Clinical Applications of Circulating Tumor Cells (CTCs)

Klaus Pantel

Director, Institute for Tumor Biology, Professor of Medicine, UKE, University Cancer Center Hamburg (UCCH)

Adjunct Professor, University of Bergen, Norway

pantel@uke.de

Klaus Pantel – Career Development Steps

since 2016	Visiting Professor of Medicine, University of Bergen, Norway
since 2015	Member of the Executive Board of the University Cancer Center Hamburg – Hubertus Wald Tumor Center
since 06/2002	Director of the new Institute of Tumor Biology, Full Professor (C4) of Medicine, University Hospital Eppendorf (UKE), University of Hamburg, Germany
01/1999 – 05/2002	Professor (C3) of Molecular Oncology; Head, Molecular Oncology, Department of Gynecology & Obstetrics, UKE, Hamburg
05/1989 – 12/1999	Group Leader, Ass. Professor, Ludwig-Maximilians-Universität Munich, Institute of Immunology; Habilitation in Immunology (1995)
05/1987 – 04/1989	Postdoctoral DFG & DKH Fellow, Wayne State University Detroit, MI, USA, Topic: Experimental Hematology – Stem Cell Regulation
1987	Dr. med., University of Cologne Subject: Mathematical Modeling in Hematopoiesis

ELBS: Translation from discovery to clinical implementation

THE EUROPEAN LIQUID BIOPSY SOCIETY

Goals:

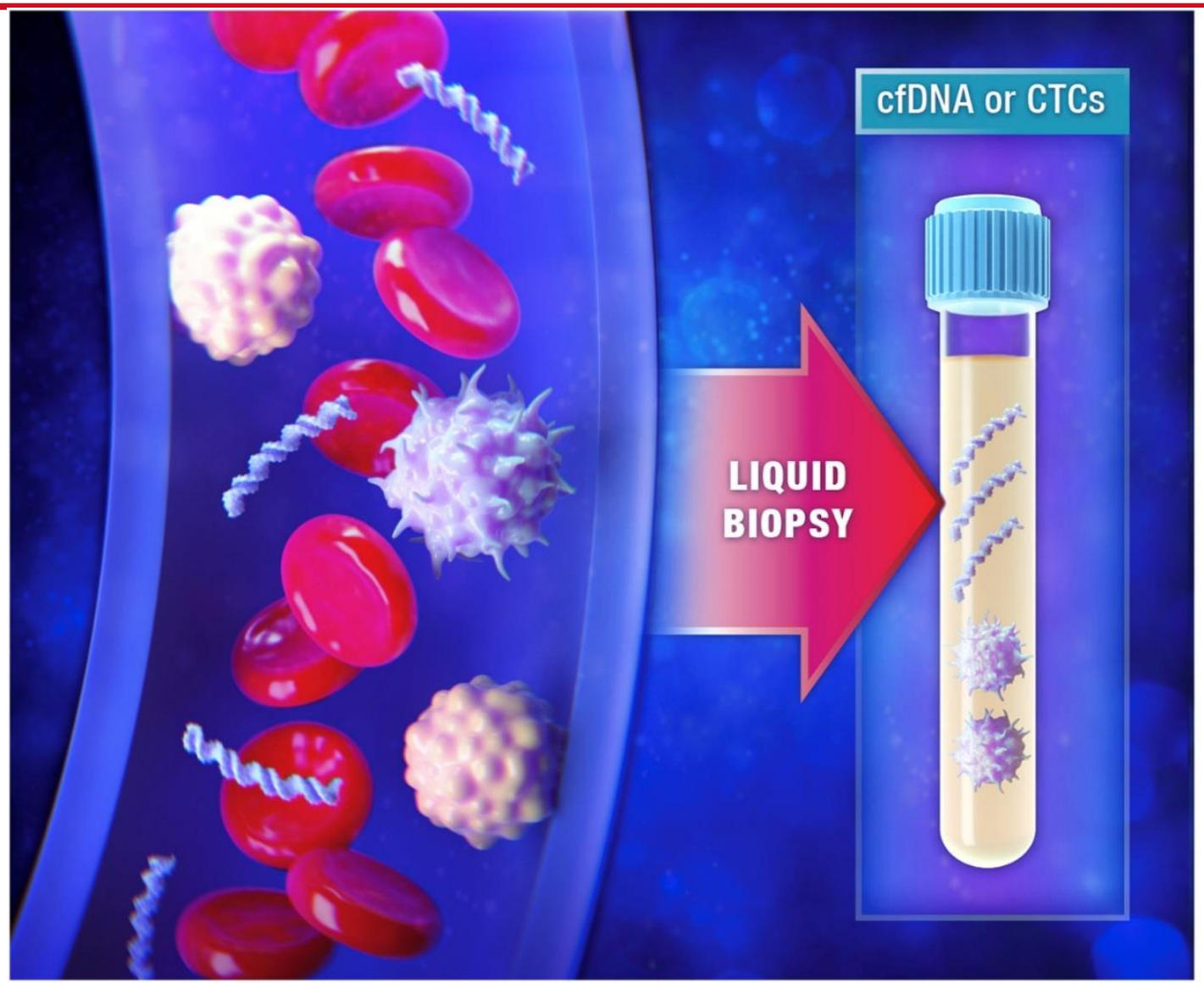
- Foster the introduction of *liquid biopsy* into clinical practice.
- Encourage interactions between academia and industry.
- Provide a partner for regulatory agencies, healthcare providers and patient advocacy groups
- Support the implementation of liquid biopsy tests into clinical trials
- Develop guidelines and provide training in *liquid biopsy*
- Disseminate the knowledge about *liquid biopsy*
- Increase visibility of Europe as leading hub for *liquid biopsy* research
- Outreach to non-EU networks

64 Institutions from Academia & Industry

Coordinator: Klaus Pantel, UKE
pantel@uke.de

ELBS is a Founding Members of the International Liquid Biopsy Standardization Alliance coordinated by the Foundation of the National Institute of Health (NIH), USA (Coordination: Dana Connors)
White Paper: Connors et al., Crit. Rev. Hematol. Oncol. 2020

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:

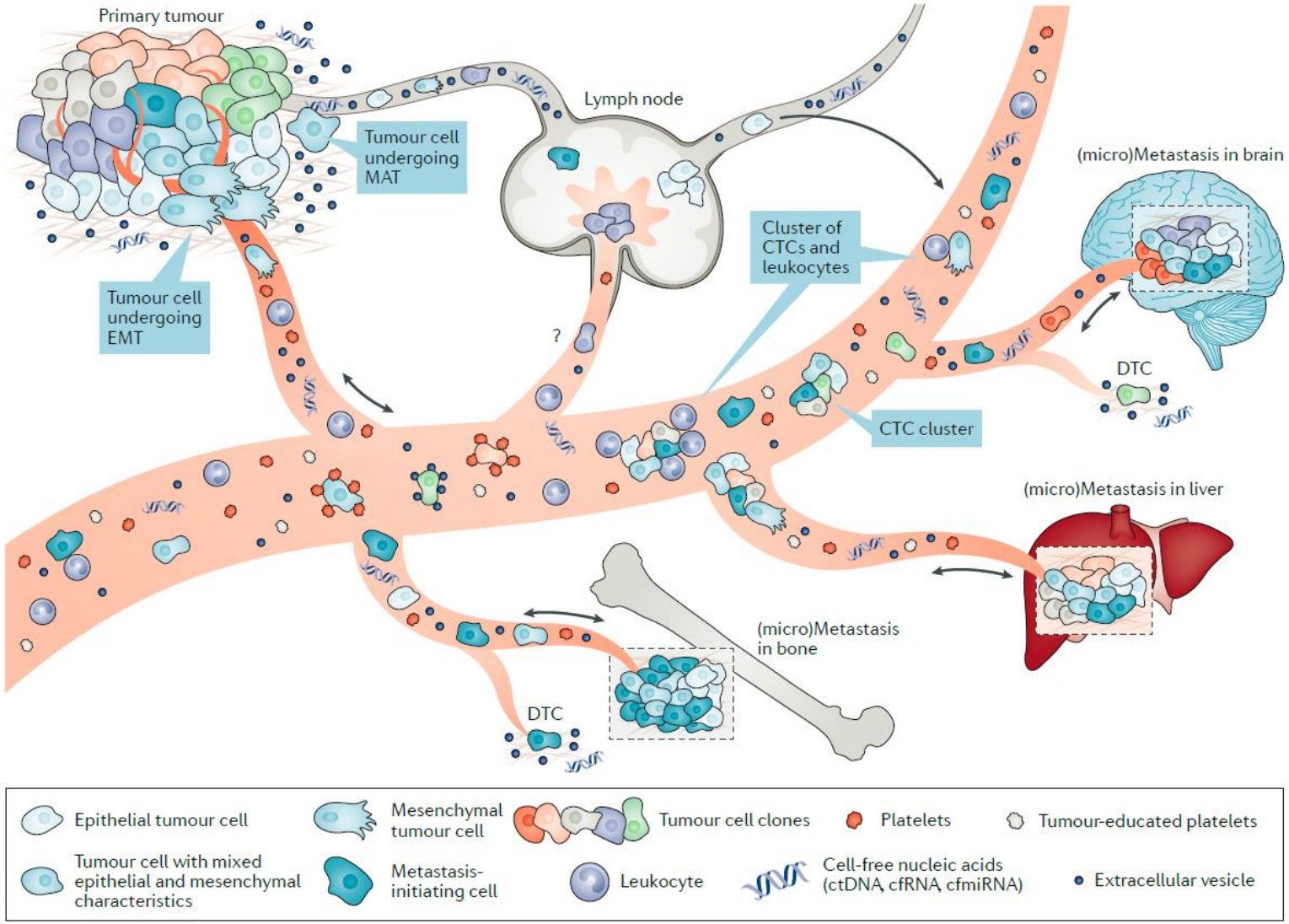
Emergence of molecular alterations associated with resistance to therapy

MINIMAL RESIDUAL DISEASE:

The presence of cfDNA or CTC in the circulation indicates that the disease is stll present

Bardelli & Pantel, *Cancer Cell*, 2017

Liquid Biopsy: Comprehensive assessment of circulating blood biomarkers



Keller and Pantel, Nature Reviews Cancer, 2019

Early Detection of Cancer

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ANNALS OF ONCOLOGY driving innovation in oncology

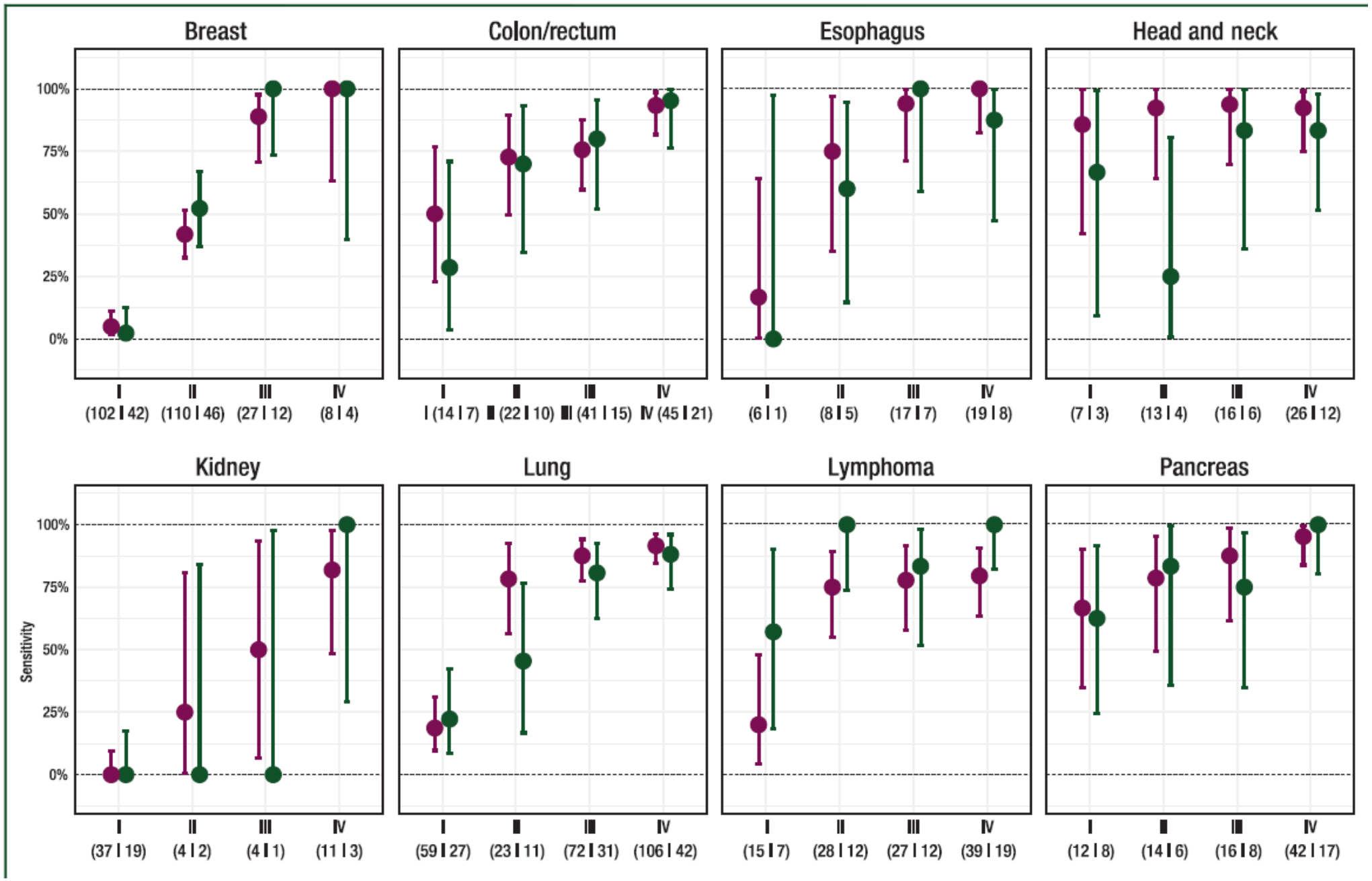
ORIGINAL ARTICLE

Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA

M. C. Liu[1†], G. R. Oxnard[2†], E. A. Klein[3], C. Swanton[4,5], M. V. Seiden[6*] & on behalf of the CCGA Consortium[‡]

[1]Division of Medical Oncology, Department of Oncology, Mayo Clinic, Rochester; [2]Lowe Center for Thoracic Oncology, Dana Farber Cancer Institute, Boston; [3]Glickman Urological and Kidney Institute, Cleveland Clinic, Cleveland, USA; [4]Cancer Evolution and Genome Instability Laboratory, The Francis Crick Institute; [5]Cancer Evolution and Genome Instability Laboratory, University College London Cancer Institute, London, UK; [6]US Oncology Research, US Oncology, The Woodlands, USA

Available online 30 March 2020


Breast
Colon/rectum
Esophagus
Head and neck
Kidney
Lung
Lymphoma
Pancreas
Sensitivity
100%
75%
50%
25%
0%
I (102 | 42)
II (110 | 46)
III (27 | 12)
IV (8 | 4)
I (14 | 7)
II (22 | 10)
III (41 | 15)
IV (45 | 21)
I (6 | 1)
II (8 | 5)
III (17 | 7)
IV (19 | 8)
I (7 | 3)
II (13 | 4)
III (16 | 6)
IV (26 | 12)
I (37 | 19)
II (4 | 2)
III (4 | 1)
IV (11 | 3)
I (59 | 27)
II (23 | 11)
III (72 | 31)
IV (106 | 42)
I (15 | 7)
II (28 | 12)
III (27 | 12)
IV (39 | 19)
I (12 | 8)
II (14 | 6)
III (16 | 8)
IV (42 | 17)

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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: Detection of prostate cancer

