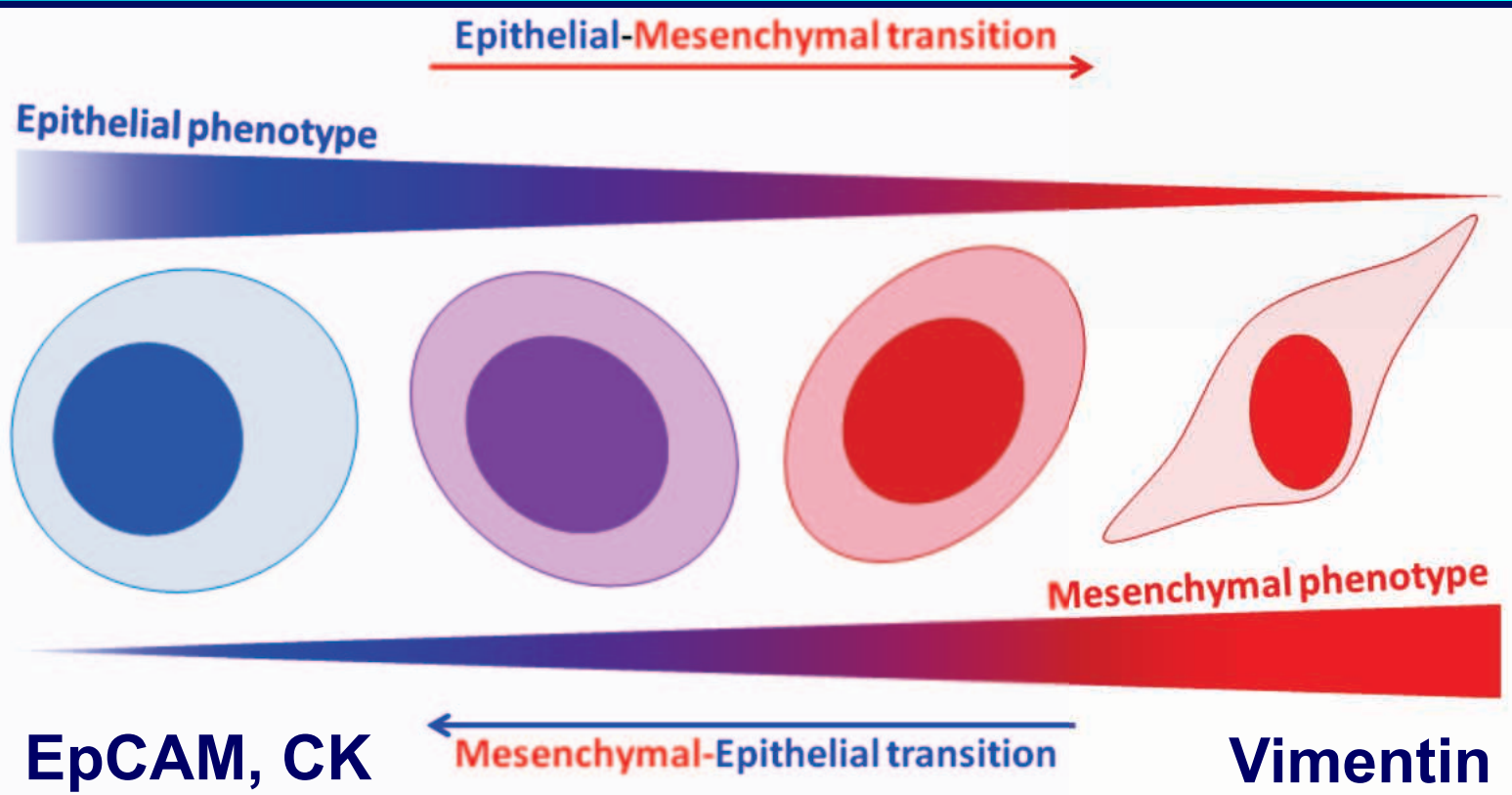
of carcinoma cells

Tumor cell dissemination, plasticity and EMT

(Bednarz-Knoll et al CMR 2012; Kang & Pantel, Cancer Cell 2013)



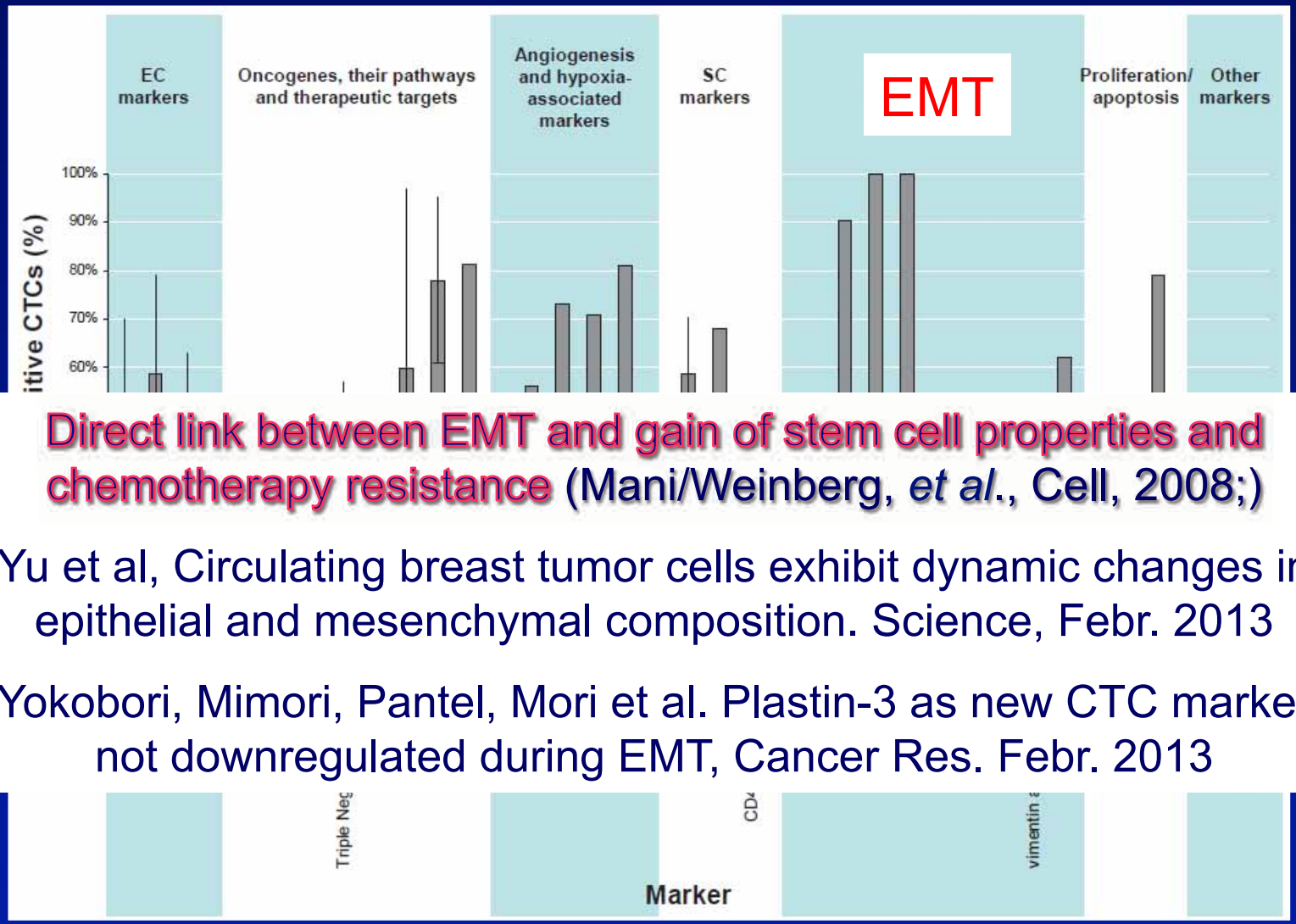
Epithelial-Mesenchymal Plasticity of CTC



