Universitätsklinikum Hamburg-Eppendorf

Institute for Tumor Biology

Klaus Pantel, MD, PhD

Circulating biomarkers: What does this mean today?

Disclosure Information

Klaus Pantel

I have the following financial relationships to disclose:

Grant/Research Support from: Veridex/Janssen

Advisory Board: Veridex/Janssen, Alere, Gilupi

CANCER

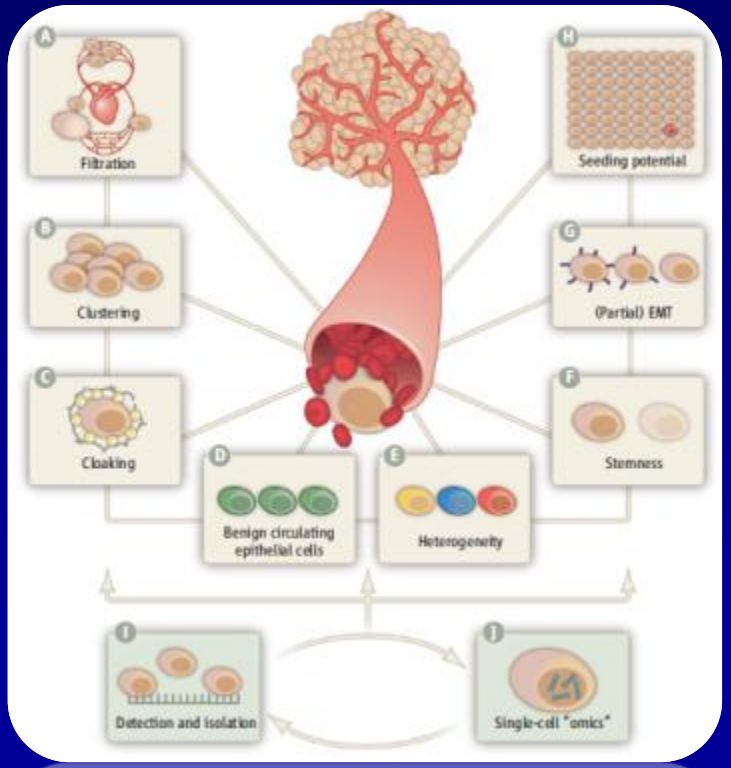
Circulating Tumor Cells

Vicki Plaks, Charlotte D. Koopman, Zena Werb

Much remains to be learned about CTCs and their clinical potential as biomarkers and therapeutic targets.

Science

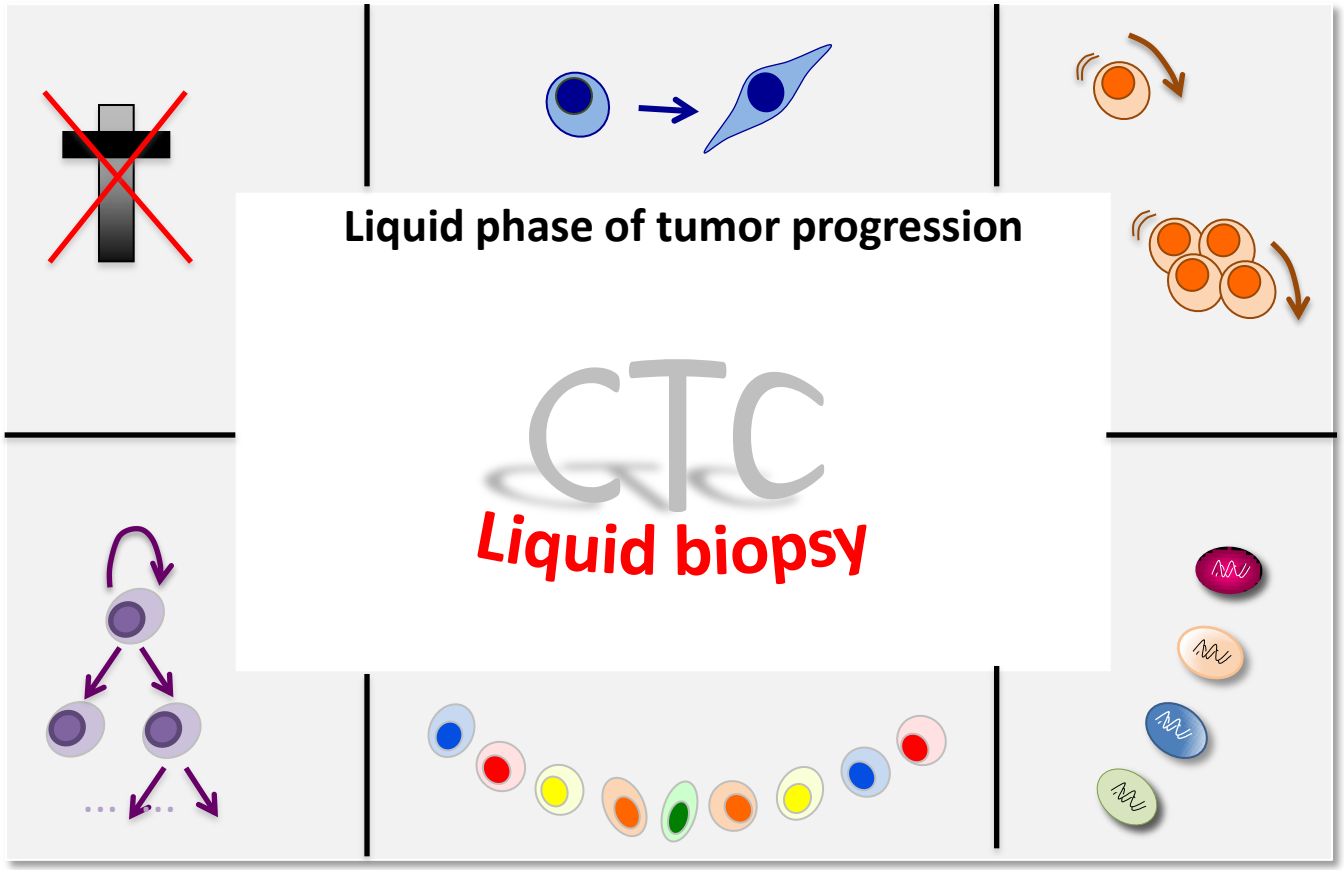
AAAS



Anoikis resistance

Epithelial-to-mesenchymal transition

Invasion/Intravasation ability
(single CTCs and/or clusters)



The technical challenge:

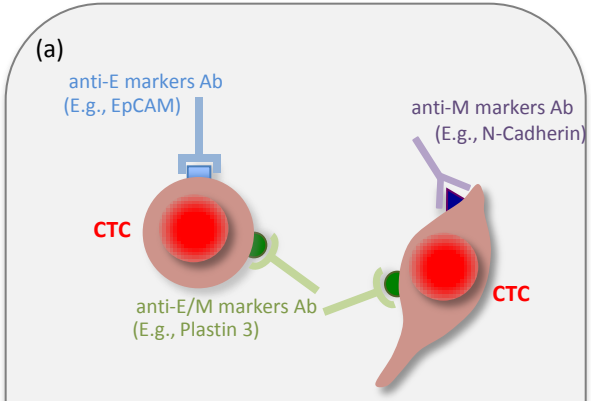
Finding one tumor cell in 10^6 – 10^8 normal blood cells

Alix-Panabieres & Pantel, *Nature Rev. Cancer* 2014

Biological properties
Protein expression

Physical properties
Label-free strategies

Positive Selection



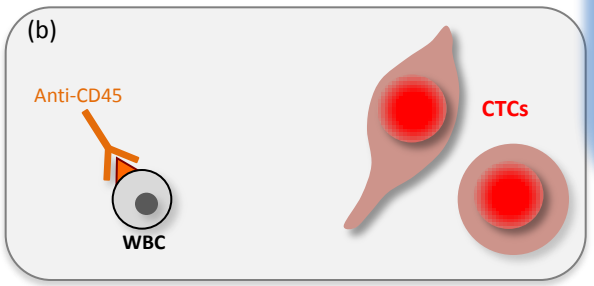
Ex vivo

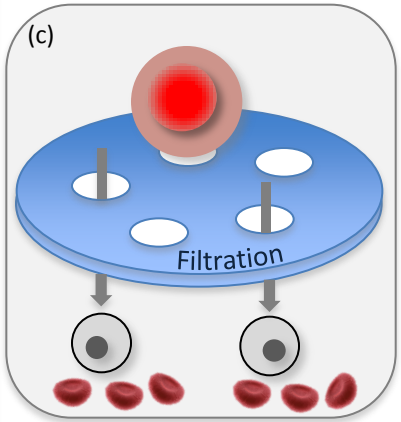
- CellSearch® system
- MagSweeper™
- EPHESIA CTC-chip
- CTC-chip
- Velcro-like device

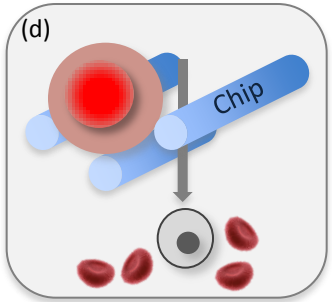
In vivo

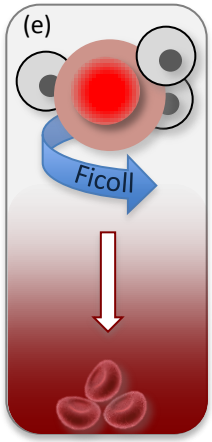
- CellCollector®
- Photoacoustic nanodetector

