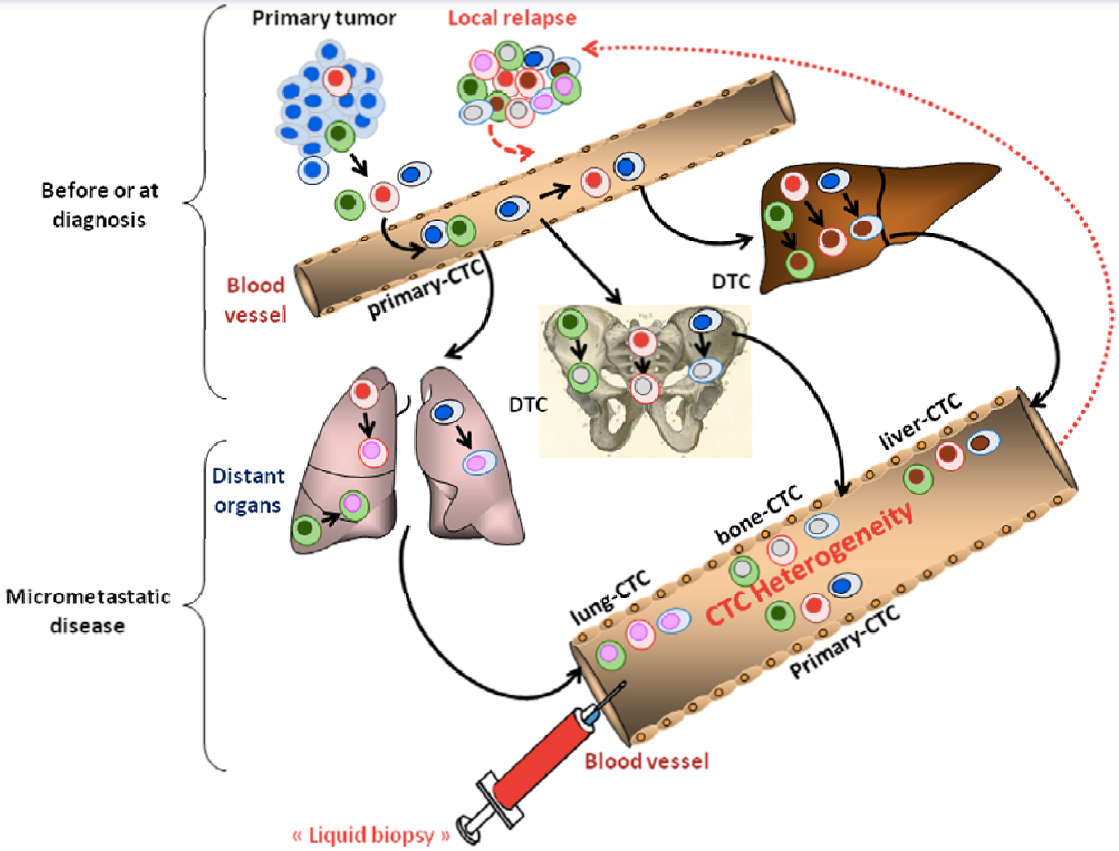
Universitätsklinikum Hamburg-Eppendorf

Institute for Tumor Biology

Klaus Pantel

"Liquid Biopsy": Current Status and Future Perspective

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*, 2012

Detection of CTC in the peripheral blood

September 2013:

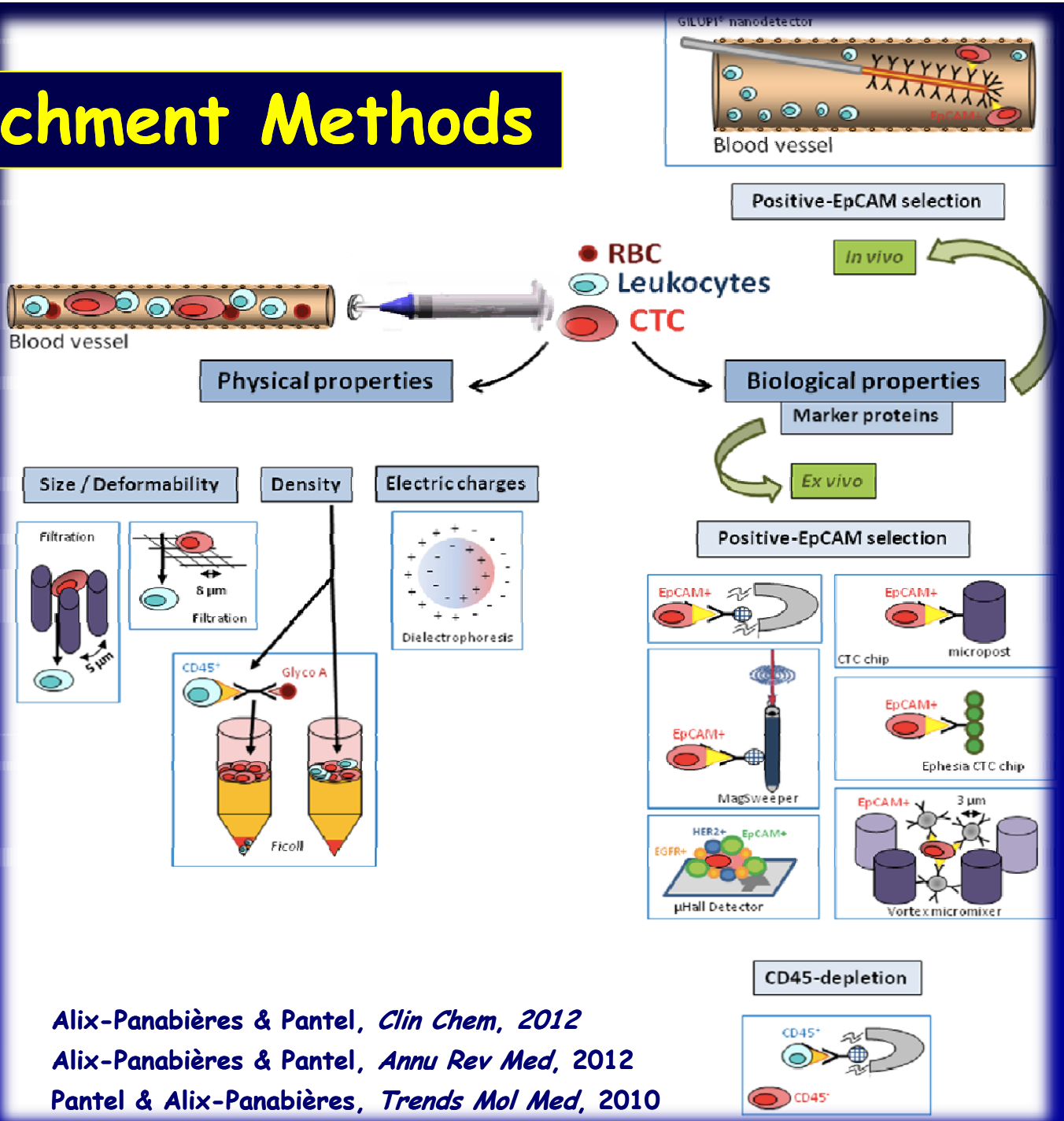
> 400 registered clinical trials with CTC as biomarkers

CTC Enrichment Methods

2013: > 50 different CTC assays !