Epithelial phenotype	Epithelial phenotype with minor mesenchymal features	Semi-mesenchymal phenotype	Mesenchymal phenotype
Epithelial markers strongly expressed	Epithelial markers moderately expressed	Epithelial markers weakly expressed	No epithelial markers
No mesenchymal markers	Mesenchymal markers weakly expressed	Mesenchymal markers moderately expressed	Mesenchymal markers strongly expressed
Detection by standard CTC technology	Detection by standard CTC technology	Limited detection by standard CTC technology	No detection by standard CTC technology

Bednarz-Knoll, Alix-Panabi□res & Pantel *Cancer & Met Rev* 2012

Expression profile of CTCs in breast cancer



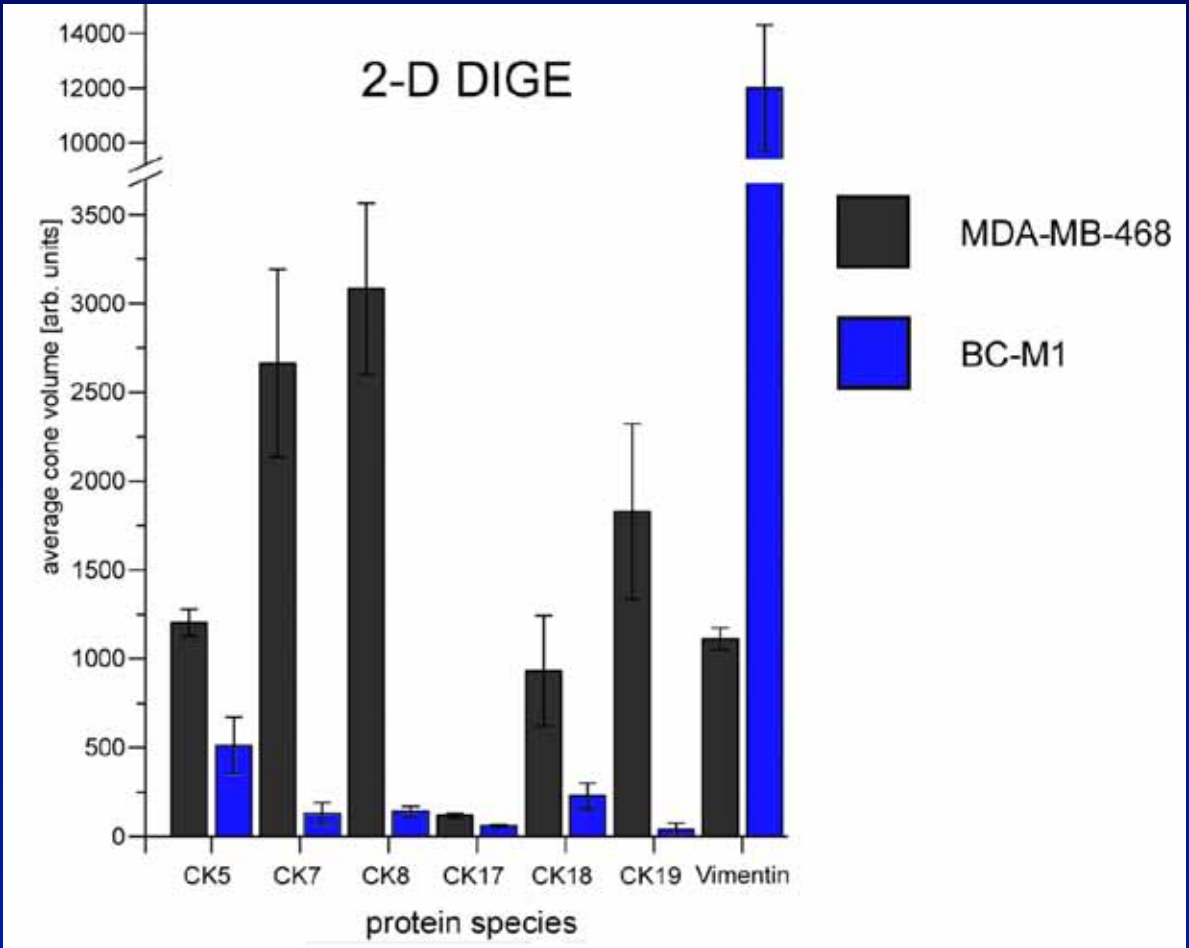
Direct link between EMT and gain of stem cell properties and chemotherapy resistance (Mani/Weinberg, *et al*., Cell, 2008;)

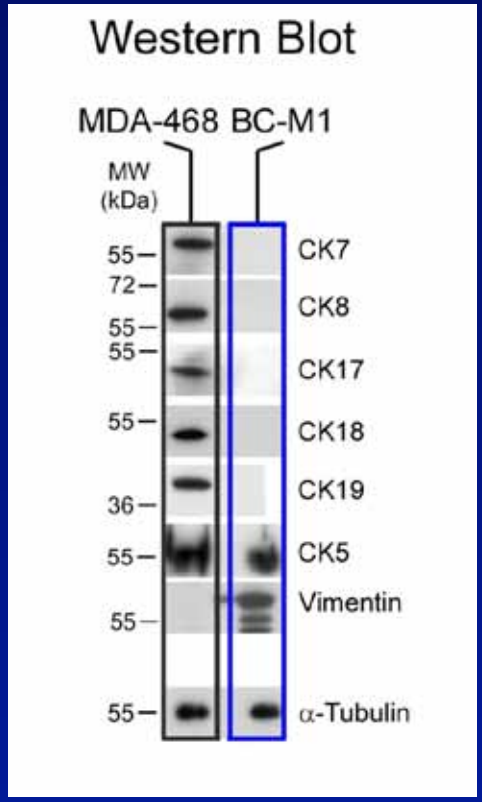
Yu et al, Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. Science, Febr. 2013

Yokobori, Mimori, Pantel, Mori et al. Plastin-3 as new CTC marker not downregulated during EMT, Cancer Res. Febr. 2013