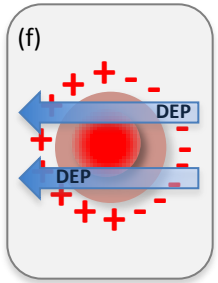
Negative Selection

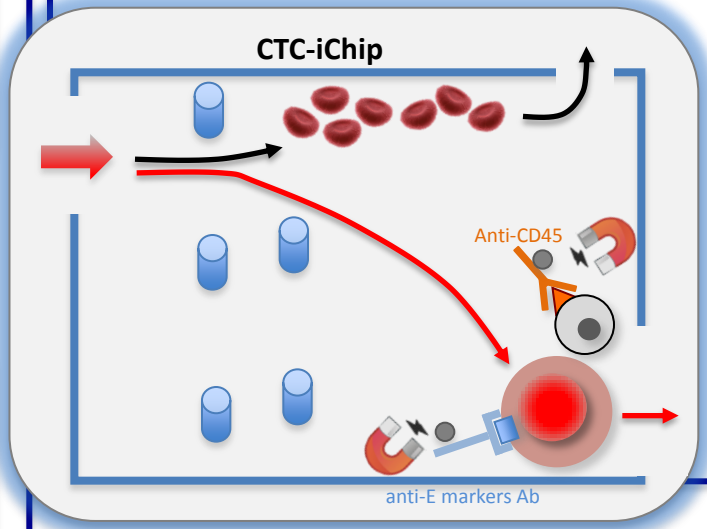


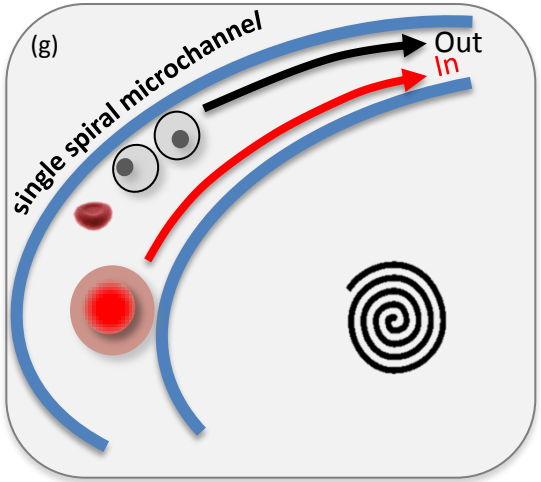


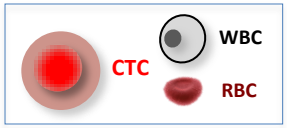








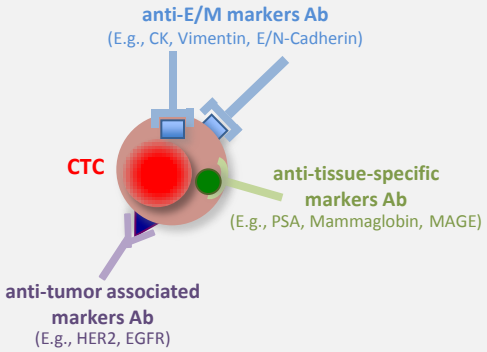




Alix-Panabieres & Pantel, *Nature Rev. Cancer* 2014

Approaches for CTC detection

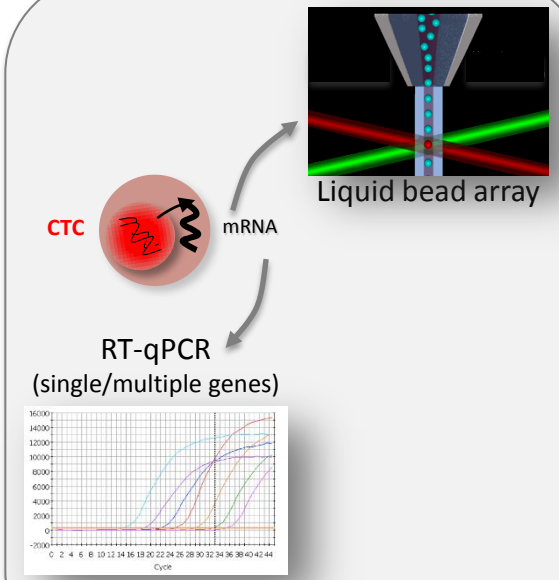
Immunocytological technologies



Technologies

- Immunocytochemistry
- CellSearch® system
- Flow Cytometry
- DEParray®

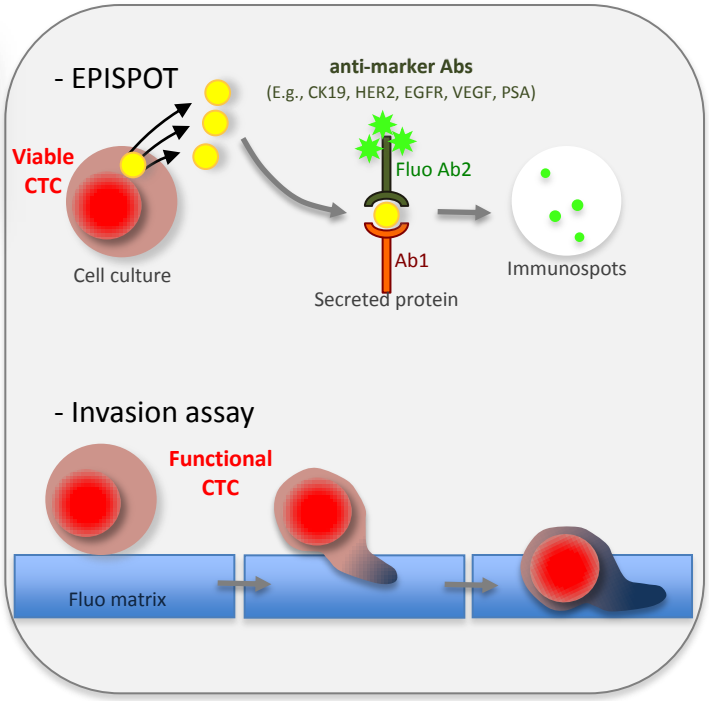
Molecular technologies



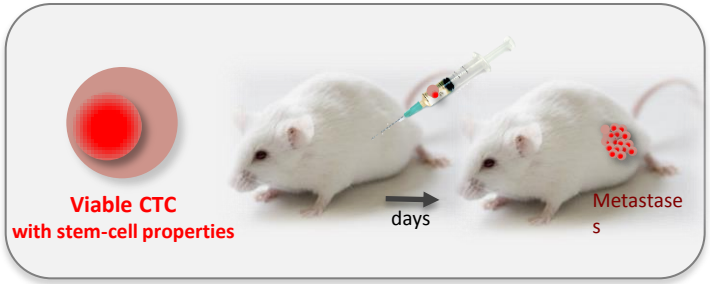
RNA-based Technologies

Functional assays

In vitro Cell Culture

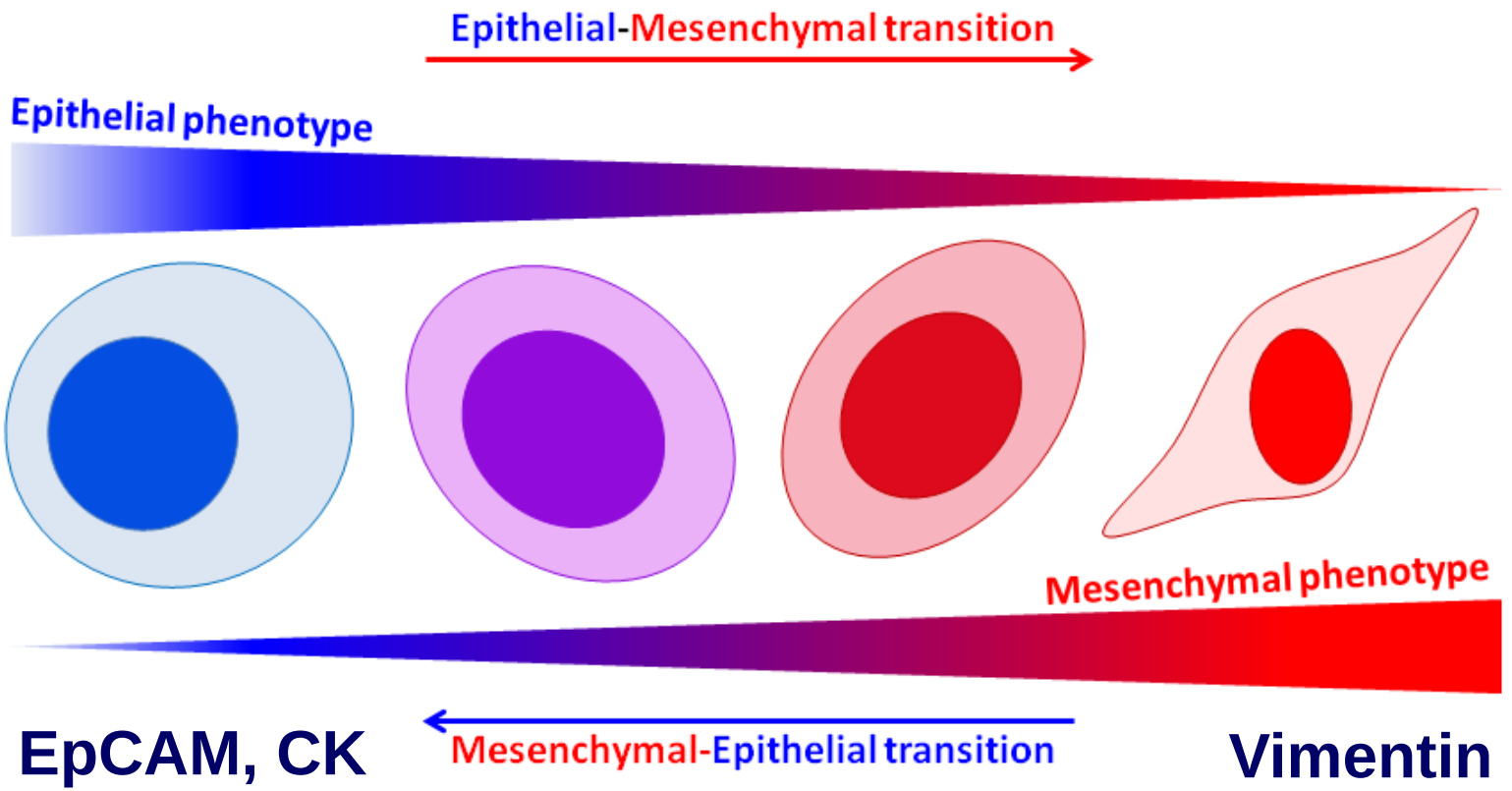


Xenotransplantation models (CDx)



Alix-Panabieres & Pantel, *Nature Rev. Cancer* 2014

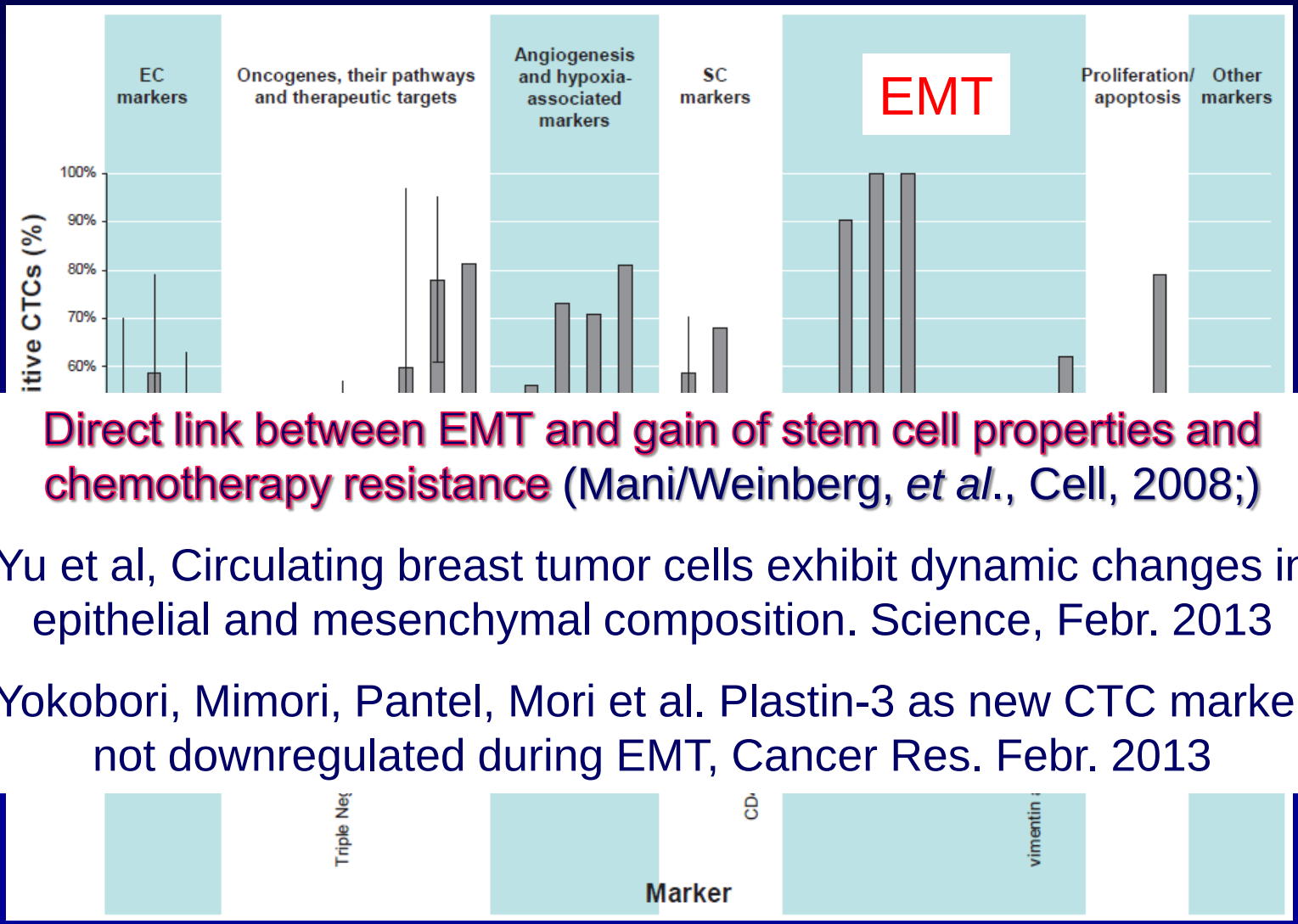
Epithelial-Mesenchymal Plasticity of CTC