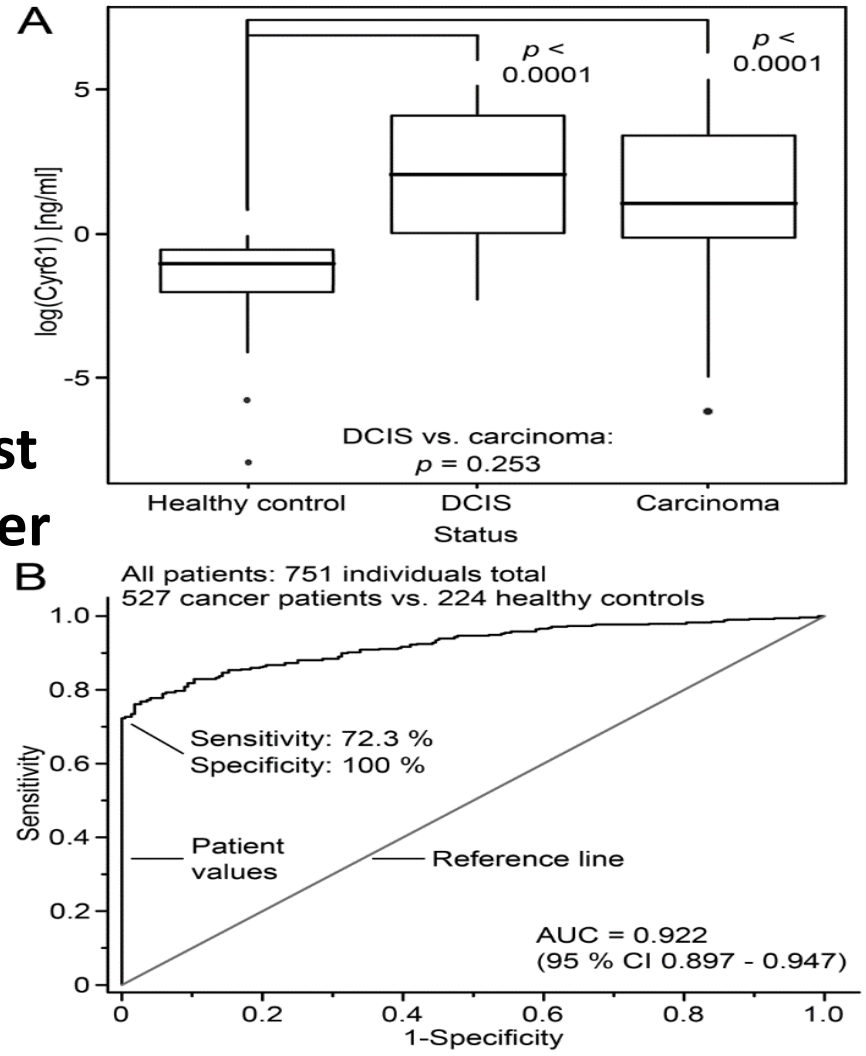
Coordinator: Klaus Pantel, Hamburg
PIs: C. Alix-Panabieres, D. Bonci, J. Budna/M. Zabel, E. Lianidou

pre-diagnostic samples / datasets

g City Health

Circulating Cyr61 protein as liquid biopsy biomarker for early detection of cancer

Breast Cancer



Breast Cancer: Bartkowiak, Heidrich, Pantel et al, Clin Chem. 2021

Lung Cancer: Ackar, Pantel et al., Mol. Oncol. 2021

Asbestos-related diseases: Bartkowiak, Pantel et al., Clin. Chem. 2020

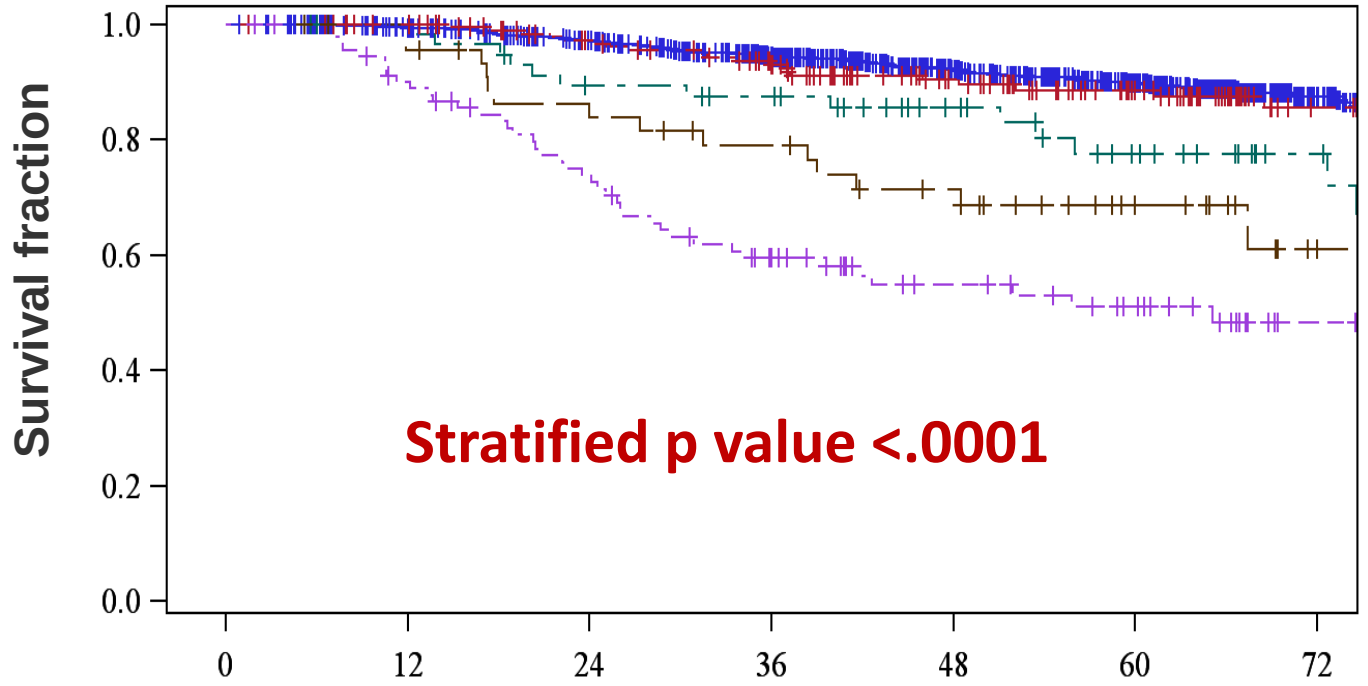
CTC Counts at Initial Cancer Diagnosis are Associated with Unfavorable Prognosis

- **Breast Cancer:** Xenidis, Lianidou, Mavroudis et al., JCO 2006, Rack, Pantel, Janni *et al. JNCI* 2014; Janni, Pantel *et al. Clin Cancer Res* 2016; Riethdorf, Pantel et al., Clin Cancer Res., 2017; Bidard, Pierga, Pantel et al, JNCI 2018
- **Bladder Cancer:** Rink, Pantel *et al. Eur Urol* 2012; Giavazzi, Pantel *et al. Int J Cancer* 2014

CTCs can be used as enrichment tool to study a high risk population cM0(i+), AJCC Cancer Staging Manual 2018 for breast cancer

- **Colorectal Cancer:** Yokobori, Mimori, Mori, Pantel *et al. Cancer Res* 2013 (incl. stage II); Deneve, Pantel, Alix-Panabieres *et al. Clin Chem* 2013; Abdallah, Pantel et al, PlosOne 2021; Heidrich, Pantel et al, Cancers 2021
- **Pancreatic Cancer:** Effenberger, Bockhorn, Pantel *et al. Clin Cancer Res* 2018; Christine Nitschke (Oral Presentation - Session 02: Thursday 1pm)
- **Merkel Cell Cancer:** Riethdorf, Pantel et al., *Clin. Chem*. 2018
- **Melanoma:** Wiltfang, Roeck, Pantel et al, Cancers, 2019

CTC before Neoadjuvant Cancer Therapy & Overall Survival in Breast Cancer



≤1 CTC/sample

≥ 5 CTCs/sample

	N pts	% events	Hazard Ratio
0 CTC	1175	9.8%	1
1 CTC	199	10.6%	1.09 [0.65-1.69]
2 CTCs	59	23.7%	**2.63** [1.42-4.54]
3-4 CTCs	47	29.8%	**3.84** [2.08-6.66]
≥ 5 CTCs	93	46.2%	**6.25** [4.34-9.09]

Bidard, Pantel *et al. JNCI* 2018

2nd ERC Advanced Investigator Grant INJURMET (PI: Klaus Pantel, 2019-2024)

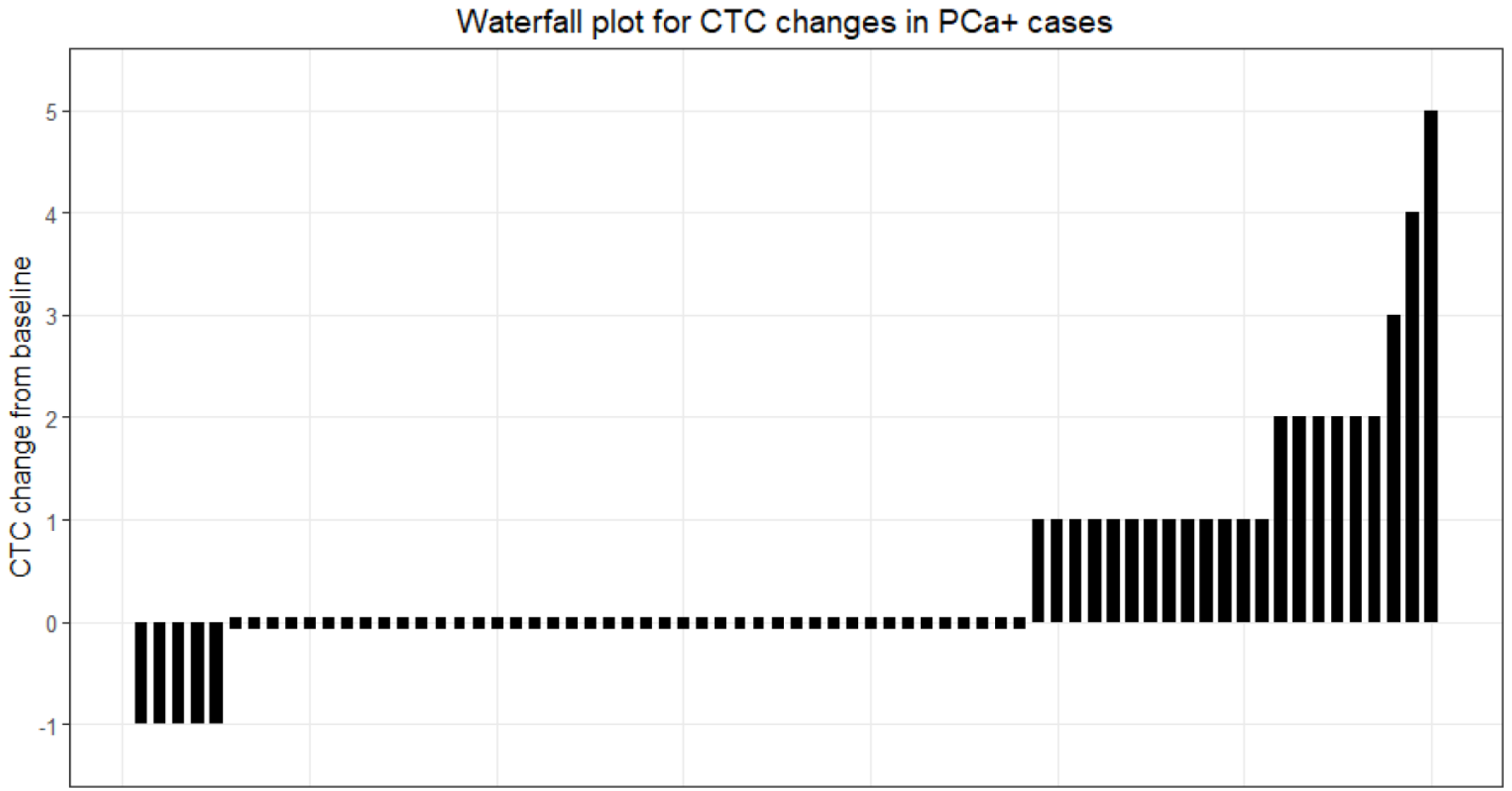
Diagnostic Biopsies, Surgery, Radiotherapy
(Breast & Prostate Cancer)

REVIEWS

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

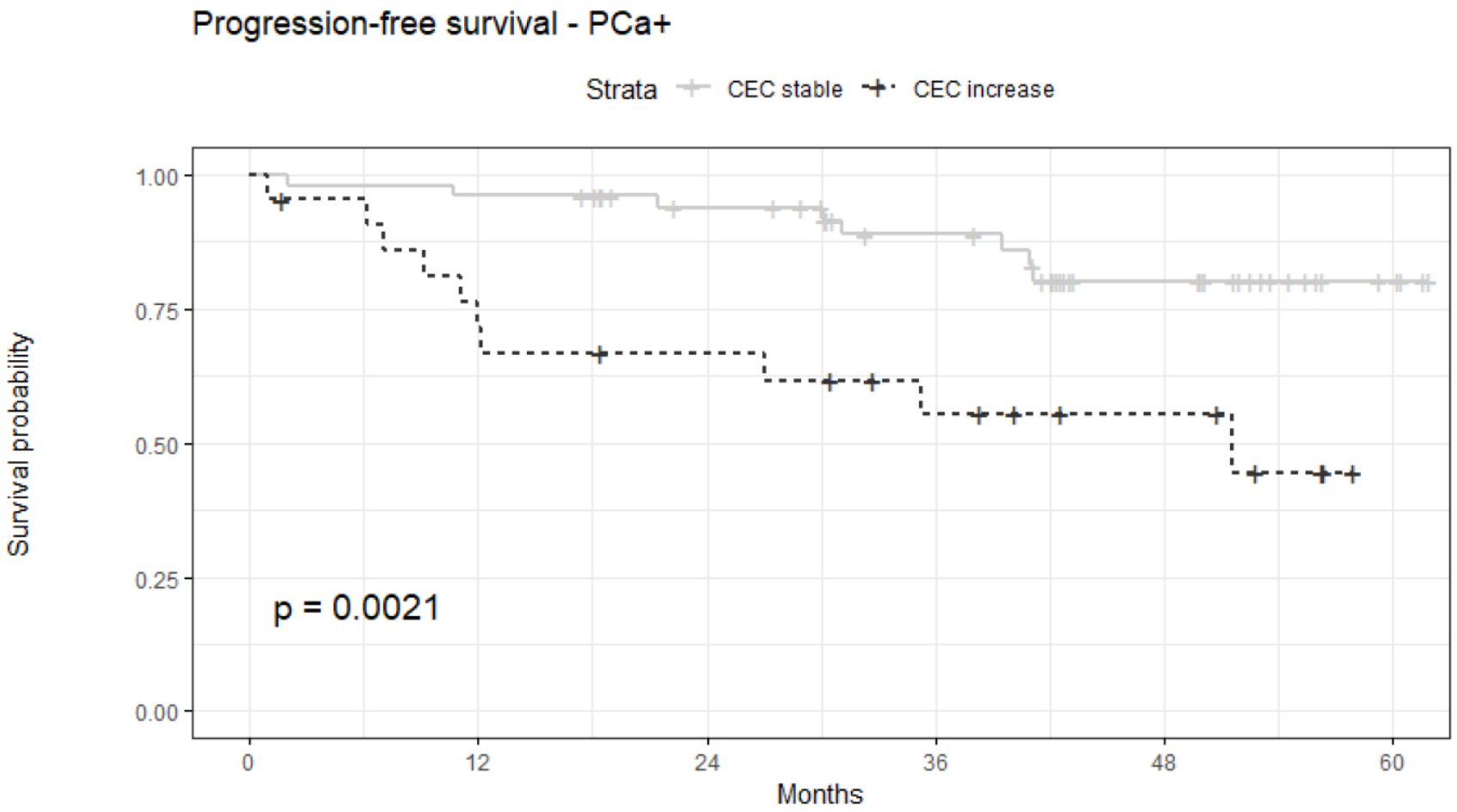
Olga A. Martin[1,2,4], Robin L. Anderson[3,4], Kailash Narayan[1,4,5] and Michael P. MacManus[1,4]