Bednarz-Knoll, Alix-Panabieres & Pantel, 2011, Breast Cancer Res, 13: 282-293

Epithelial-Mesenchymal Transition in DTC line BC-M1





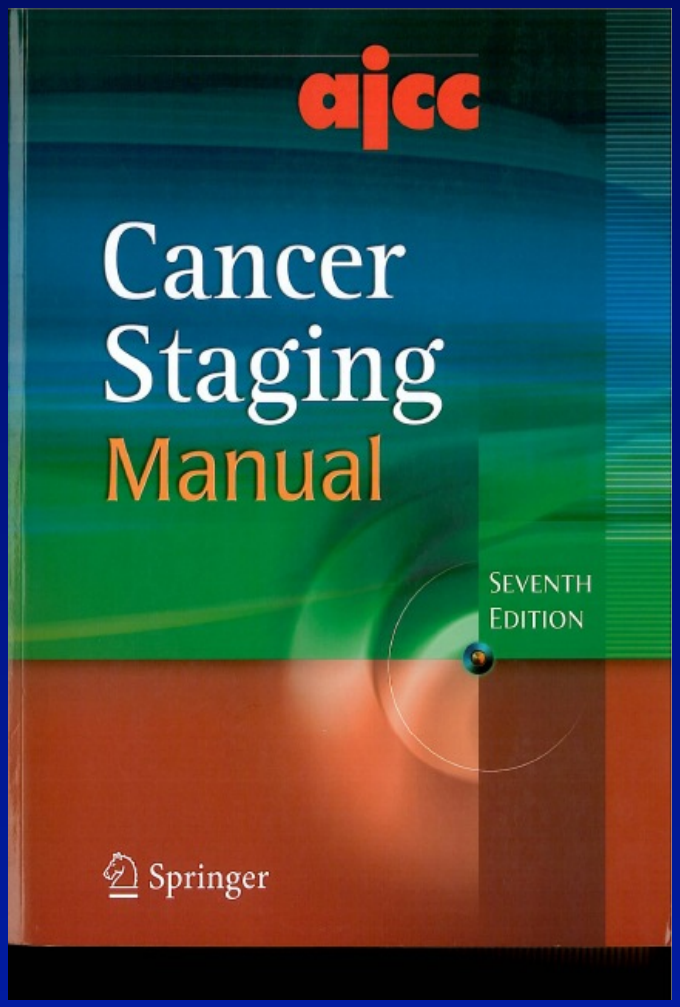
Universitätsklinikum Hamburg-Eppendorf

Bartkowiak *et al. J Prot Res*, 2009

Willipinski-Stapelfeldt *et al. Clin Cancer Res*, 2005

CTC in Clinical Studies

TNM 2010: CTC in new cM0(i+) Classification

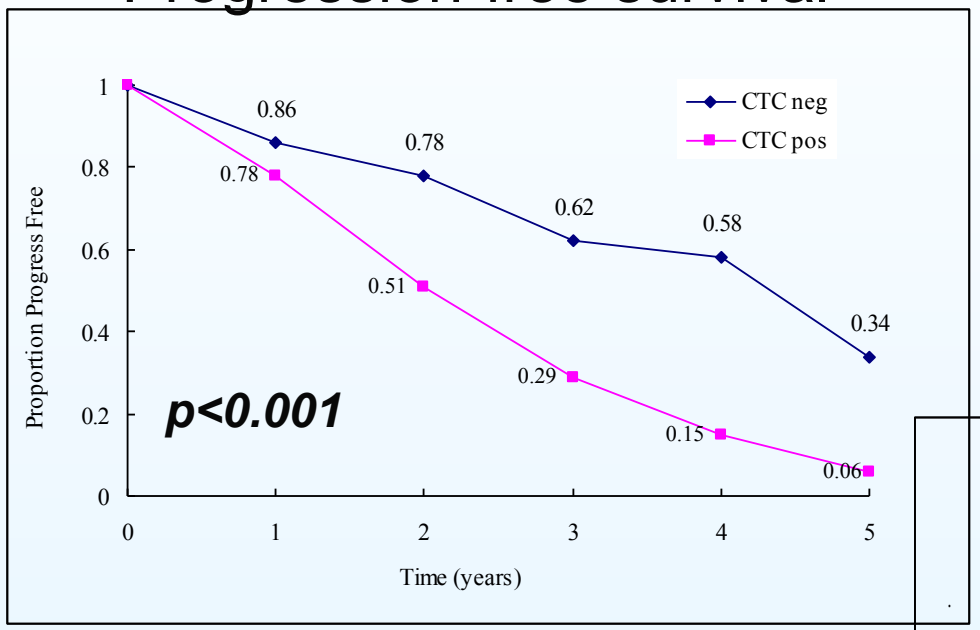


Distant Metastases (M)

M0	No clinical or radiographic evidence of distant metastases
cM0(i+)	No clinical or radiographic evidence of distant metastases, but deposits of molecularly or microscopically detected tumor cells in circulating blood, bone marrow, or other nonregional nodal tissue that are no larger than 0.2 mm in a patient without symptoms or signs of metastases
M1	Distant detectable metastases as determined by classic clinical and radiographic means and/or histologically proven larger than 0.2 mm

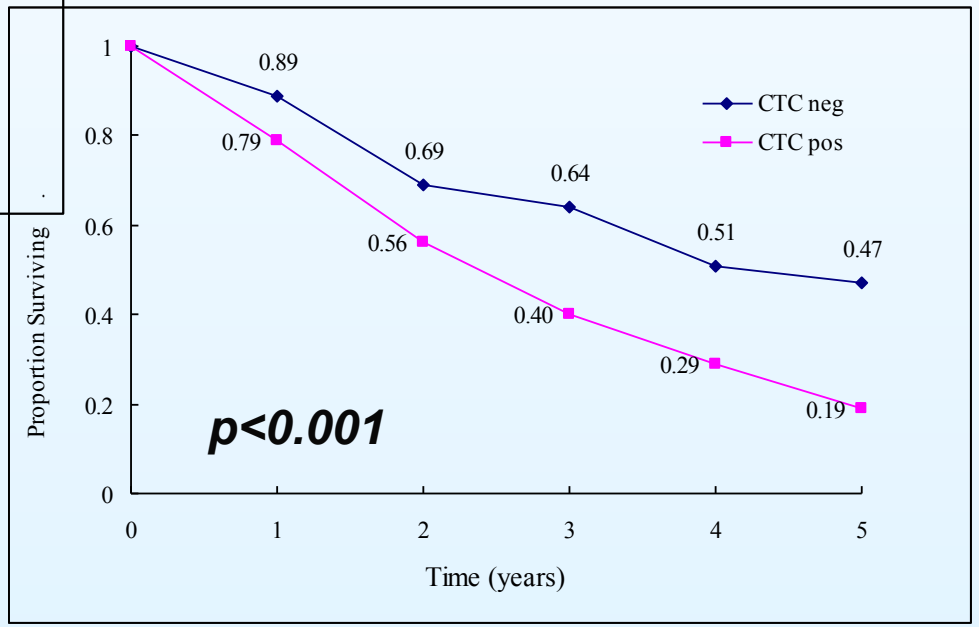
Meta-Analysis of 49 studies comprising 6815 breast cancer patients