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-Panabières & Pantel *Cancer & Met Rev* 2012

Expression profile of CTCs in breast cancer



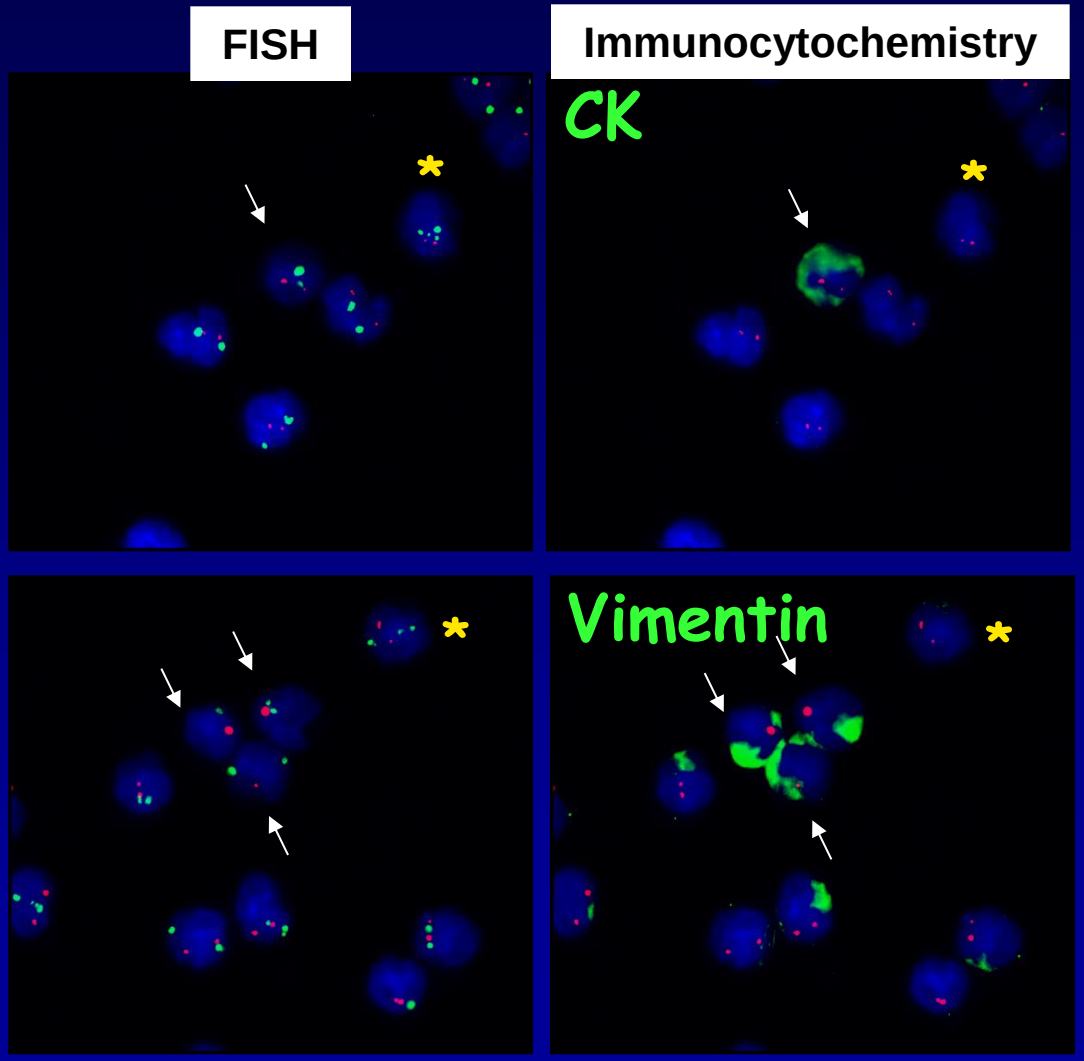
Direct link between EMT and gain of stem cell properties and chemotherapy resistance (Mani/Weinberg, *et al*., Cell, 2008;)

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

EMT in prostate cancer: BRCA1 gene loss in vimentin-positive CTC



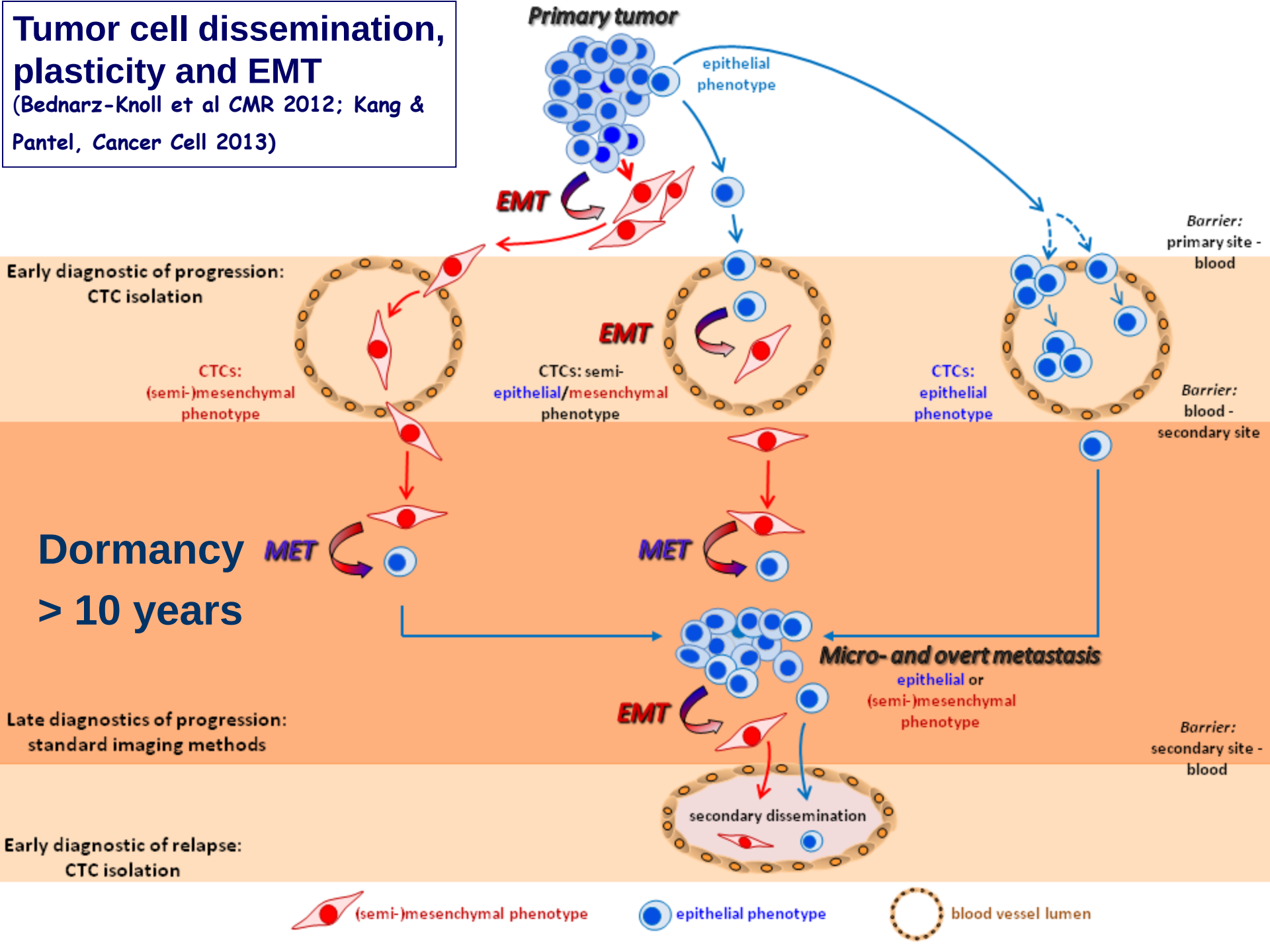
CK-pos. CTC

VIM-pos. CTC: EMT !

Bednarz/Pantel et al., Clin Cancer Res 2010

Tumor cell dissemination, plasticity and EMT

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

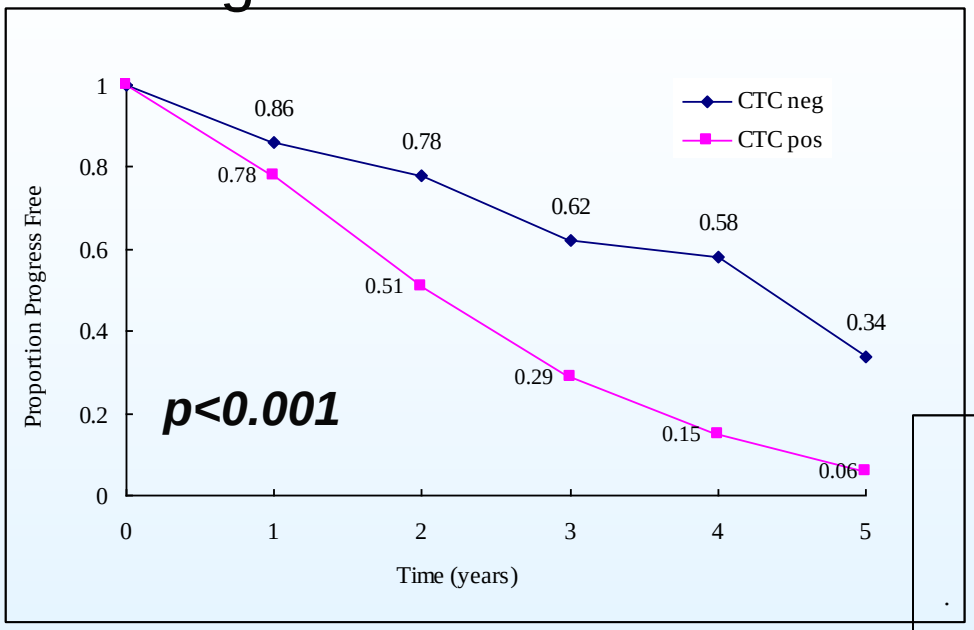


Prognostic relevance of CTC in cancer patients:

Is the CTC count relevant for the development & progression of metastases?