The technical challenge:

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

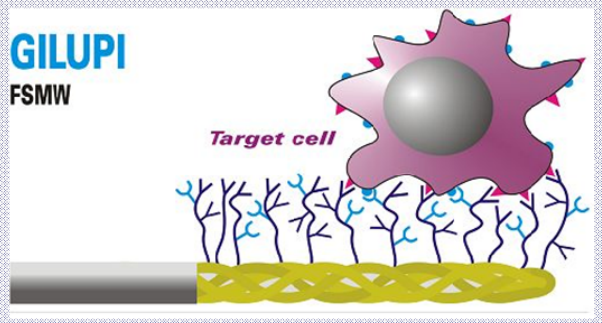


Alix-Panabières & Pantel, *Clin Chem*, 2012

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

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

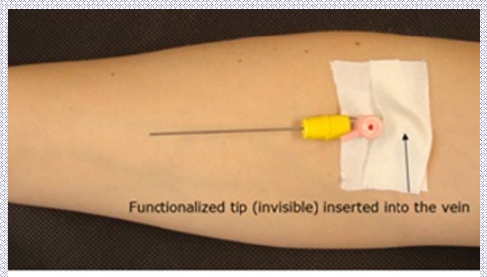
New approach: In vivo capture of CTC (1.5 L blood)

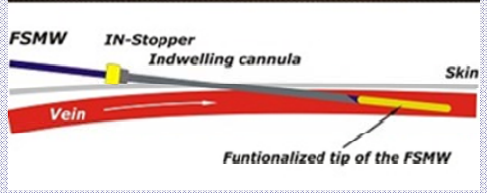


Nanodetector

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

30 minutes exposure time in a vein





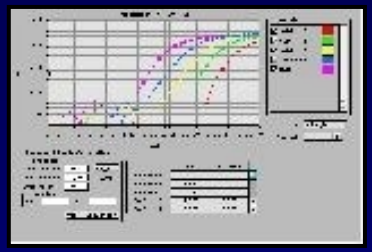
Diagnostics
- *cytology*
- *PCR, etc.*

Result ← *Decision*

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

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

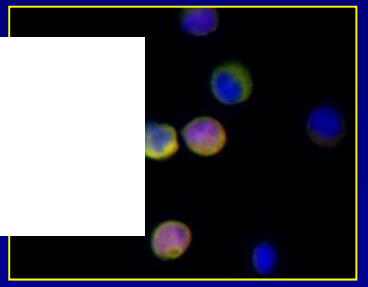
mRNA

DNA

intra-cytoplasmic proteins

> 13,000 publications on CTC in PubMed 2013

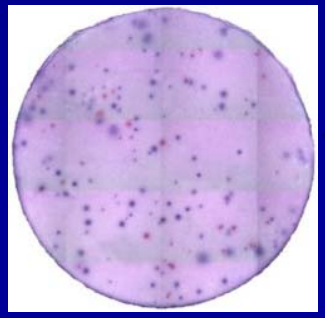
mRNA



Immunocytochemistry

Tumor cell

secreted proteins by VIABLE cells



EPISPOT assay

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

Design of robust automated systems for reproducible CTC detection

CellSearch™ System (FDA-cleared)

cellsave™ Preservative Tube

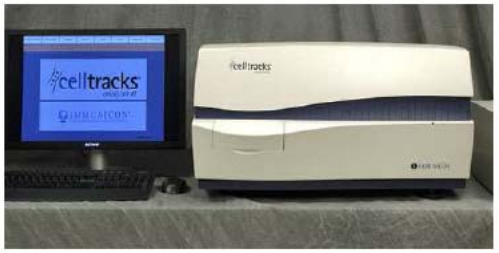
celltracks™ autoprep system

MagNest™

CellSearch™ Epithelial Cell Kit

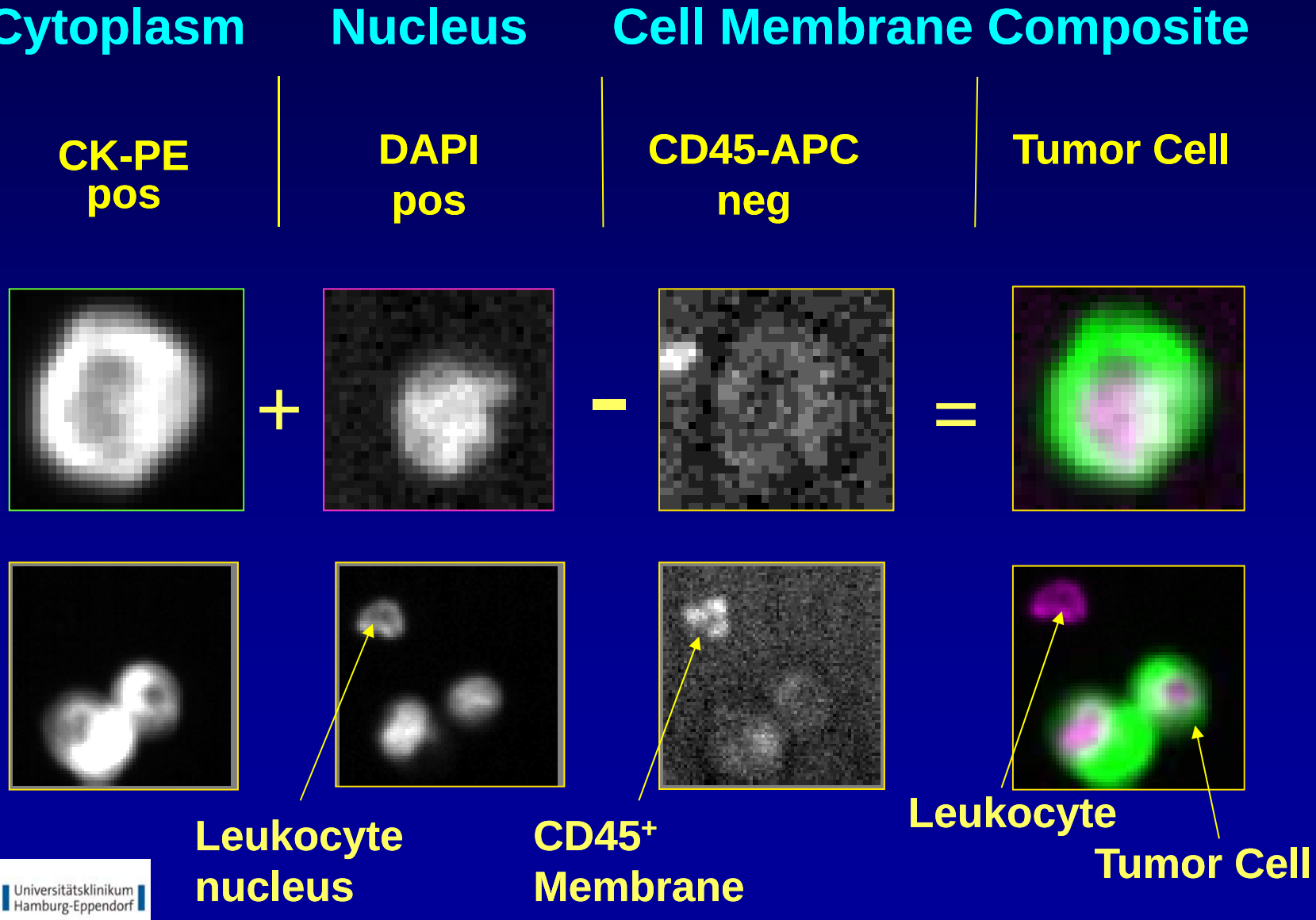
Enrichment of CTC with anti-EpCAM ferro fluids