Increase in numbers of circulating epithelial cells in blood after biopsy of men subsequntly diagnosed with prostate cancer



Joosse, Kuske,…, Alix-Panabieres,Riethdorf, Schlomm, Pantel et al., Clin. Chem, 2019

Progression-free survival in 74 prostate cancer patients with (n=22) and without (n=52) increase in numbers of circulating epithelial cells in blood after biopsy



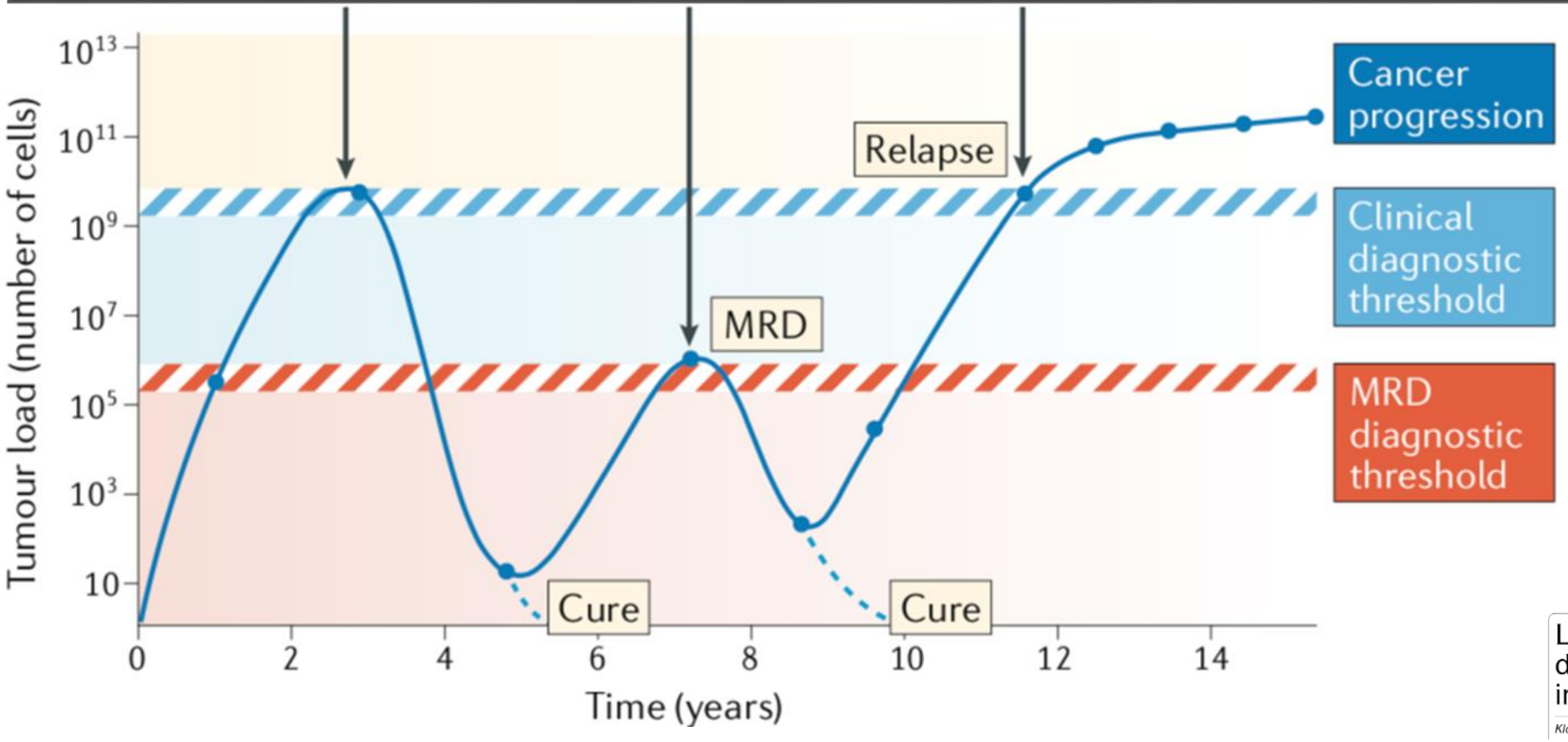
Joosse, Kuske,…, Alix-Panabieres,Riethdorf, Schlomm, Pantel et al., Clin. Chem, 2019

Minimal Residual Disease (MRD)

Tumor evolution: Dynamic changes in tumour burden in cancer patients

Challenge: Detect Minimal Residual Disease (MRD) and monitor tumor evolution in individual cancer patients as prerequisite for early intervention

(Alix-Panabieres & Pantel, Nature Rev. Cancer 2014; Bardelli & Pantel, Cancer Cell 2017; Pantel & Hayes, *Nature Rev. Clin. Oncol*. 2018; Pantel & Alix-Panabieres, *Nature Rev. Clin. Oncol*. 2019; Keller & Pantel, *Nature Rev. Cancer* 2019, Hofbauer, Pantel et al, *Nature Rev. Clin. Oncol*. 2020)



Imaging (CT, MRI)

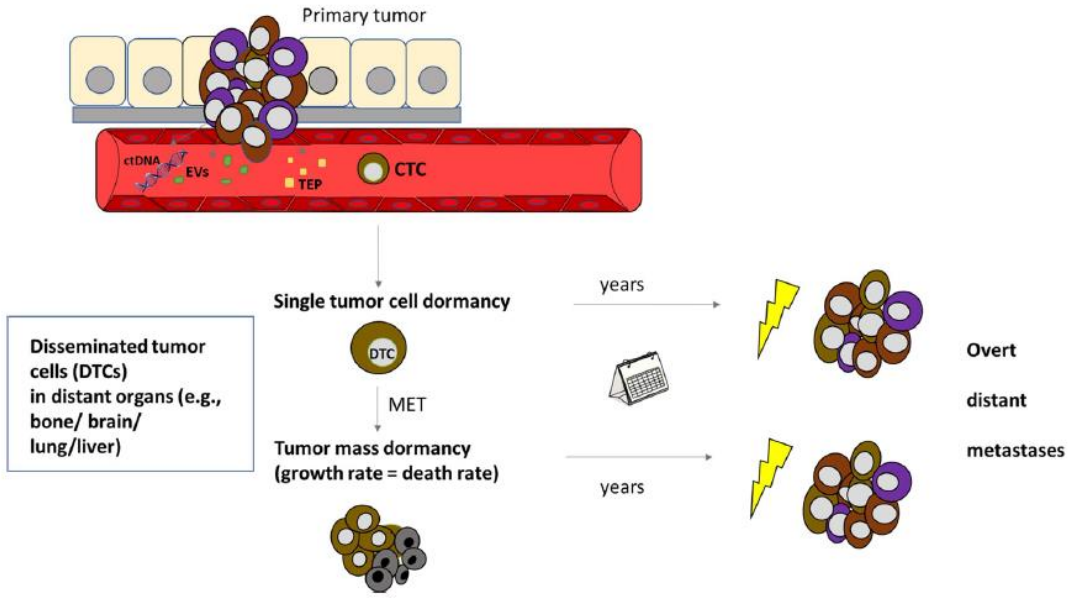
Liquid biopsy

nature REVIEWS CLINICAL ONCOLOGY

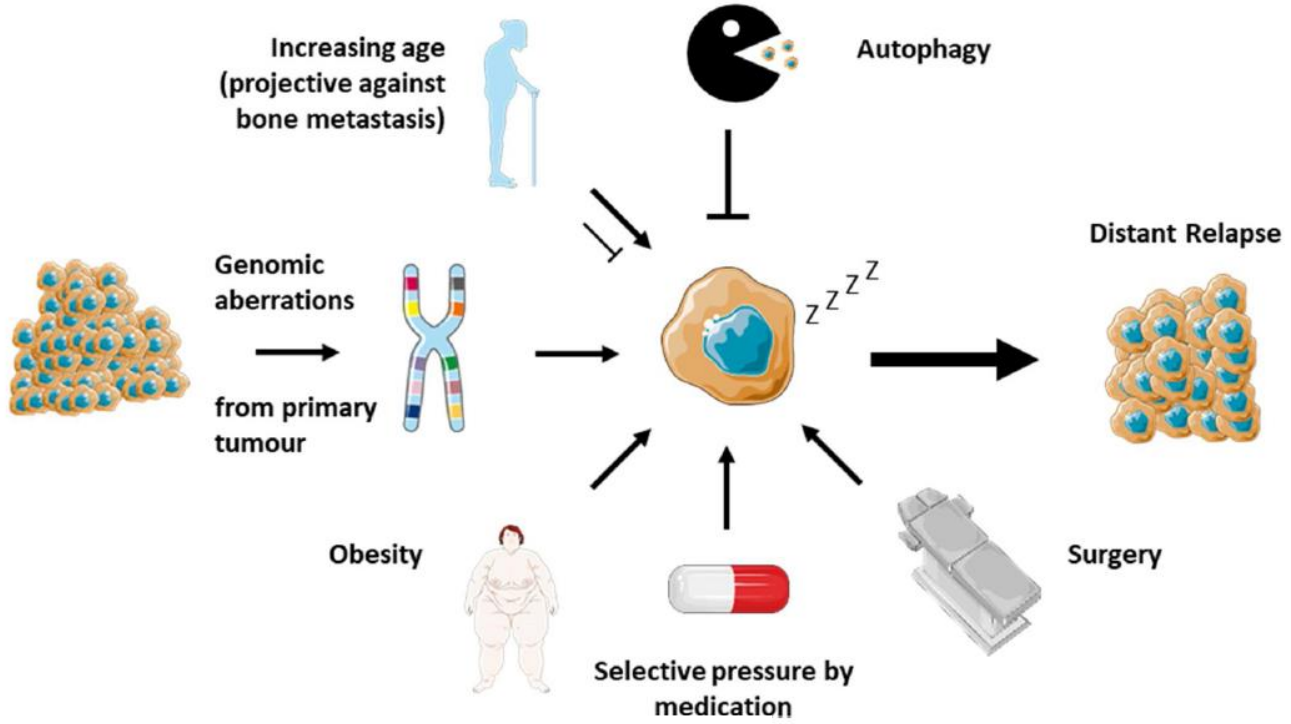
Liquid biopsy and minimal residual disease — latest advances and implications for cure

Klaus Pantel[1] * and Catherine Alix-Panabières[2]

Cancer dormancy & MRD



Werner, Heidrich, Pantel, Sem. Cancer Biol. 2022



cancers

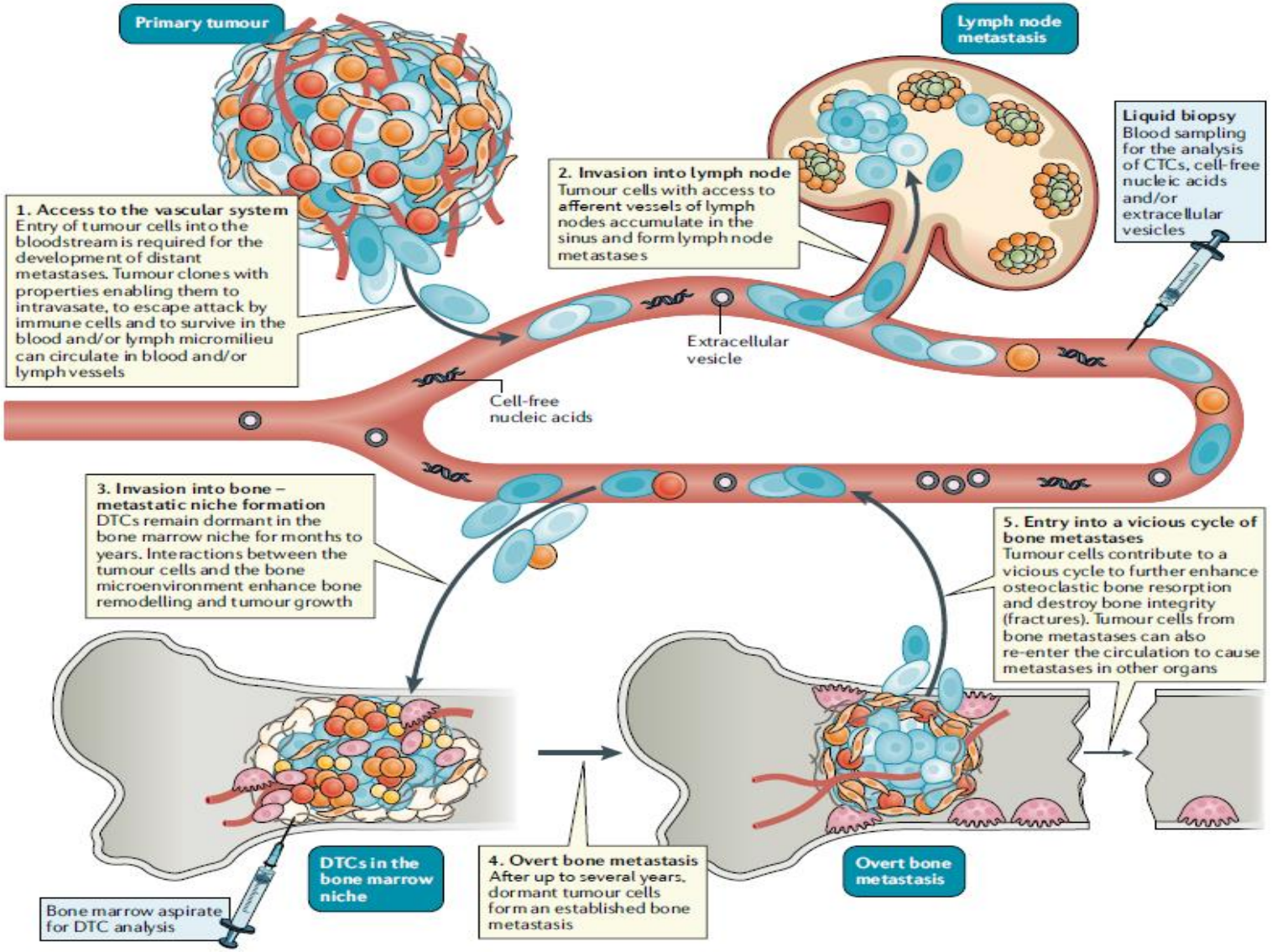
MDPI

Article

Metastatic Breast Cancer Recurrence after Bone Fractures

Nadia Obi [1,†], Stefan Werner [2,3,†], Frank Thelen [4], Heiko Becher [1] and Klaus Pantel [2,*]

Biology of Micrometastasis in Bone Marrow



Bone marrow is a reservoir for micrometastatic tumor cells

DFG SPP microBONE Consortium

(Hofbauer, Pantel et al, Nat. Rev. Clin. Oncol. 2021; Werner, Heidrich, Pantel, Sem. Cancer Biol. 2022)

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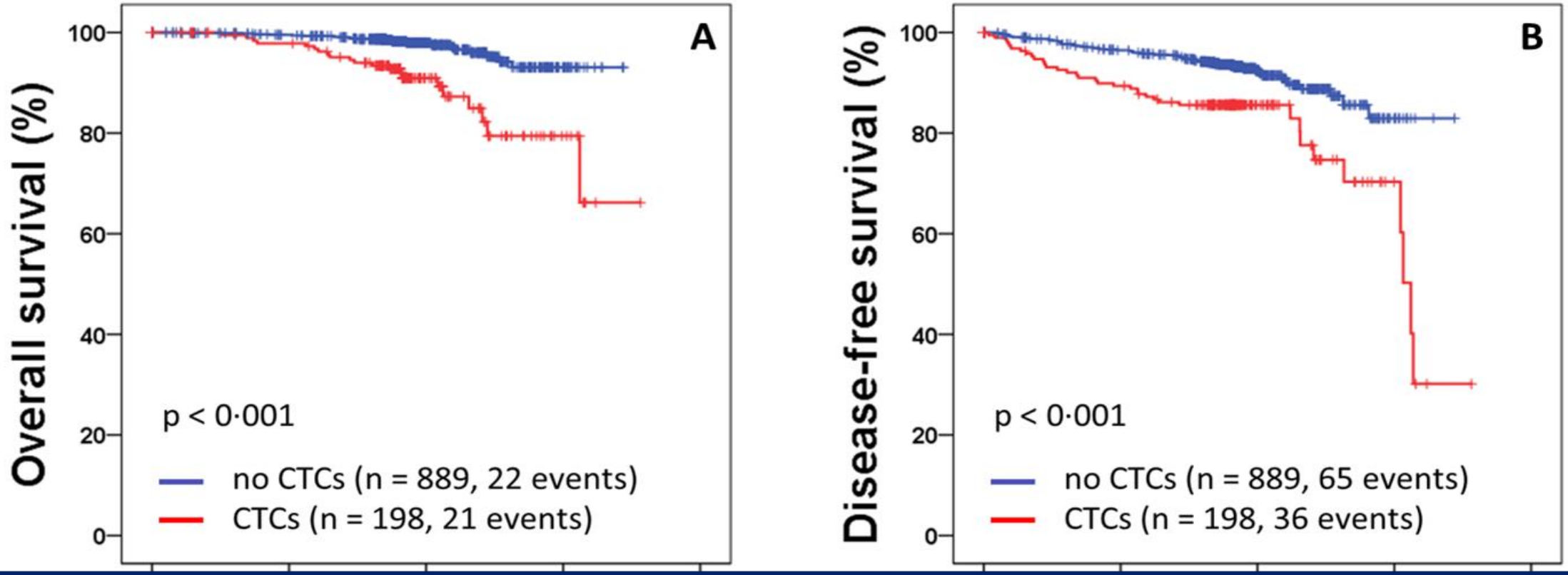
Can we detect MRD in the peripheral blood by CTC analyses?