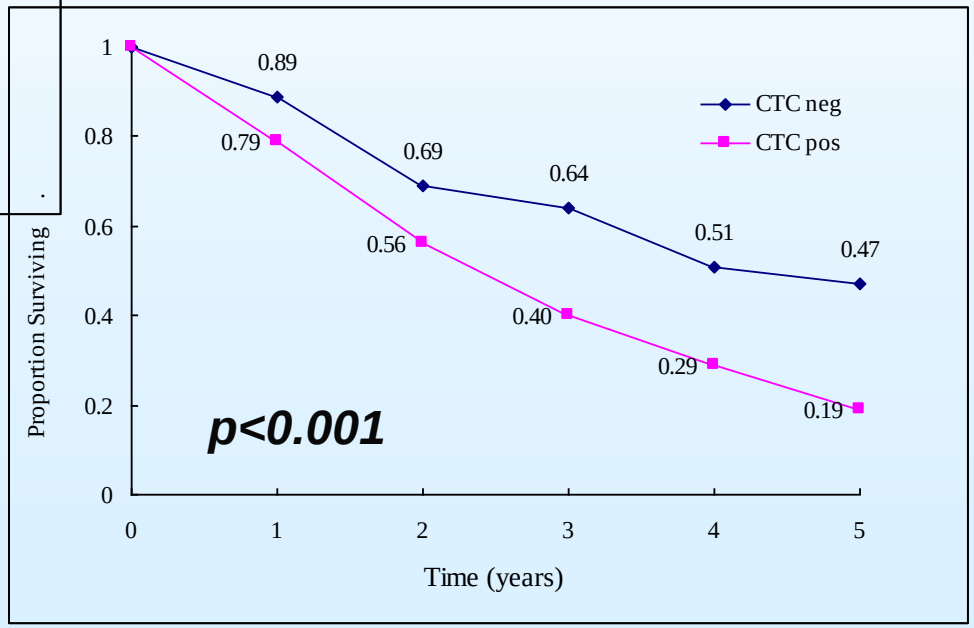
Meta-Analysis of prognostic influence of CTCs in breast cancer (49 studies, 6815 patients)

Progression-free survival



CTC detection: ICC & RT-PCR

Overall survival



M0 & M1 patients

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

THE LANCET Oncology 2014

The Lancet Oncology, Volume 15, Issue 4, Pages 406 - 414, April 2014
doi:10.1016/S1470-2045(14)70069-5 Cite or Link Using DOI

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This article can be found in the following collections: Oncology (Breast cancer, Translational oncology)
Published Online: 11 March 2014

Clinical validity of circulating tumour cells in patients with metastatic breast cancer: a pooled analysis of individual patient data

17 centres provided data for 1944 eligible patients from 20 studies

Meta-analysis on raw data.

CTCs vs. conventional tumor markers (PFS, p values) in metastatic breast cancer patients

Model used as reference	(								
	baseline			3-5 weeks			6-8 weeks		
	CTCBL	CA15-3BL	CEABL	CTC3-5	CA15-3 BL + CA15-3 3-5	CEABL + CEA 3-5	CTC6-8	CA15-3 BL + CA15-3 6-8	CEABL + CEA 6-8
N patients	1193	914	593	436	357	289	279	215	170
CP	6 E-10	.10	.04						
CP +CTCBL		.32	.12	5 E -05	.25	.35	9 E-05	.40	*Few events*
CP +CTCBL + CTC3-5					.26	.41			
CP +CTCBL + CTC6-8								.36	*Few events*

Bidard, Pierga, Michels, Pantel et al, Lancet Oncology 2014, **European Pooled Analysis of CTCs in metastatic BC (n=1944)**

CTCs in early stage cancer patients

Challenge: Very low number of CTCs

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 ***

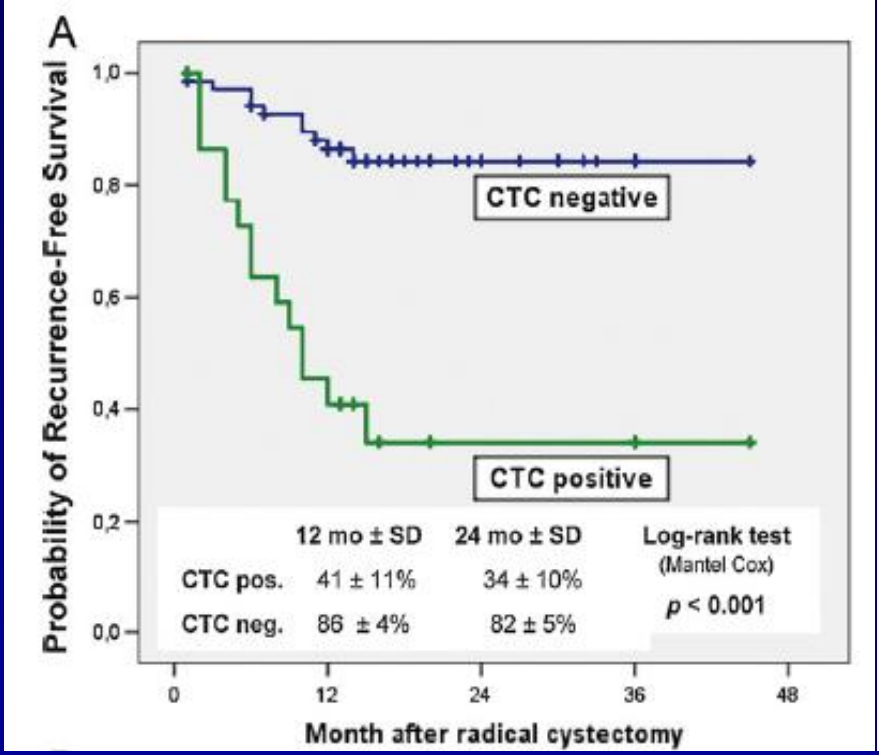
Rack, Janni, Pantel et al, JNCI 2014

*** P < 0.05**

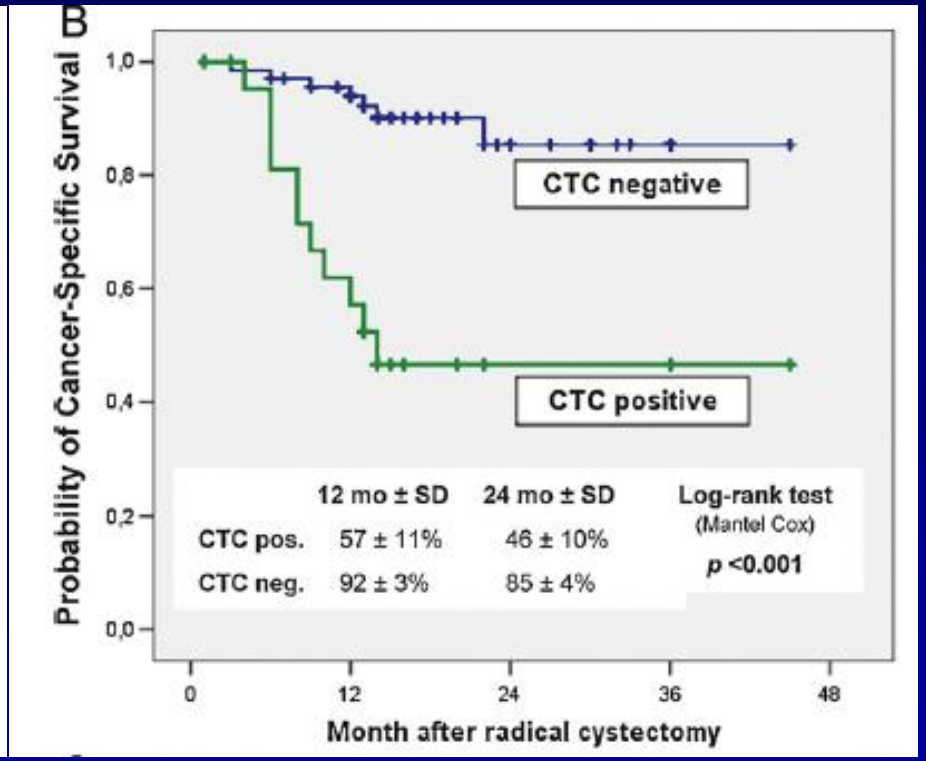
Prognostic value of CTC in urinary bladder cancer

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

CTCs in early stage cancer patients

Head & Neck Cancer:

CTCs: 10/80 Pts (12.5%); DTCs: 18/90 Pts (20.0%)

(Grobe, Riethdorf, Pantel et al., CCR 2014)

Testicular Germ Cell Tumors:

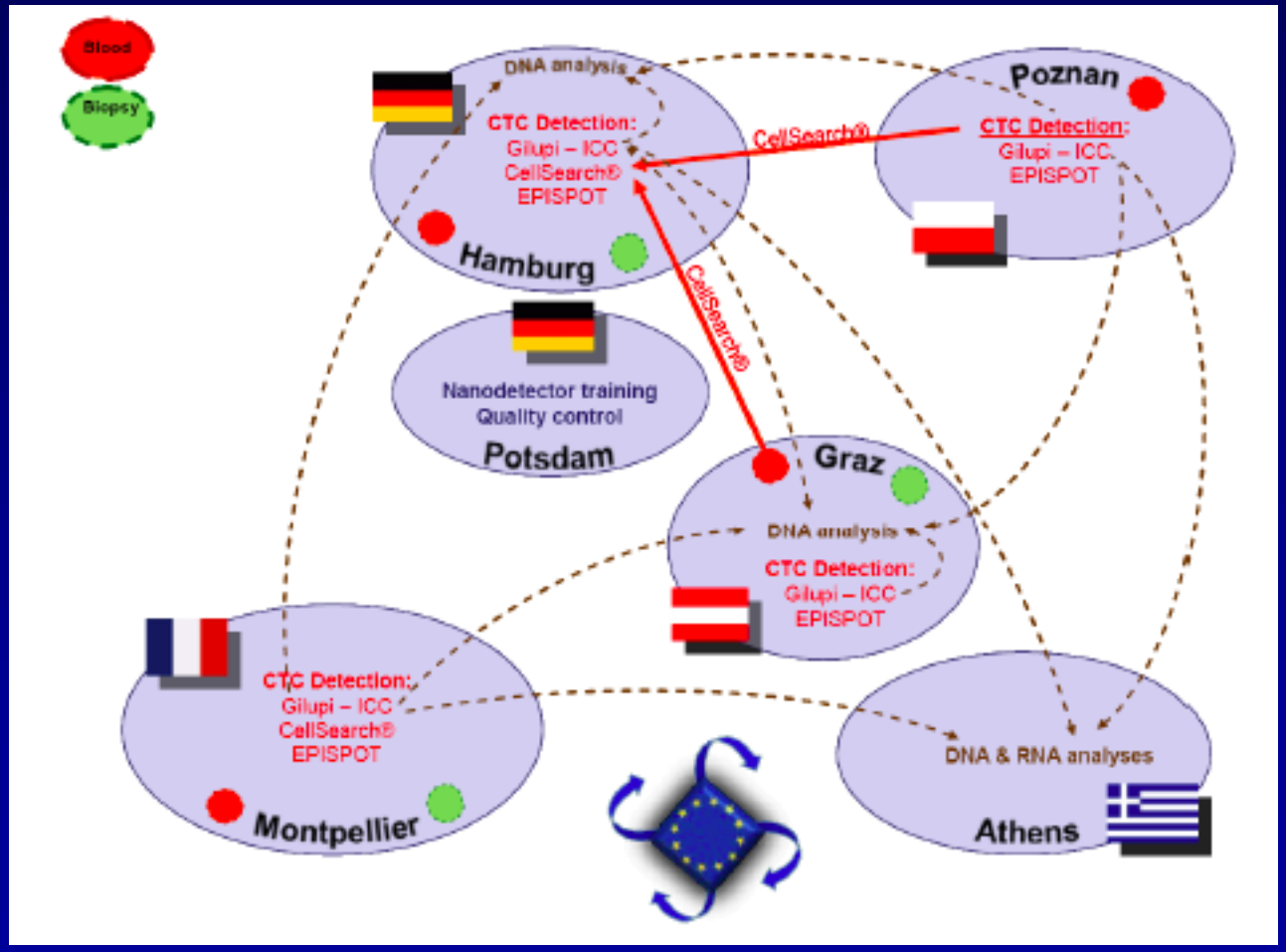
25 of 143 Pts (17.5%)

