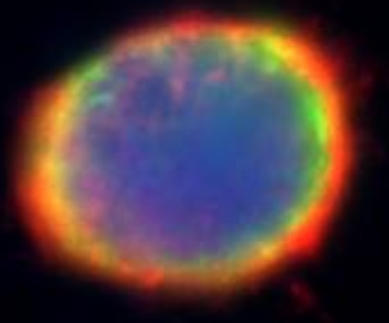
Universitätsklinikum Hamburg-Eppendorf

Liquid Biopsy – Prostate Cancer



Prof. Dr. med. Klaus Pantel

Institut für Tumorbiologie
UKE/UCCH, Hamburg, Germany

Liquid Biopsy: The Concept

Definition: Liquid Biopsy* - Analysis of tumor cells (CTCs) or their products (e.g., DNA, miRNA, extracellular vesicles) in blood or other body fluids

Rationale: Tissue biopsies are invasive and some locations are difficult to access (e.g., lung or brain)

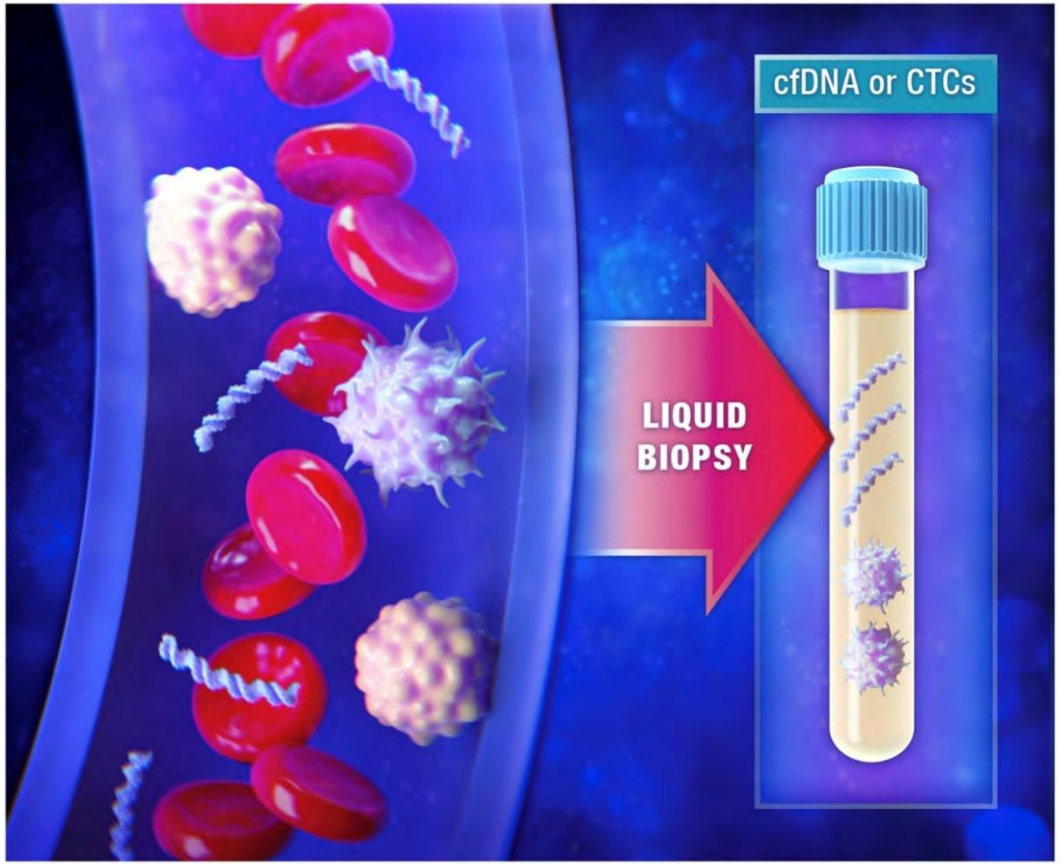
Single biopsy can miss relevant tumor clones due to intra-patient tumor heterogeneity

Sequential tissue biopsies in individual patients for real-time monitoring of therapy response are less feasable in clinical practise

Vision: Comprehensive and real-time tumor information by the analyses of blood (or other body fluids)

*Pantel & Alix-Panabieres, *Trends Mol. Med.* 2010

Liquid Biopsy: Clinical Applications



DIAGNOSIS:
Genotyping cfDNA in the blood to determine the tumor profile

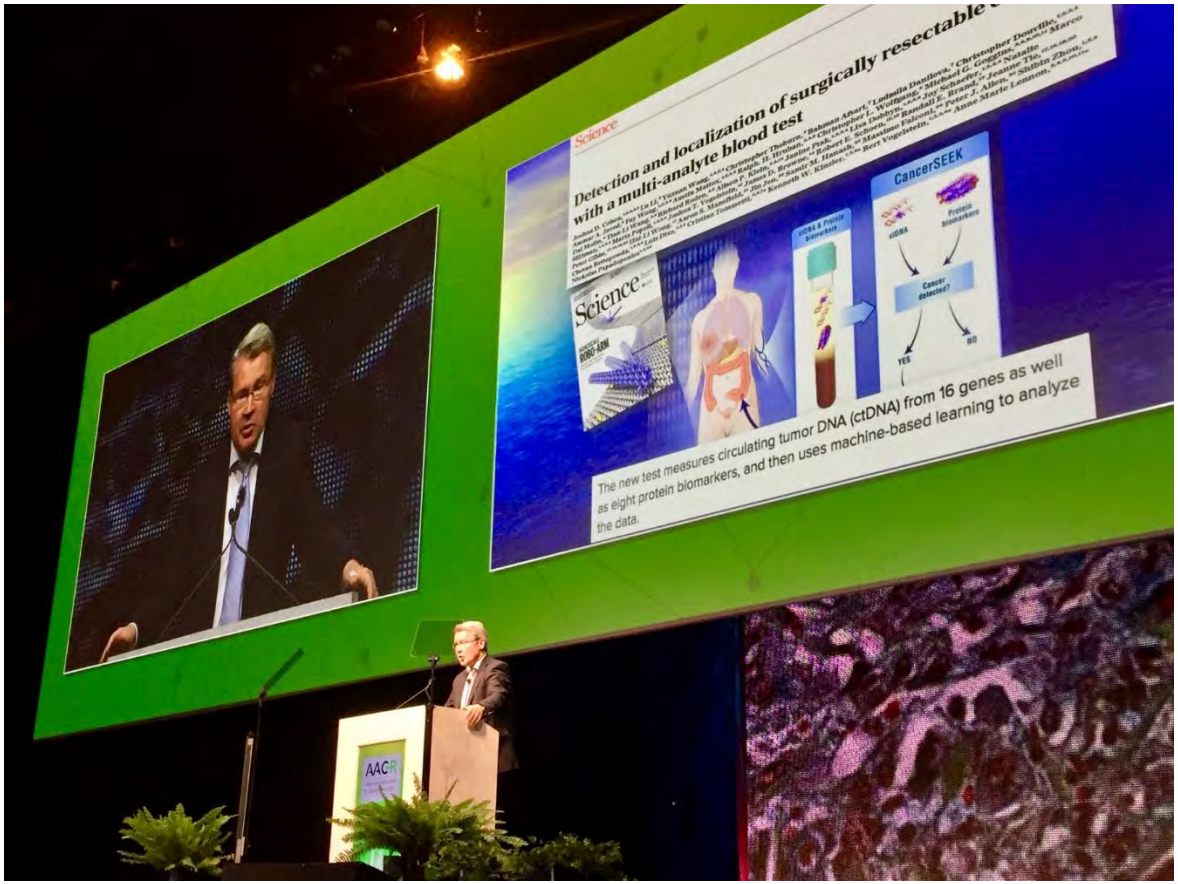
RESPONSE AND FOLLOW UP:
Analysis of cfDNA and CTC for real time monitoring of response to treatment

TUMOR EVOLUTION:
Emergence of molecular alterations associated with resistance to therapy

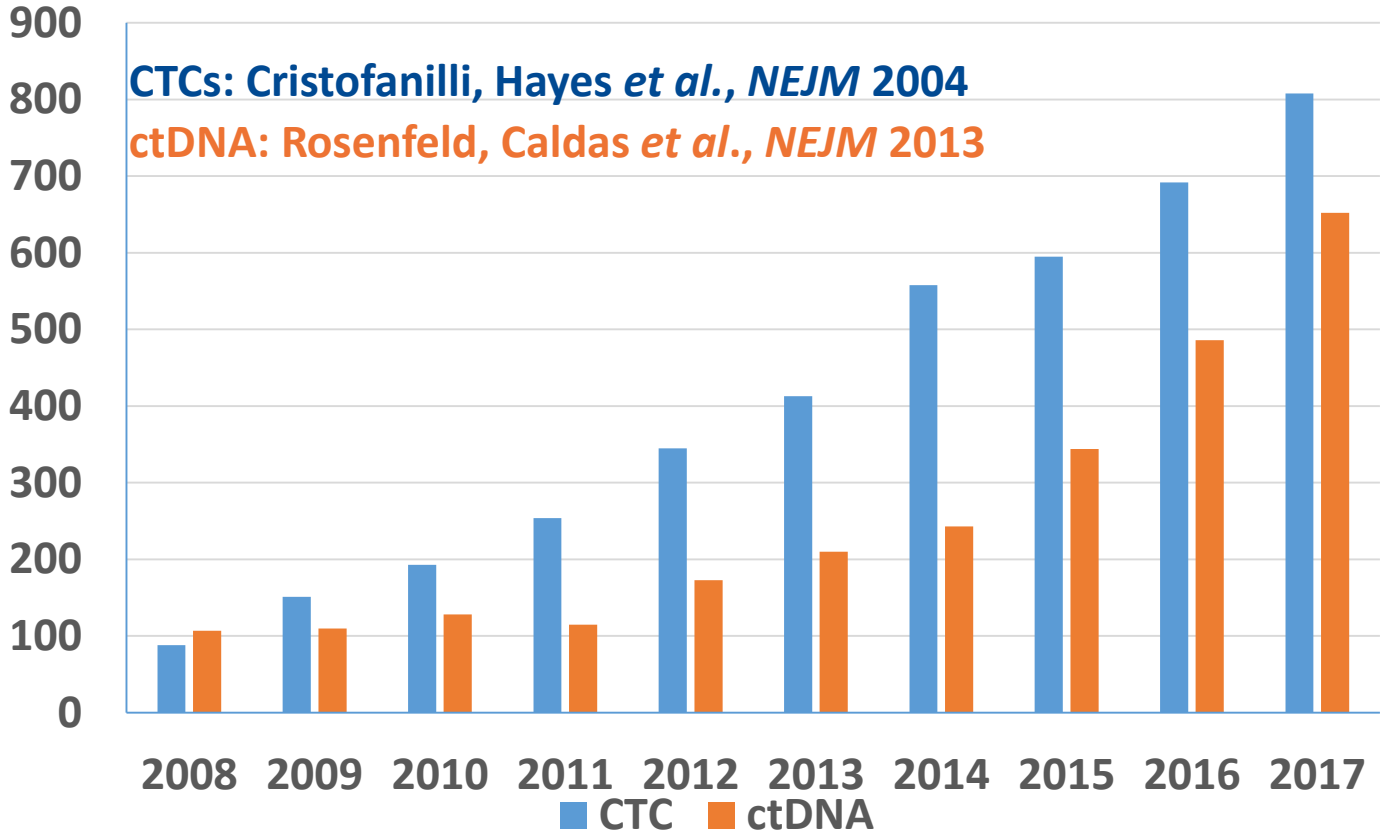
MINIMAL RESIDUAL DISEASE:
The presence of cfDNA or CTC in the circulation indicates that the disease is stll present

Bardelli & Pantel, *Cancer Cell*, 2017

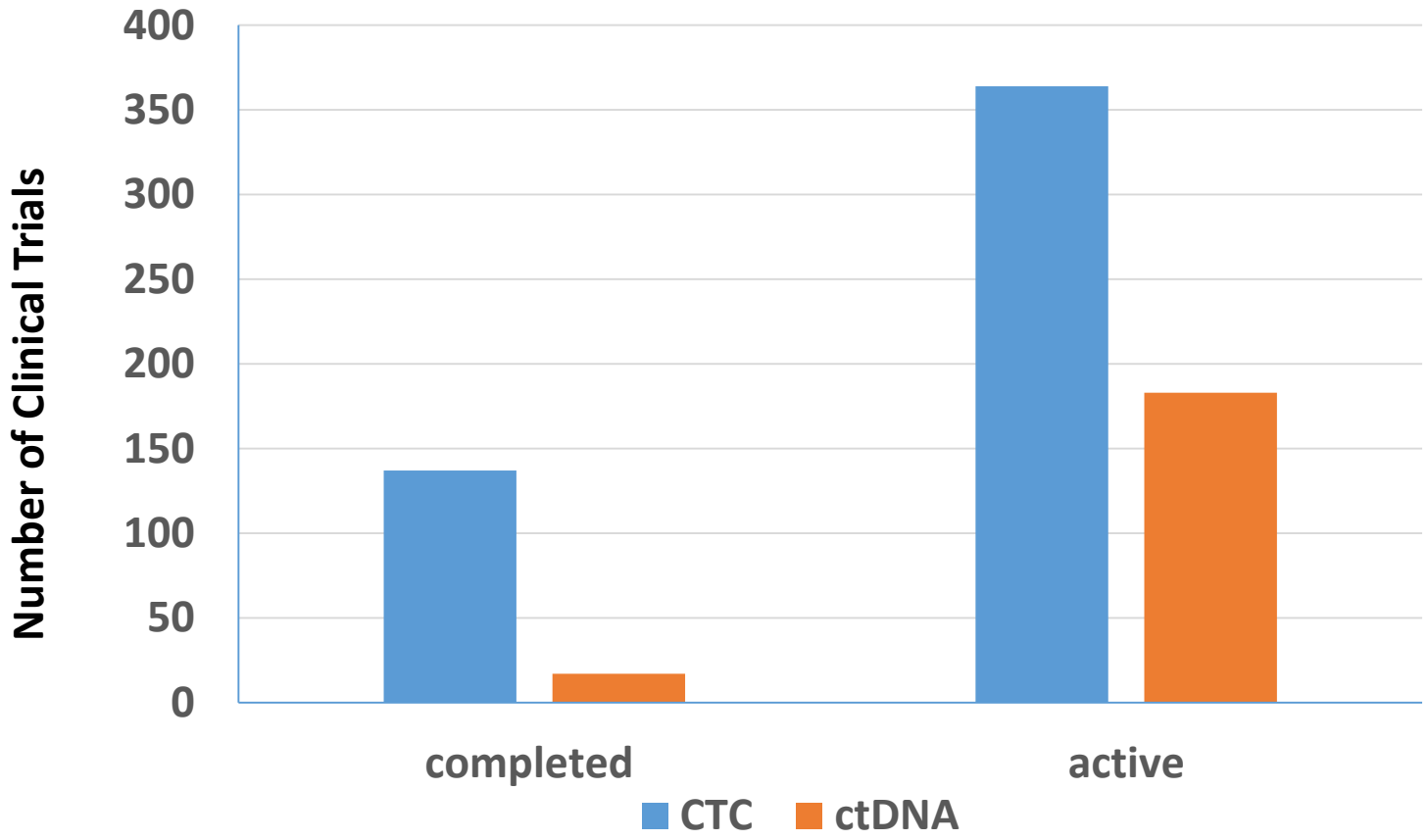
Annual AACR Meeting in Chicago, Open Plenary Session, 15 April 2018