CTCs in high-risk early breast cancer patients during follow-up



Need for „Post-Adjuvant“ Clinical Trials

(Pantel & Hayes, *Nature Rev. Clin. Oncol*. 2018)

Figure 2 CTCs detected 2 years after adjuvant chemotherapy

Trapp, Janni, Pantel *et al. (SUCCESS study group), JNCI 2018*

Research

JAMA Oncology | Original Investigation

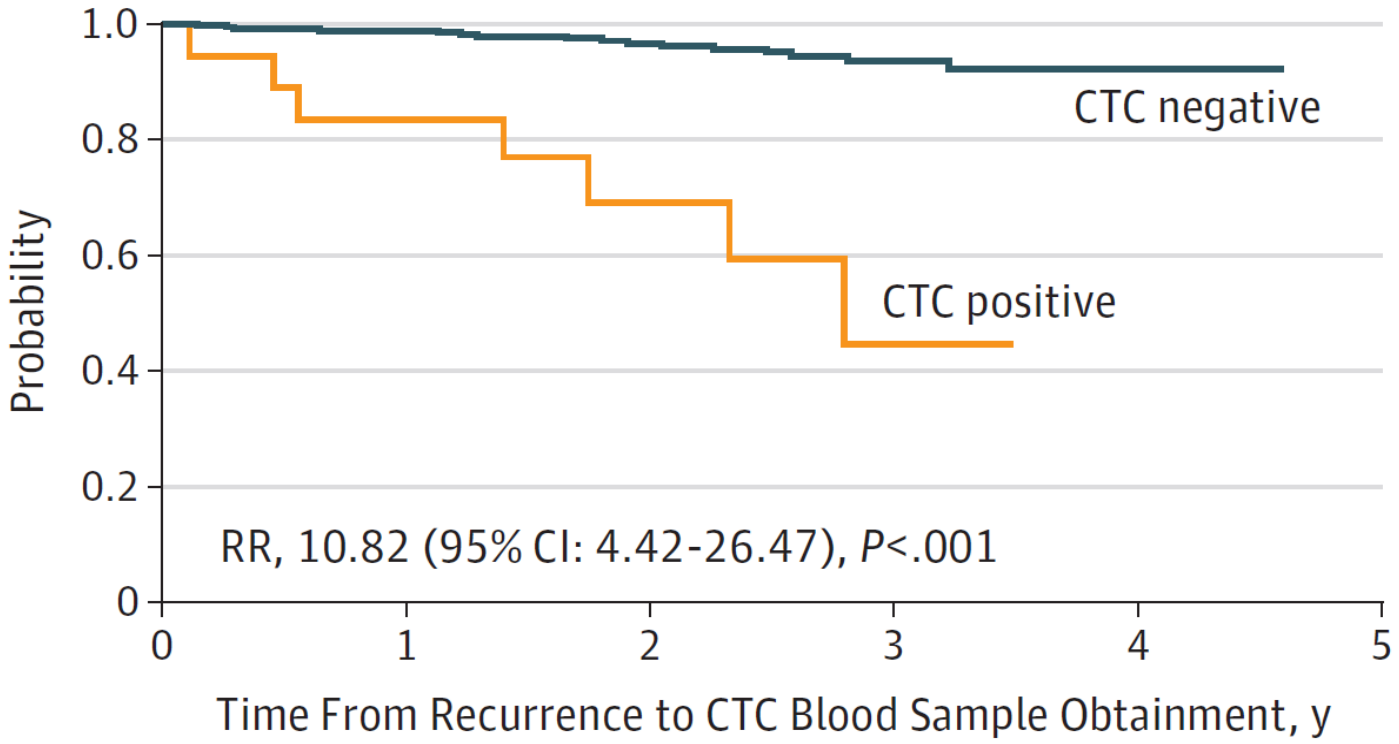
Association of Circulating Tumor Cells With Late Recurrence of Estrogen Receptor–Positive Breast Cancer

A Secondary Analysis of a Randomized Clinical Trial

Joseph Sparano, MD; Anne O'Neill, MS; Katherine Alpaugh, PhD; Antonio C. Wolff, MD; Donald W. Northfelt, MD; Chau T. Dang, MD; George W. Sledge, MD; Kathy D. Miller, MD

Blood was analyzed approx. 5 years after cancer diagnosis

Figure 2. Time to Recurrence by Circulating Tumor Cell (CTC) Assay Result Among Patients With Hormone Receptor–Positive Breast Cancer



No. at risk

CTC negative	335	306	211	102	16	0
CTC positive	18	13	7	3	0	0

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Liquid Biopsy in Metastatic Patients

Monitoring of CTC & ctDNA counts

CTC & ctDNA Characterization

CTCs vs. conventional tumor markers (PFS, p values) in metastatic breast cancer patients (n=1944) receiving chemotherapy

Model used as referen-ce	(								
	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

Clinical Utility of CTCs

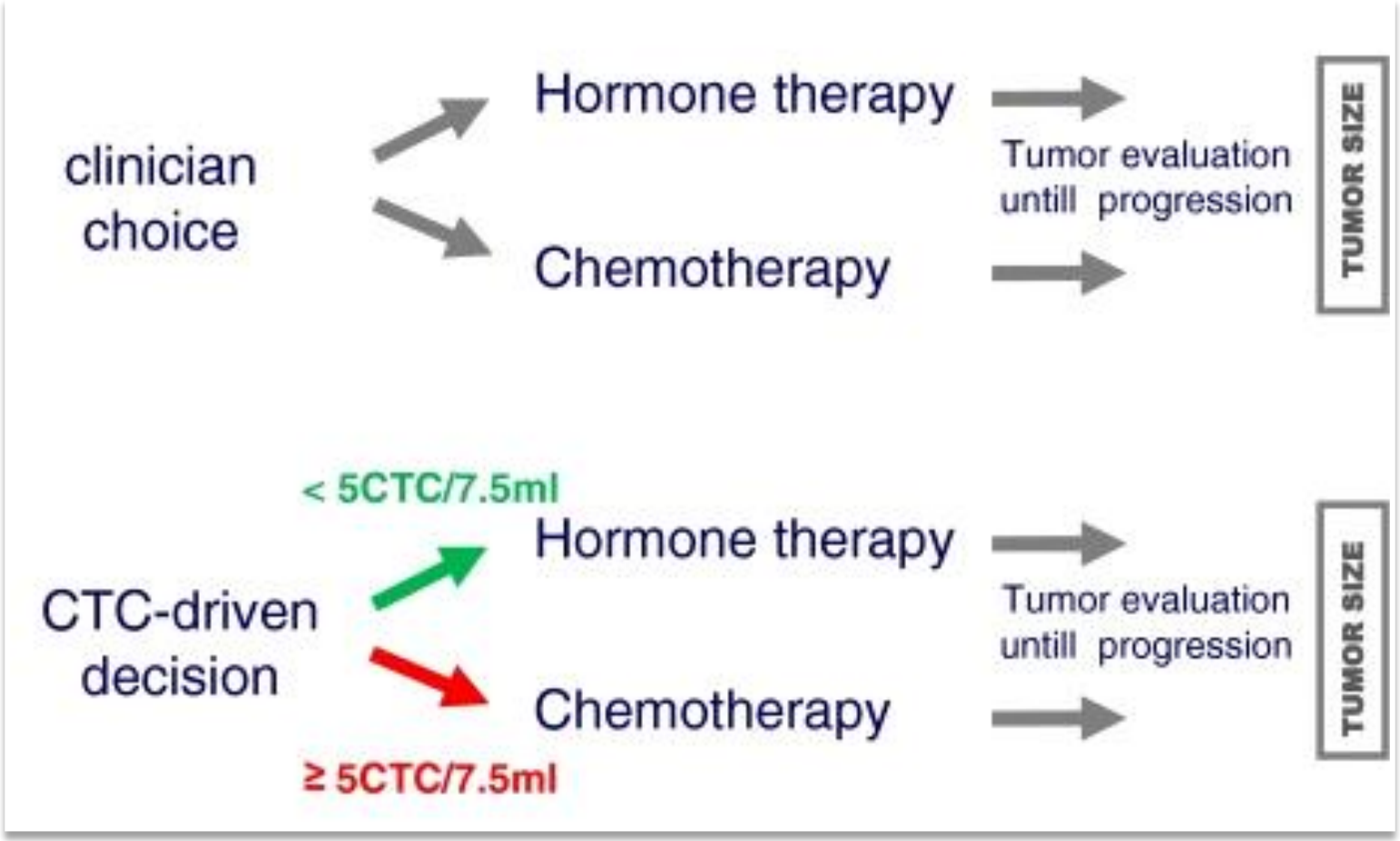
JAMA Oncology | Original Investigation

Efficacy of Circulating Tumor Cell Count–Driven vs Clinician-Driven First-line Therapy Choice in Hormone Receptor–Positive, ERBB2-Negative Metastatic Breast Cancer

The STIC CTC Randomized Clinical Trial

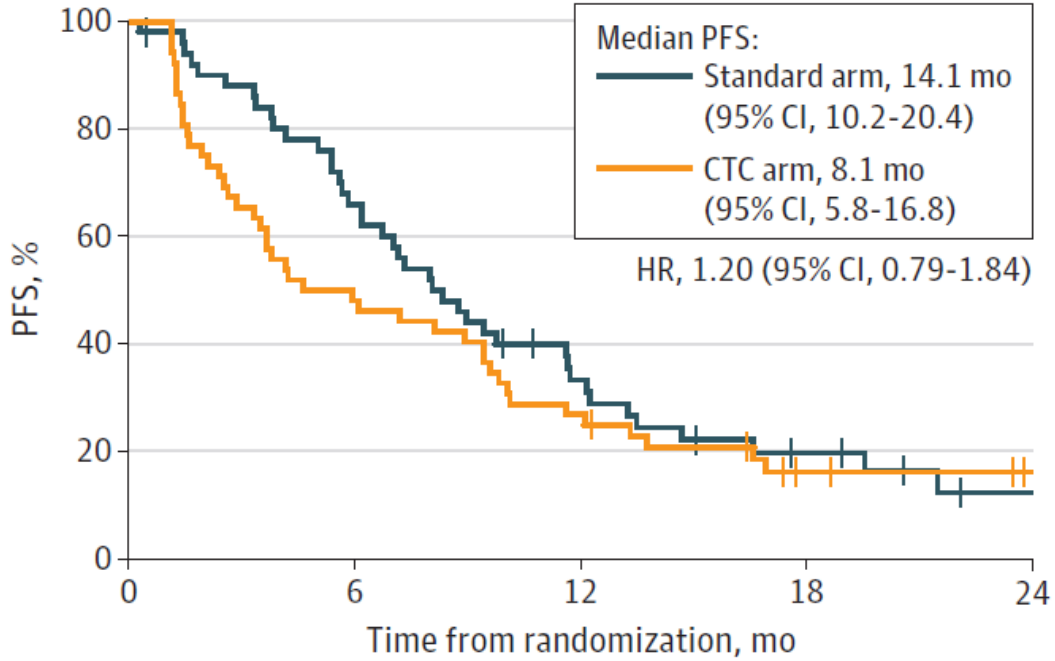
François-Clément Bidard, MD, PhD; William Jacot, MD, PhD; Nicolas Kiavue, MBBS; Sylvain Dureau, PharmD; Amir Kadi, PhD; Etienne Brain, MD, PhD; Thomas Bachelot, MD; Hugues Bourgeois, MD; Anthony Gonçalves, MD, PhD; Sylvain Ladoire, MD, PhD; Hervé Naman, MD; Florence Dalenc, MD, PhD; Joseph Gligorov, MD, PhD; Marc Espié, MD; George Emile, MD; Jean-Marc Ferrero, MD; Delphine Loirat, MD, PhD; Sophie Frank, MD; Luc Cabel, MD; Véronique Diéras, MD; Laure Cayrefourcq, MSc; Cécile Simondi, MSc; Frédérique Berger, MSc; Catherine Alix-Panabières, PhD; Jean-Yves Pierga, MD, PhD

STIC CTC Metabreast Interventional Study:
CTC-driven therapy choice in metastatic breast cancer



Subgroups with **discordant** risk estimates (Clin^{high} CTC^{low} et Clin^{low} CTC^{high})

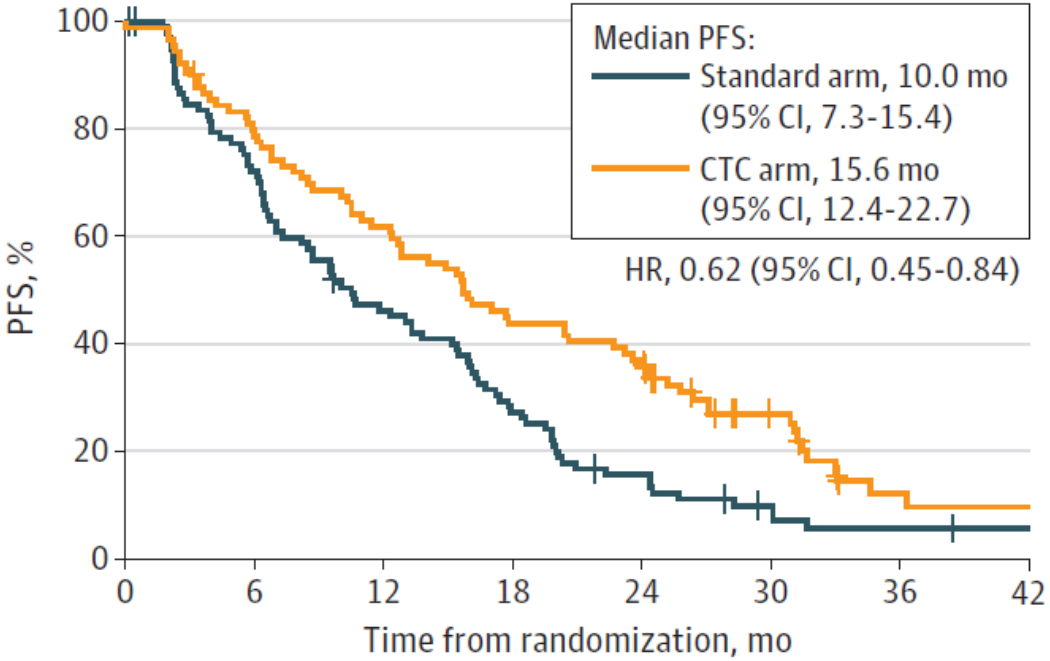
C PFS in the Clin^{high} CTC^{low} subgroup