(Nastaly, Riethdorf, Pantel et al., CCR 2014)

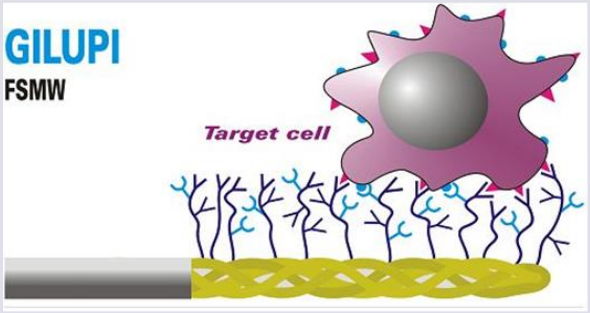
ERA-NET TRANSCAN: **CTC-SCAN Project**

High-risk Prostate Cancer (stage M_0)

Partners: Germany, France, Greece, Poland, Austria



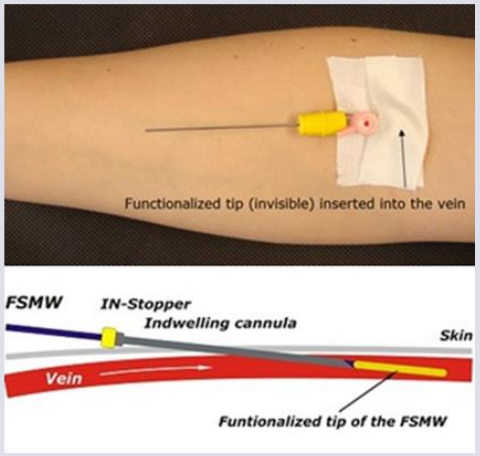
New approach: *In vivo* capture of CTC



Nanodetector

Insertion into patient´s vein at the doctor´s office

30 minutes exposure time in a vein

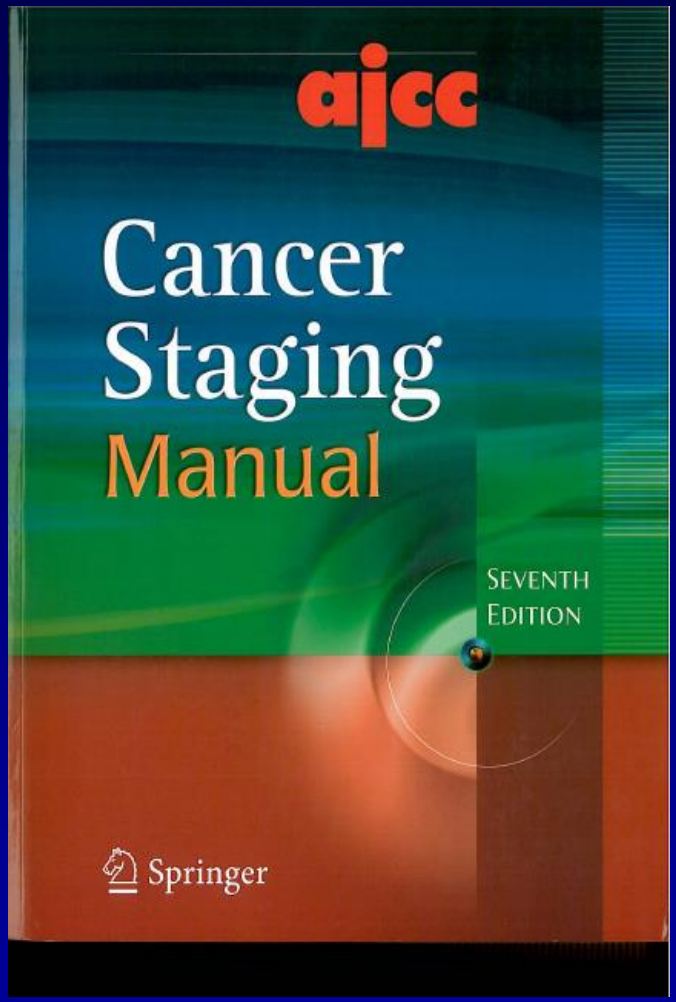


Diagnostics
- *cytology*
- *PCR, etc.*

Result ← *Decision*

Proof of principle data in breast, prostate, colon and lung cancer

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

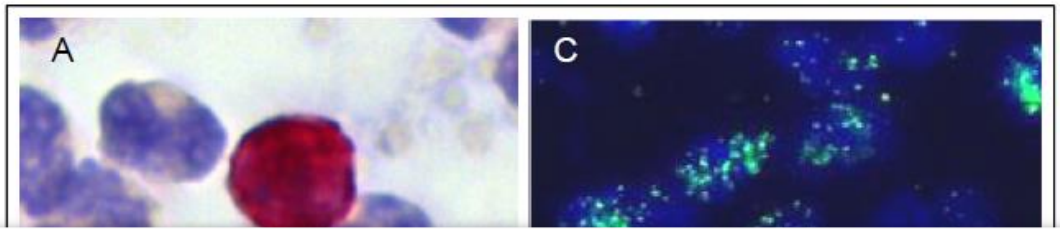


Distant Metastases (M)	
M0	No clinical or radiographic evidence of distant metastases
cM0(i+)	No clinical or radiographic evidence of distant metastases, but deposits of molecularly or microscopically detected tumor cells in circulating blood, bone marrow, or other nonregional nodal tissue that are no 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

Hematogeneous dissemination of tumor cells in patients with primary brain tumors (paradigm shift)

Hematogeneous spread of primary brain tumors: Detection of CTCs in glioma patients (~20%)

GFAP stain



EGFR amplification

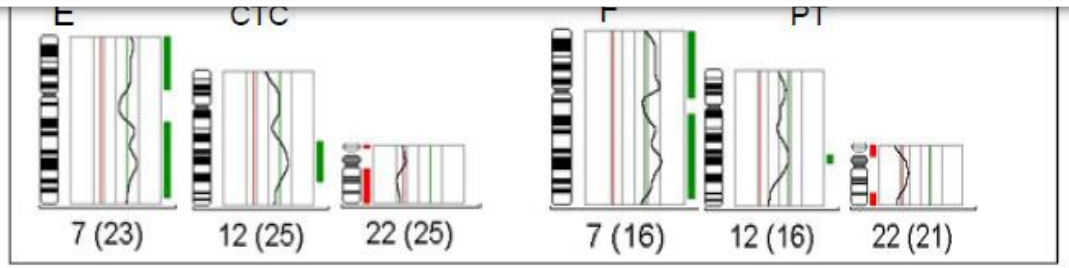
TRANSLATIONAL RELEVANCE:

Glioma patients with CTCs may not be used as transplant donors

CTCs may serve as liquid biopsy

Single Cell isolation

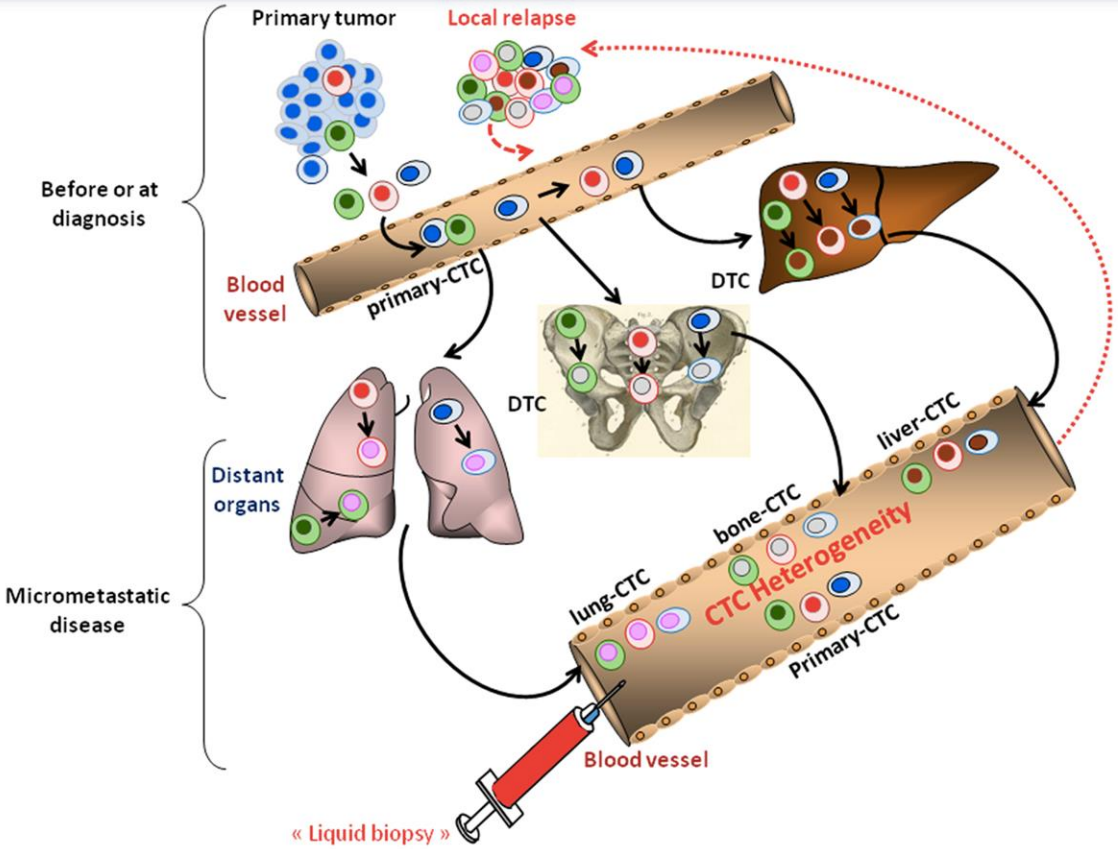
Single Cell CGH



Müller, Westphal, Pantel *et al*., Science TM, *2014*

Interventional clinical studies based on enumeration and/or characterization of CTC

CTC as Liquid Biopsy for metastatic cells



Genotype/Phenotype: CTCs ≠ Primary Tumor

Patient { Stratification / Monitoring } → Personalized Therapies

DRUG RESISTANCE ?