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

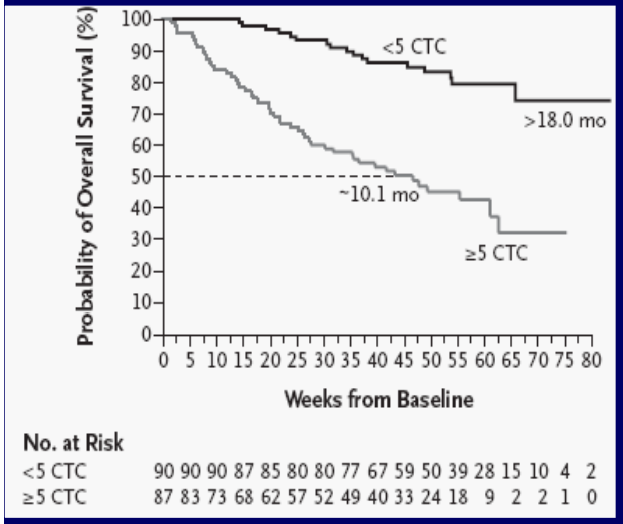
CellSearch™ System: Images of Tumor Cells



Prognostic value of CTC counts for survival in cancer patients with advanced disease

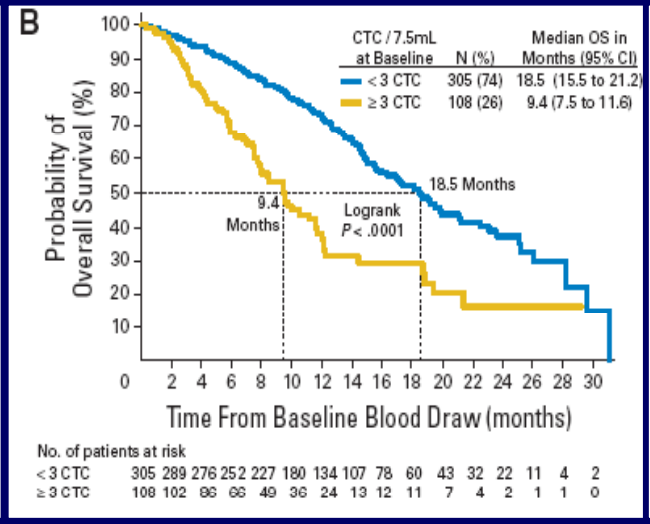
Breast Cancer

Christofanilli, NEJM, 2004



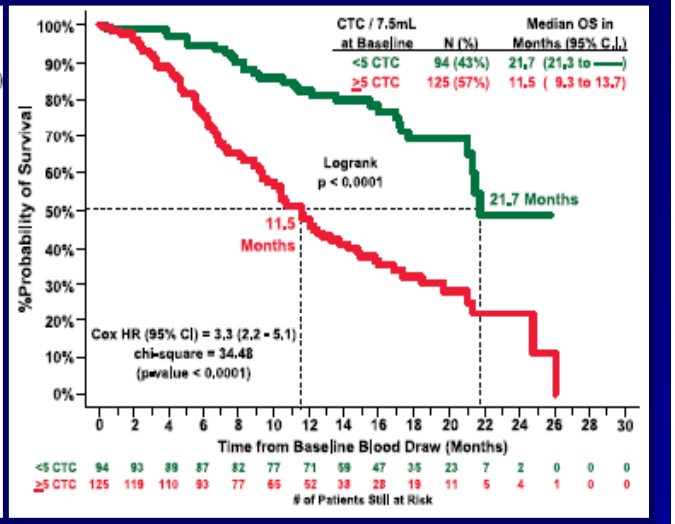
Colorectal Cancer

Cohen, JCO, 2008



Prostate Cancer

De Bono, Clin Can Res, 2008



→ FDA clearance

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