No. at risk (patients censored)

Standard arm	51(0)	42(1)	30(1)	19(2)	11(3)	8(4)	5(7)	2(8)
CTC arm	52(0)	33(0)	24(0)	15(0)	11(1)	7(2)	4(5)	2(7)

D PFS in the Clin^{low} CTC^{high} subgroup



No. at risk (patients censored)

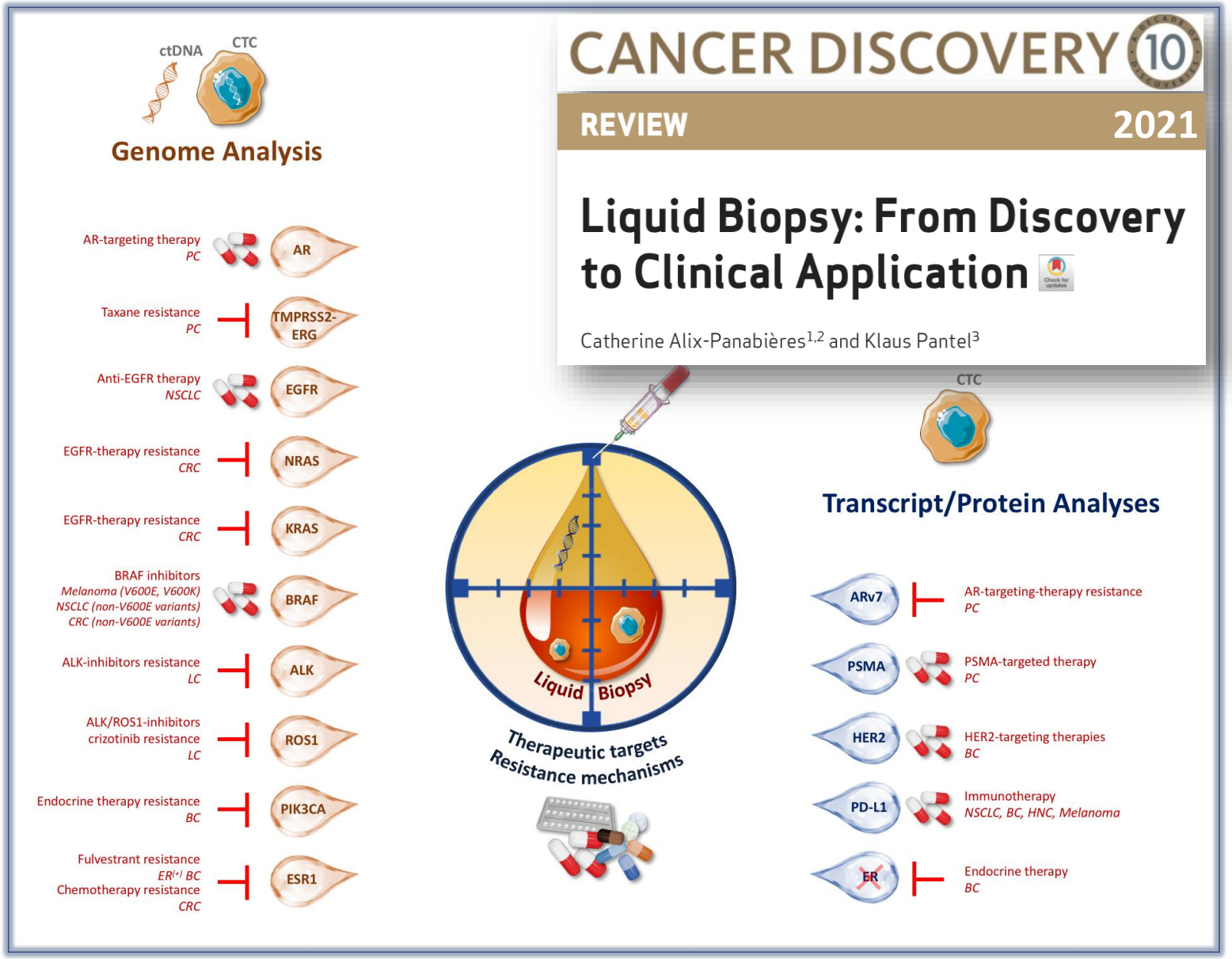
Standard arm	99(0)	70(2)	44(3)	26(3)	14(4)	7(6)	4(6)	3(7)
CTC arm	90(0)	71(1)	55(1)	39(1)	33(1)	16(10)	5(13)	4(13)

Small subgroup
No statistically different PFS

Larger subgroup
Statistically different PFS, favoring the CTC arm
(=patients treated with chemotherapy)

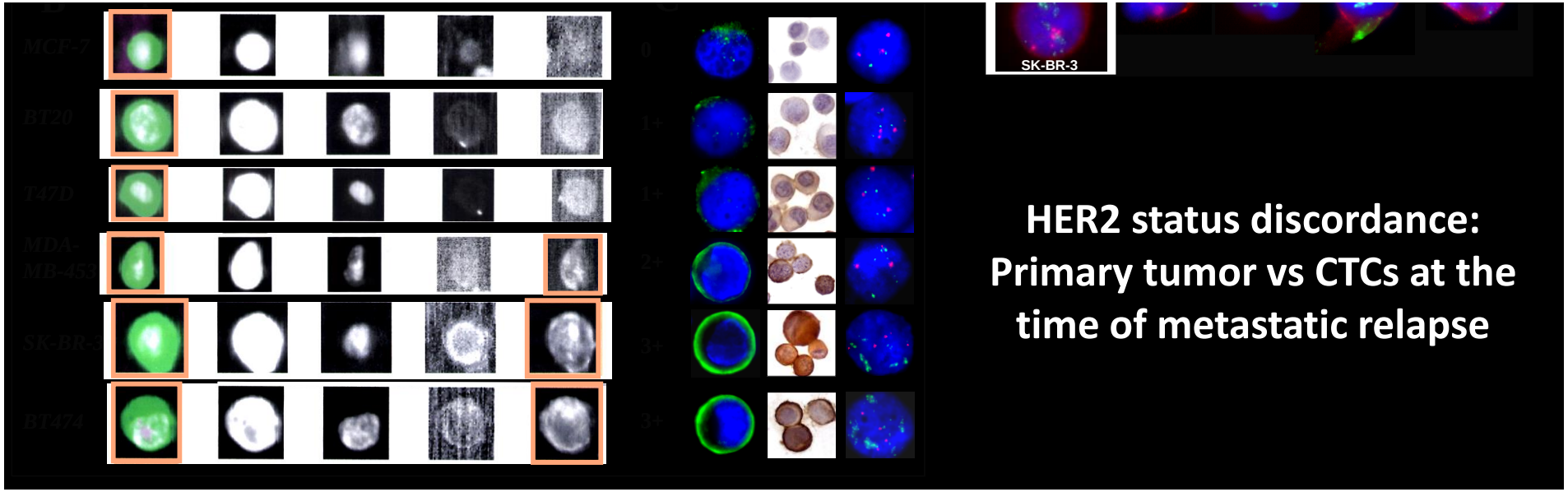
CTC count should be included in the decision algorithm for HR+ HER2- MBC patients

Therapeutic Targets and Resistance Biomarkers



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

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



HER2 status discordance: Primary tumor vs CTCs at the time of metastatic relapse

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**

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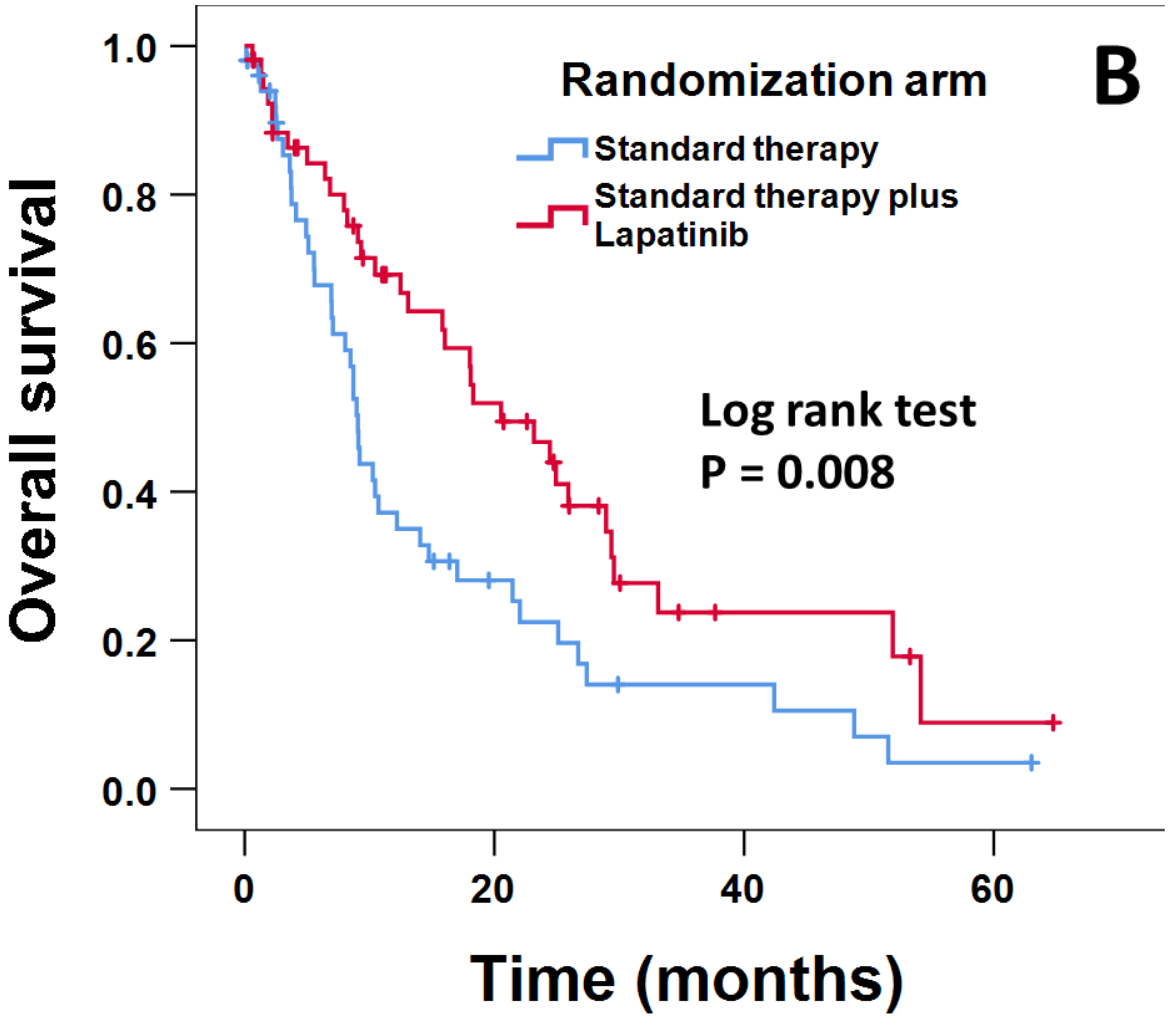
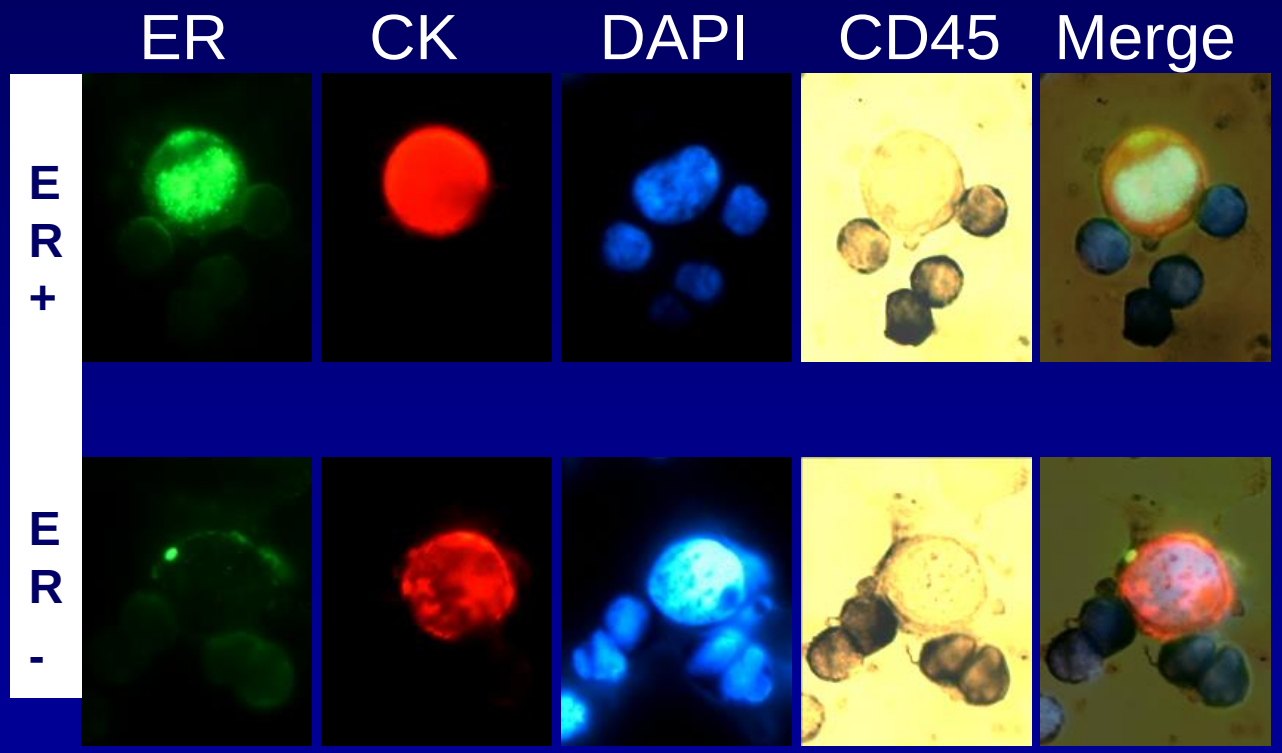
Survival of metastatic breast cancer patients with HER2- primary tumors but HER2+ CTCs receiving standard therapy with and without Lapatinib