CTCs	Treatments
PROTEINS	
ER+	Endocrine therapy
Her2/*neu*+	Trastuzumab
DNA MUTATIONS	
KRAS mutations	EGFR targeted therapies
PI3K mutations	HER2/*neu* targeted therapies

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

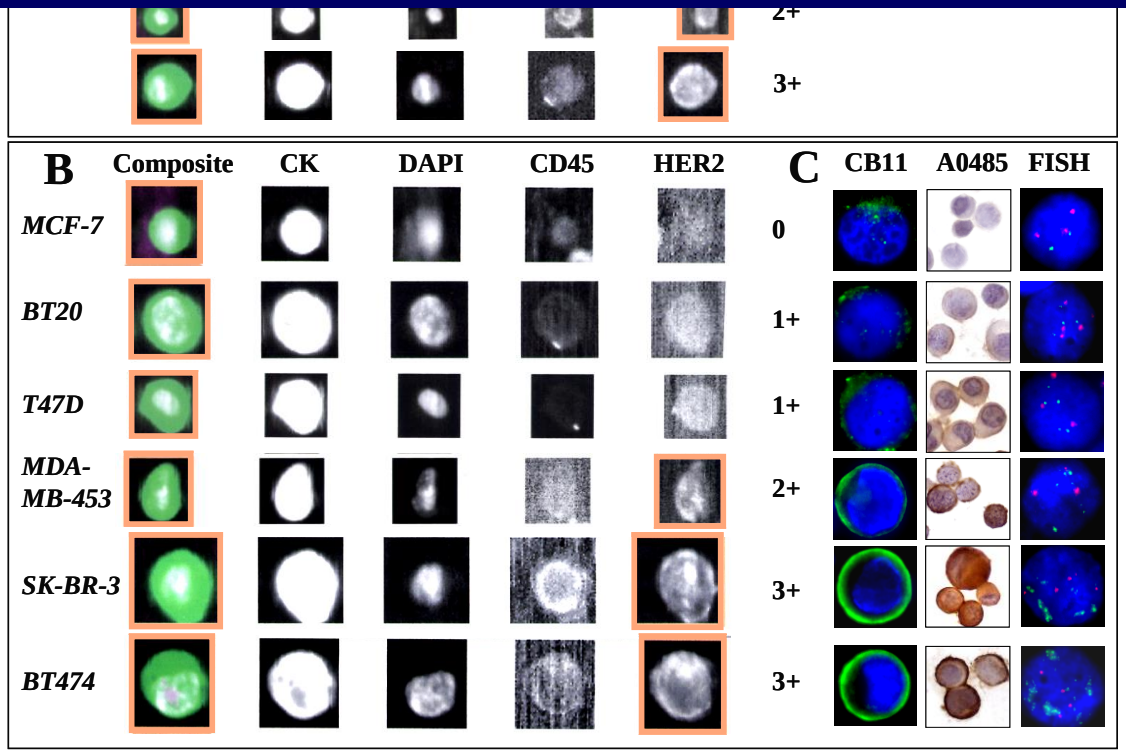
Biopsy of metastases is an invasive and sometimes dangerous procedure.

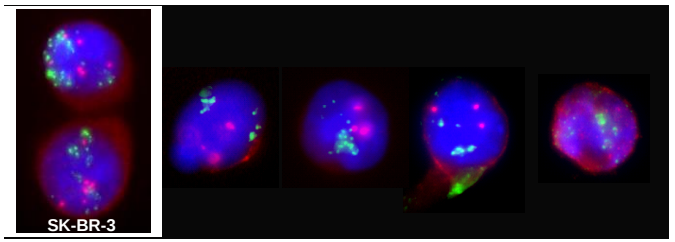
Can the molecular characterizaton of CTC reveal representative information on metastatic cells located at different sites ?

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



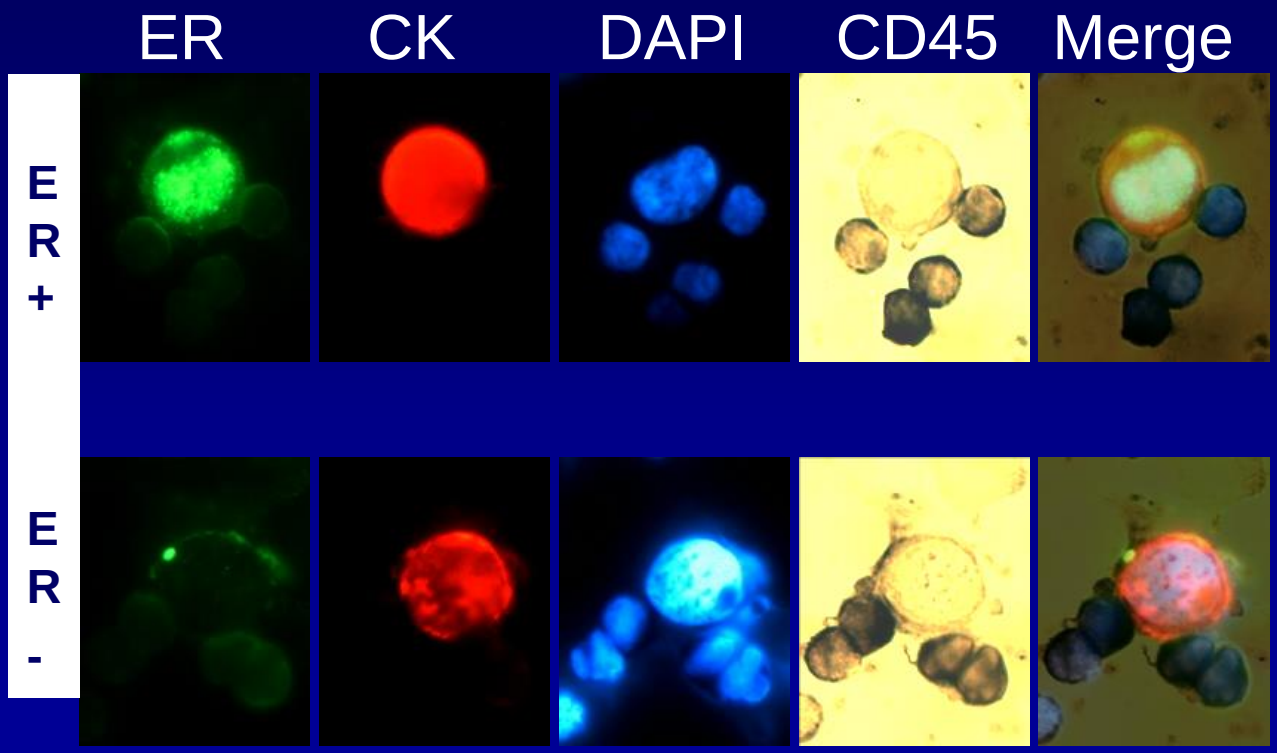


Discordance between HER2 status of primary tumor and CTC

Riethdorf/Pantel *et al.*, ***Clinical Cancer Res*** **2010** - **Fehm/Pantel** *et al*., ***Breast Cancer Res Treat*** **2010**
Ignatiadis/Sotiriou *et al*, ***PlosONE*****, 2011** - **Ignatiadis/Pantel** *et al*, ***SABCS*****, 2011**

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

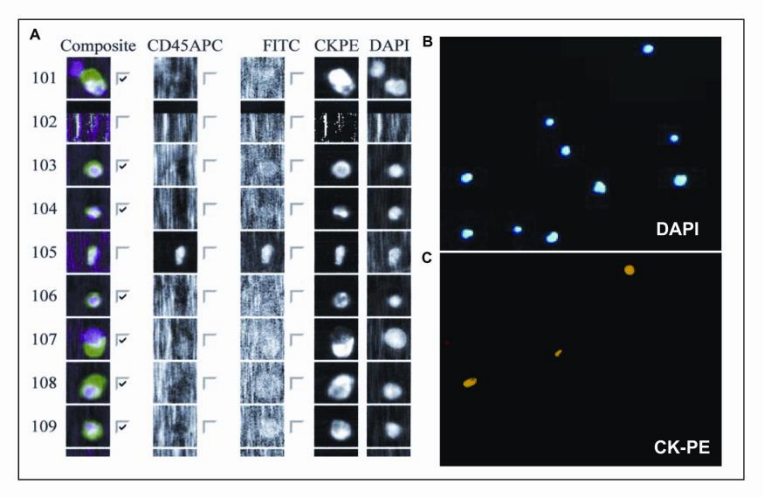
Babayan, Joosse, Pantel et al., PLOS ONE 2013



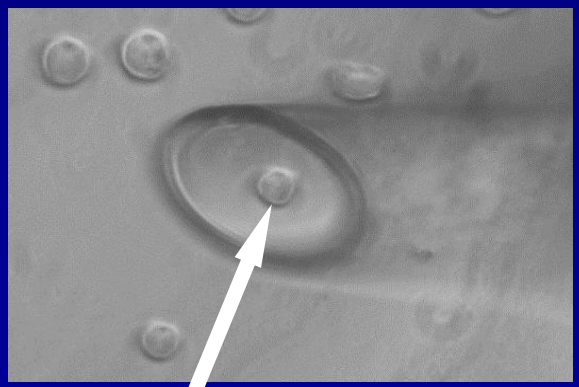
ER-negative CTCs may survive endocrine therapy