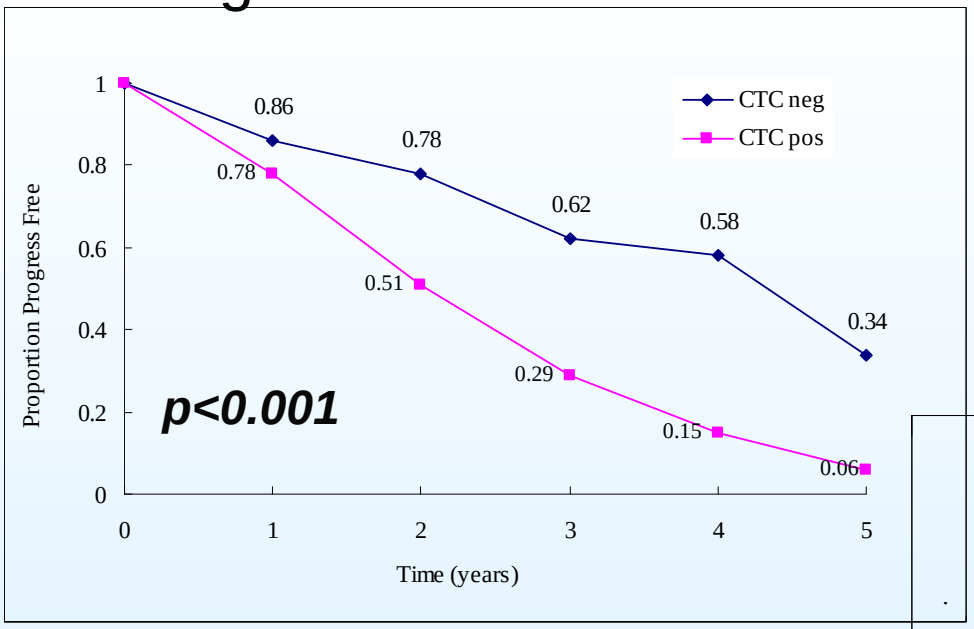
SUCCESS

*** P < 0.05**

KLINIKUM

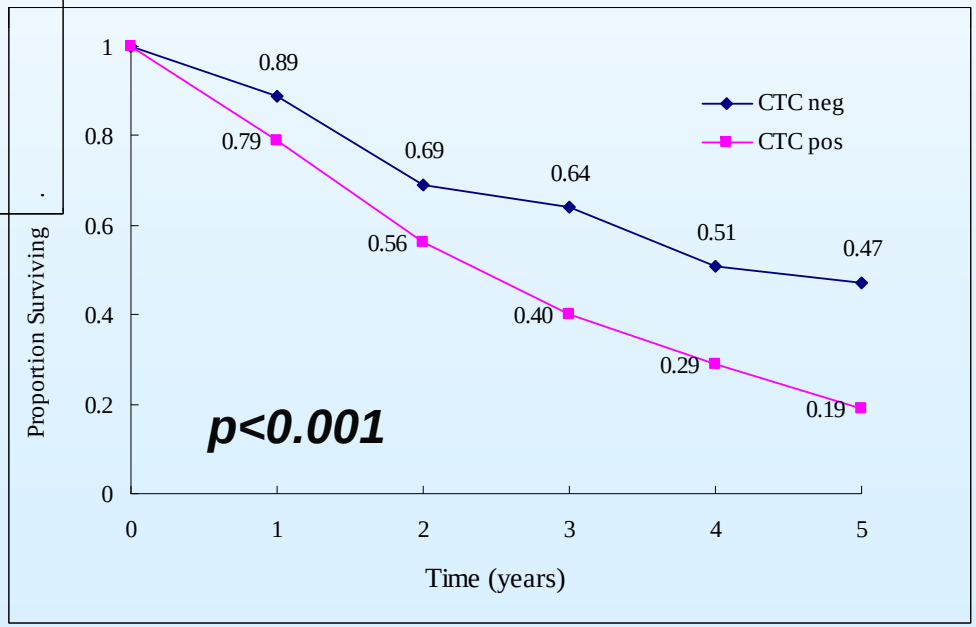
Meta-Analysis of 49 studies comprising 6815 breast cancer patients

Progression-free survival



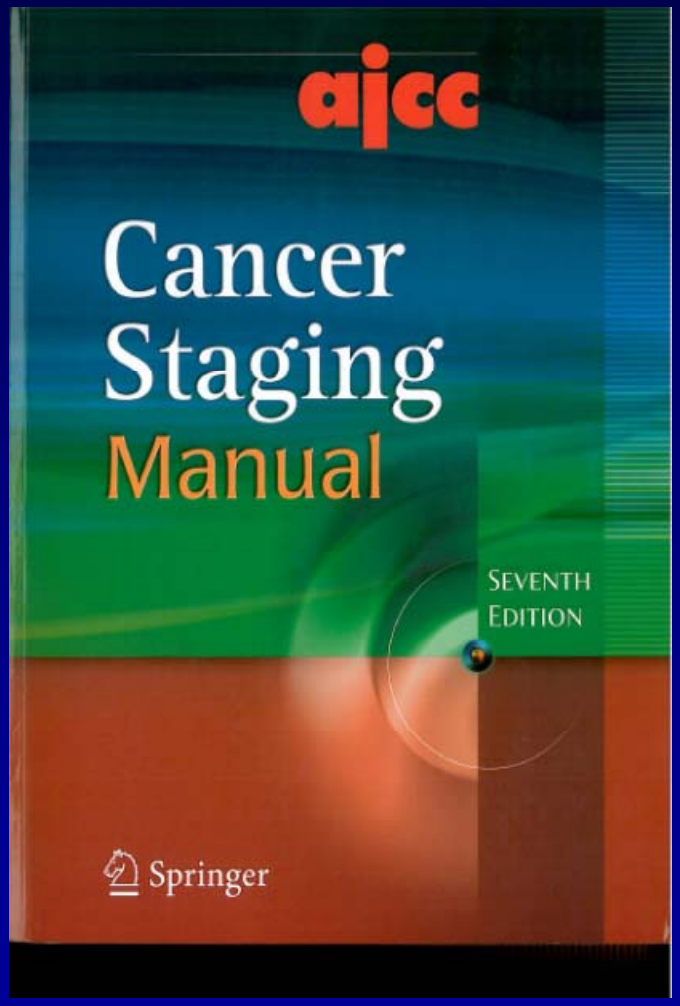
CTC detection: ICC & RT-PCR

Overall survival



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

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

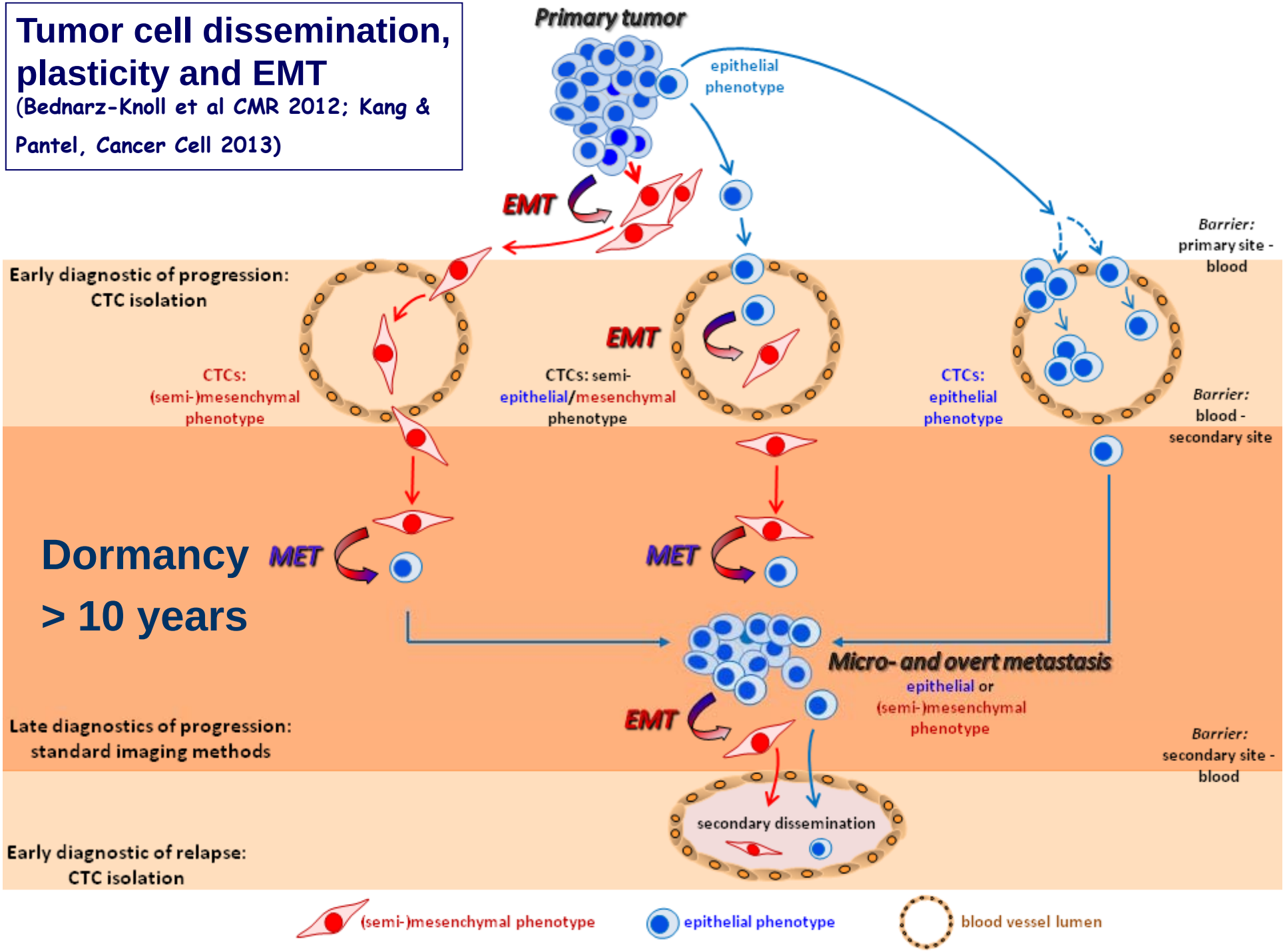
Challenge of CTC detection:

Epithelial-Mesenchymal Transition (EMT)

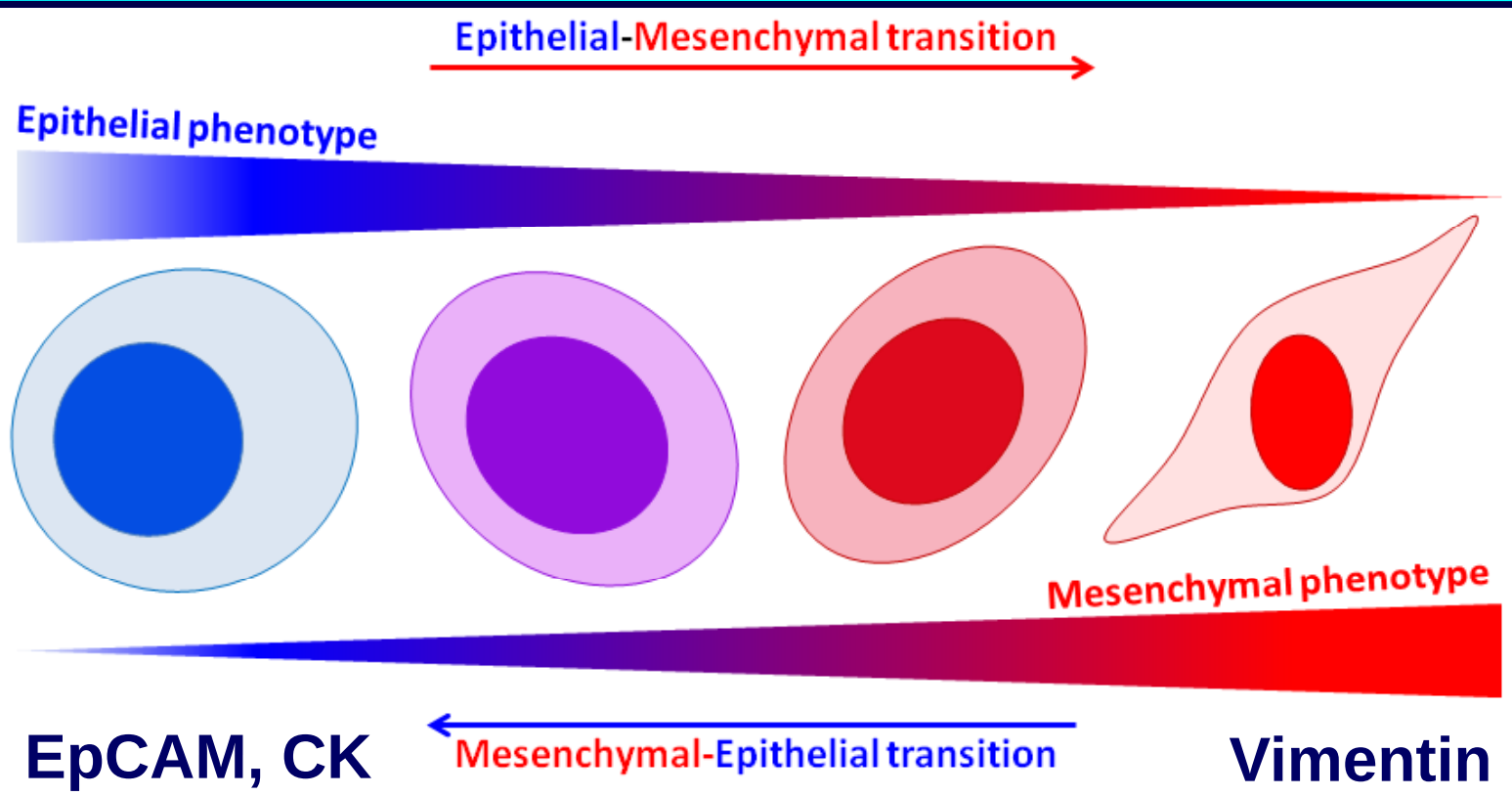
of carcinoma cells

Tumor cell dissemination, plasticity and EMT

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



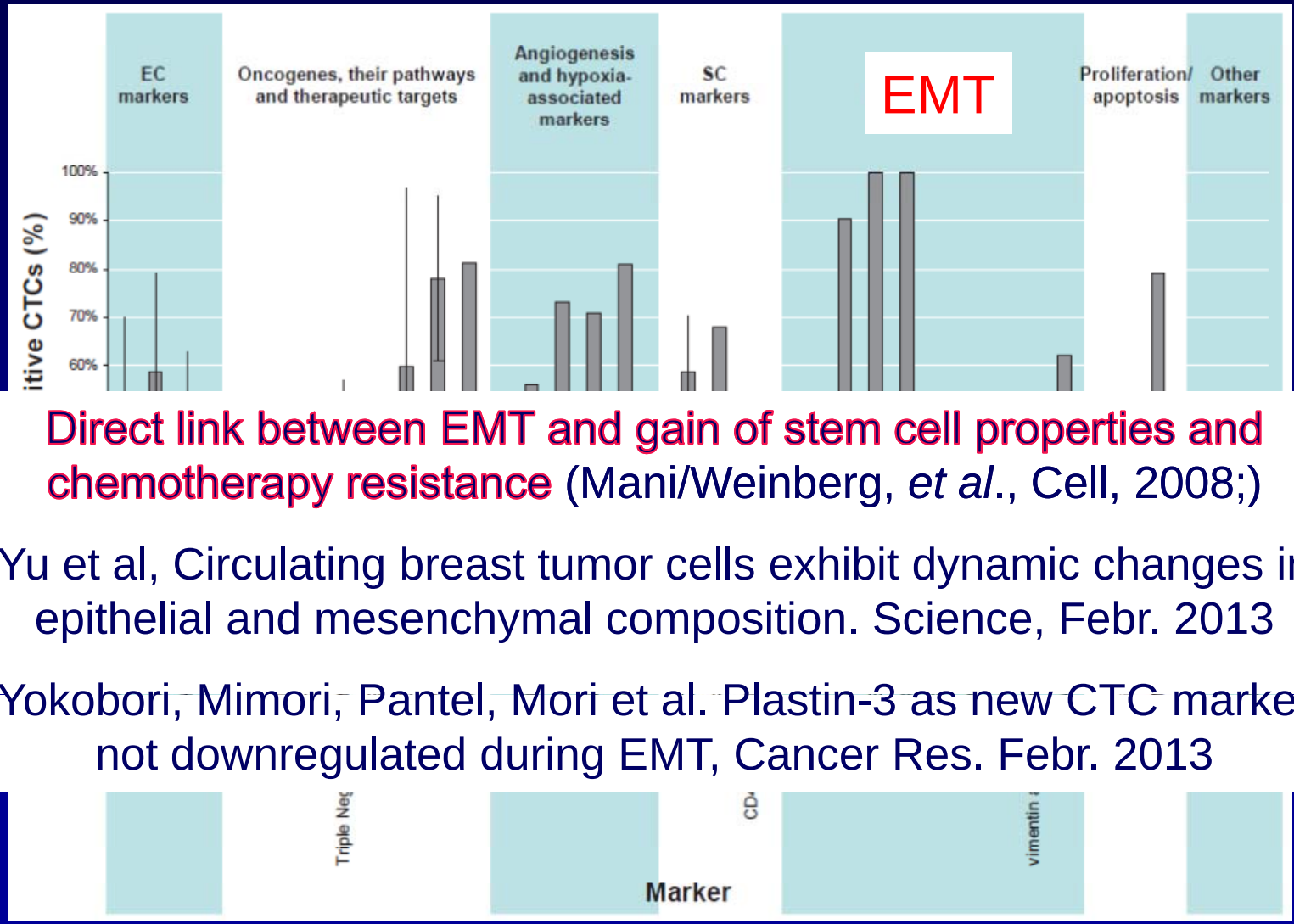
Epithelial-Mesenchymal Plasticity of CTC