Publications of the Last 10 Years – CTC and ctDNA in PubMed



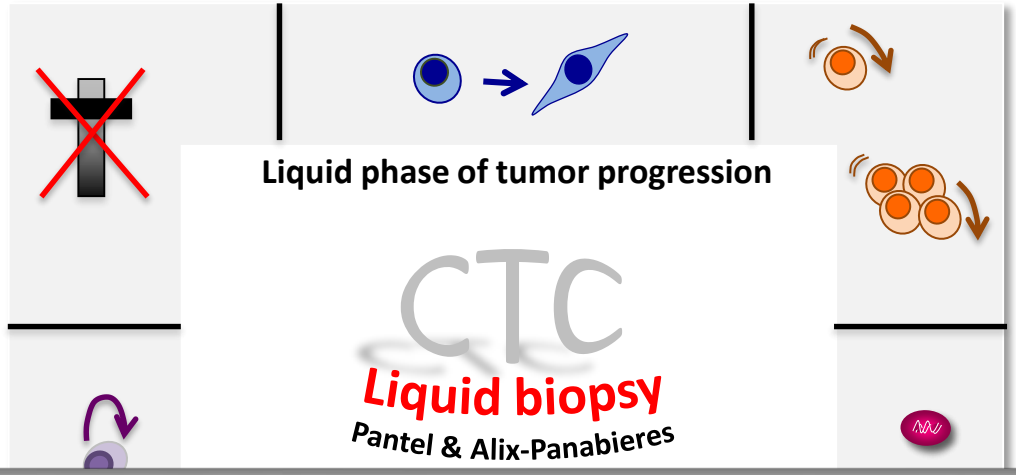
Clinical Trials - CTC and ctDNA in ClinicalTrial.Gov



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

Principle of CTC assays:

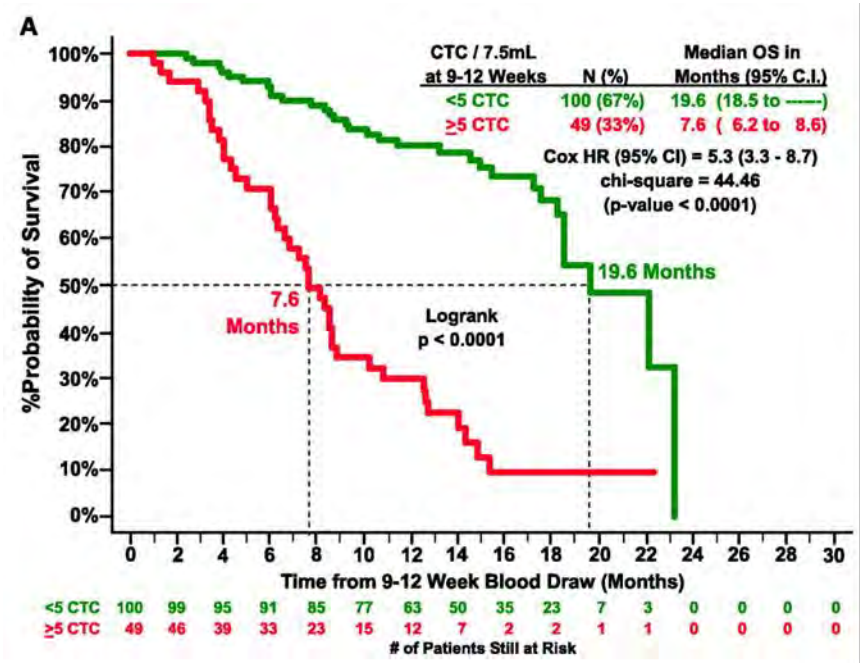
CTC enrichment followed by CTC detection

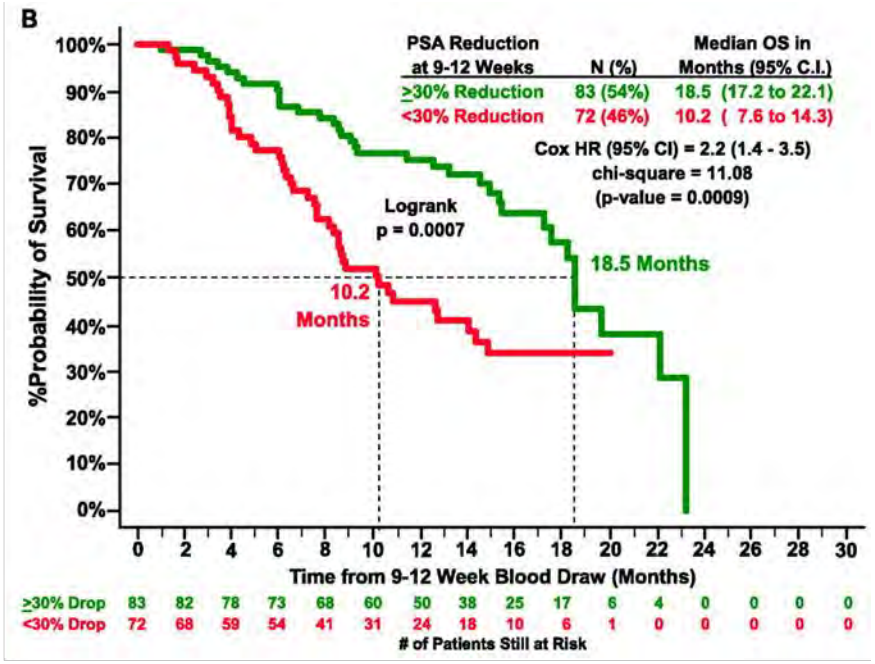
Alix-Panabieres & Pantel, *Nature Rev. Cancer* 2014

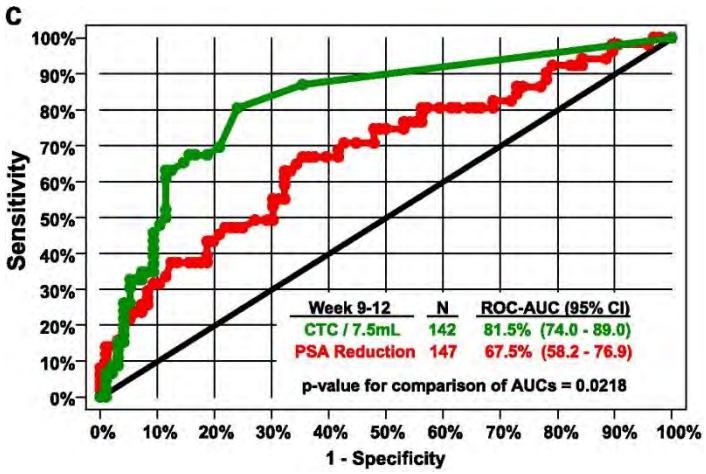
Monitoring of CTCs in CRPC:

Can early changes in CTC counts predict the efficacy of therapeutic interventions (e.g., chemotherapy, hormonal therapy)?

CTC count and PSA reduction for prostate cancer prognosis



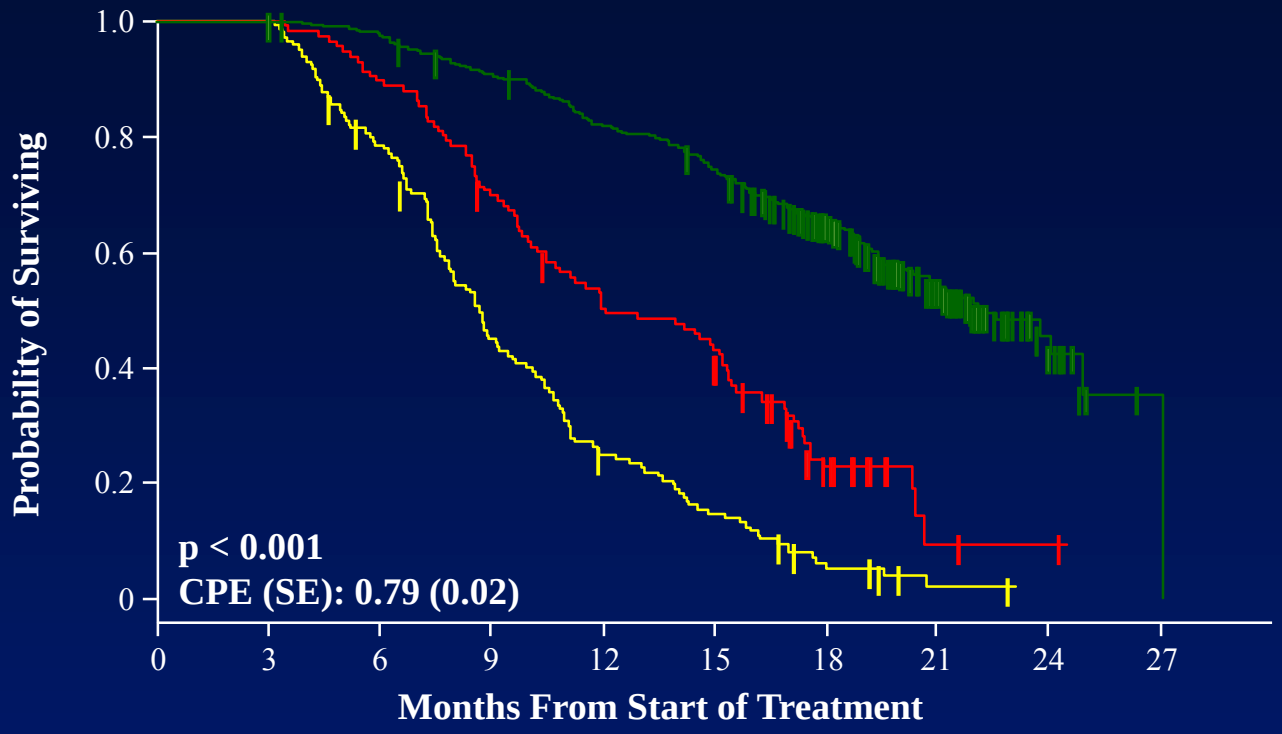




De Bono *et al.* (2008) *Clin Cancer Res*

Simon Joosse (s.joosse@uke.de)

Impact of CTCs & LDH level on survival in prostate cancer patients treated with abiraterone



No. at risk										
High risk	145	**145**	112	63	35	20	7	1	0	0
Intermediate risk	116	116	104	80	57	49	17	2	1	0
Low risk	450	450	439	405	364	329	238	110	14	1

- The surrogate discriminates low-risk from high-risk patients

Scher et al, J Clin Oncol 2015

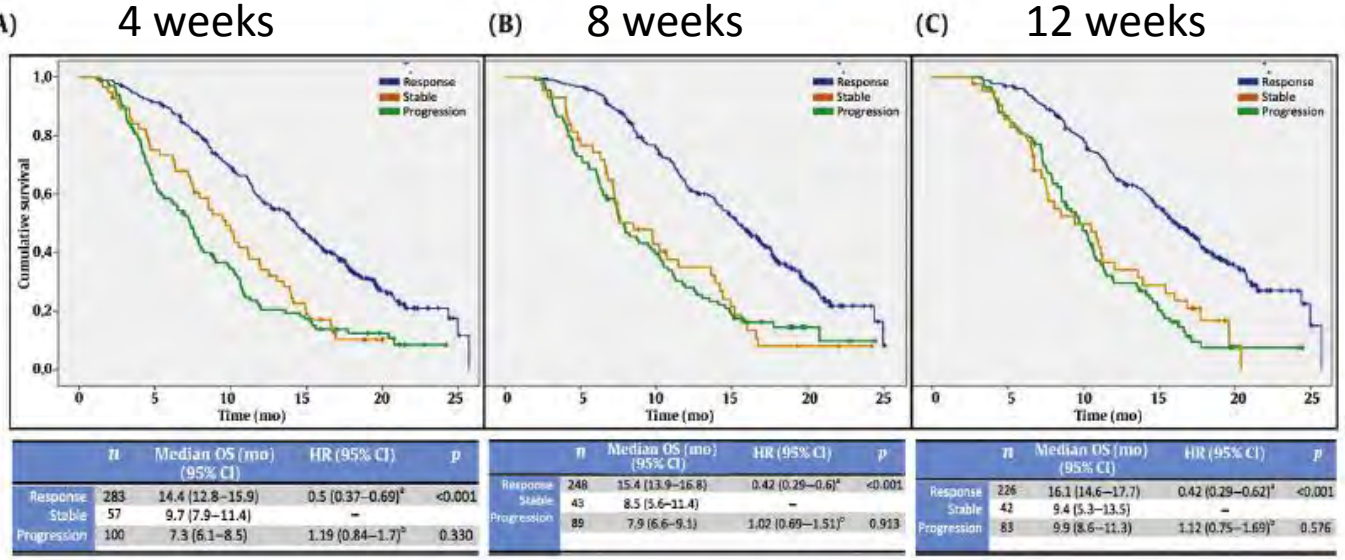
European Association of Urology

Platinum Priority – Prostate Cancer
Editorial by XXX on pp. x–y of this issue

Decline in Circulating Tumor Cell Count and Treatment Outcome in Advanced Prostate Cancer

David Lorente [a,h], David Olmos [b,i], Joaquin Mateo [a], Diletta Bianchini [a], George Seed [a], Martin Fleisher [c], Daniel C. Danila [c], Penny Flohr [a], Mateus Crespo [a], Ines Figueiredo [a], Susana Miranda [a], Kurt Baeten [d], Arturo Molina [e], Thian Kheoh [f], Robert McCormack [e], Leon W.M.M. Terstappen [g], Howard I. Scher [c], Johann S. de Bono [a,]*

OS related to CTC response (response: 30% decline)