Progression-free survival



CTC detection: ICC & RT-PCR

Overall survival



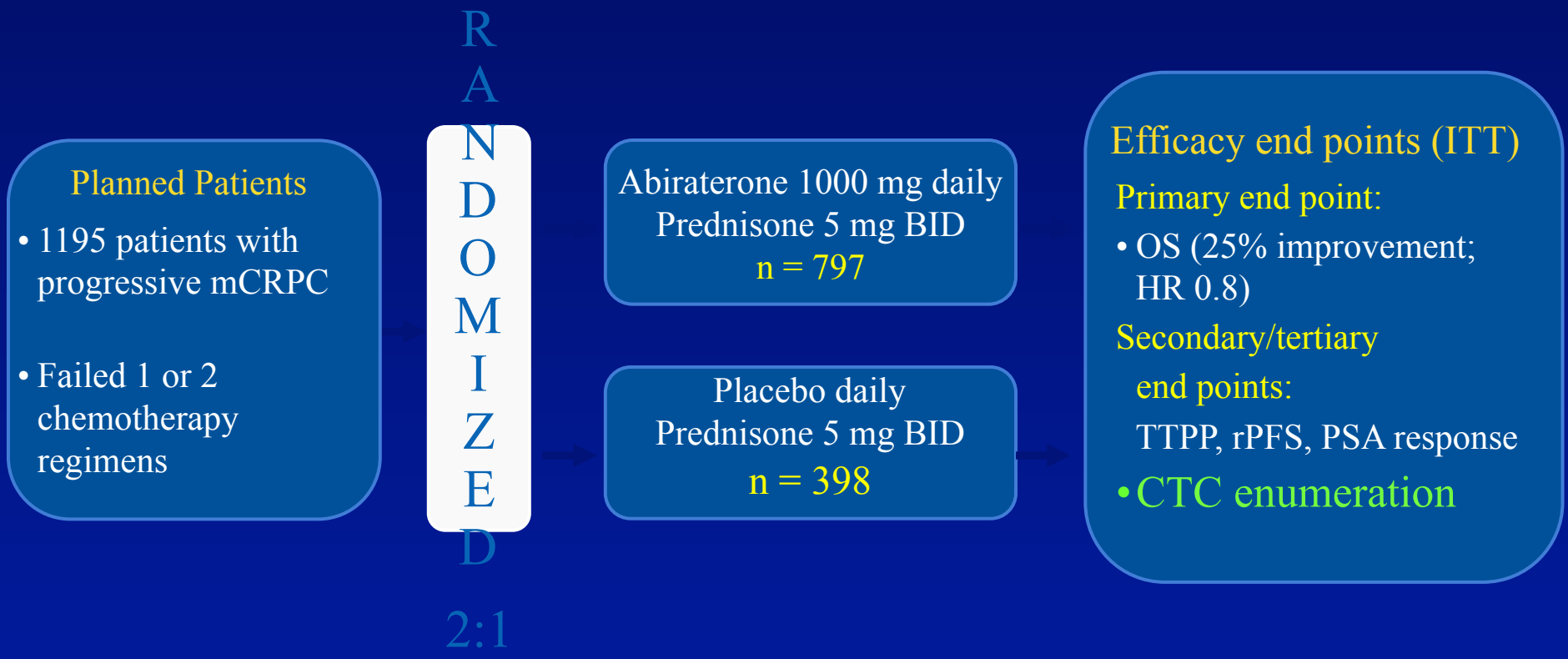
Zhang L, Riethdorf S…Pantel K, *Clin Cancer Res*, 2012.

Real-time monitoring of CTC (surrogate marker)

- Urgent need for biomarkers to tailor systemic therapy in individual cancer patients, such as the blood glucose test for directing insulin treatment of diabetes

Alix-Panabières, Riethdorf, Pantel, Clin Cancer Res, 2008

Abiraterone Phase III Study with CTC as secondary end point in patients with metastatic prostate cancer



HR, hazard ratio; ITT, intent to treat;

H. Scher, ASCO 2011

Detection of CTC in early stage cancer patients (low CTC counts):

Is the ability to release cancer cells into the circulation relevant for the development of distant metastases?

Prognostic impact of CTC in breast cancer patients without overt metastases

San Antonio Breast Cancer Symposium – Cancer Therapy and Research Center at UT Health Schience Center – December 8 – 12, 2010

Multivariate Analysis for DFS for different CTC cut-offs

Variable	Hazard Ratio adjusted for treatment		
	0 vs. ≥ 1	0, 1 vs. ≥ 2	0-4 vs. ≥ 5
CTCs in blood pos/neg	**1.878 ***	**2.825 ***	**4.035 ***
Hormone receptor status pos/neg	**2.073 ***	**2.020 ***	**3.273 ***
Lymph Node Involvement pos/neg	**1.698 ***	**1.664 ***	**1.574 ***
Grading G1 vs. G2-3	**2.961 ***	**3.182 ***	**3.245**
Tumor size T1 vs. T2-4	**1.629 ***	**1.655 ***	**2.573 ***