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-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

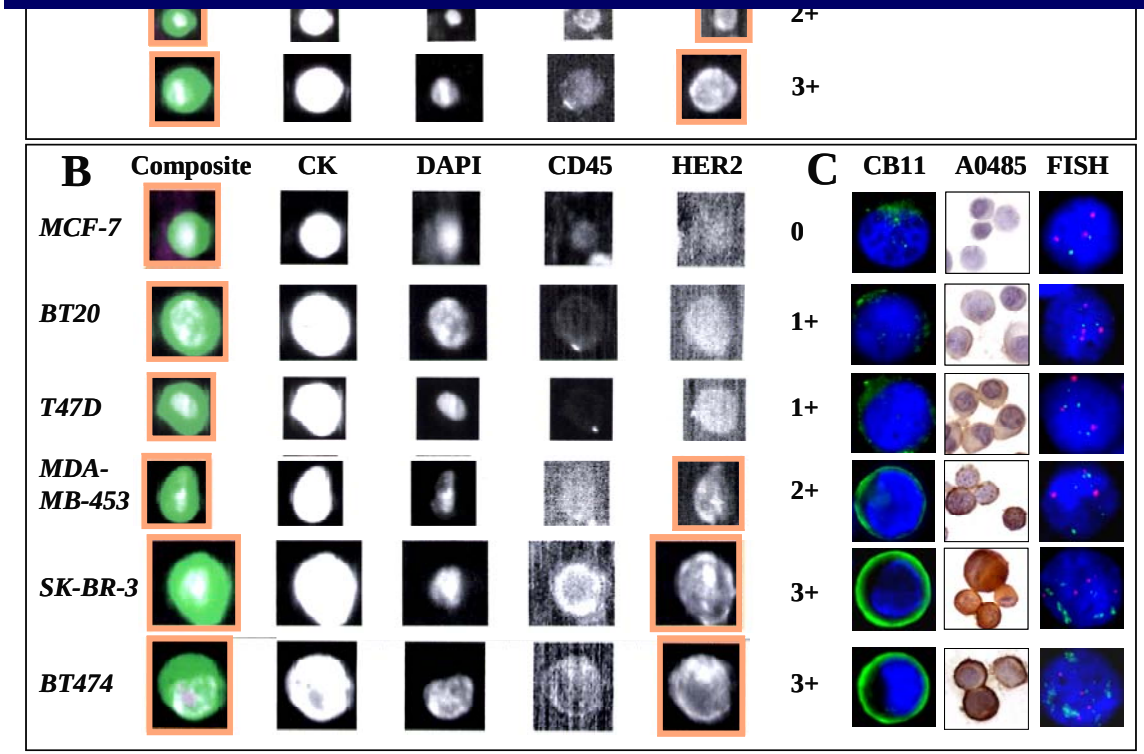
Bednarz-Knoll, Alix-Panabieres & Pantel, 2011, Breast Cancer Res, 13: 282-293

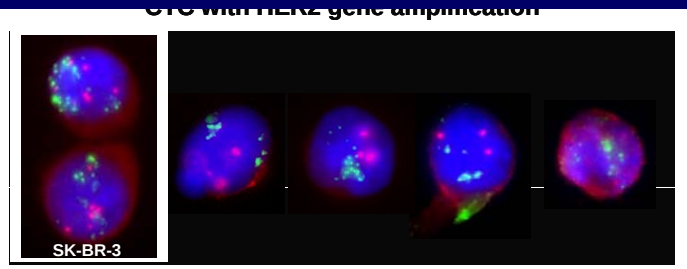
Molecular Characterization of CTC for therapeutic targets

(„real-time liquid biopsy“)