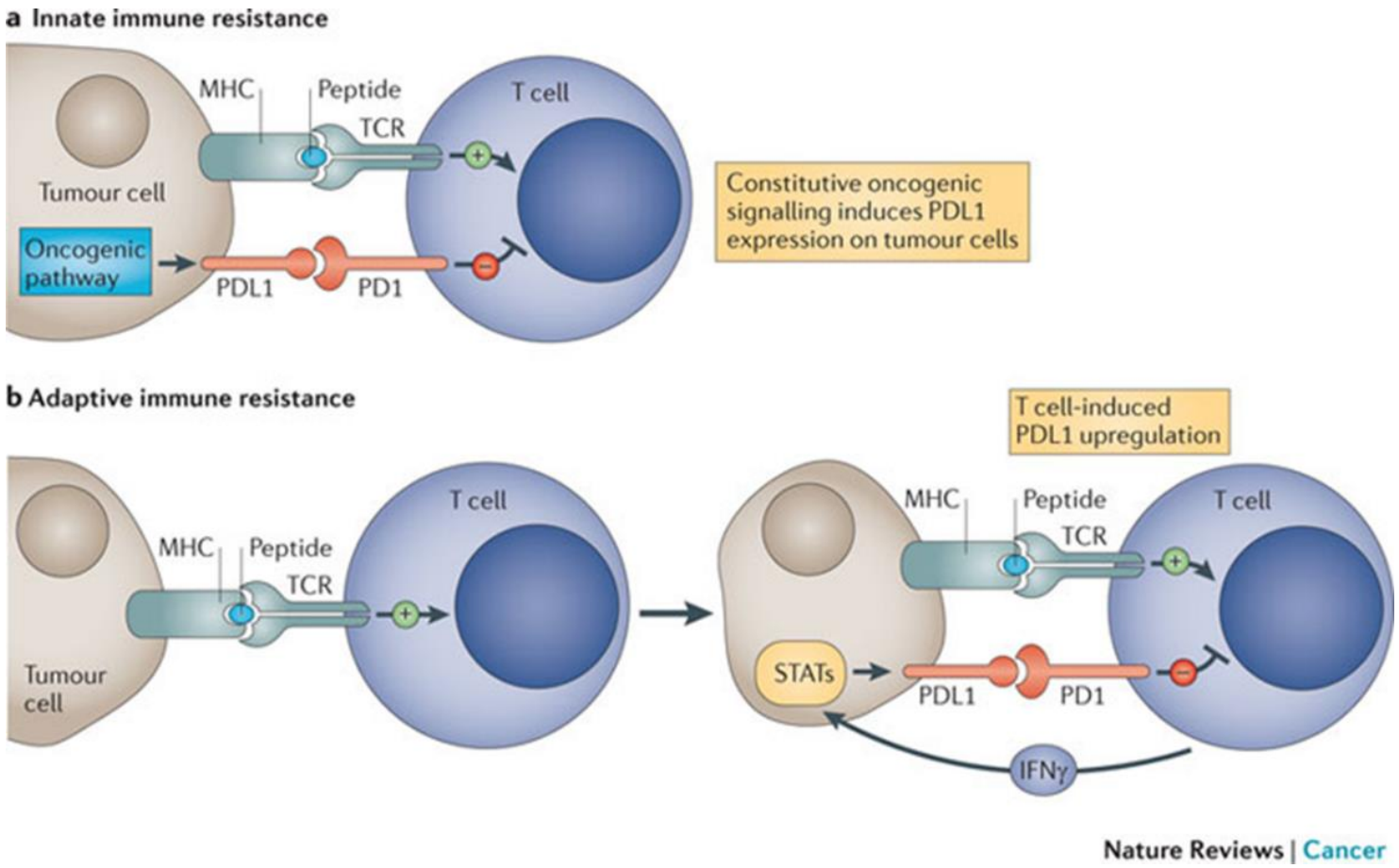
Heterogeneity of ER status in CTCs of breast cancer patients with ER-positive primary tumors

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



ER-negative CTCs may survive endocrine therapy

PD1-PDL1 mediated immune blockade as cancer target



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Combined CTC & ctDNA analysis

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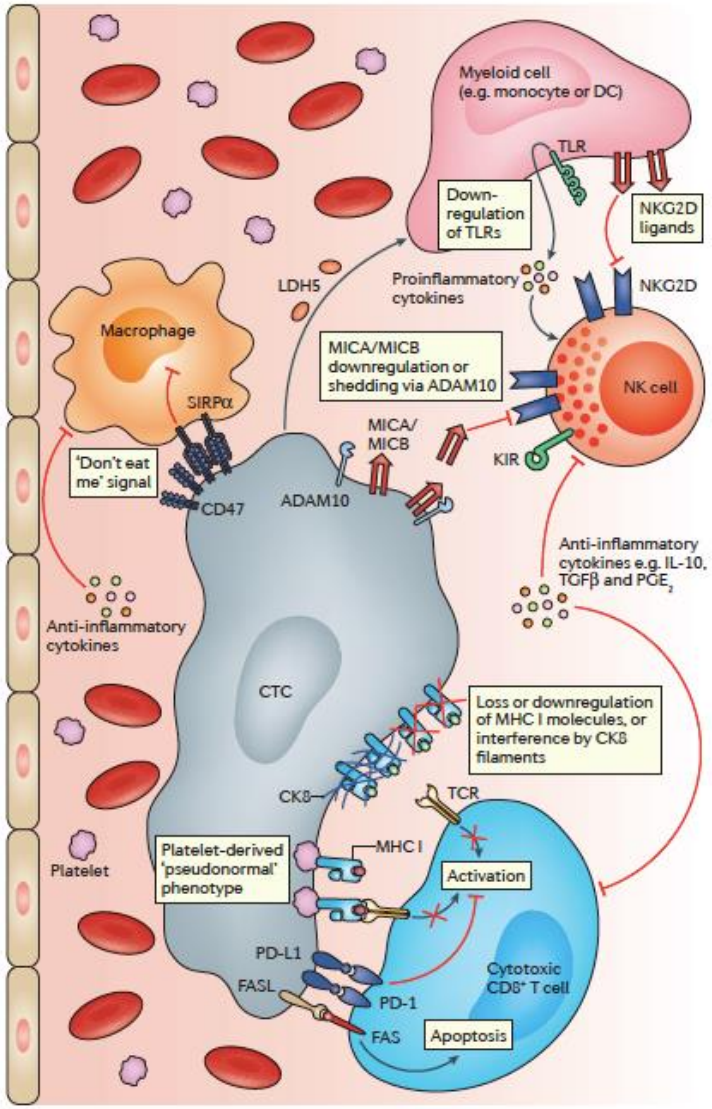


Circulating and disseminated tumour cells — mechanisms of immune surveillance and escape

Malte Mohme[1,2], Sabine Riethdorf[1] and Klaus Pantel[1]

nature REVIEWS CLINICAL ONCOLOGY

Prediction of **immune checkpoint inhibition therapy** in **NSCLC (M. Reck)** and **Melanoma (Ch. Gebhardt)**:

Monitoring of ctDNA & CTC concentrations

Combination of immune markers on **CTCs** (e.g., MHC class I & PDL1 expression) and tumor mutational burden (TMB) on **ctDNA**

German representative of the international network on cancer immunotherapy of **Tasuku Honjo**, Kyoto (Nobel Price 2018)

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

REVIEW

Liquid biopsy in the era of immuno-oncology: is it ready for prime-time use for cancer patients?

P. Hofman[1,2,3*], S. Heeke[1,2], C. Alix-Panabières[4,5] & K. Pantel[6]

Annals of Oncology 30: 1448–1459, 2019
doi:10.1093/annonc/mdz196
Published online 22 June 2019

CANCER RESEARCH | REVIEW

Current and Future Clinical Applications of ctDNA in Immuno-Oncology

Julia-Christina Stadler[1,2,3], Yassine Belloum[1], Benjamin Deitert[1], Mark Sementsov[1], Isabel Heidrich[1,2,3], Christoffer Gebhardt[2], Laura Keller[1], and Klaus Pantel[1]

THE LANCET Oncology

PD-L1 expression by circulating breast cancer cells

Rachel David

PD-L1 expression by circulating breast cancer cells

Metastatic breast cancer cells express PD-L1, the ligand for the immune checkpoint receptor PD-1, according to new research. The findings suggest that it might be possible to predict which patients will benefit from PD-1 blockade, a promising therapeutic approach in cancer.

The team, led by Catherine Alix-Panabières (University of Montpelier, Montpelier, France), used a tool called CellSearch to detect circulating tumour cells (CTCs) in blood samples from hormone-receptor-positive, HER2-negative patients with metastatic breast cancer. They assessed the samples for expression of PD-L1 with an antibody validated using western blotting and flow cytometry.

The analysis showed that PD-L1 expression is common among CTCs derived from breast tumours. Although the sample size was small (one male patient and 15 female patients), 11 of 16 samples had a subpopulation of cells expressing PD-L1, either at low or high levels.

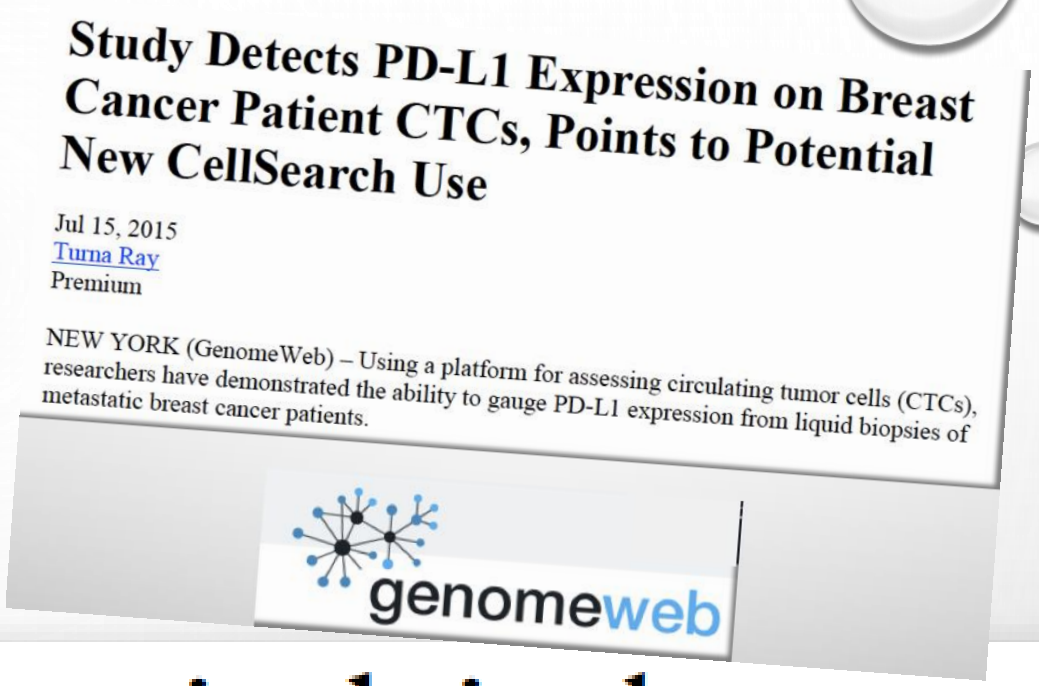
This high frequency of PD-L1 expression contrasts with previous work suggesting that breast cancer cells have low PD-L1 expression. According to Alix-Panabières, this discrepancy could be the result of higher PD-L1 expression by CTCs than by primary tumours, and potential differences in methods.

The team now wants to investigate whether their technique could be used as a "liquid biopsy" to identify patients who will benefit from treatment with PD-1 inhibitors—molecules that bind PD-1 and interfere with the checkpoint that enables cancer cells to evade the immune system. This technique could help to avoid exposing patients to any toxicities associated with PD-1 inhibitor treatment.

"It would be nice to see whether we can really stratify patients, and decide whether they will start, or not, immunotherapy", she commented. Additionally, the technique offers opportunities to "monitor patients during immunotherapy".

Daniel Hayes (University of Michigan Comprehensive Cancer Center, Ann Arbor, MI, USA) agreed that this could be a valuable method to determine "entry into clinical trials and as a pharmacodynamic monitoring tool". However, he cautioned that further research is necessary to assess the clinical usefulness of measuring PD-L1 expression by CTCs, since "it is not clear that tissue PD-L1 measurement in cancer cells is sufficiently predictive for patient selection for PD-L1 inhibitor therapy".

Rachel David



Study Detects PD-L1 Expression on Breast Cancer Patient CTCs, Points to Potential New CellSearch Use

Jul 15, 2015
Turna Ray
Premium

NEW YORK (GenomeWeb) – Using a platform for assessing circulating tumor cells (CTCs), researchers have demonstrated the ability to gauge PD-L1 expression from liquid biopsies of metastatic breast cancer patients.

genomeweb

Frequent expression of PD-L1 on circulating breast cancer cells

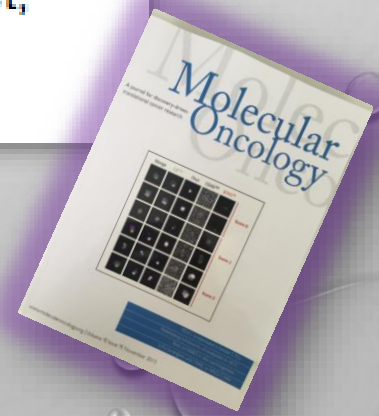
2015

Martine Mazel, William Jacot, Klaus Pantel, Kai Bartkowiak, Delphine Topart,

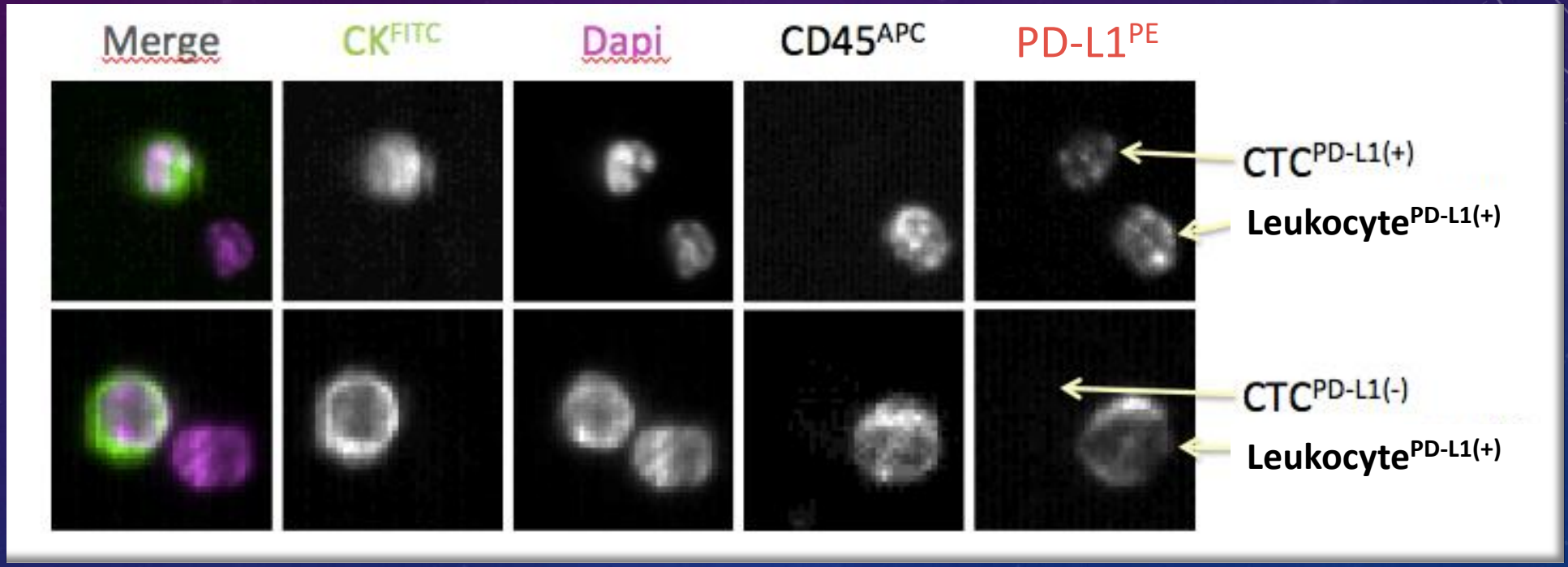
Laure Cayrefourcq, Delphine Rossille, Thierry Maudelonde, Thierry Fest,

Catherine Alix-Panabières

Molecular Oncology



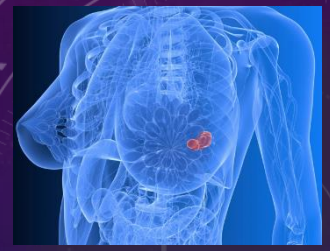
METASTATIC BREAST CANCER PATIENTES



Mazel *et al.*, *Mol Oncol* 2015
(Editorial by R. David in *Lancet Oncol.* 2015)