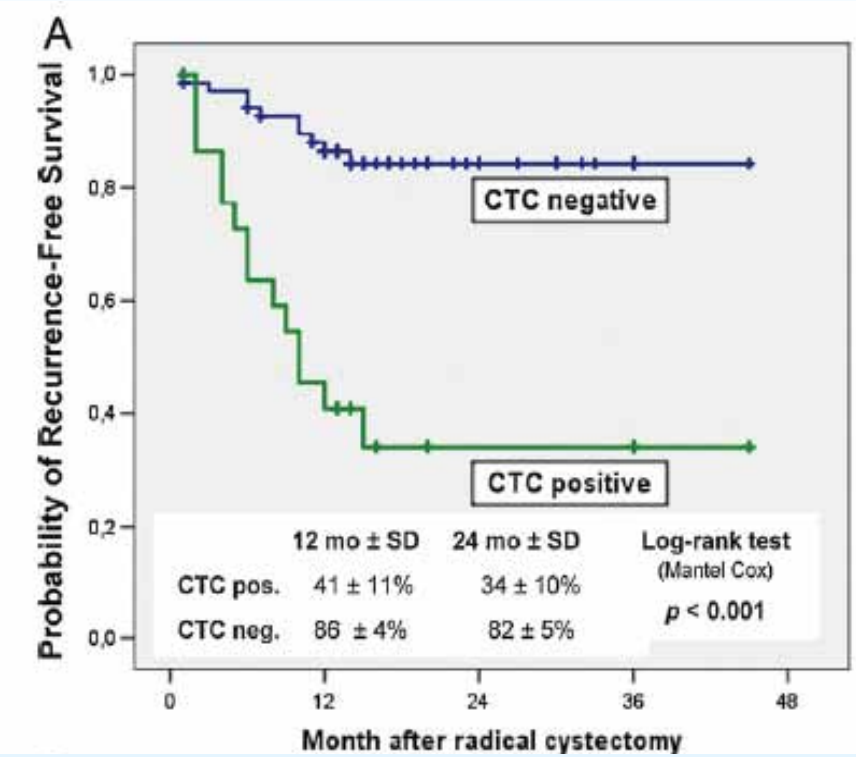
Rack, Janni et al, unpublished

*** P < 0.05**

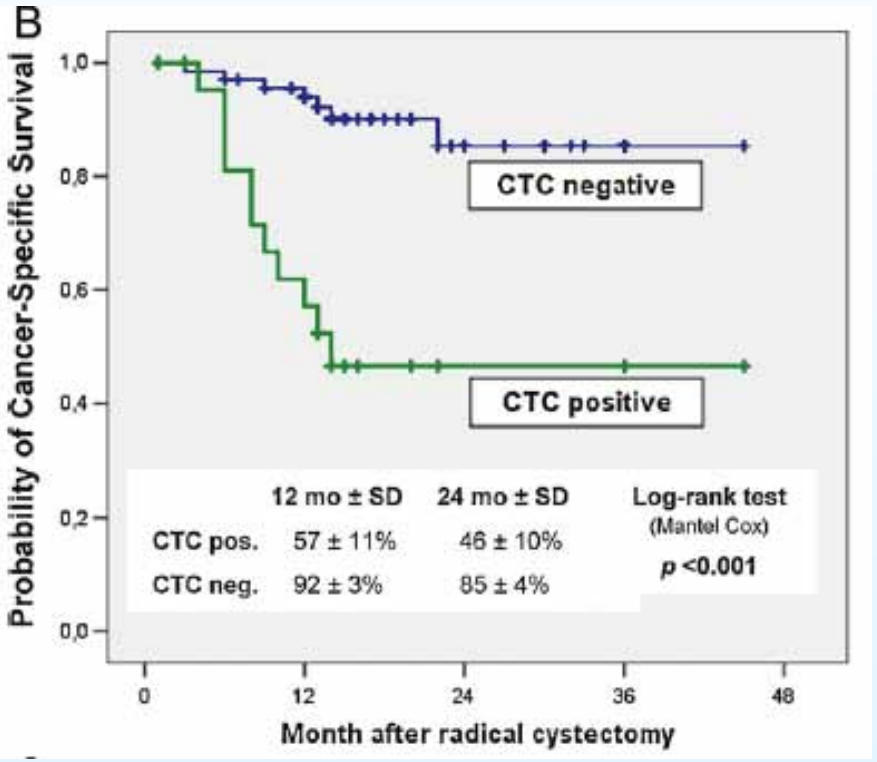
Prognostic value of CTC in urinary bladder cancer

Survival outcomes: Independent prognostic factor

Median Follow-up: 18 months



DFS HR: 4.6

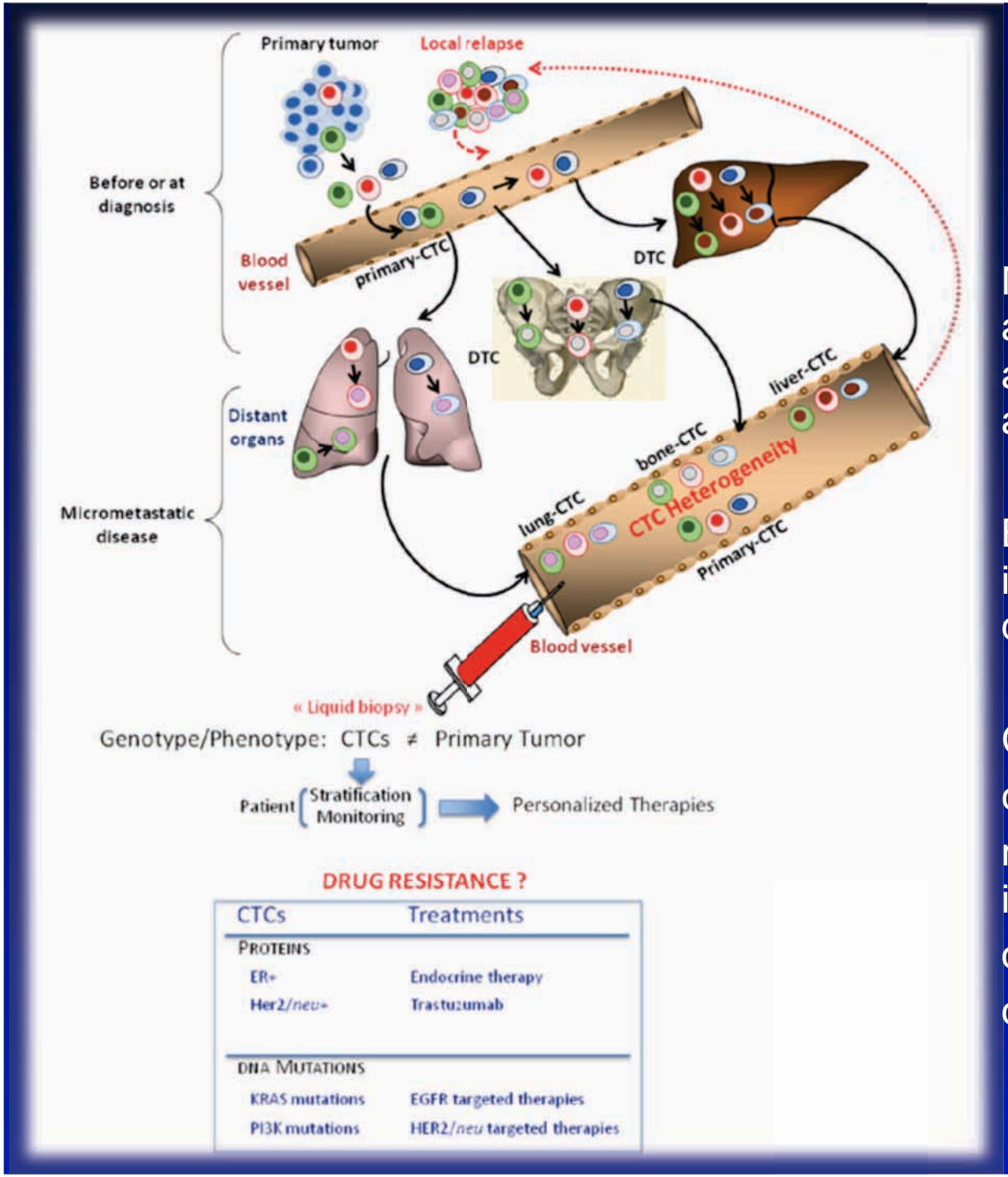


CSS HR: 5.2

Rink, Pantel, Riethdorf *et al.*, Eur Urol 2012

Molecular Characterization of CTC

CTC as Liquid Biopsy for metastatic cells



Metastasis evolve many years after primary tumor resection and can harbor unique genomic alterations.

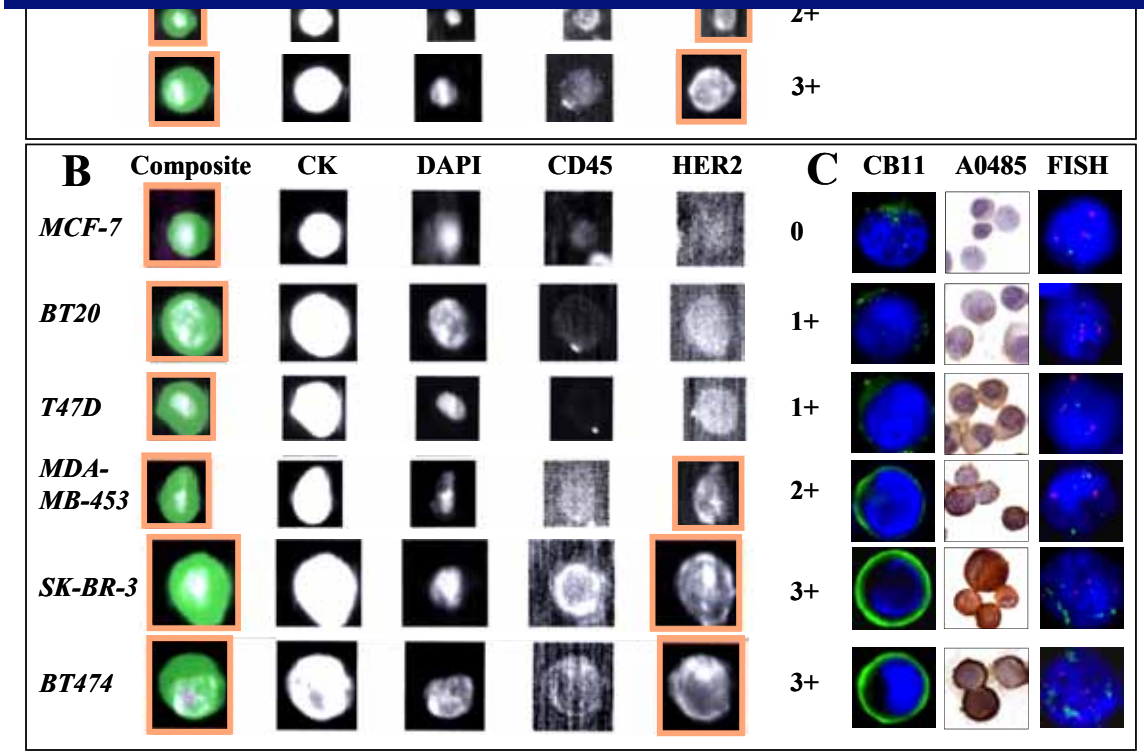
Biopsy of metastases is an invasive and sometimes dangerous procedure.