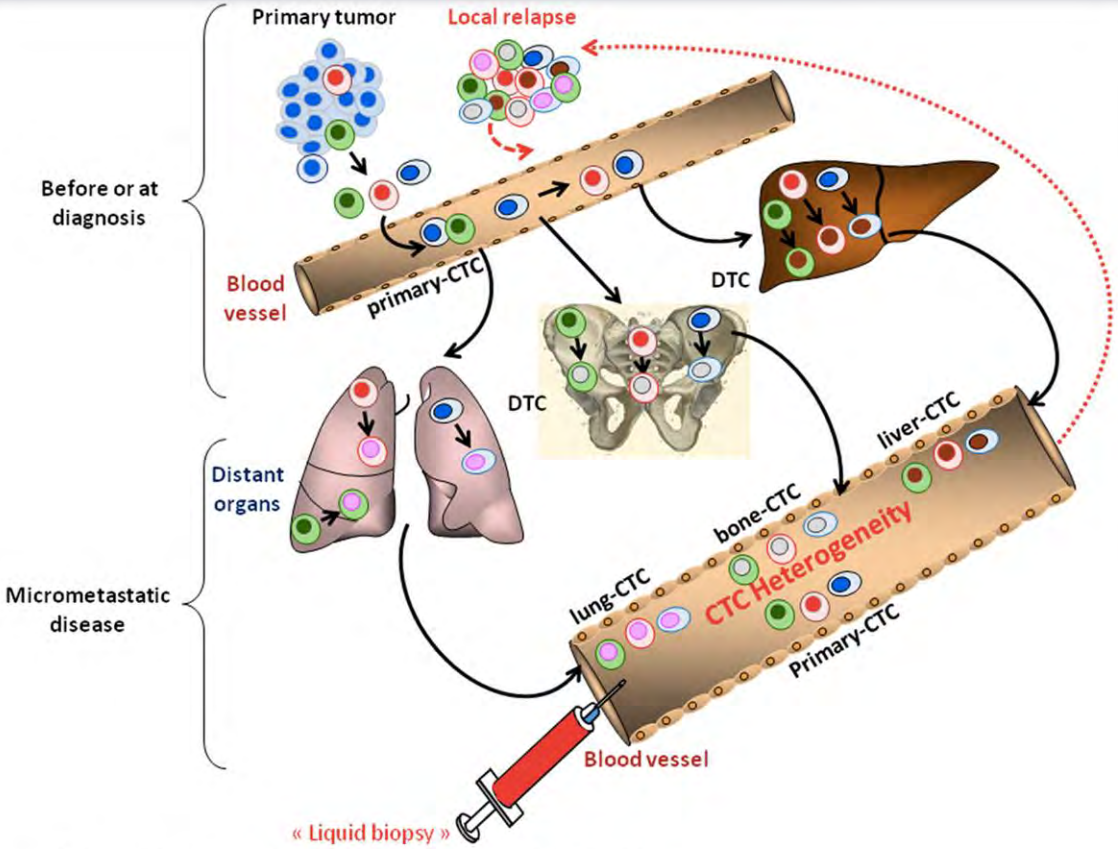
	n	Median OS (mo) (95% CI)	HR (95% CI)	p
Response	283	14.4 (12.8–15.9)	0.5 (0.37–0.69)*	<0.001
Stable	57	9.7 (7.9–11.4)	–	
Progression	100	7.3 (6.1–8.5)	1.19 (0.84–1.7)*	0.330

	n	Median OS (mo) (95% CI)	HR (95% CI)	p
Response	248	15.4 (13.9–16.8)	0.42 (0.29–0.6)*	<0.001
Stable	43	8.5 (5.6–11.4)	–	
Progression	89	7.9 (6.6–9.1)	1.02 (0.69–1.51)*	0.913

	n	Median OS (mo) (95% CI)	HR (95% CI)	p
Response	226	16.1 (14.6–17.7)	0.42 (0.29–0.62)*	<0.001
Stable	42	9.4 (5.3–13.5)	–	
Progression	83	9.9 (8.6–11.3)	1.12 (0.75–1.69)*	0.576

Fig. 3 – Overall survival (OS) according to circulating tumor cell (CTC) response at (A) 4 wk, (B) 8 wk, and (C) 12 wk. The hazard ratio (HR) and 95% confidence interval (CI) were determines using Cox regression with CTC response as the categorical variable and stable disease as the reference covariable.

Liquid Biopsy Concept for Metastatic Patients



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.

Intra-patient heterogeneity of metastases at different sites

CTC/ctDNA might reveal representative information on metastatic cells located at different sites

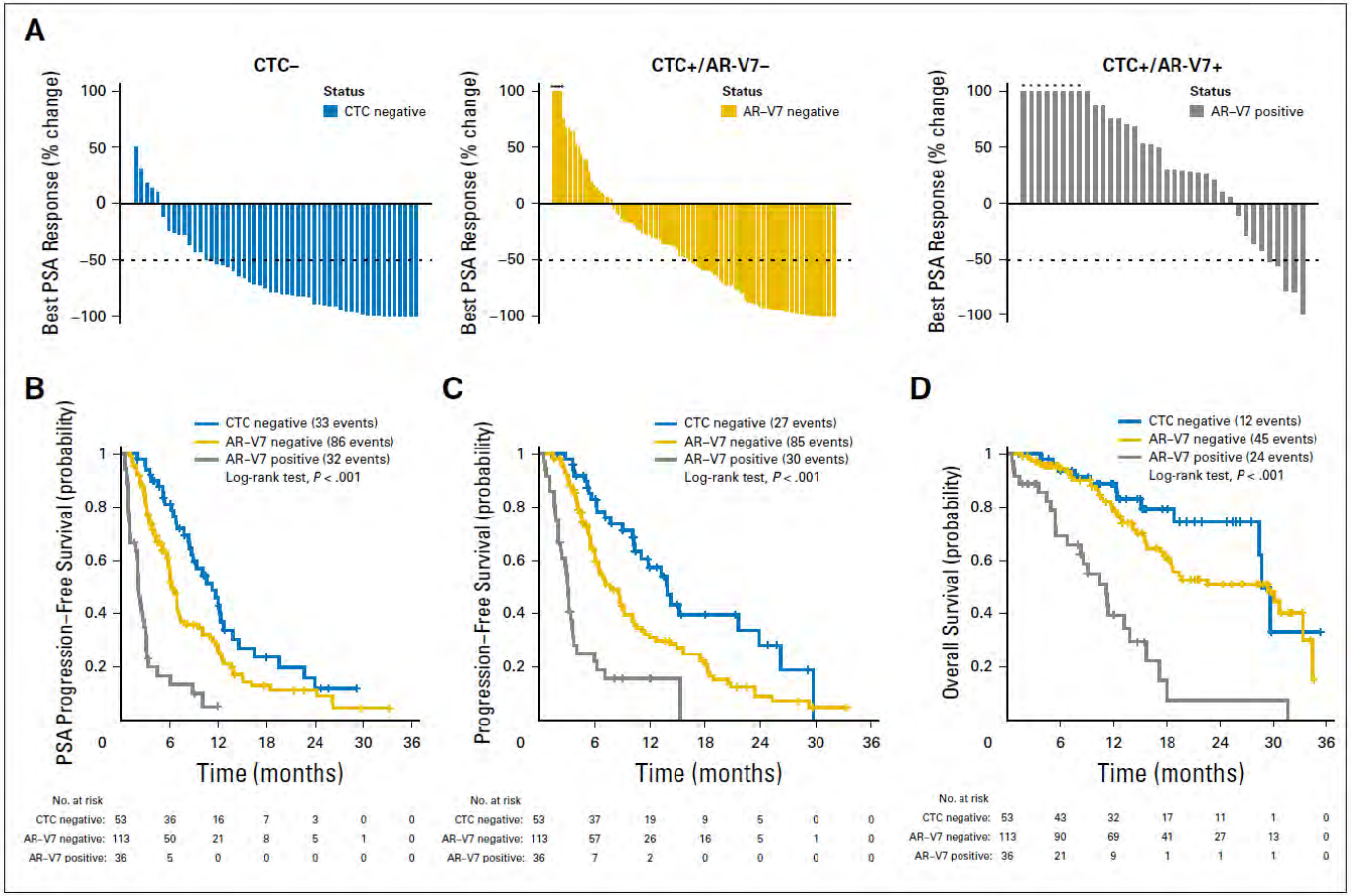
Alix-Panabières & Pantel, *Clin Chem, 2013; Pantel & Alix-Panabieres, Cancer Res. 2013*

CTC characterization

(DNA, RNA, proteins)

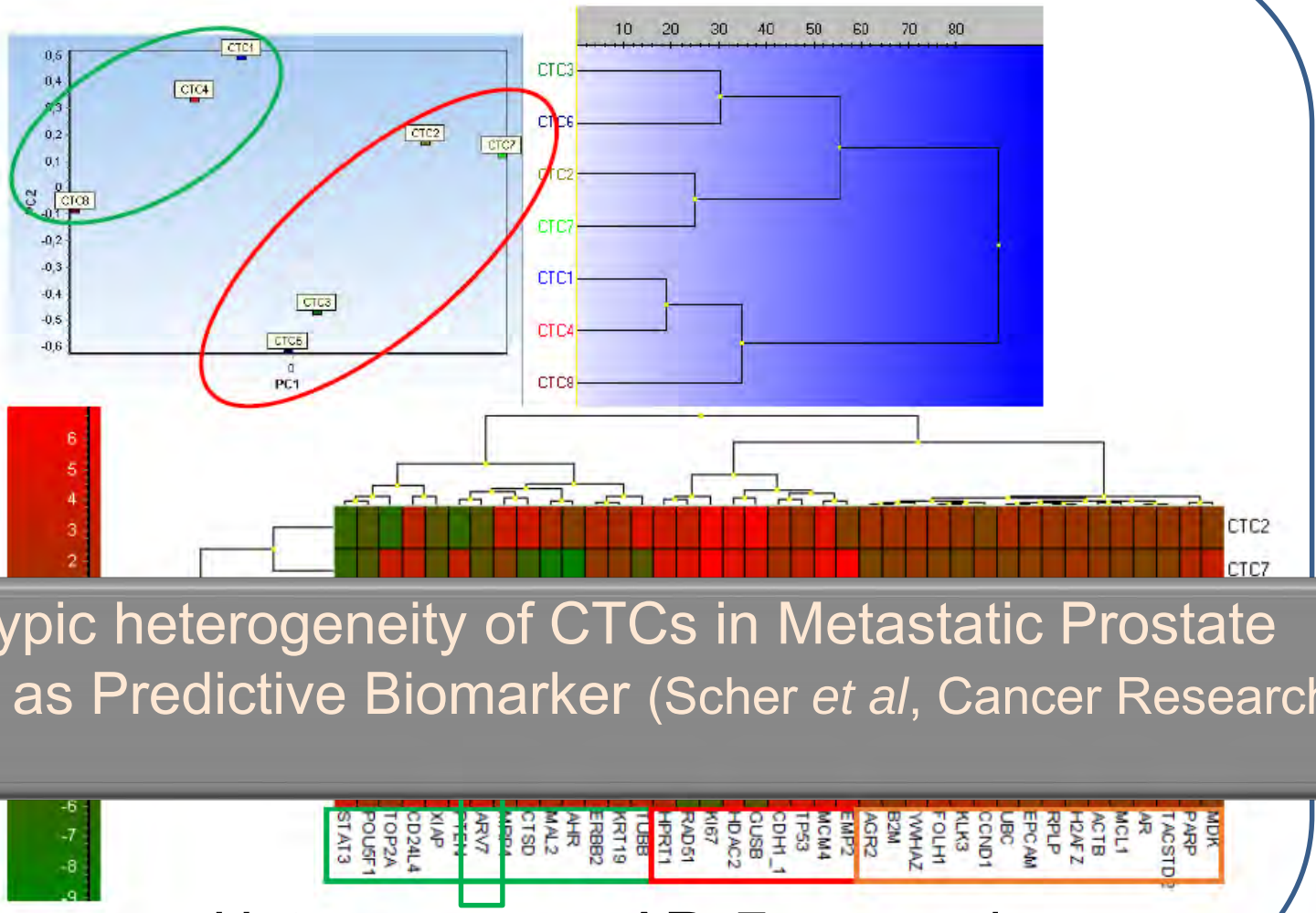
- Therapeutic targets
- Resistance mechanisms

Clinical outcomes of prostate cancer patients starting treatment with arbiraterone or enzalutamide according to CTC & ARv7 status



Antonarakis et al. *J. Clin. Oncol.* 2017

Multiplex mRNA Profiling of single CTCs captured from a prostate cancer patient

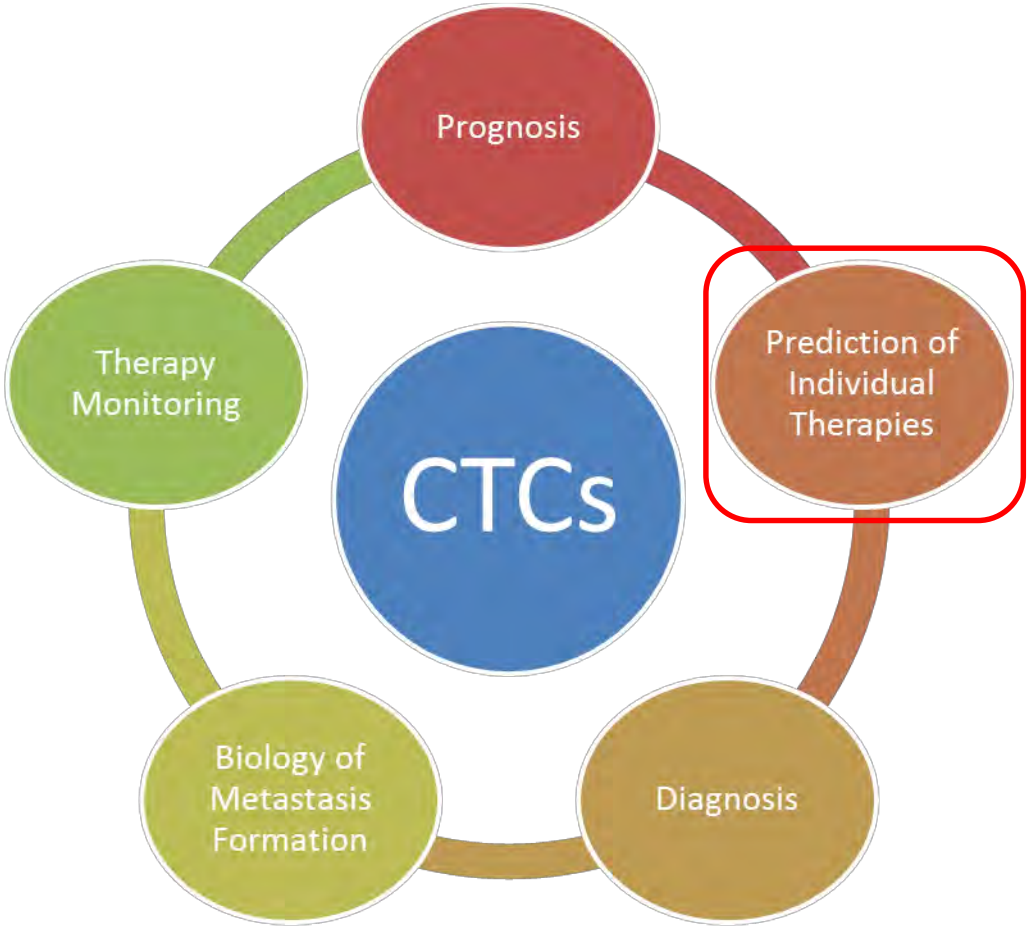


Phenotypic heterogeneity of CTCs in Metastatic Prostate Cancer as Predictive Biomarker (Scher *et al*, Cancer Research, 2017)

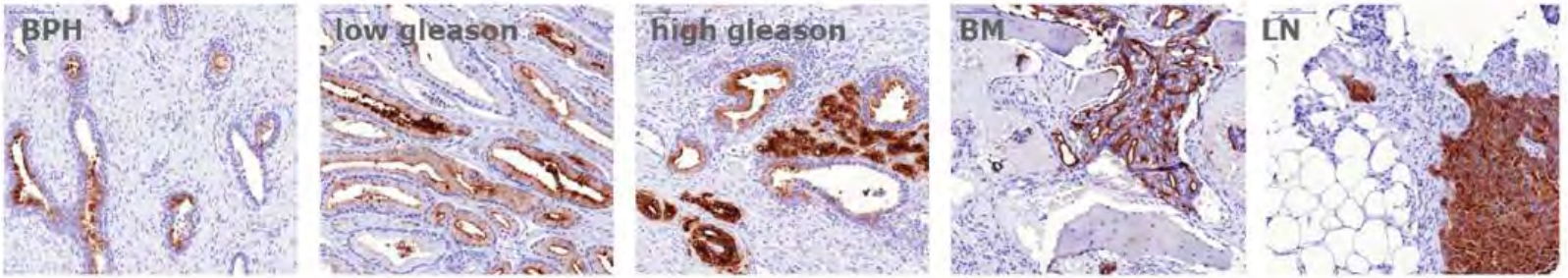
Heterogeneous ARv7 expression

Gorges et al, Clin Chem, 2016

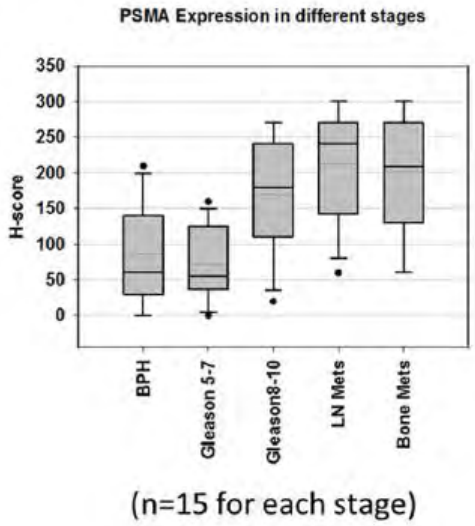
PSMA on CTCs as predictive future biomarker?