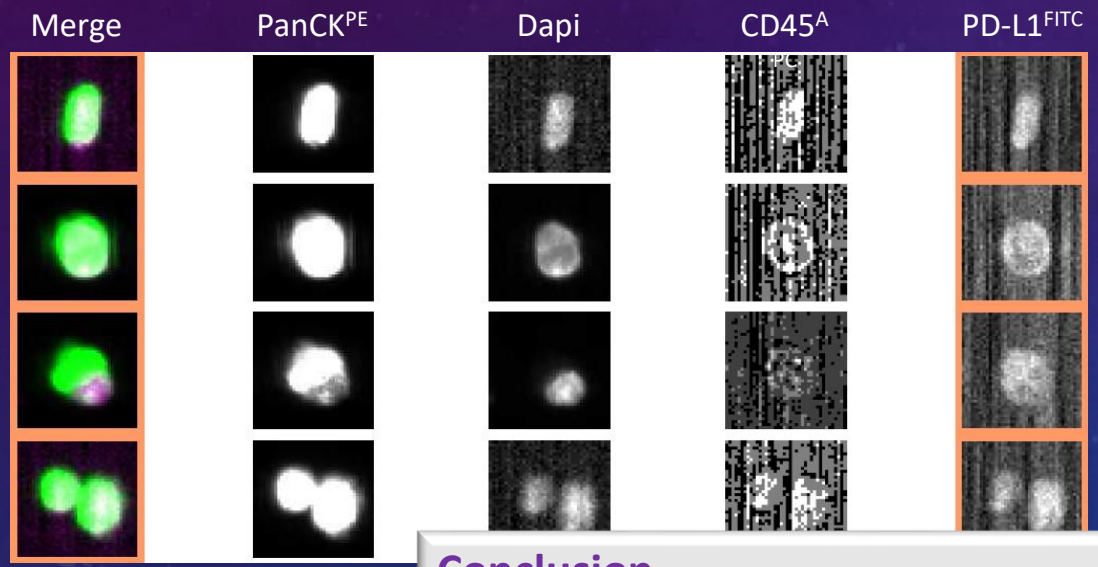
PD-L1 is frequently expressed on CTCs (> 60% of patients) in metastatic breast cancer patients

CTC Detection & PD-L1 Expression
METASTATIC BREAST CANCER



ALCINA 1

- **72 MBC patients**
- Median Age 65 yrs old (range 35-87)
- Subtypes of breast cancer: **ER$^{(+)}$/HER2$^{(-)}$** 69.4%; **HER2$^{(+)}$** 18.1%, **TN** 12.5%



Clinical Chemistry 0:0
1093–1101 (2020)

Clinical Chemistry 2020

Cancer Diagnostics

Clinical Correlations of Programmed Cell Death Ligand 1 Status in Liquid and Standard Biopsies in Breast Cancer

William Jacot, Martine Mazel, Caroline Mollevi, Stéphane Pouderoux, Véronique D'Hondt, Laure Cayrefourcq, Céline Bourgier, Florence Boissiere-Michot, Fella Berrabah, Evelyne Lopez-Crapez, François-Clément Bidard, Marie Viala, Thierry Maudelonde, Séverine Guiu, and

Conclusion

- ❑ PD-L1 expression : tumor *vs* CTCs → no correlation (p=0.589)
- ❑ Presence of CTC$^{PD\text{-}L1(+)}$: independent biomarker for shorter PFS

CTC Detection & PD-L1 Expression
Metastatic Non-Small Lung Cancer

ALCINA 1

- **54 MNSCLC patients**
- Mean Age 64.5 yrs old
- 57.4% were men and 86% were smokers

Clinical Chemistry 00:0
1–10 (2021)

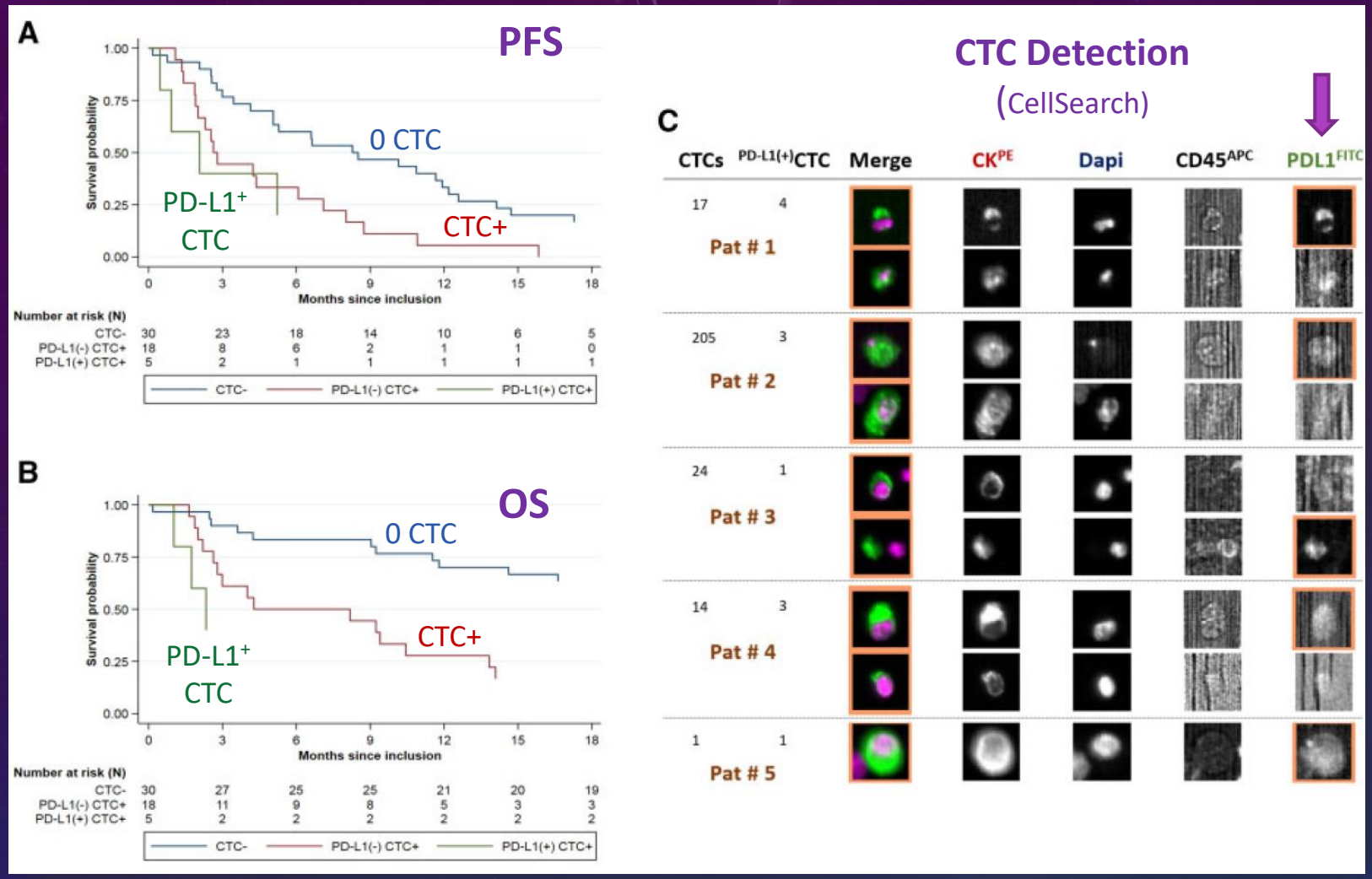
Clinical Chemistry **2021**

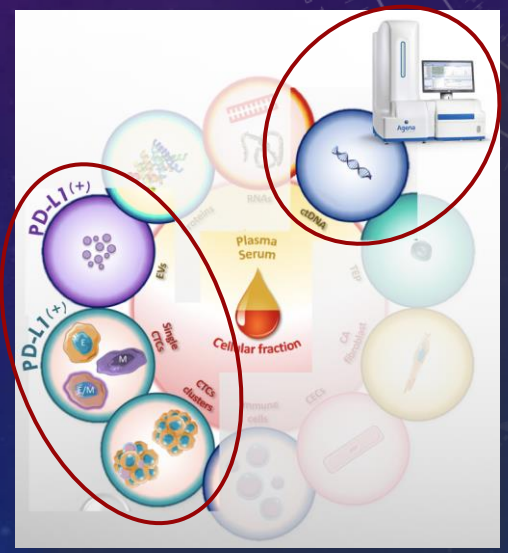
Cancer Diagnostics

Programmed Cell Death Ligand 1-Expressing Circulating Tumor Cells: A New Prognostic Biomarker in Non-Small Cell Lung Cancer

Léa Sinoquet,[a] William Jacot,[a,b] Ludovic Gauthier,[c] Stéphane Pouderoux,[a] Marie Viala,[a] Laure Cayrefourcq,[d,e] Xavier Quantin,[a,b] and Catherine Alix-Panabières[d,e,*]

- CTCs detected in 23/53 patients (43.4%)
- $CTC^{PD\text{-}L1(+)}$ observed in 5 patients (9.4%).



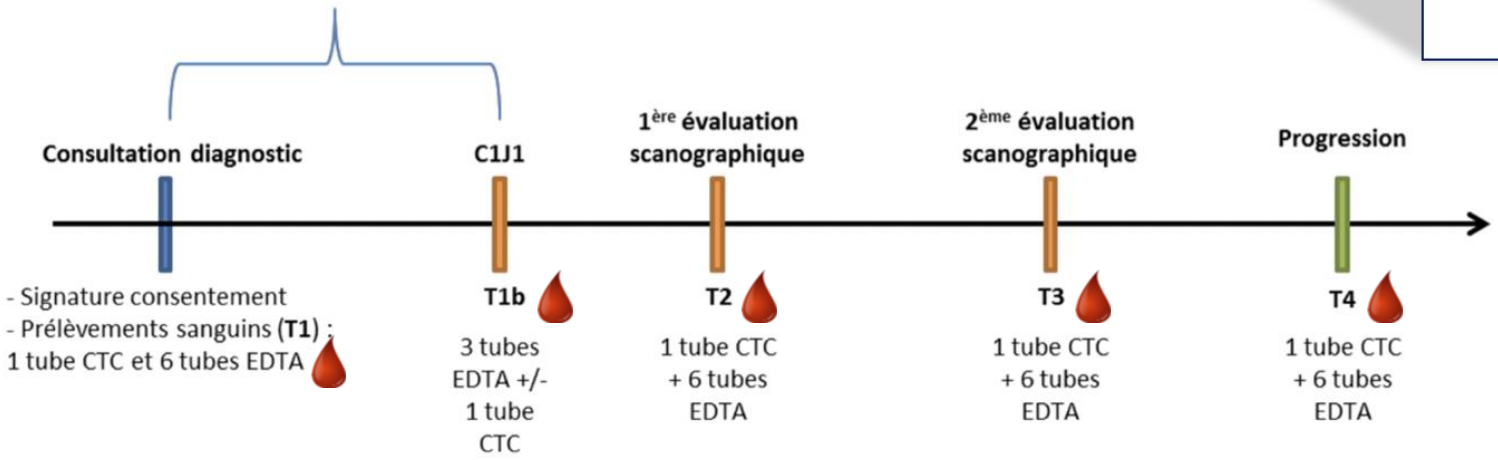


Conclusion

- PD-L1 expression: Low concordance between tissue & liquid biopsies (53.7%)
- PFS & OS are worse in patients **with CTCs**; worse **with PD-L1⁺ CTCs**
- PD-L1 expression on tumor tissue was not associated with PFS and OS.

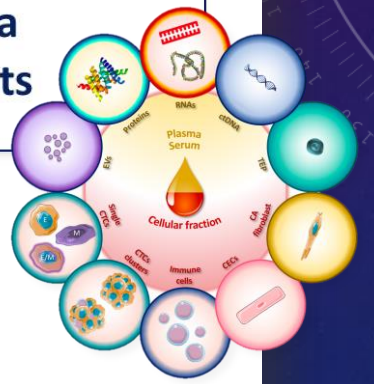
ALCINA2 Clinical trial overview

CTC number > 50 in SLCL
CTC number > 5 in NSCLC

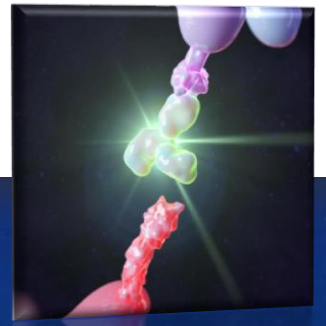


Biobanking

CTCs
Plasma
Platelets

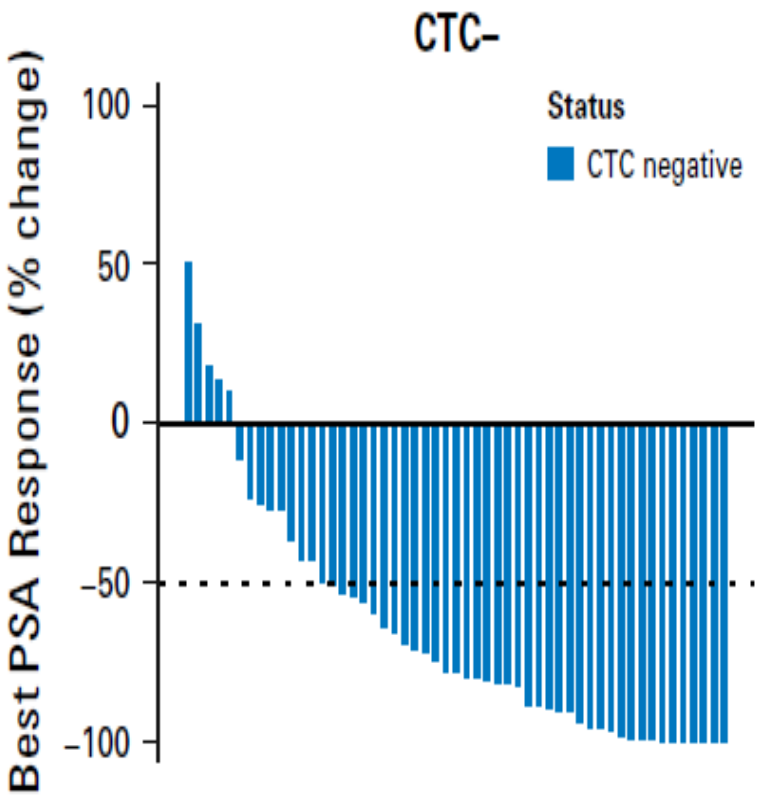


Cohort n= 60 NSCLC + 50 healthy samples
Lung cancer patients in context of Immunotherapy

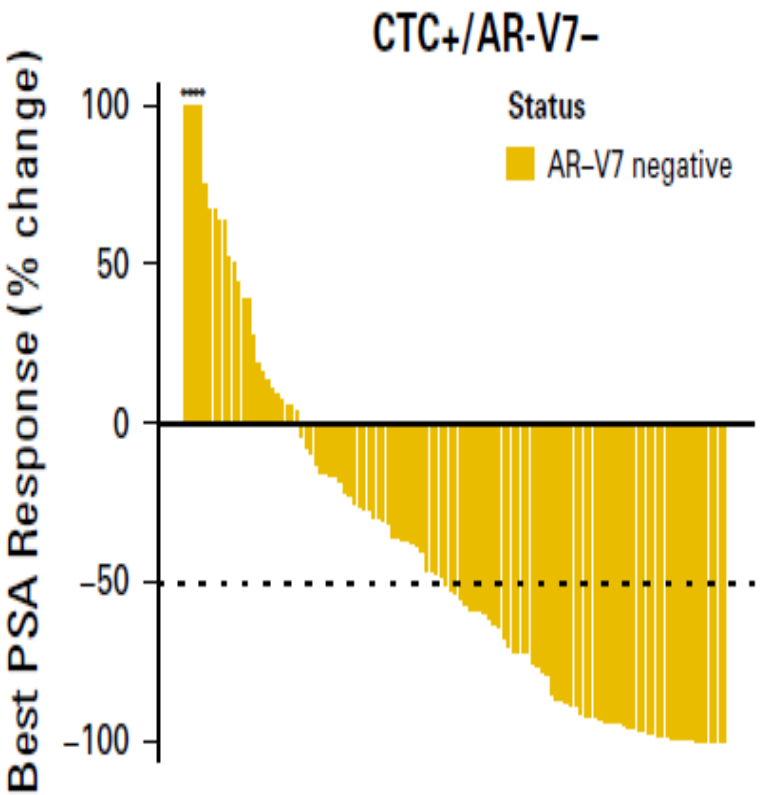


Androgen Receptor Variant 7 (AR-V7) Expression in CTCs: Predictive marker for Abiraterone or Enzalutamide Therapy in Prostate Cancer