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



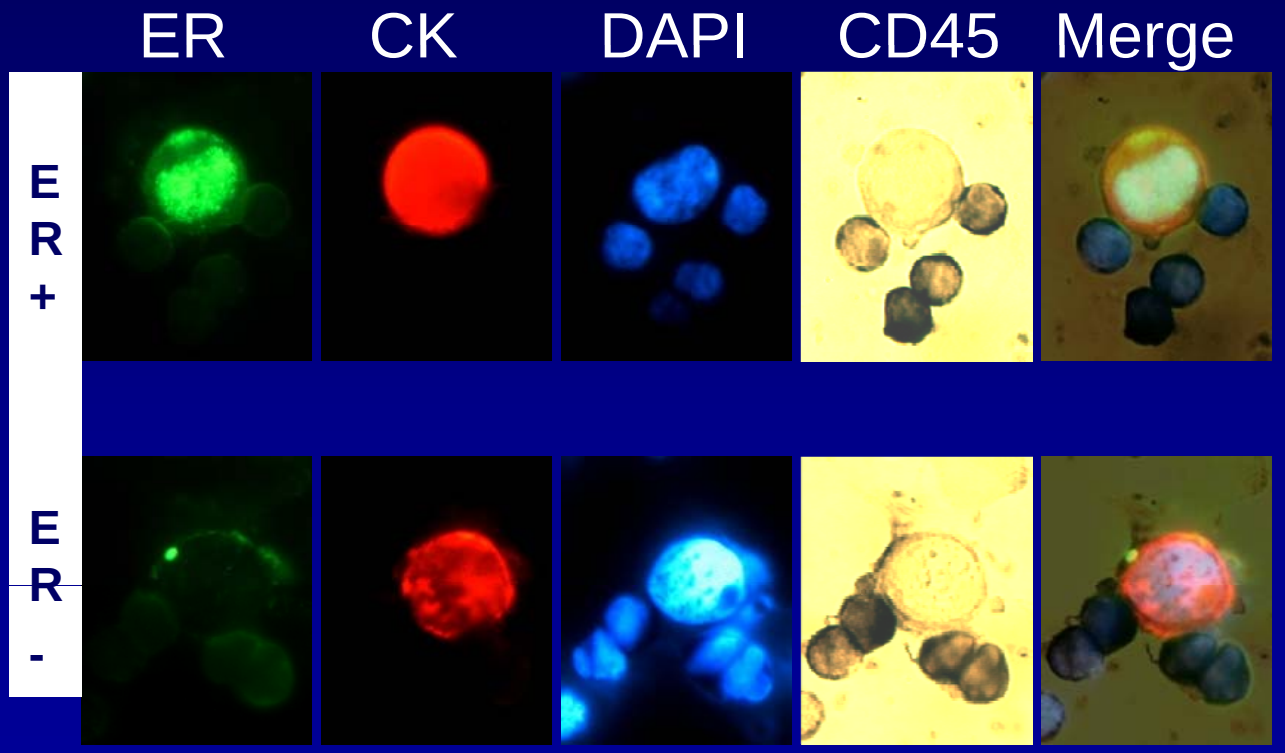


Discordance between HER2 status of primary tumor and CTC

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

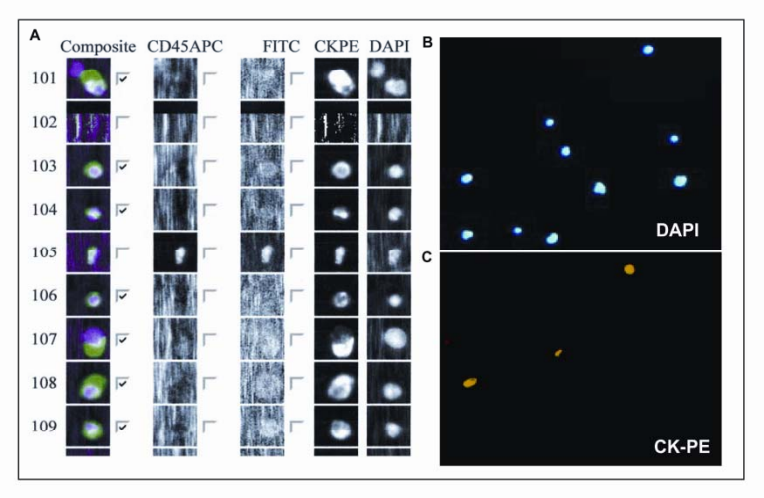
Babayan, Joosse, Pantel et al., PLOS ONE 2013



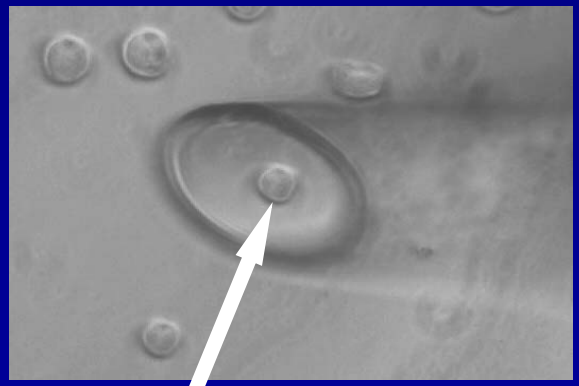
ER-negative CTCs may survive endocrine therapy

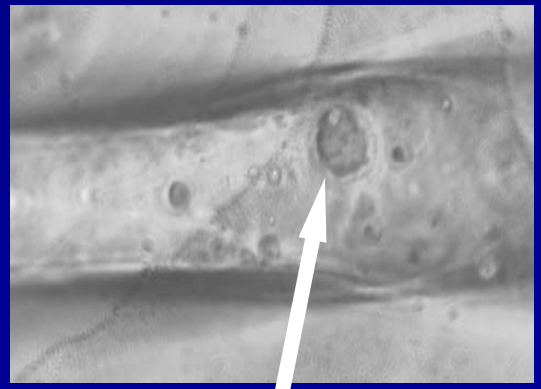
Genomic Characterization of single CTC

CTC detection



CTC isolation





CTC

Capillary

CTC

WGA +

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

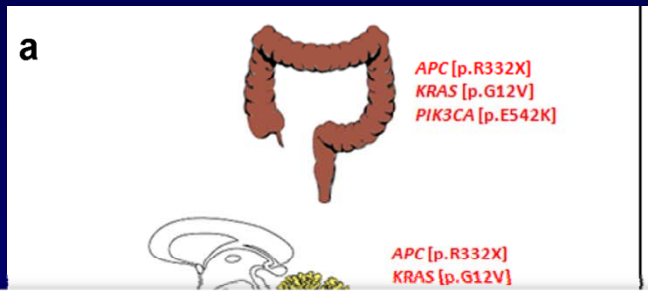
Detection of mutations in genes relevant for resistance of targeted therapies (*eg*, EGFR inhibition)

Patient	No. Of CTCs	*P53*			*KRAS*			*BRAF*			*PIK3CA*		
		WT	MUT	n.a.	WT	MUT	n.a.	WT	MUT	n.a.	WT	MUT	n.a.
1	38	18	2	18	13	7	18	14	-	24	8	9	21
2	14	10	-	4	9	-	5	9	-	5	6	1	7
3	6	5	-	1	5	-	1	1	-	5	4	1	1
4	11	9	2	-	7	-	4	2	-	9	4	-	7
5	28	7	15	6	21	-	7	20	-	8	20	3	5
6	10	6	-	4	5	-	5	4	-	6	5	3	2
7	4	-	-	4	1	-	3	-	1	3	1	2	1
8	4	4	-	-	4	-	-	4	-	-	4	-	-
9	2	2	-	-	2	-	-	2	-	-	2	-	-
10	3	2	-	1	2	1	-	1	-	2	-	1	2
11	13	2	4	7	7	-	6	7	-	6	6	-	7
12	5	3	-	2	3	-	2	2	-	3	2	1	2
13	3	2	-	1	2	-	1	2	-	1	2	-	1
Total	141	70	23	48	81	8	52	68	1	72	64	21	56

Gasch, Pantel, Riethdorf et al., Clin. Chem. 2012

Distribution of mutations in primary tumor, metastases and CTC

CRC patient #6



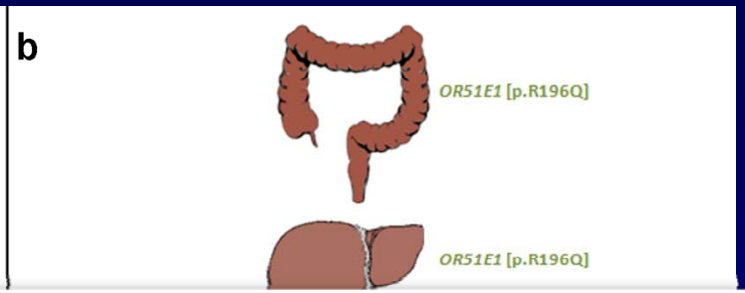
LAMA1 [p.H1002Y]
NF1 [p.R135W]
PIK3CA [p.E542K]
TP53 [p.R141C]

TP53 [p.R141C]

GNAS [p.G869D]
GUCY1A2 [p.H439Y]
KRAS [p.G12V]
NF1 [p.R135W]
PIK3CA [p.E542K]
TP53 [p.R141C]