PSMA-Spiegel nimmt mit Progressions- und Metastasierungsgrad zu

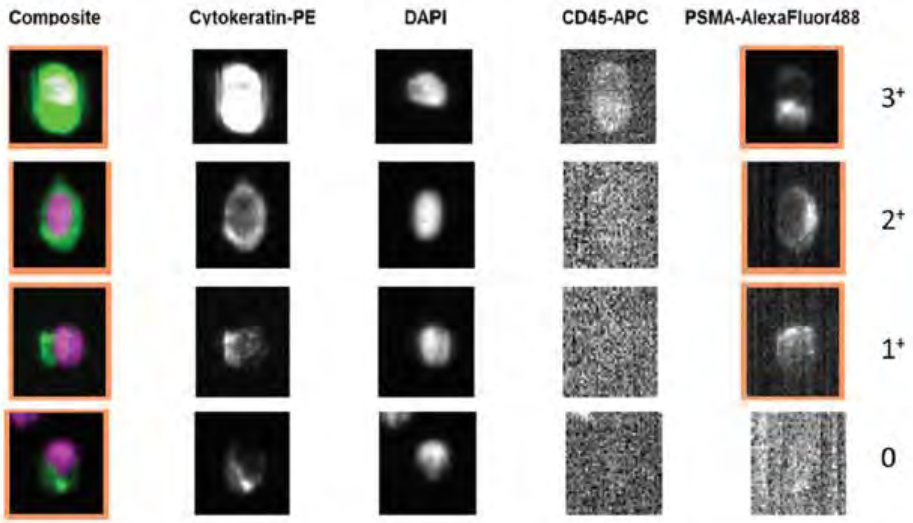


B



(n=15 for each stage)

C



Gorges, Riethdorf, König, Pantel, et al. (Oncotarget 2016)

Heterogenität in CTCs bzw. CTCs und Tumorgewebe

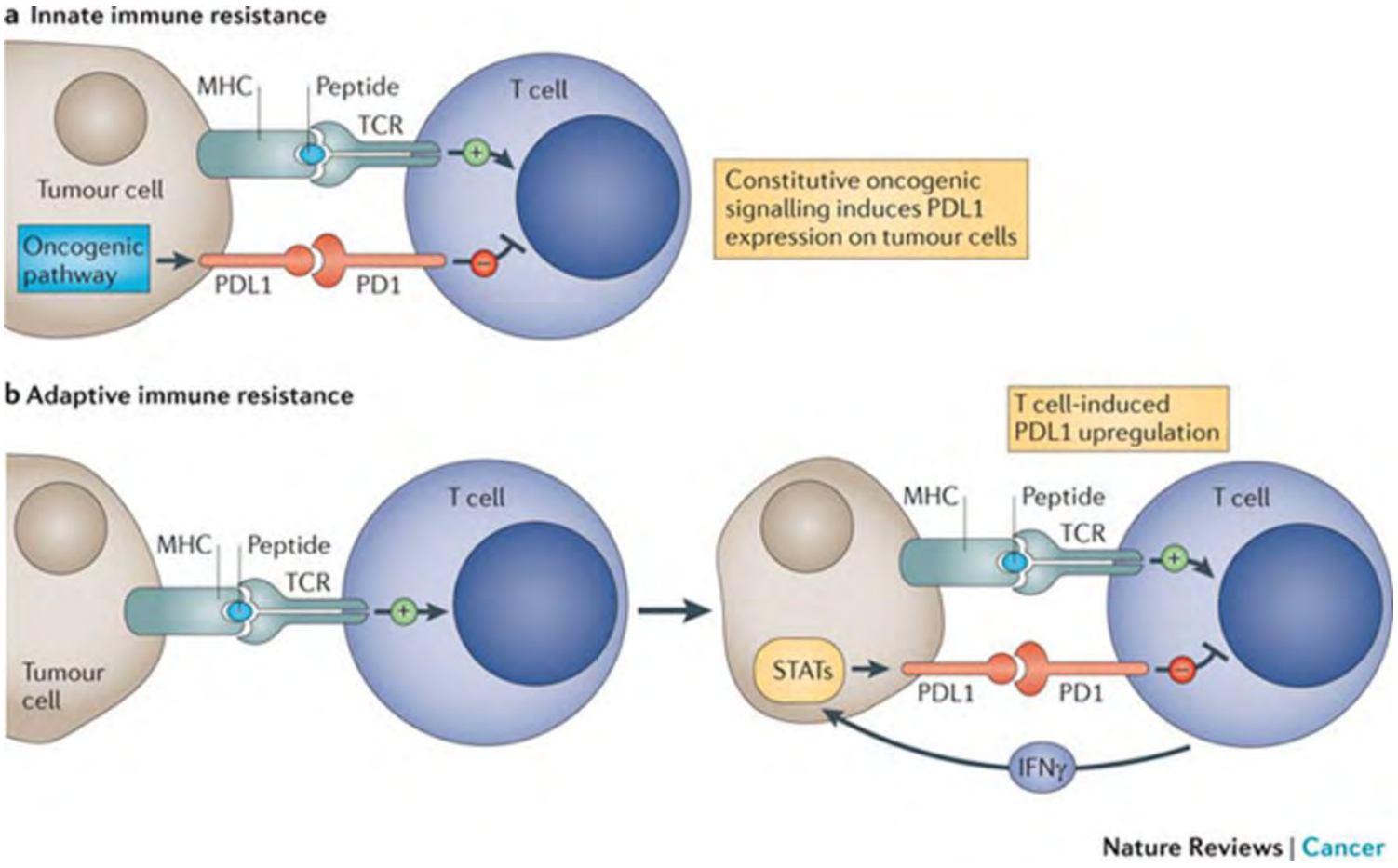
												Circulating tumor cells						Primary tumor
												Intensity of PSMA immunostaining						
ID	Age	T	N	M	Initial Gleason	Initial PSA ng/ml	tPSA	AP	LDH	HB	Actual therapy	Total No.	neg.	weak	moderate	strong	PSMA positive CTCs	PSMA Mean
1	66	4	1	1	5+4	99	79.88	72	198	10.8	DXI, nsAA	5	5	0	0	0	0 %	65 %
12	62	2	1	1	4+3	43	52.46	-	-	-	nsAA	0	0	0	0	0	0 %	25 %
13	76	3	-	1	4+5	9.5	31.13	82	179	15	CC	0	0	0	0	0	0 %	55 %
14	65	2	-	1	3+4	63	-	118	225	9.1	Abirateron	2	2	0	0	0	0 %	-
15	76	3	0	1	5+5	8.21	854.46	1460	618	11	DXI	426	400	26	0	0	4.50 %	50 %
16	49	3	1	1	4+5	31	18.92	540	157	13.9	DXI, CC	0	0	0	0	0	0 %	-
17	77	2	1	1	4+5	3.1	15.56	402	-	11.1	Abirateron, CC	24	21	3	0	0	12.50 %	7.50 %
18	90	3	-	1	4+5	433	117.59	105	219	10.1	Abirateron	6	6	0	0	0	0 %	90 %

Ga-68-PSMA-11 in high-risk prostate cancer study:

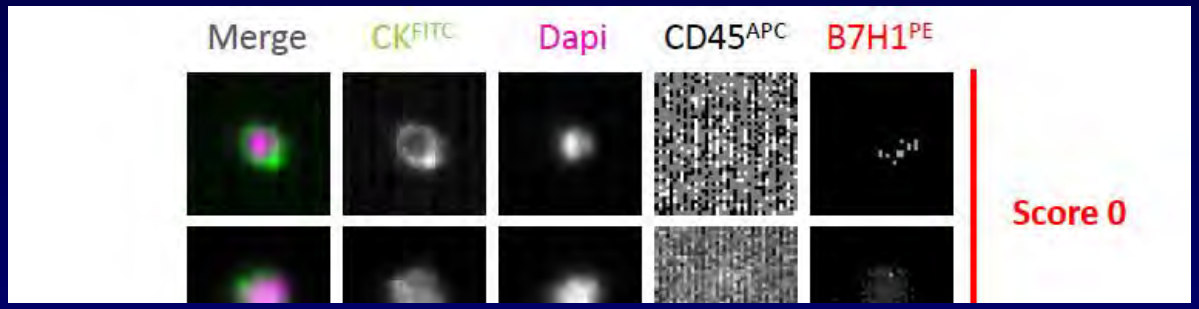
An open-label, single-arm, rater-blinded, multicenter phase 1/2 study to assess safety and diagnostic accuracy and radiotherapeutic implications of pre-operative Ga-68-PSMA-11 PET/CT imaging in comparison to histopathology, in newly diagnosed prostate cancer (PCA) patients at high risk for metastasis, scheduled for radical prostatectomy (RP) with extended pelvic lymph node dissection (EPLND).
Ga-68-PSMA-11 in high-risk prostate cancer

Gorges, Riethdorf, König, Pantel, et al. (Oncotarget 2016)

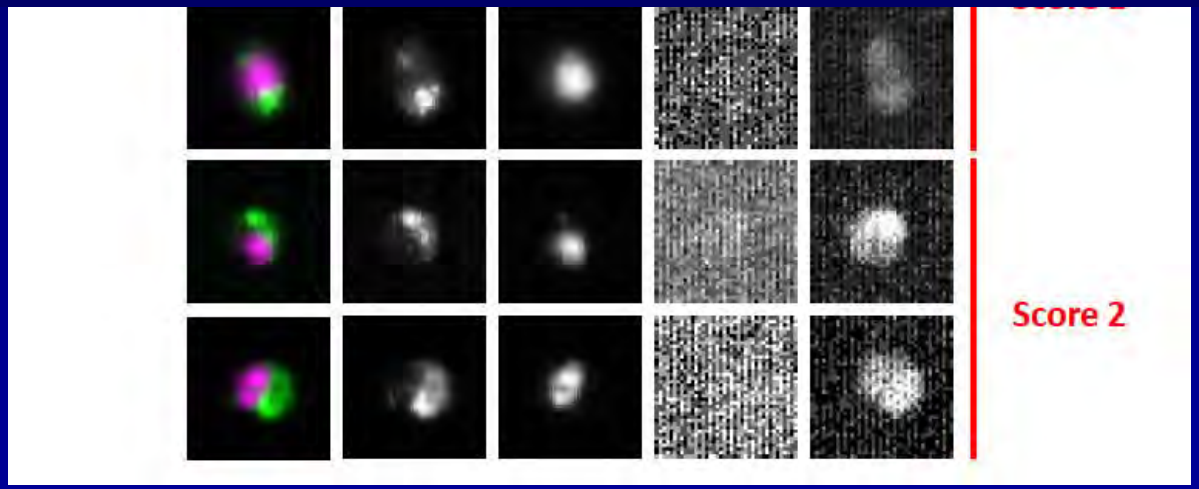
PD1-PDL1 mediated immune blockade as cancer target



PD-L1 expression on CTCs in breast cancer

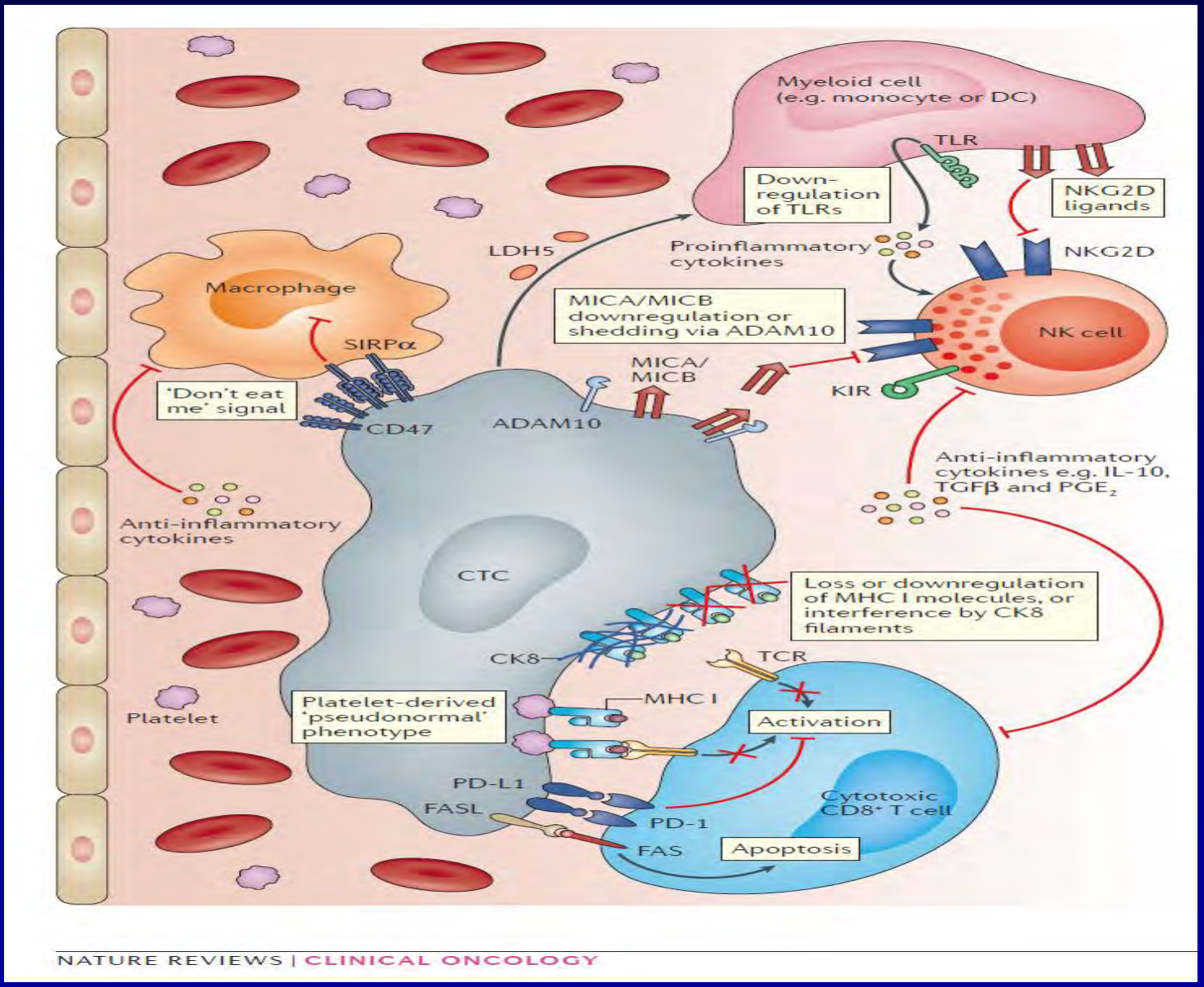


PD-L1 is frequently expressed on CTCs (> 60% of patients) in metastatic breast cancer patients