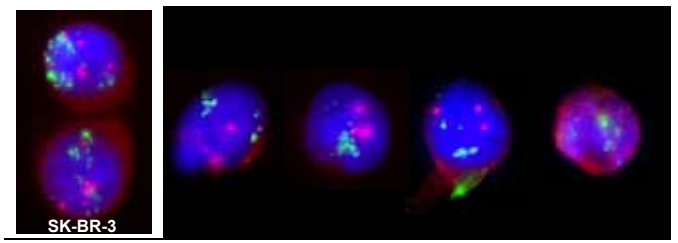
Can the molecular characterizaton of CTC reveal representative information on metastatic cells located at different sites ?

Alix-Panabières & Pantel, *Clin Chem, 2013; Pantel & Alix-Panabieres, Cancer Res. 2013*

Detection of therapeutic targets on CTC: HER2 oncogene in breast cancer

DETECT-III study: Anti-HER2 therapy (lapatinib) in metastatic breast cancer patients with HER2-negative primary tumors and HER2-positive CTC



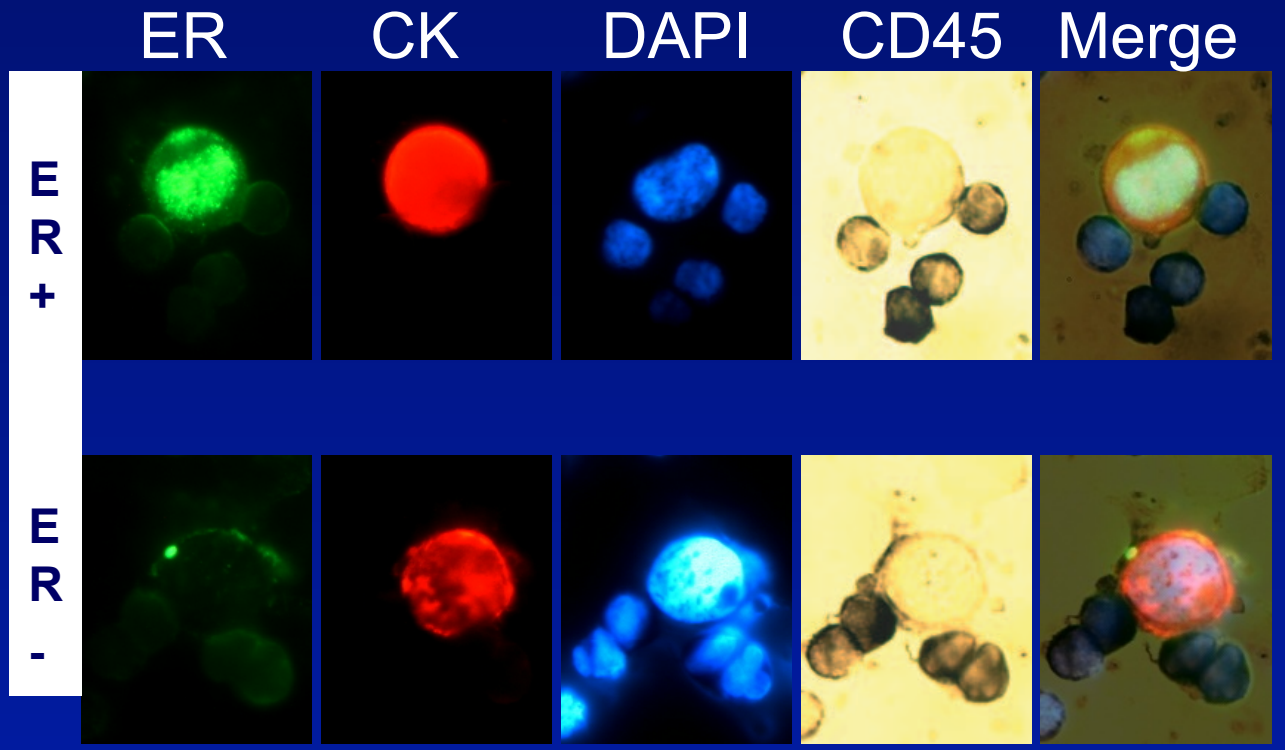


Discordance between HER2 status of primary tumor and CTC

Riethdorf/Pantel *et al.*, ***Clinical Cancer Res*** 2010 - Fehm/Pantel *et al.*, ***Breast Cancer Res Treat*** 2010

Heterogeneity of ER status in CTCs of breast cancer patients with ER-positive primary tumors

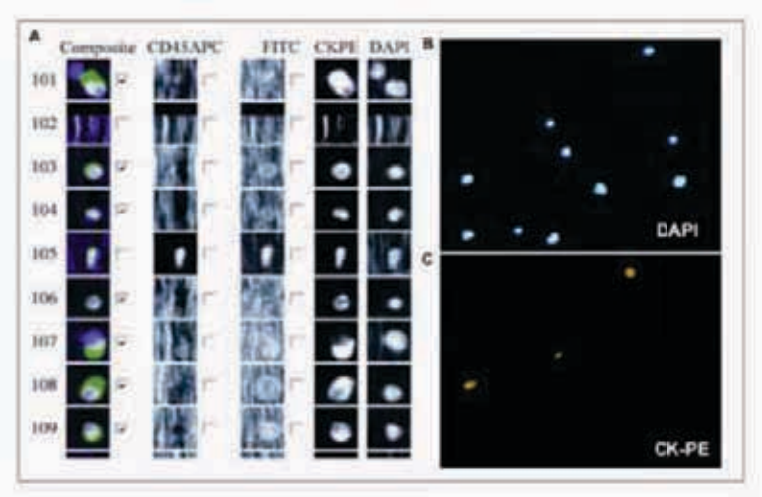
Babayan, Joosse, Pantel et al., PLOS ONE 2013



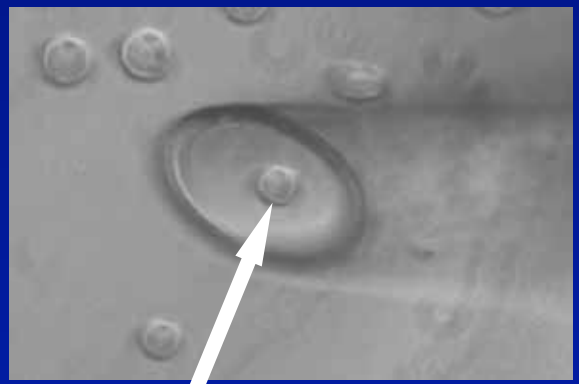
ER-negative CTCs may survive endocrine therapy