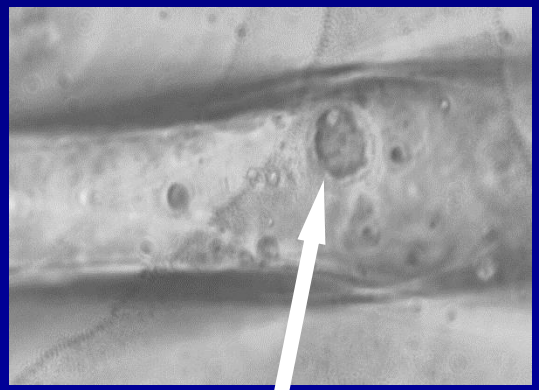
Genomic Characterization of single CTC

CTC detection



CTC isolation





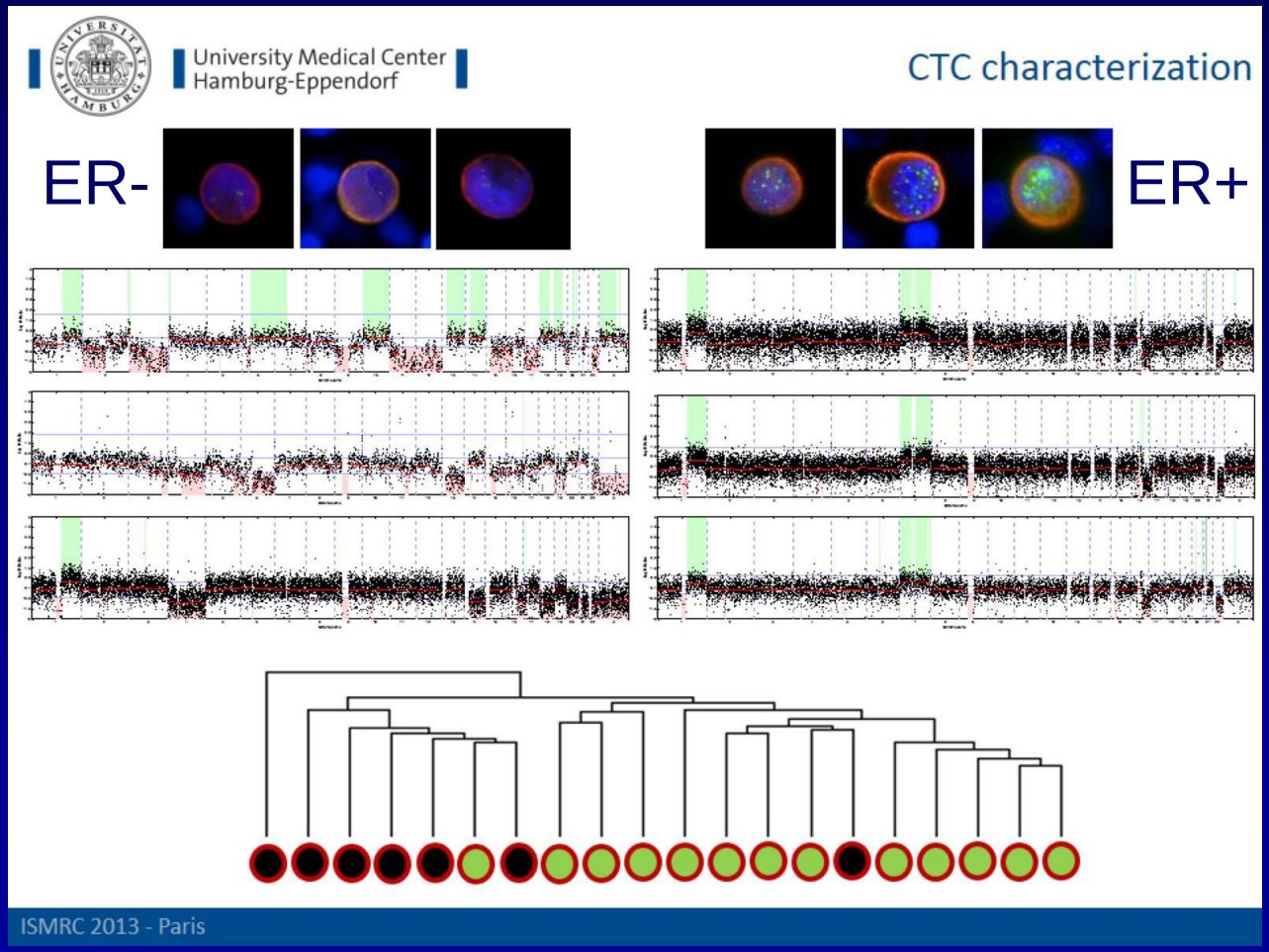
CTC

Capillary

CTC

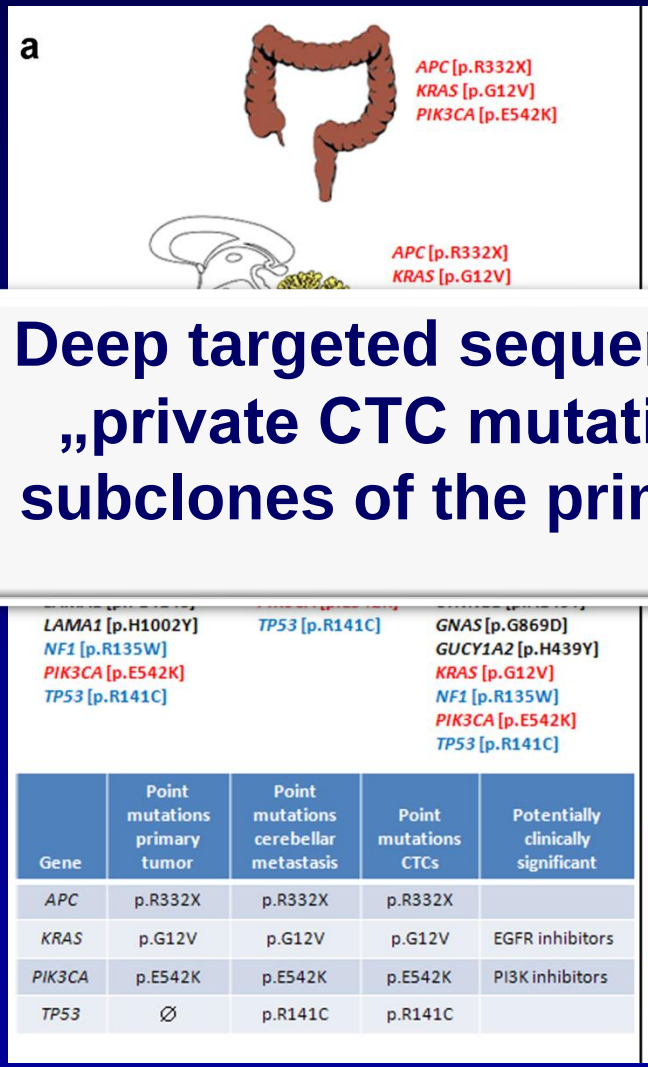
- WGA +
- Mutation analysis
- CGH (conv./array)
- NextGen Sequencing

Different genomic profiles of ER+ and ER- CTCs in breast cancer patients



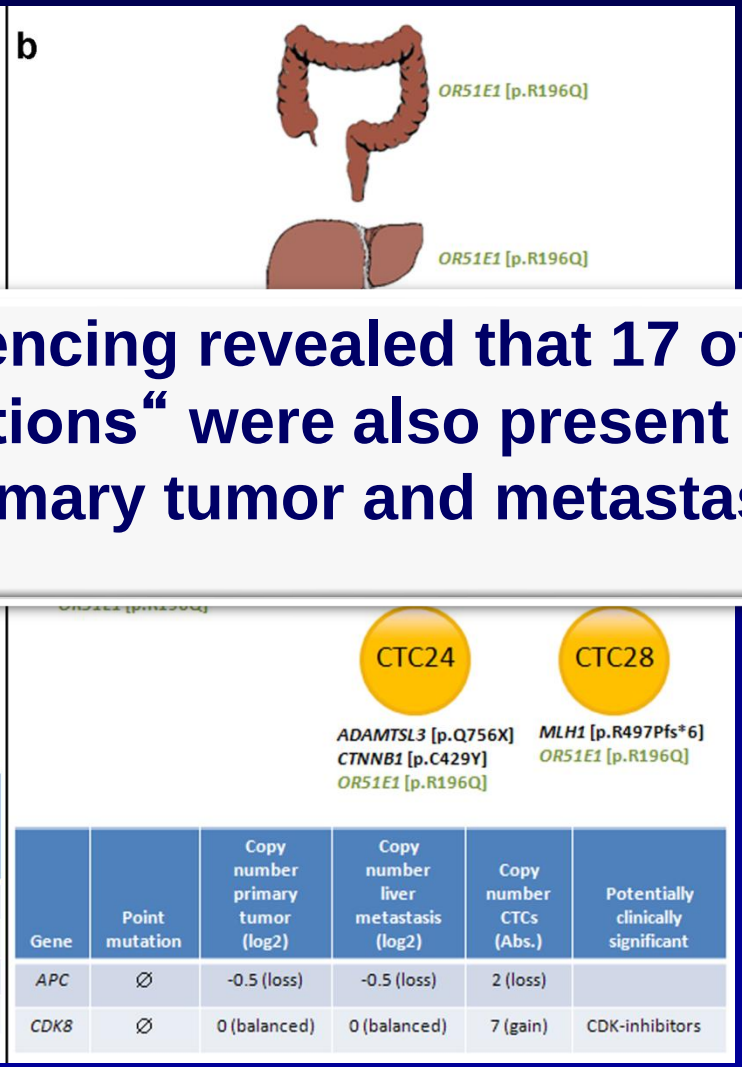
Distribution of mutations in primary tumor, metastases and CTC

CRC patient #6



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	

CRC patient #26



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

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

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

Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay

Irène Baccelli, Andreas Schneeweiss, Sabine Riethdorf, Albrecht Stenzinger, Anja Schillert, Vanessa Vogel, Corinna Klein, Massimo Saini, Tobias Bäuerle, Markus Wallwiener, Tim Holland-Letz, Thomas Höfner, Martin Sprick, Martina Scharpff, Frederik Marmé, Hans Peter Sinn, Klaus Pantel, Wilko Weichert & Andreas Trumpp

nature biotechnology

Potential Metastasis-initiating Cells:

$EPCAM^{low}$, $CD44^{+}$, $CD47^{+}$ and $cMET^{+}$

Functional analyses of CTCs in xenograft assays

ARTICLES

nature medicine

Tumorigenicity and genetic profiling of circulating tumor cells in small-cell lung cancer

Cassandra L Hodgkinson[1,7], Christopher J Morrow[1,7], Yaoyong Li[2], Robert L Metcalf[1], Dominic G Rothwell[1], Francesca Trapani[1], Radoslaw Polanski[1], Deborah J Burt[1], Kathryn L Simpson[1], Karen Morris[1], Stuart D Pepper[3], Daisuke Nonaka[4], Alastair Greystoke[1,4,5], Paul Kelly[1], Becky Bola[1], Matthew G Krebs[1], Jenny Antonello[1], Mahmood Ayub[1], Suzanne Faulkner[1], Lynsey Priest[1], Louise Carter[1], Catriona Tate[1], Crispin J Miller[2,6], Fiona Blackhall[4,5,8], Ged Brady[1,8] & Caroline Dive[1,8]

Science
AAAS

Ex vivo culture of circulating breast tumor cells for individualized testing of drug susceptibility

Min Yu *et al.*

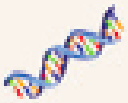
Science **345**, 216 (2014);
DOI: 10.1126/science.1253533

Review

Cancer Research

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

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

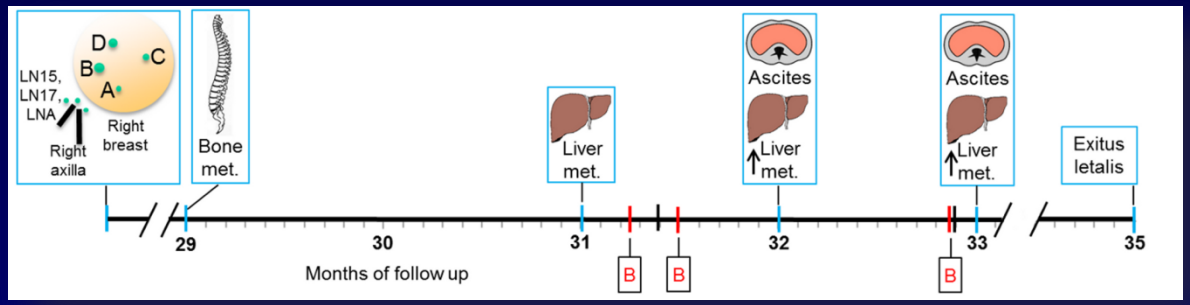
Targets	CTCs 	ctDNA  <  < 
Origins	Selected viable tumor cells leaving actively the primary tumor and/or metastases	Necrotic and apoptotic tumor cells

Why should the analysis of DNA fragments released from apoptotic/necrotic cells reveal important information on resistant tumor cells surviving therapy?