Mazel, Pantel, Alix-Panabieres et al, Mol. Oncol. 2015
(Editorial by R. David in Lancet Oncol. 2015)

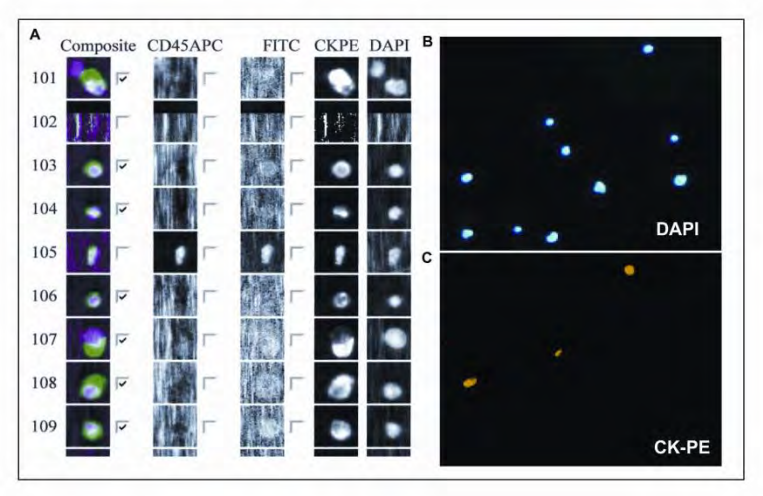
Immune escape mechanisms of CTCs in the peripheral blood



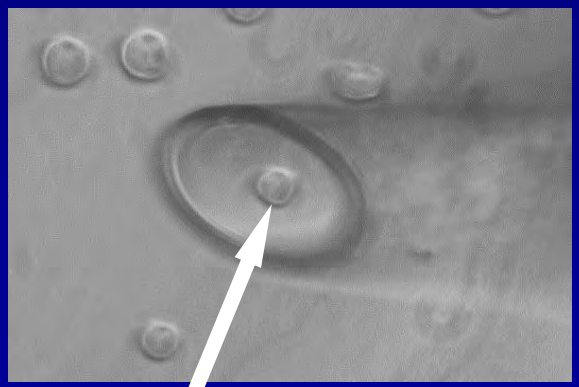
Mohme, Riethdorf & Pantel, Nature Rev Clin Oncol 2016

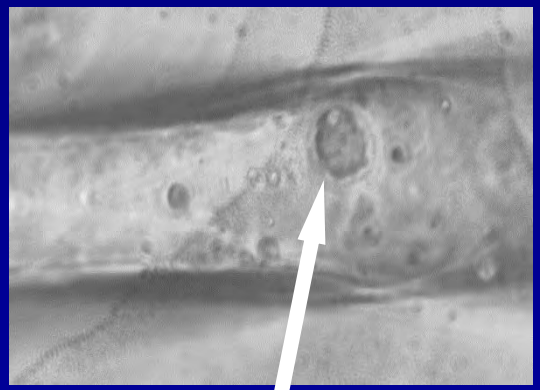
Genomic Characterization of single CTC

CTC detection



CTC isolation





CTC

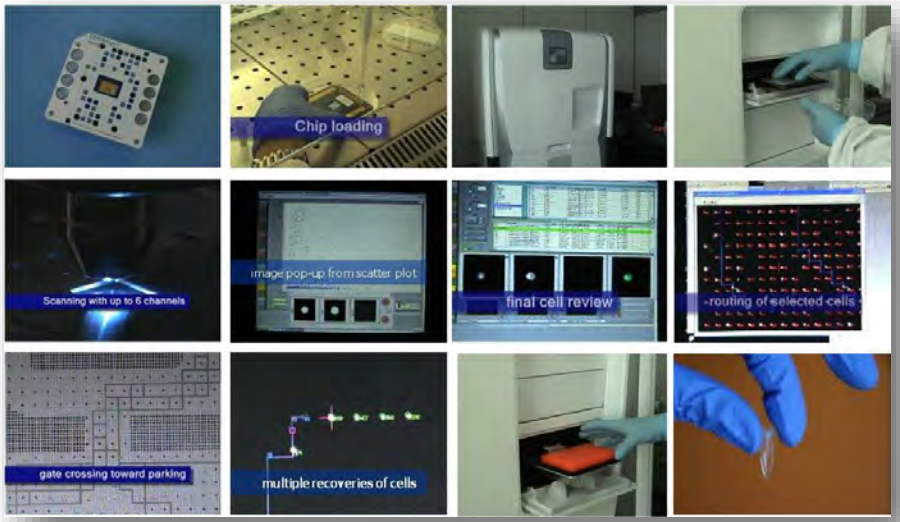
Capillary

CTC

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

UKE-Workflow for Genomic Characterization of Single CTCs

Automated individual CTC sorting with DEPArray™



Whole genome amplification & NGS

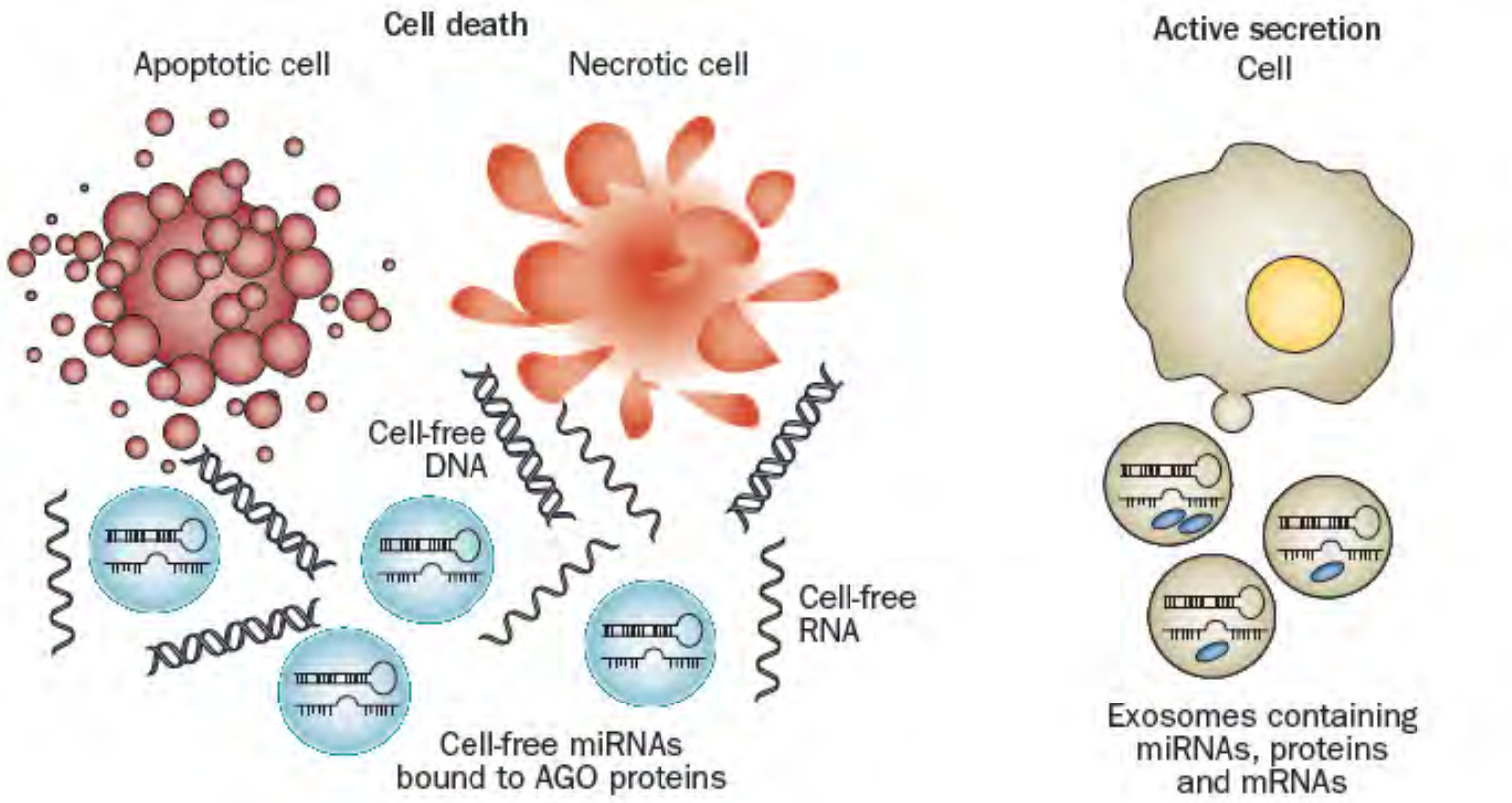
www.impactjournals.com/oncotarget/ Oncotarget, Advance Publications 2016

Comparative study of whole genome amplification and next generation sequencing performance of single cancer cells

Anna Babayan[1], Malik Alawi[2,3], Michael Gormley[4], Volkmar Müller[5], Harriet Wikman[1], Ryan P. McMullin[6], Denis A. Smirnov[4], Weimin Li[4], Maria Geffken[7], Klaus Pantel[1], Simon A. Joosse[1]

[1]Department of Tumor Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[2]Bioinformatics Core, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[3]Heinrich-Pette-Institute, Leibniz-Institute for Experimental Virology (HPI), Hamburg, Germany

Cell-free ctDNA/miRNA and exosomes as Blood-Based Biomarkers



Schwarzenbach, Pantel et al., Nature Rev. Cancer 2011; Nature Rev. Clin. Oncol. 2014; Pantel et al., Nature Med. 2013; Speicher & Pantel, Nature Biotech. 2014; Joosse & Pantel, Cancer Cell, 2015; Alix-Panabieres & Pantel, Cancer Discovery, 2016; Bardelli & Pantel, Cancer Cell, 2017

Cancer-ID is a project funded by the Innovative Medicines Initiative Joint Undertaking (IMI JU).

CellPress

Cancer Cell
Perspective

Liquid Biopsies, What We Do Not Know (Yet)

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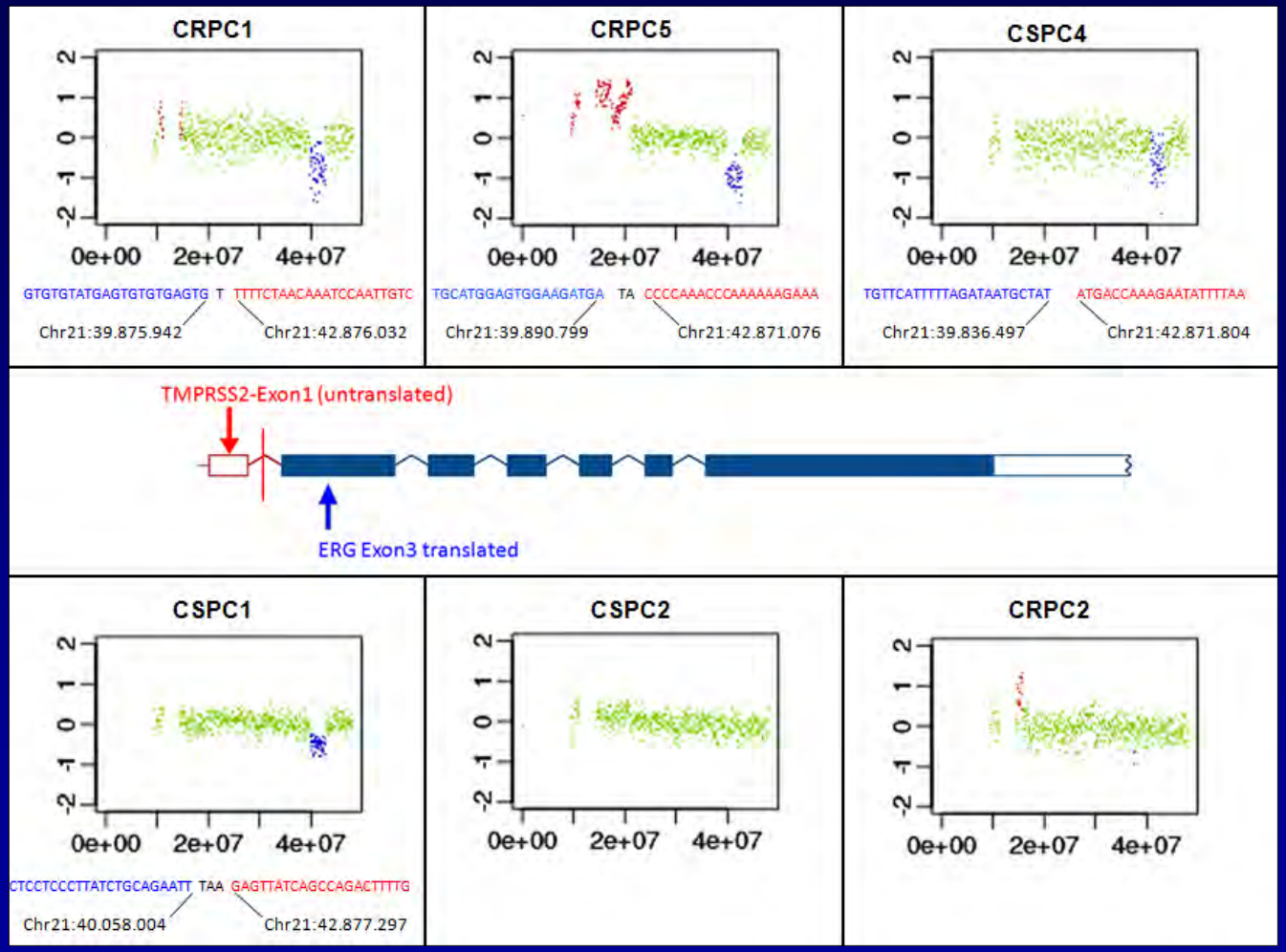
[3]Department of Tumor Biology, Center of Experimental Medicine, University Cancer Center Hamburg, University Medical Center Hamburg-Eppendorf, Martinistraβe 52, 20246 Hamburg, Germany
*Correspondence: alberto.bardelli@unito.it (A.B.), pantel@uke.de (K.P.)
http://dx.doi.org/10.1016/j.ccell.2017.01.002

Chun FK, Muller I, Lange I, Friedrich MG, Erbersdobler A, Karakiewicz PI, Graefen M, Pantel K, Huland H, Schwarzenbach H. Circulating tumour-associated plasma DNA represents an independent and informative predictor of prostate cancer. ***BJU Int.*** 2006;98: 544-8.