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



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

CTC24

ADAMTSL3 [p.Q756X]
CTNNB1 [p.C429Y]
OR51E1 [p.R196Q]

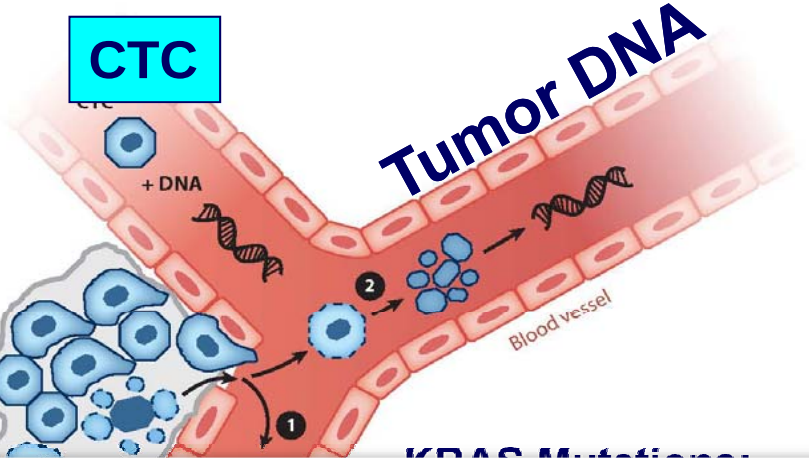
CTC28

MLH1 [p.R497Pfs*6]
OR51E1 [p.R196Q]

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

Tumor-associated circulating cell-free nucleic acids in blood



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

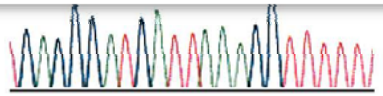
Prostate Cancer: Schwarzenbach, Alix-Panabieres, Pantel et al., Clin Cancer Res 2009; **Breast cancer**: Dawson et al, NEJM, 2013; **Colon Cancer**: Heitzer, Pantel et al, Int J Cancer, 2013

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)

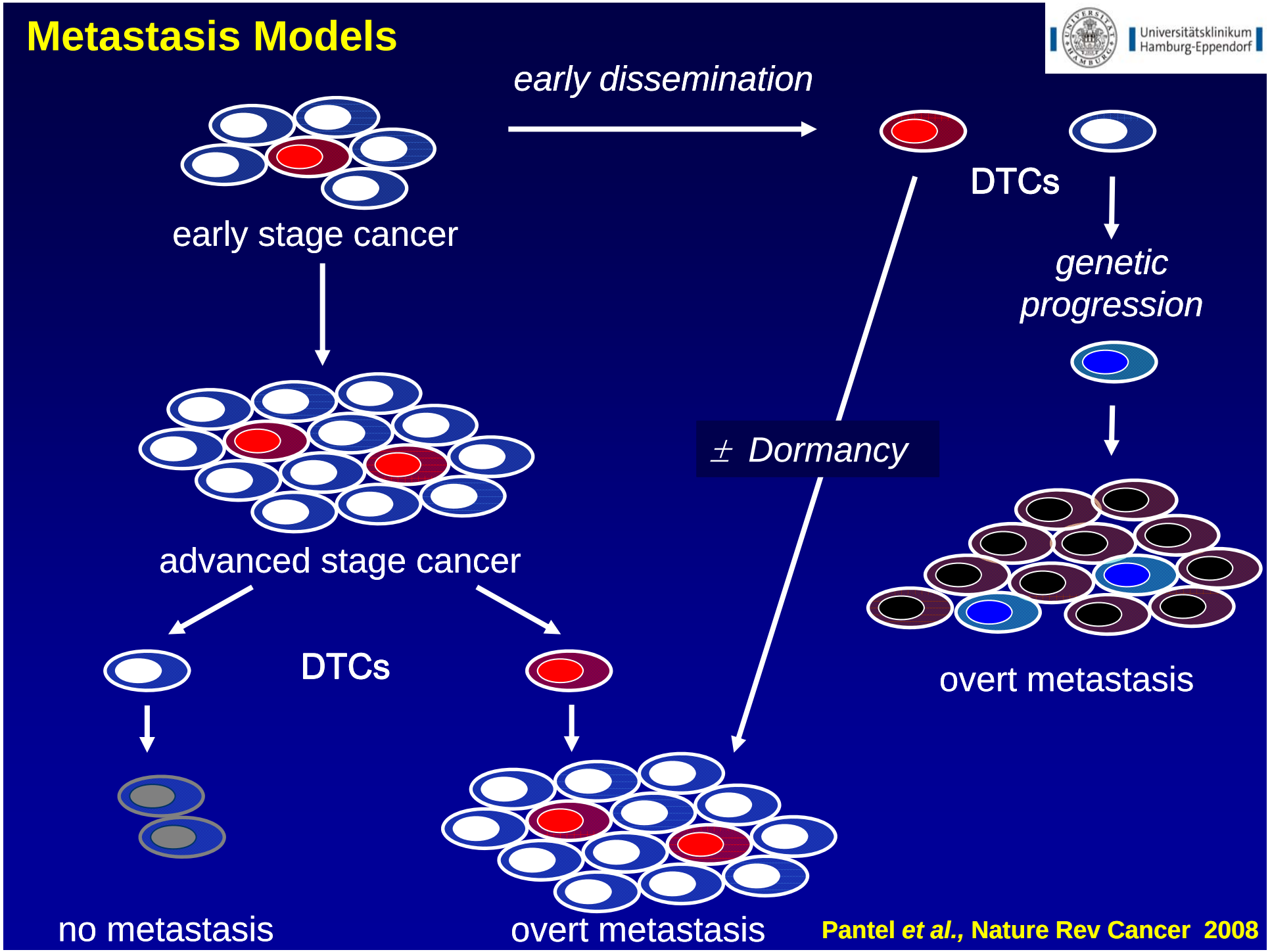


Schwarzenbach/Hoon/Pantel, *Nat Rev Cancer,* 2011, Alix-Panabières/Schwarzenbach/Pantel, *Annu Rev Med*, 2012; Pantel & Alix-Panabieres, Cancer Res., 2013

Aims of Research on Circulating Tumor Cells

- 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

Metastasis Models



Pantel *et al.*, Nature Rev Cancer 2008

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

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
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http://dx.doi.org/10.1016/j.ccr.2013.04.017

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

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Roggenbuck-Stiftung

Micrometastasis Research Network at UCCH/UKE

EU-Consortium-DISMAL

Start: November 2005 Coordinator: Klaus Pantel

Free University of Amsterdam
Medical Center (The Netherlands)

University Medical Center
Hamburg-Eppendorf (Germany)

Imperial College London
(United K

Radium Hospital Oslo,

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(United King

Cancer
Center,

University o
(The Nether

ity
(Austria)

Netherland
Cancer Ins
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-Pette-
Germany)

Lapeyro
Montpel

hotonics

SME 3 Agendia,
(The Netherlands)

Leiden University Medical
Center (The Netherlands),

ERC Advanced Investigator Grant „DISSECT“ (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