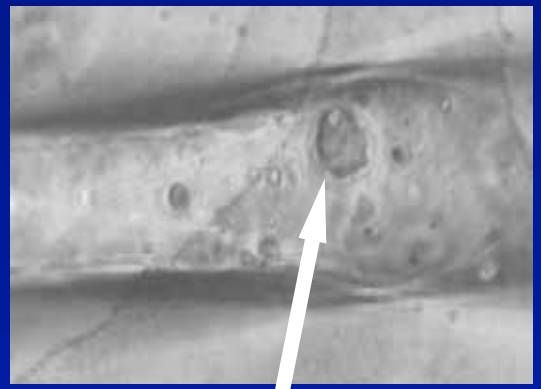
Genomic Characterization of single CTC

CTC detection



CTC isolation





CTC

Capillary

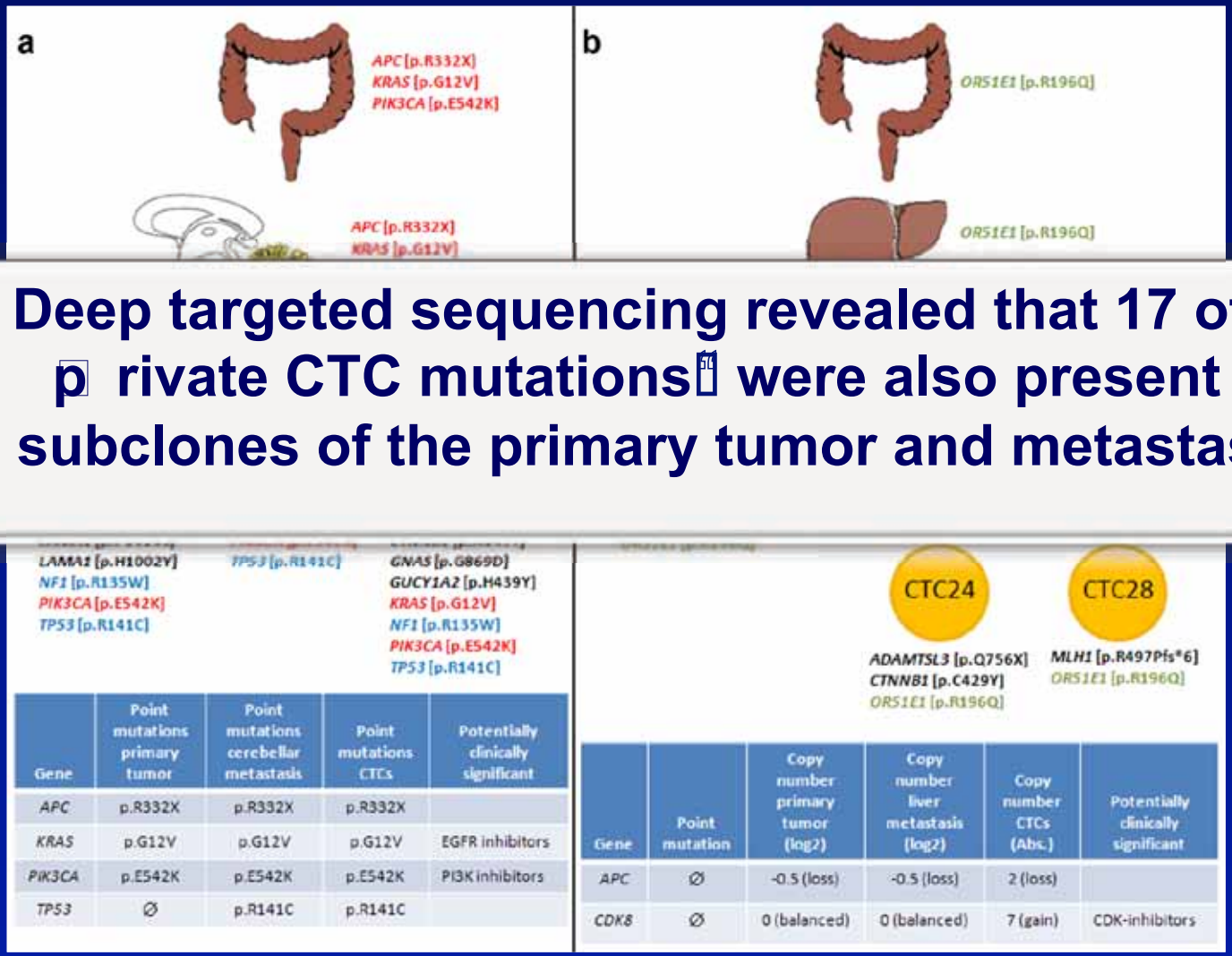
CTC

WGA +

- Mutation analysis
- CGH (conv./array)
- NextGen Sequencing

Distribution of mutations in primary tumor, metastases and CTC

CRC patient #6 **CRC patient #26**



Deep targeted sequencing revealed that 17 of 20 p rivate CTC mutations were also present in subclones of the primary tumor and metastases

Gene	Point mutations primary tumor	Point mutations cerebellar metastasis	Point mutations CTCs	Potentially clinically significant
APC	p.R332X	p.R332X	p.R332X	
KRAS	p.G12V	p.G12V	p.G12V	EGFR inhibitors
PIK3CA	p.E542K	p.E542K	p.E542K	PI3K inhibitors
TP53	∅	p.R141C	p.R141C	