TMPRSS-ERG-associated 3 Mb deletion on chromosome 21 and mapping of the breakpoint on ctDNA in prostate cancer



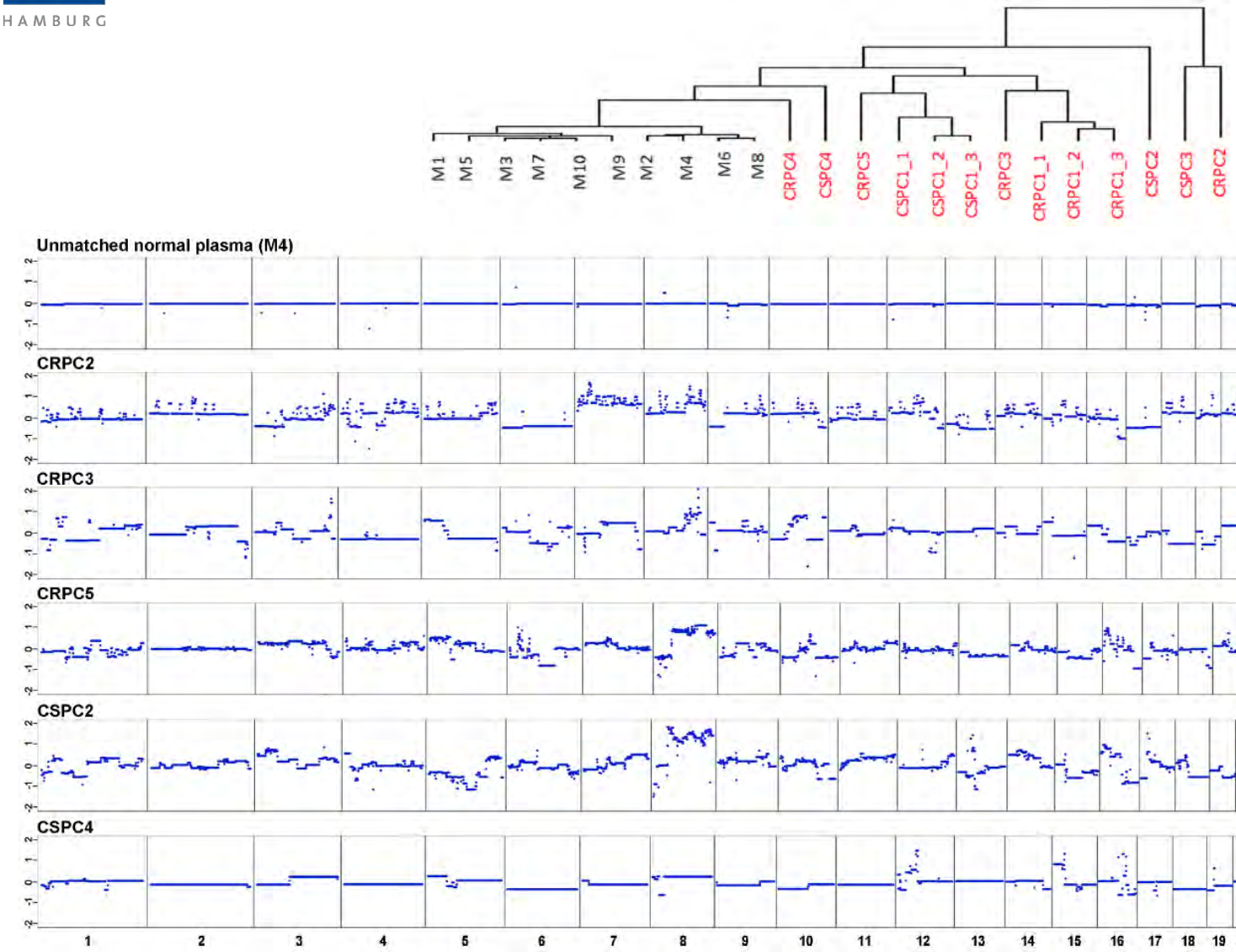
Heitzer/Pantel/Speicher et al. Genome Medicine 5 (2013), 30

ctDNA characterization

- Druggable genomic aberrations
- Resistance-inducing aberrations

Prostate cancer

Next Generation Sequencing (NGS) of plasma DNA



Mutations

MLL3

-

TP53

BRCA2*
HNF1A

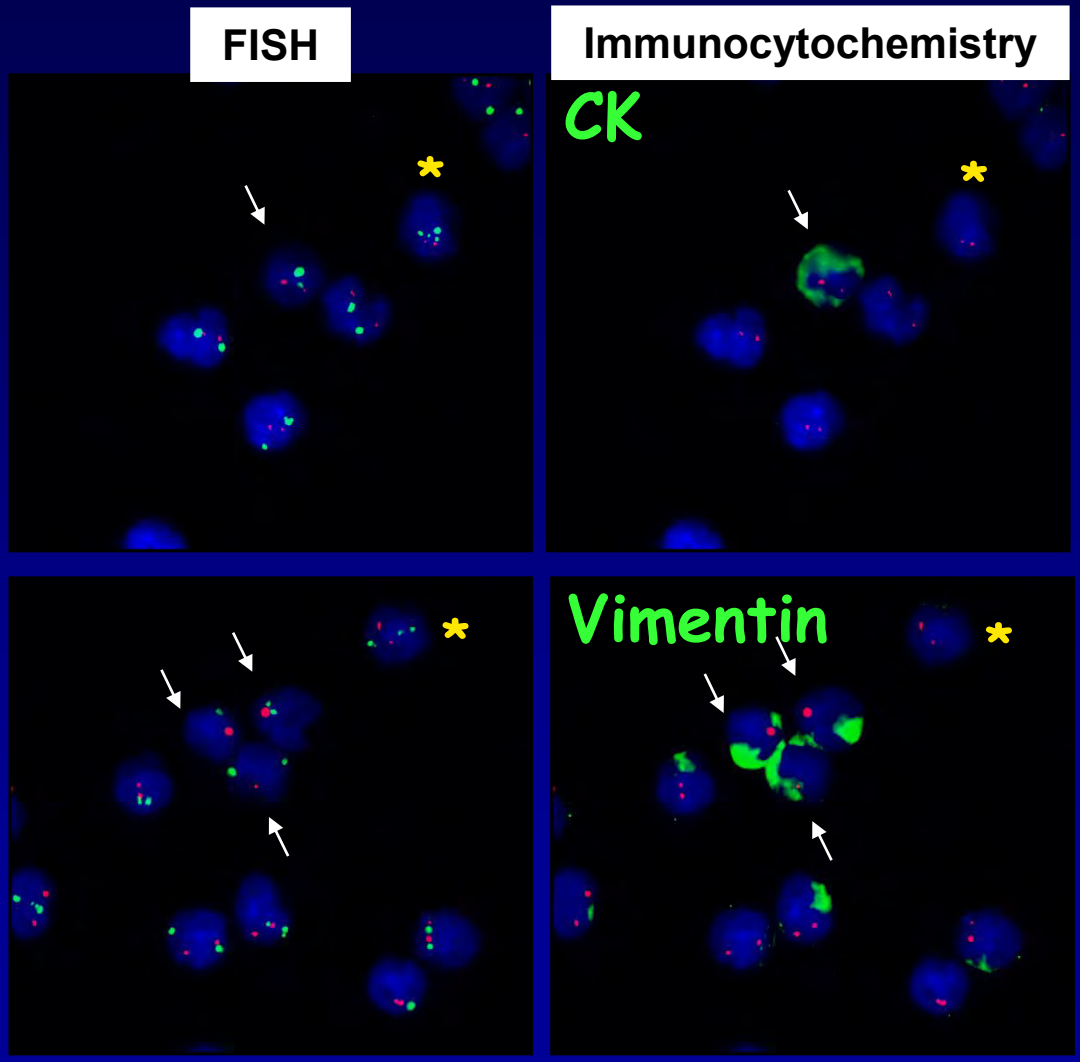
BRCA1*

*important information for PARP inhibitor therapy

Heitzer *et al.* (2013) *Genome Med*

Simon Joosse (s.joosse@uke.de)

BRCA1 Deletion in CTCs: Possible Predictor for Olaparib-Therapy ?



CK-pos. CTC

VIM-pos. CTC

Bednarz/Pantel et al., Clin Cancer Res 2010

CTC detection in early stage cancer: Identification of Minimal Residual Disease

Challenge: Low concentration of CTCs & ctDNA

CTC Counts are Associated with Unfavorable Prognosis

- **Breast Cancer:** Rack, Pantel, Janni *et al. JNCI* 2014; Janni *et al. Clin Cancer Res* 2016; Riethdorf, Pantel et al CCR 2017, Bidard, Pantel et al, JNCI 2018
- **Bladder Cancer:** Rink, Pantel *et al. Eur Urol* 2012; Giavazzi, Pantel *et al. Int J Cancer* 2014
- **Head & Neck Cancer:** Grobe, Riethdorf, Pantel *et al. Clin Cancer Res* 2014
- **Testicular Germ Cell Tumors:** Nastaly, Riethdorf, Pantel *et al. Clin Cancer Res* 2014
- **Colorectal Cancer:** Yokobori, Mimori, Mori, Pantel *et al. Cancer Res* 2013; Deneve, Pantel, Alix-Panabieres *et al. Clin Chem* 2013
- **Pancreatic Cancer:** Effenberger, Bockhorn, Pantel *et al. Clin Cancer Res* 2018

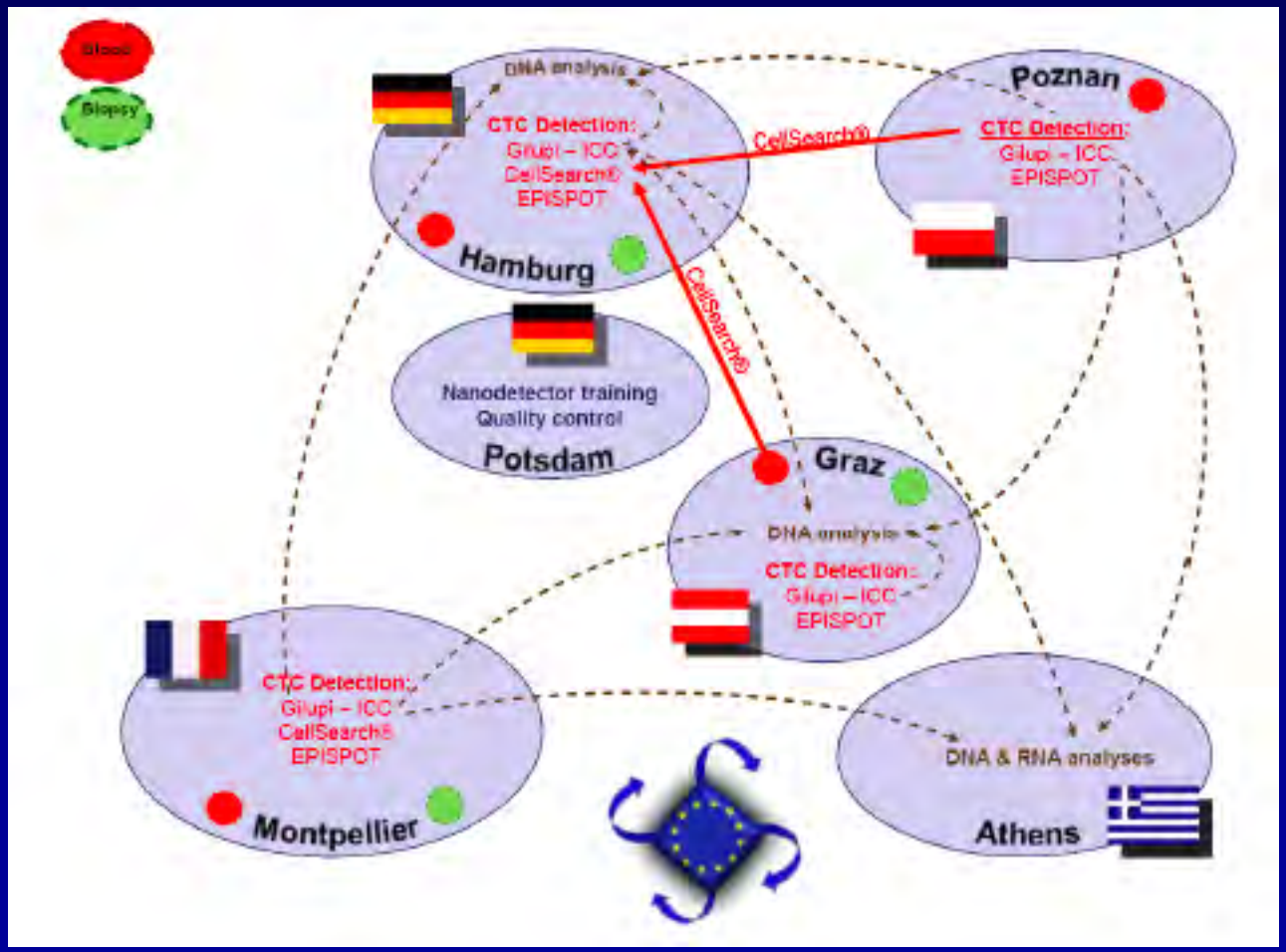
Conclusion: Survival of CTCs in the Blood is an Important Hallmark of Metastatic Progression in Cancer Patients

ERA-NET TRANSCAN: CTC-SCAN Project

High-risk Prostate Cancer (stage M_0)

Partners: Germany, France, Greece, Poland, Austria

Coordinator: K. Pantel, Hamburg



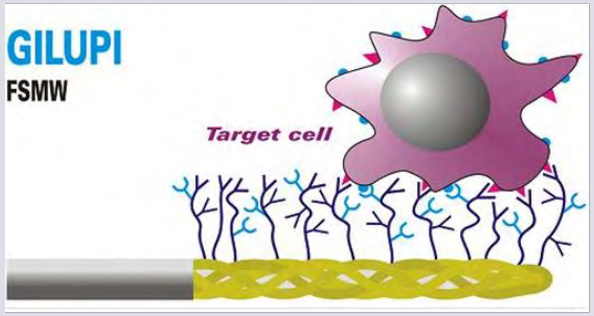
CTC detection in 87/107patients (81 %)

5 CTCs in 21.5%

Candidates for adjuvant therapy?