(Schwarzenbach, Hoon, Pantel, Nat. Rev. Cancer 2011; Pantel *et al*., Nature Med., 2013; Speicher & Pantel, Nat. Biotech. 2014)

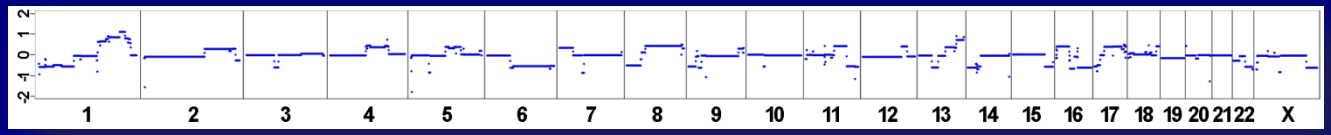
Comparative analysis of CTCs and ctDNA in breast cancer

1. Progressive disease with increasing liver metastases and ascites – no chemoT



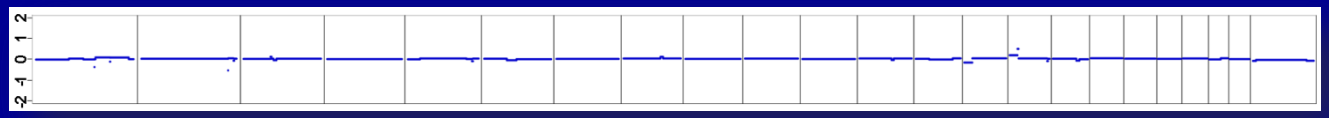
2. Excessive numbers of CTCs (~50.000/7.5 ml) in three blood samples; each with multiple homogeneous copy number changes and mutations in CTCs

CTC



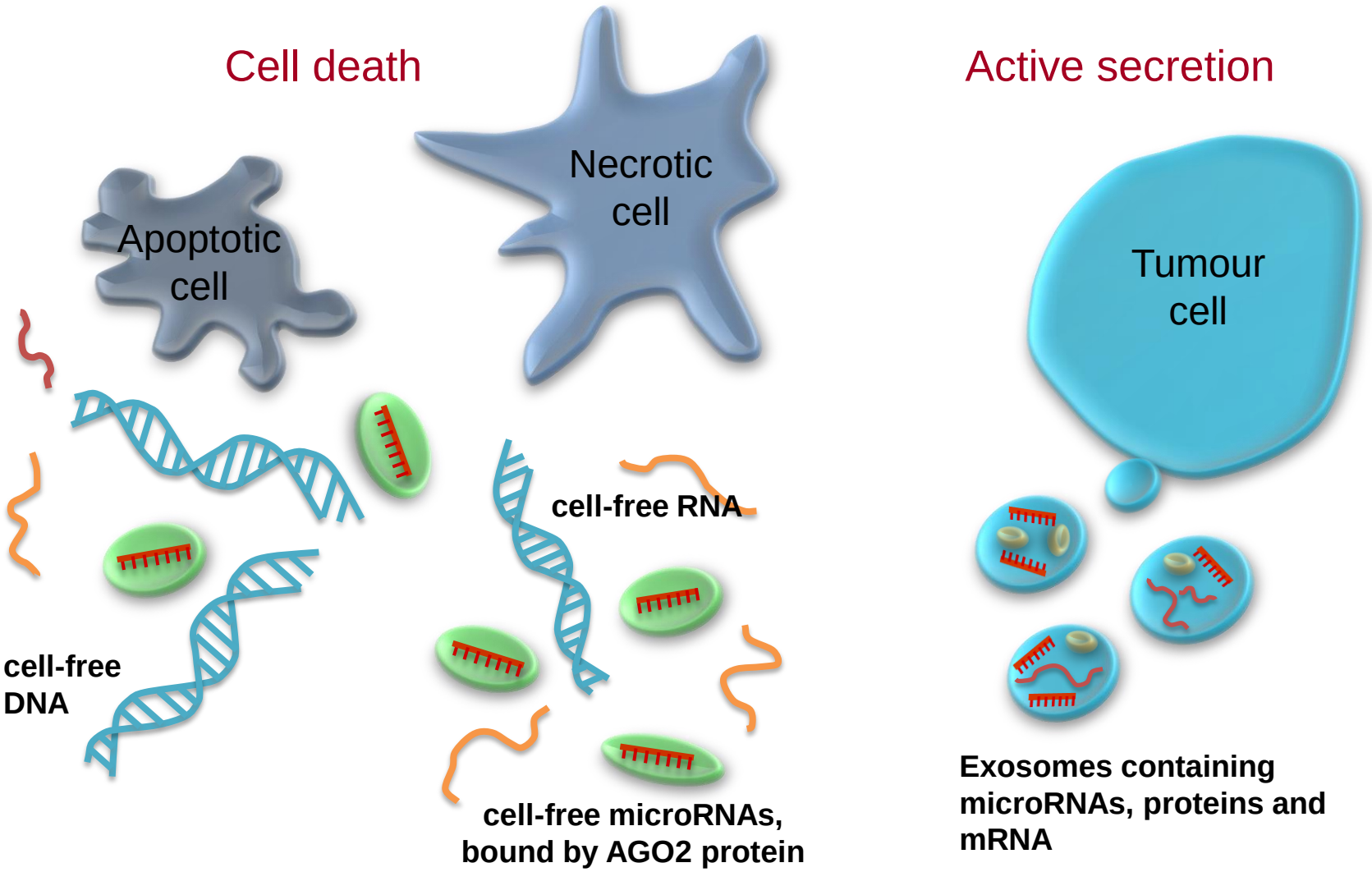
3. However, very low concentration of ctDNA fragments at each measurement

ctDNA

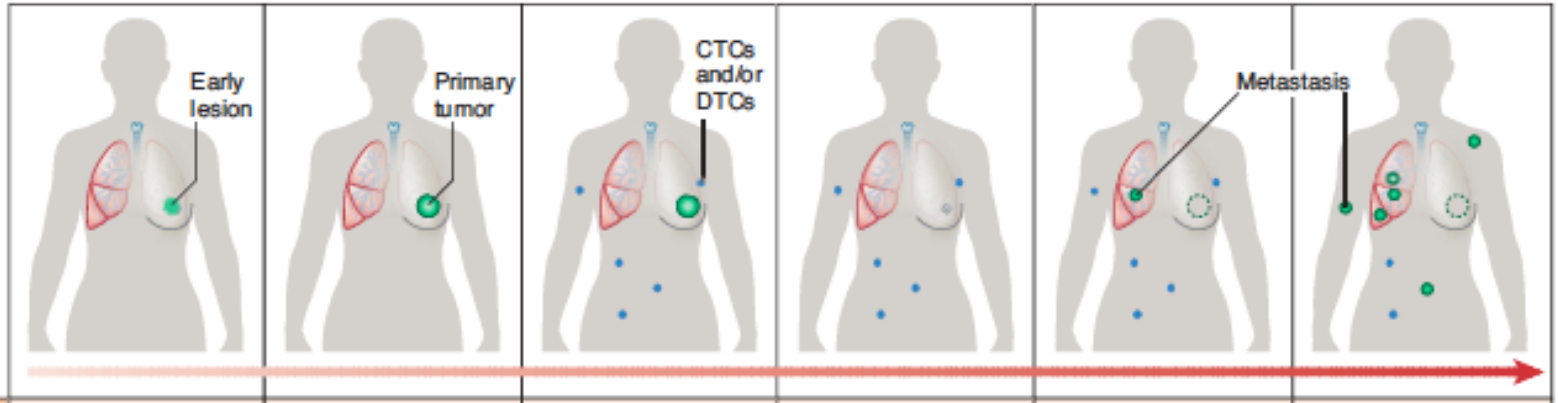


Heidary, Speicher, Pantel et al, *Breast Cancer Res. 2014*

Release of microRNAs into the blood circulation



Schwarzenbach, Nishida, Calin, Pantel, Nat. Rev. Clin. Oncol. 2014



Status	Pre-neoplasm Subclinical	Primary (–) CTCs and/or DTCs	Primary (+) CTCs and/or DTCs	Dormancy	Oligometastases	Systemic metastases
Focus	Management of primary tumor		Prevention of metastasis		Treatment of metastasis	
Challenge	Early detection and prevention Identify high-risk patients		Prevent local and distant relapse Drug resistance of DTCs		Early detection of relapse Heterogeneity and drug resistance	
New tools	Diagnostic markers	Prognostic markers	Profiling of primary tumor, metastases, CTCs and/or DTCs for accurate targeting Biomarkers and imaging technologies for disease monitoring Biomarkers for therapeutic efficacy			
Possible treatment strategies	Prophylactic treatment Vaccination	Surgery, radiotherapy (+) Systemic therapy		Targeted therapy against driver oncogenes and their pathways tailored by genetic makeup of tumor cells		
			Long-term adjuvant treatment (for high-risk patients): • Metronomic chemotherapy and anti-angiogenesis • Targeting common driver oncogenes and pathways • Immunotherapy • Targeting dormancy-related survival and CSC signaling and niche components		Systemic therapy Immunotherapy Stroma-targeting treatments Palliative radiation and/or surgery	
					Surgery stereotactic radiotherapy	
Possible new targets		DTC and/or CTC survival pathways; stem cell features; tumor-stroma crosstalk and niche factors Activation of metastasis-suppressive signaling				

Debbie Maizels

Universitätsklinikum Hamburg-Eppendorf

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Institute of Tumor Biology - **Klaus Pantel**

ERC Advanced Investigator Grant „DISSECT“ (2011-2016)

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- He
- Harriet Wikman/Michaela Wrage
- Katharina Effenberger
- Juliane Hannemann/Simon Joosse
- Kai Bartkowiak, Natalia Bednarz-Koll

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Sander-Stiftung

Roggenbuck-Stiftung

Micrometastasis Research Network at UCCH/UKE

EU-Consortium-DISMAL

Start: November 2005 Coordinator: Klaus Pantel

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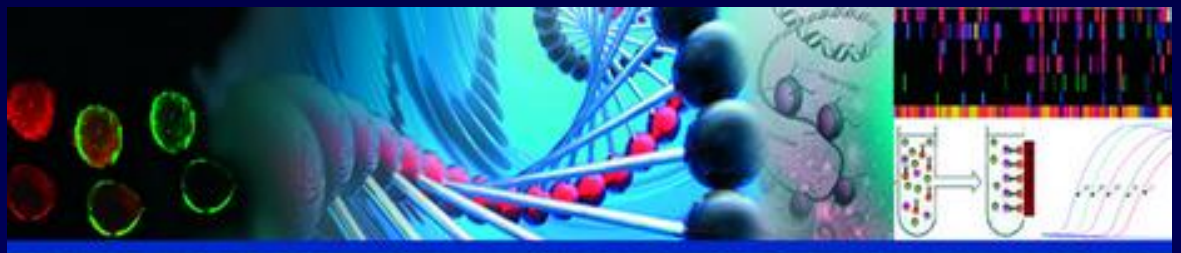

Advances in Circulating Tumour Cells (ACTC): from Basic Research to Clinical Practice

Save the Date

October 8th - 11th, 2014

Crete, Greece

Organizers:

- *Evi S. Lianidou, Department of Chemistry, University of Athens, Greece*
- *Dimitris Mavroudis, School of Medicine, University of Crete - Department of Medical Oncology, University General Hospital of Heraklion, Greece*
- *Klaus Pantel, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany*
- *Hellenic Oncology Research Group*

www. actc2014.org

Organizers:

Evi Lianidou, Athens

Dimitris Mavroudis, Crete

Klaus Pantel, Hamburg

Klaus Pantel, MD, PhD

See you in 2015 in Hamburg, Germany!