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

Heitzer, Riethdorf, Pantel, Speicher et al, Cancer Res. 2013

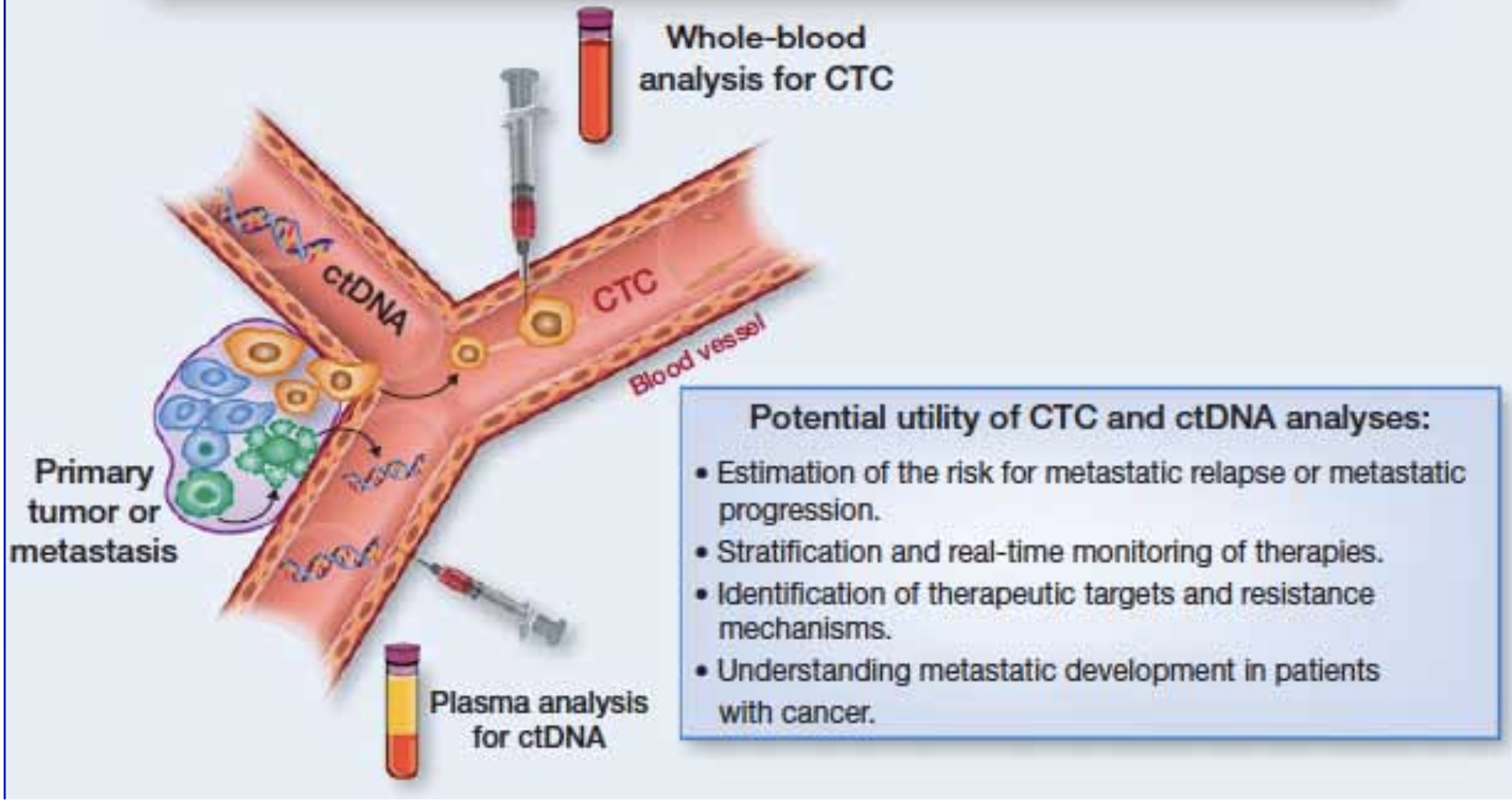
CTC and Other Circulating Markers

Review

Cancer Research

Real-Time Liquid Biopsy in Cancer Patients: Fact or Fiction?

Klaus Pantel[1] and Catherine Alix-Panabières[2,3]



Review

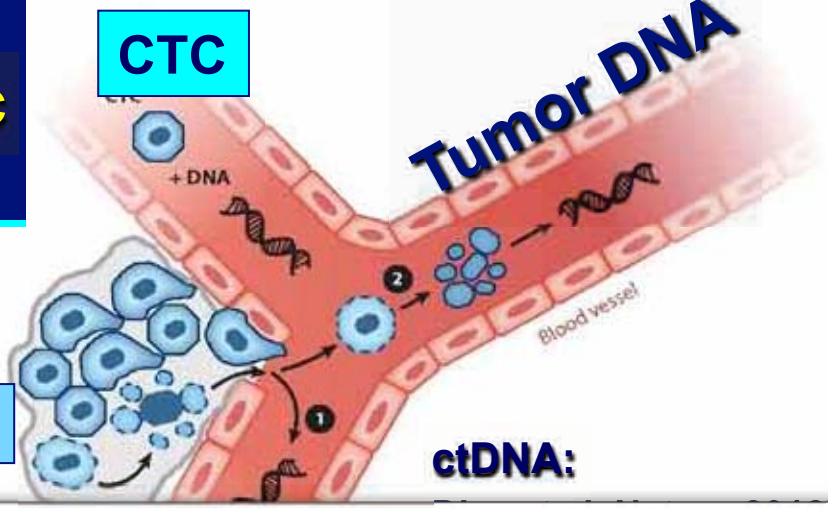
Cancer Research

Real-Time Liquid Biopsy in Cancer Patients: Fact or Fiction?

Klaus Pantel[1] and Catherine Alix-Panabières[2,3]

Targets	CTCs 	ctDNA  <  < 
Origins	Selected viable tumor cells leaving actively the primary tumor and/or metastases	Necrotic and apoptotic tumor cells
Definition	Tumor cells as a real-time liquid biopsy of the tumor and/or metastases	Fragmented genomes released from dying tumor cells of the primary tumor and/or metastases and/or CTC
Analytes	DNA, RNA (mRNA/microRNA), and protein functional studies (*in vitro*, *in vivo*)	DNA
Technologies	Immunocytologic and molecular assays (including next-generation sequencing), cell culture, and xenotransplantation	Molecular DNA assays (including next-generation sequencing)

Tumor-associated circulating cell-free nucleic acids in blood



ctDNA:

Correlation **CTC** & Circulating Tumor **DNA:**

Prostate Cancer: Schwarzenbach, Alix-Panabieres, Pantel et al., Clin Cancer Res 2009; **Breast cancer**: Dawson et al, NEJM, 2013; **Colon Cancer**: Heitzer, Pantel et al, Int J Cancer, 2013

Correlation **CTC** & Circulating **microRNA:**

Breast Cancer: Madhavan, Pantel et al Clin Cancer Res 2012

BUT: ctDNA is released from apoptotic/necrotic cells

Isolation of CTC allows in-depth molecular & functional characterization of viable cells including xenotransplantion into immunodeficient mice (Baccelli, Pantel et al, Nat. Biotech., 2013; Pantel et al., Nature Med., 2013)

Schwarzenbach/Hoon/Pantel, *Nat Rev Cancer,* 2011, Alix-Panabières/Schwarzenbach/Pantel, *Annu Rev Med*, 2012; Pantel & Alix-Panabieres, Cancer Res., 2013

Summary: Aims of Research on DTC & CTC

- Estimation of the risk for metastatic relapse or metastatic progression (prognostic information)
- Stratification & real-time monitoring of therapies
- Identification of therapeutic targets and resistance mechanisms (biological therapies)
- Understanding the biology of metastatic development

Universitätsklinikum Hamburg-Eppendorf

Center of Experimental Medicine

Institute of Tumor Biology - **Klaus Pantel**

- Sabine Riethdorf/Christin Gasch
- Heidi Schwarzenbach
- Harriet Wikman/Michaela Wrage
- Katharina Effenberger
- Simon Joosse, Anna Babayan
- Kai Bartkowiak, Natalia Bednarz-Koll

Grant Support:

DFG
BMBF
EU / ERC
Dt. Krebshilfe
Sander-Stiftung
Roggenbuck-Stiftung

Micrometastasis Research Network at UCCH/UKE

EU-Consortium-DISMAL

Start: November 2005 Coordinator: Klaus Pantel

Free University of Amsterdam

University Medical Center

Imperial
(United

ital Oslo,

SME 1 App
(United Kin

Cancer
Center,

University
(The Nethe

ity
(Austria)

Netherlan
Cancer In
(The Neth

-Pette-
Germany)

Lapeyr
Montpe

hotonics

Catherine Alix-Panabieres, Montpellier:

Prix cancer Gallet et Breton 2012

Klaus Pantel, Hamburg:

ERC Advanced Investigator Grant
D ISSECT (2011-2016)

ERA-NET TRANSCAN: CTC-SCAN Project (2013 2016)

ISMRC 2013

First Announcement

9th International Symposium on Minimal Residual Cancer

September 24-27, 2013

Pullman Paris Bercy, France