Kuske et al, Nature Scientific Reports, 2016

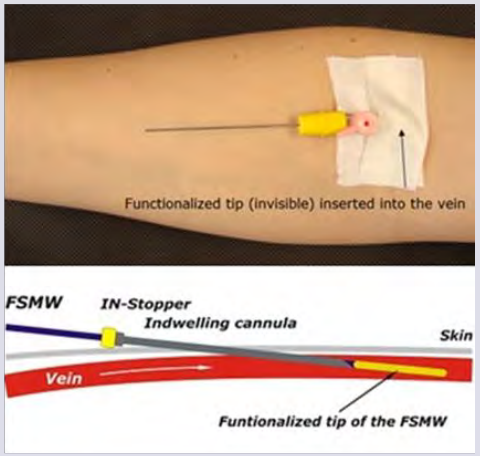
New approach: *In vivo* capture of CTC



Nanodetector

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

30 minutes exposure time in a vein



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

Result ← *Decision*

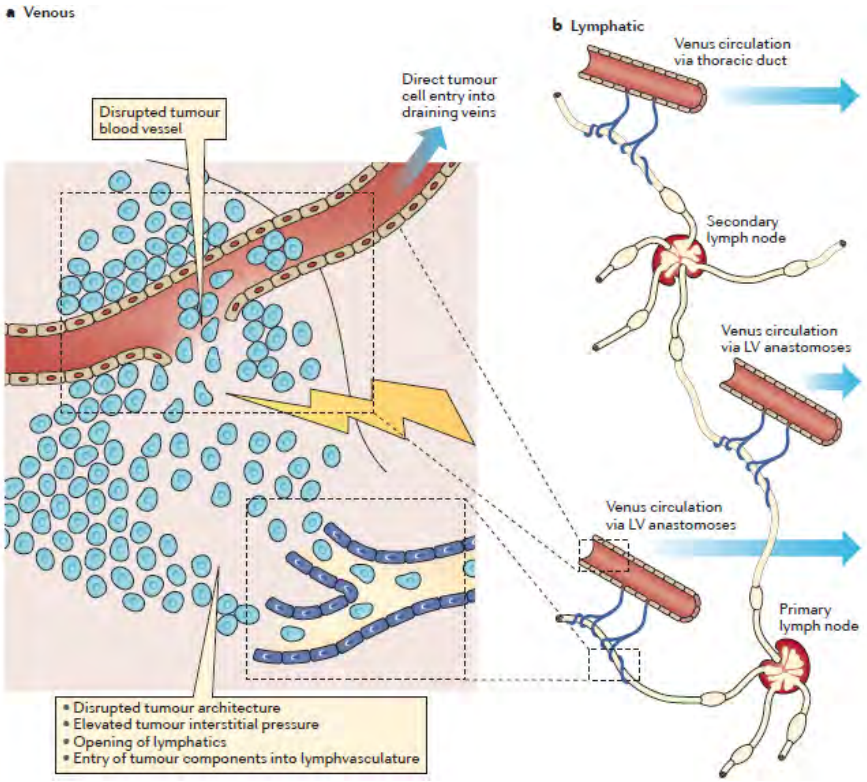
Lung cancer: Gorges, Pantel et al., CCR 2016;

Prostate Cancer: Kuske, Gorges, Schlomm, Beyer, Pantel et al., Nature Scientific Reports, 2016; Markou, Pantel, Lianidou et al, Clin Chem 2018;

Does the mobilization of circulating tumour cells during cancer therapy cause metastasis?

Olga A. Martin[1,2,4], Robin L. Anderson[3,4], Kailash Narayan[1,4,5] and Michael P. MacManus[1,4]

CTCs and radiotherapy



- **Locoregional radiotherapy can reduce the risk of distant metastasis (e.g. in early breast cancer)**

  vs.

- **Radiotherapy can mobilize viable tumor cells into the circulation**
- **In early stage of fractionated radiotherapy (such as 2,0-6,0 Gy in 1-3 fractions): tumor cells are much more likely to survive if they escape into the circulation**
- **Irradiated tumor cells: increased genomic instability and plasticity -> can become more radioresistent**

Martin et al, Nature reviews/ clinical oncology, January 2017

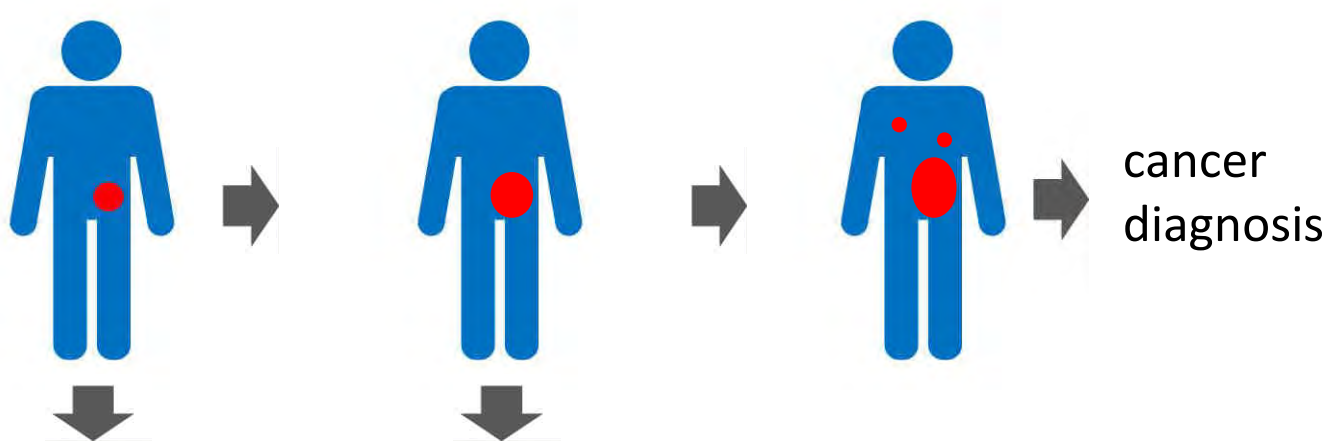
Challenges of Early Cancer Detection

- Very low concentrations of CTCs and ctDNA in patients with early malignant lesions
- Tumor-associated mutations on cfDNA in ageing individuals

Hamburg City Health Study (start: 2015)

Biomaterial repository:

- 45 000 individuals between 45 and 74 years
- Biomaterials: blood cells, DNA, RNA, plasma, serum, urine, tooth plaques, skin punch, pluripotent stem cells (skin)
- Network research on 270 Mio. datasets
- 2021: >400 prostate, >150 breast/colon/lung cancer



pre-diagnostic samples / datasets

Liquid Biopsy Tumor Markers: A Growing Family

EU Marie Curie Network:
European Liquid Biopsy Academy (**ELBA**)

Start: January 2018, Focus: Detection of Lung Cancer

Coordinator: Tom Würdinger, Amsterdam
Deputy Coordinator: Klaus Pantel, Hamburg

New ERA-NET TRANSCAN Project:

PROLIPSY

Start: June 2018, Focus: High-risk prostate cancer

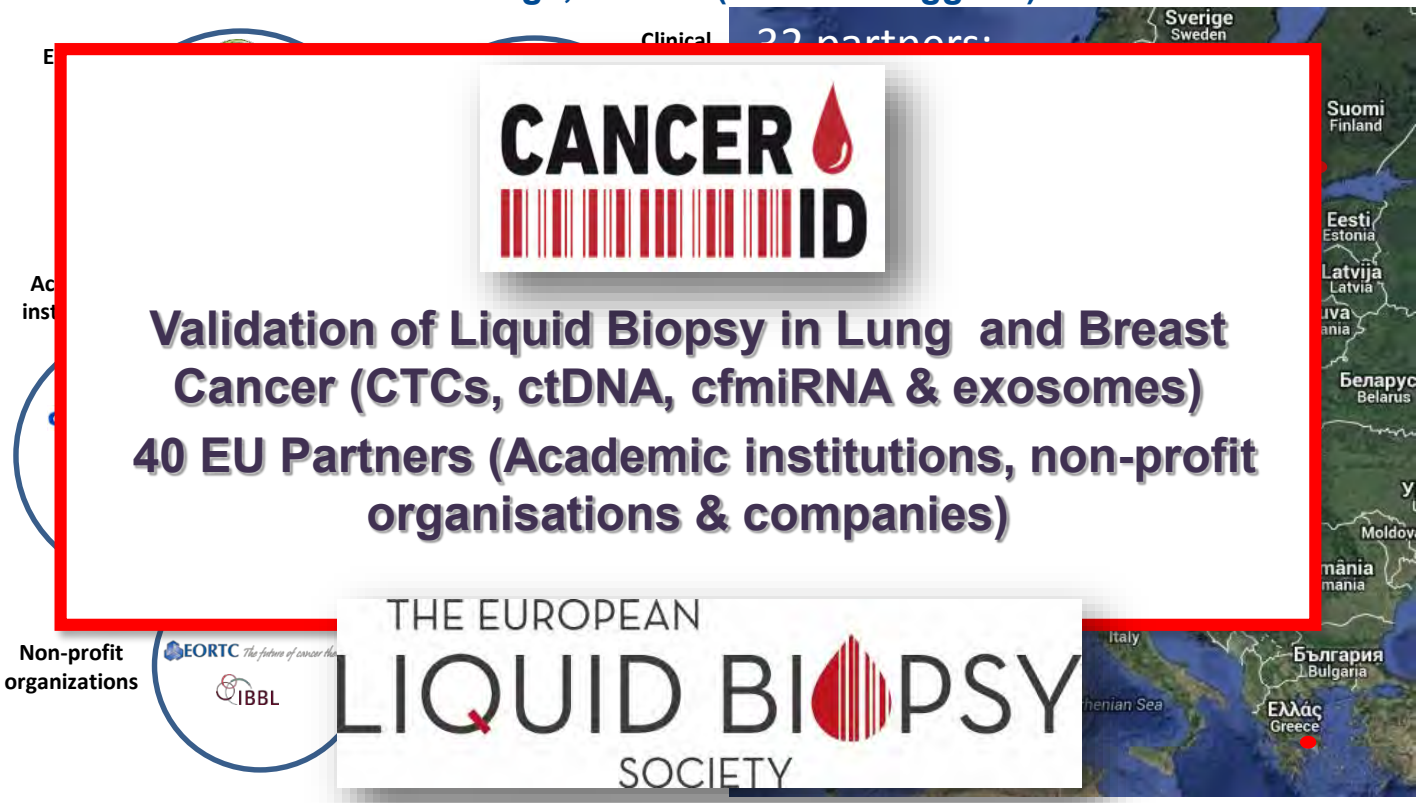
Coordinator: Klaus Pantel, Hamburg

er *et al., Cancer*

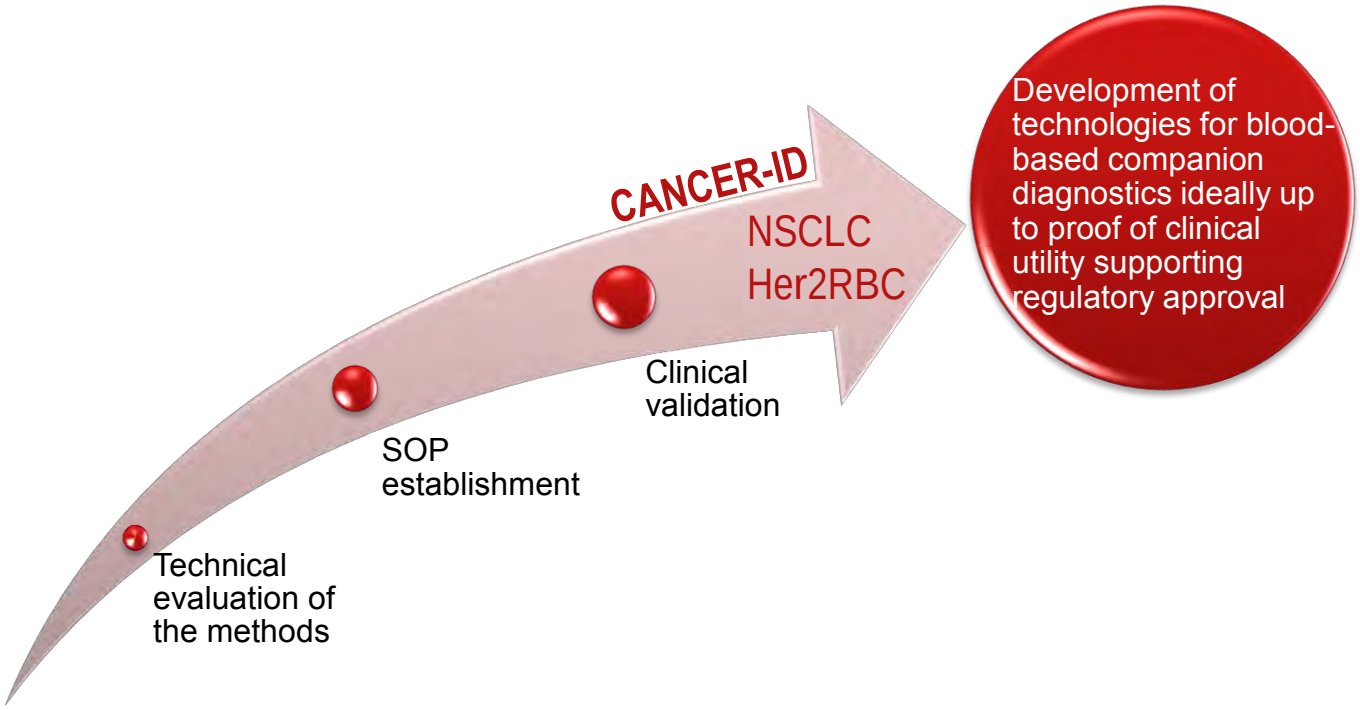
Joosse & Pantel, *Cancer Cell 2015*

CANCER-ID EU Consortium 2015-2020

Scientific Management: Klaus Pantel, UKE (Leon Terstappen)
Coordination: Thomas Schlange, BAYER (Barbara Baggiani)

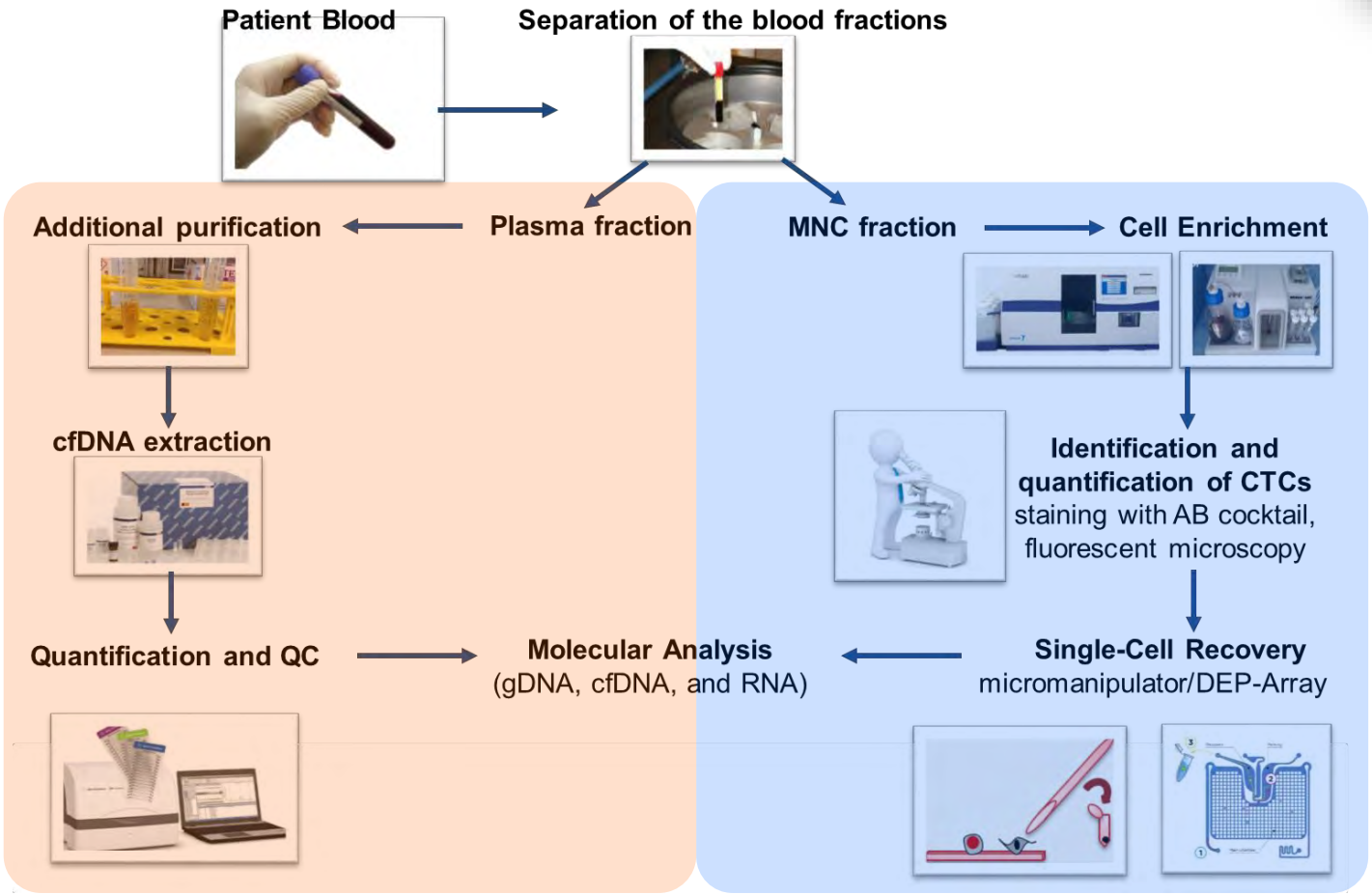


Cancer-ID philosophy

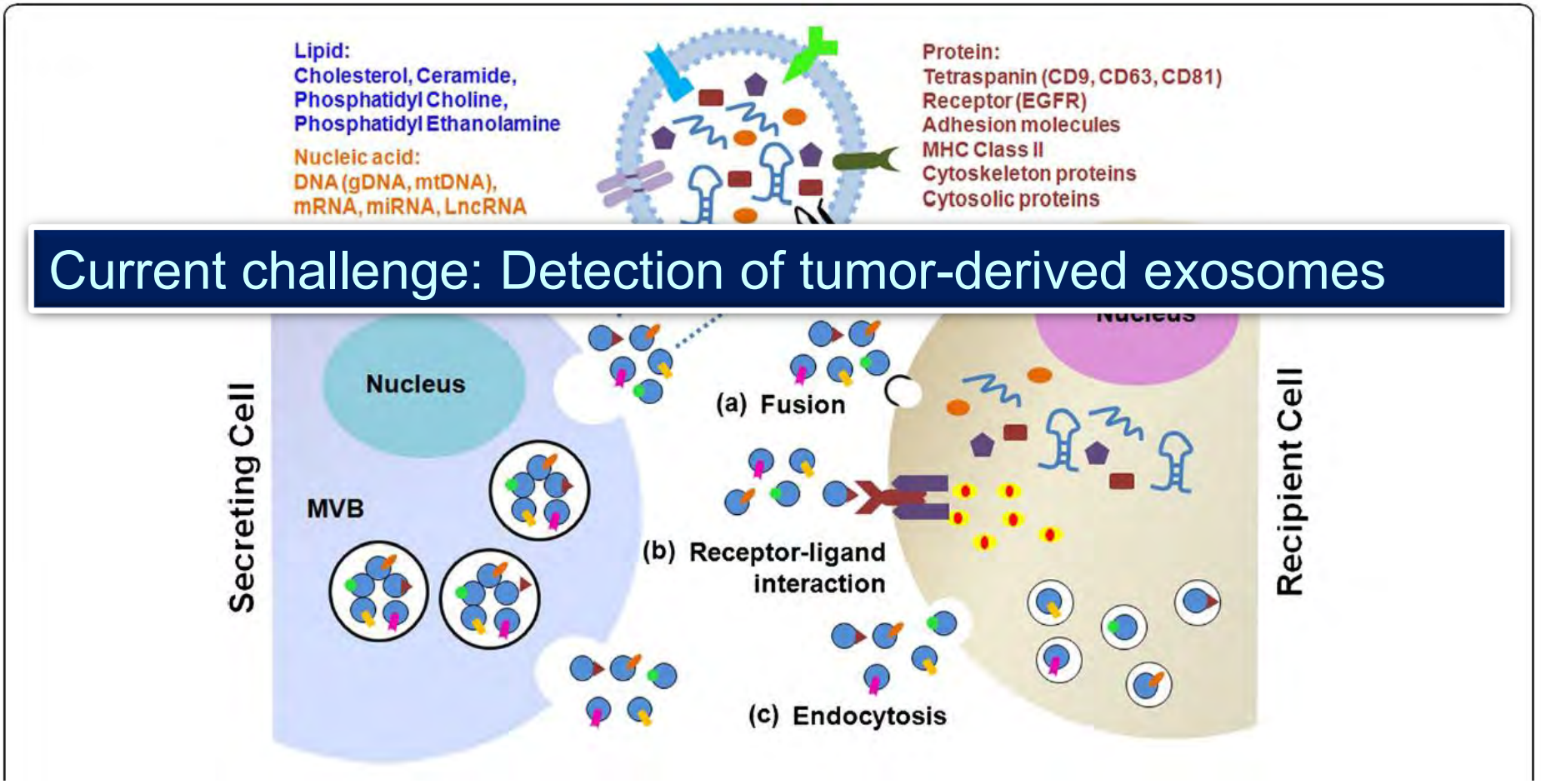


Cancer-ID is a project funded by the Innovative Medicines Initiative Joint Undertaking (IMI JU) under grant agreement nr. 115749.

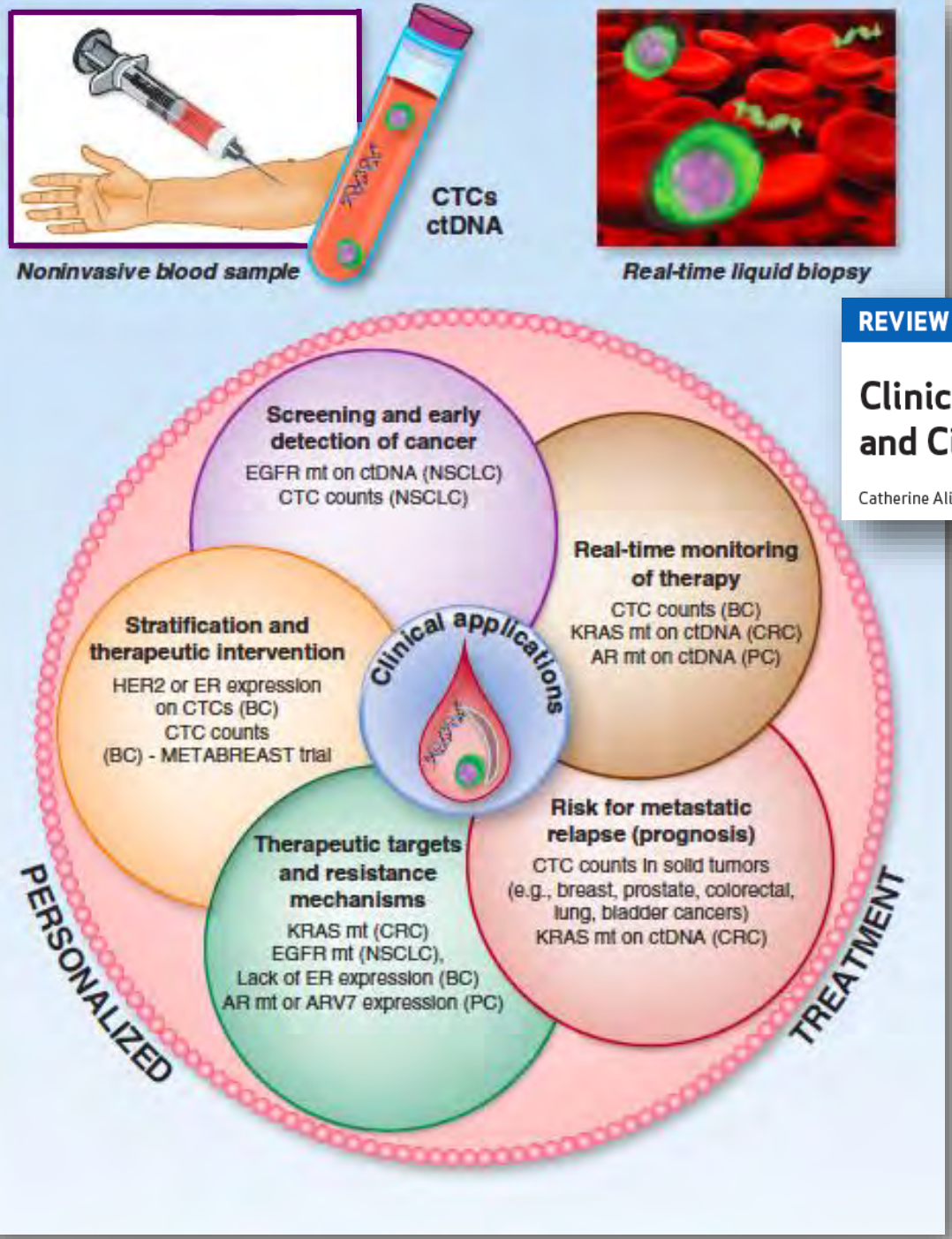
Key Aim: Combined Analysis of CTCs and cfDNA



Exosomes: Biogenesis, release, structure and uptake



Hoshino, Pantel, Lyden et al. Nature 2015: **Exosomes guide organ-specific metastasis**
Meng, Pantel, Schwarzenbach et al., Oncotarget 2016: **Exosomes in early detection of ovarian cancer**



CANCER DISCOVERY

2016

REVIEW

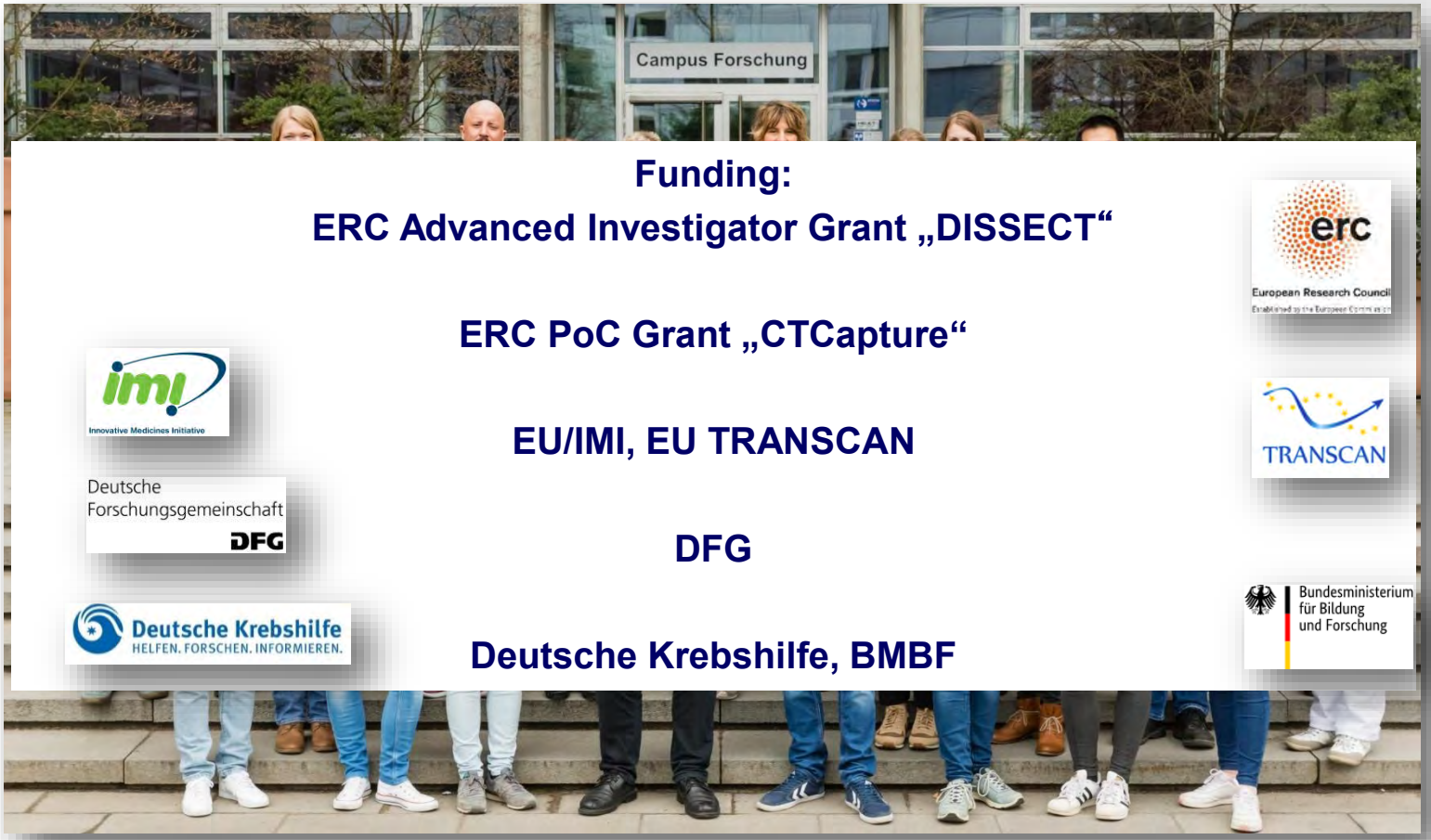
Clinical Applications of Circulating Tumor Cells and Circulating Tumor DNA as Liquid Biopsy

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

CTCs, ctDNA and exosomes provide complementary information for liquid biopsy

Micrometastasis Research Network at UCCH/UKE

Center of Experimental Medicine
Institute of Tumor Biology - THE TEAM !



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ERC PoC Grant „CTCapture“

EU/IMI, EU TRANSCAN

DFG

Deutsche Krebshilfe, BMBF