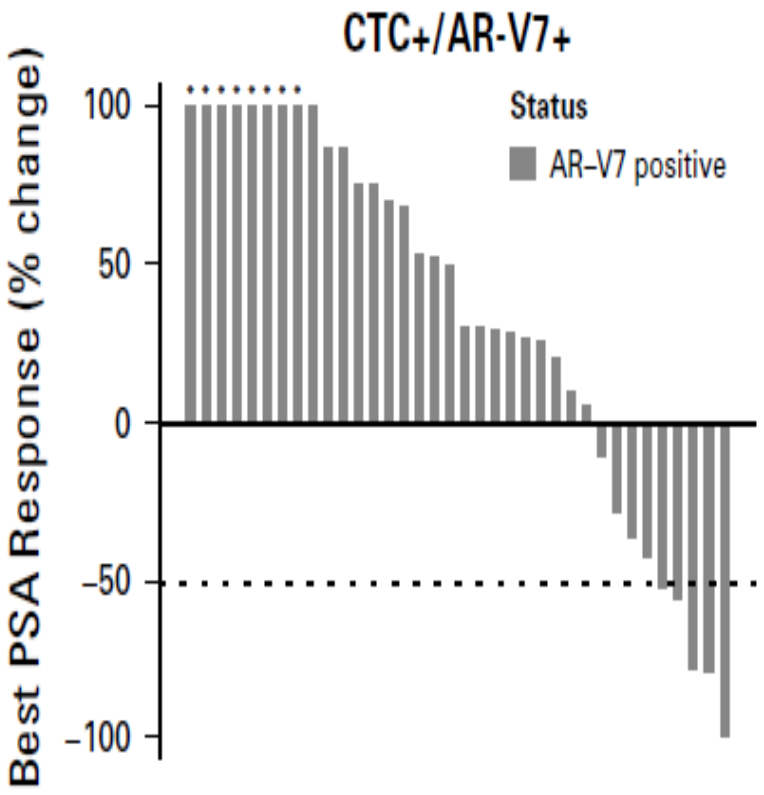
Good Response



Moderate Response

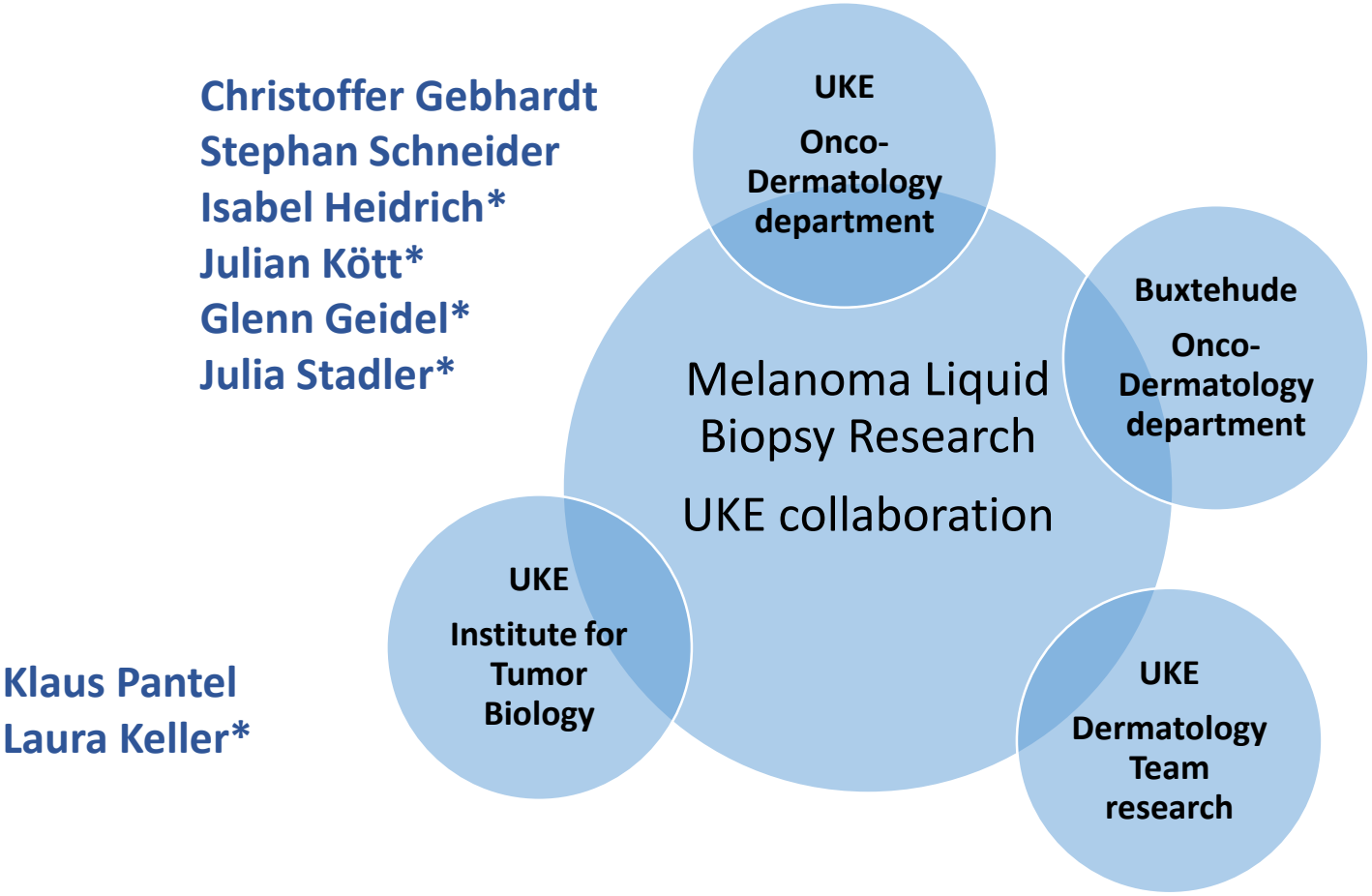


Poor Response



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

Northern German LiquiMel Melanoma Biobank Consortium



Christoffer Gebhardt
Stephan Schneider
Isabel Heidrich*
Julian Kött*
Glenn Geidel*
Julia Stadler*

Peter Mohr
Rüdiger Greinert
Beate Volkmer

Klaus Pantel
Laura Keller*

Christian Gorzelanny
Alexander Bauer

*Young researchers supported by DKH Mildred-Scheel Nachwuchszentrum, UCCH-Stipendium, Hiege-Stiftung

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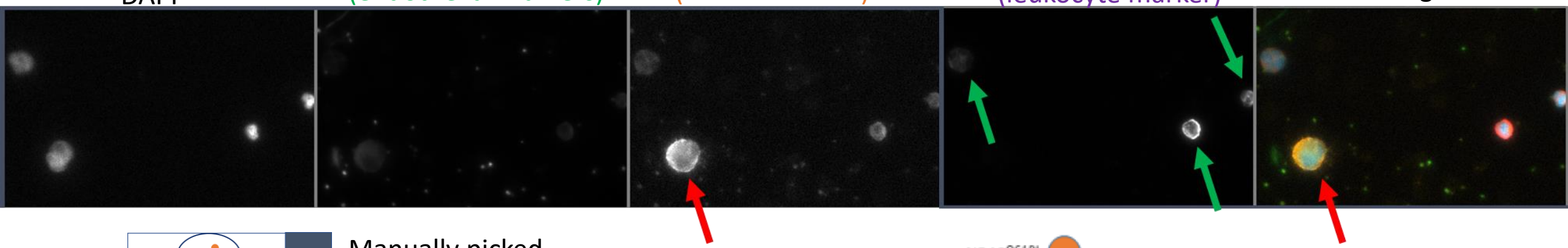
UKE HAMBURG

Intra-Patient Heterogeneity of Circulating Tumor Cells and Circulating Tumor DNA in Blood of Melanoma Patients

Katharina Gorges [1,†], Lisa Wiltfang [2,†], Tobias M. Gorges [2], Alexander Sartori [3], Lina Hildebrandt [4], Laura Keller [2], Beate Volkmer [5], Sven Peine [6], Anna Babayan [2], Ingrid Moll [7], Stefan W. Schneider [4], Sören Twarock [1], Peter Mohr [5], Jens W. Fischer [1] and Klaus Pantel [2,*]

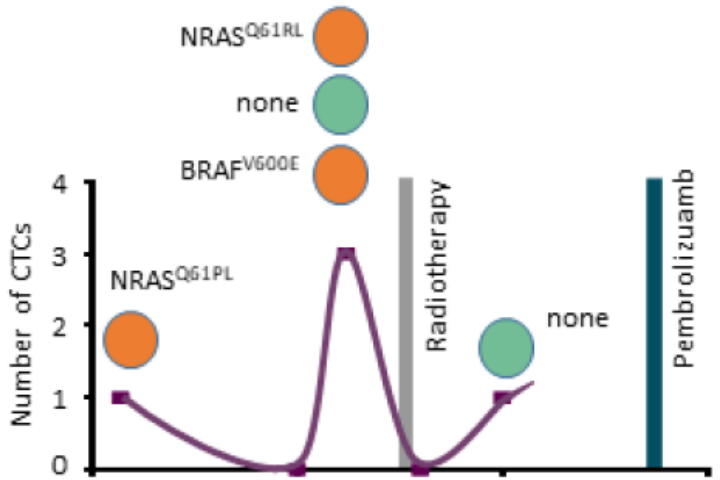
Molecular characterization of CTC



Manually picked

Whole Genome Amplification

Mutation detection



ctDNA Mutation Detection in Melanoma

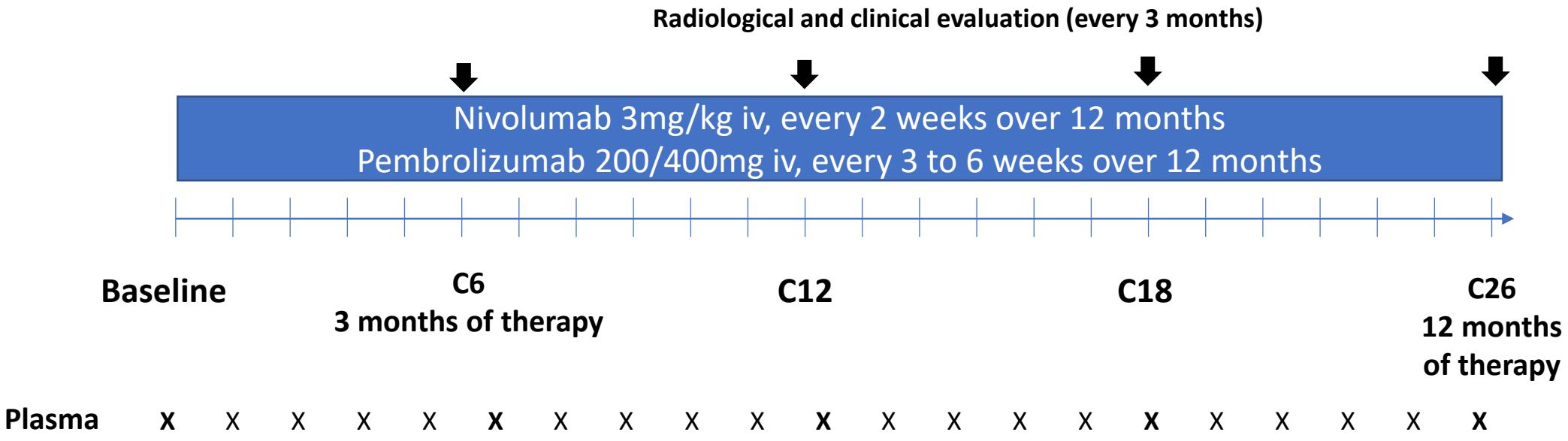
Gene	# mutations
BRAF	12
CDKN2A	1
CTNNB1	5
DPH3	2
IDH1	2
KIT	7
MAP2K1	8
NRAS	20
RAC1	1
RPS27	1
RQCD1	1
SDHD	3
YAE1D1	2
13 Genes	**65**

UltraSEEK Melanoma Panel v1.0

- Single multiplexed PCR reaction
- ≥0.1% mutation frequency detection
- MassARRAY™ System

Schneegans, Pantel, Wikman et al. Mol. Oncol. 2020

Monitoring MRD in tumor-resected melanoma patients (stage III) undergoing immune checkpoint inhibition therapy

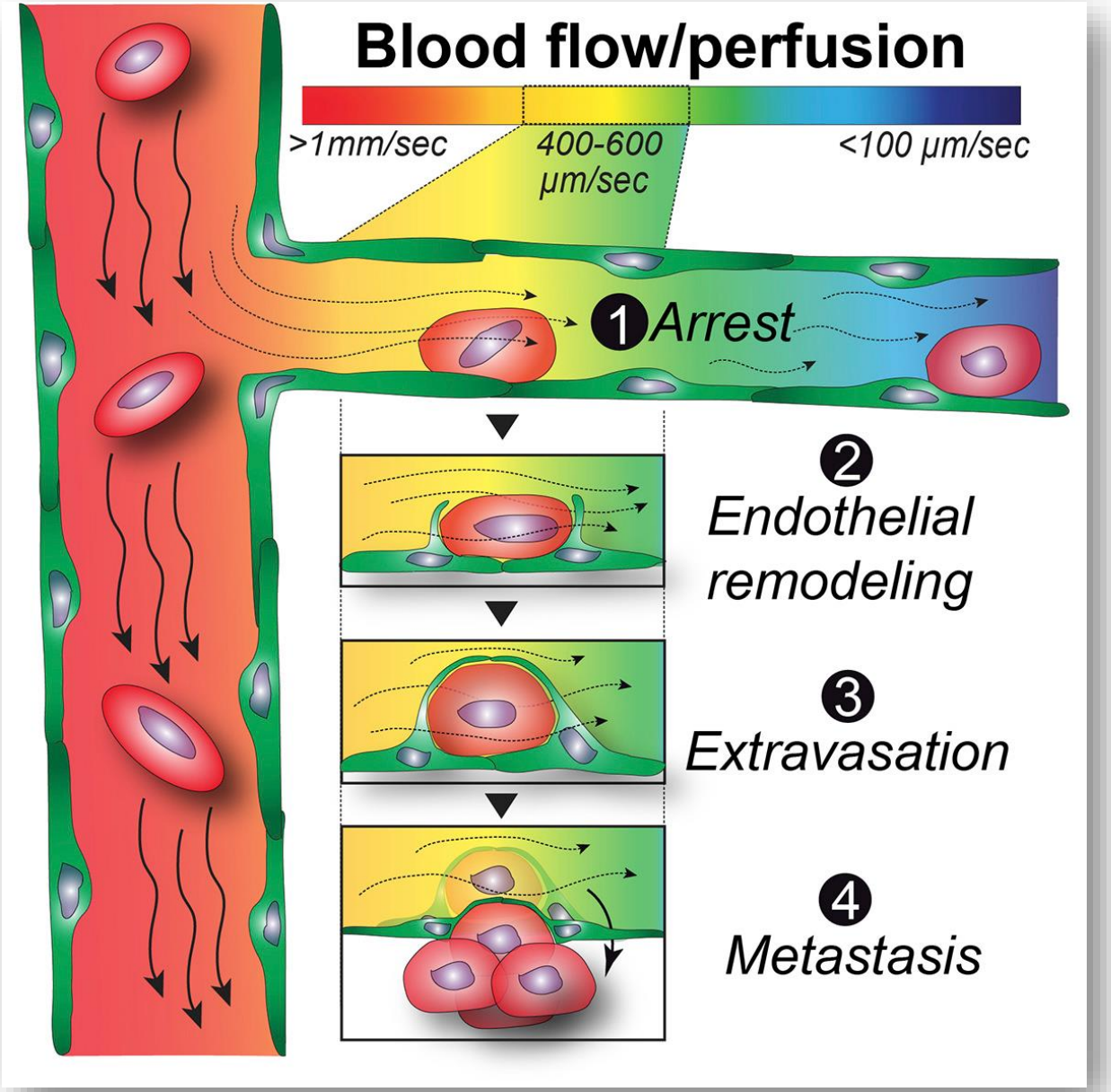


Combined liquid biopsy analysis (CTCs, ctDNA, EVs, proteins) to evaluate minimal residual disease

Tissue origin of CTCs

– Brain Metastases -

Hemodynamic Forces Tune the Arrest, Adhesion,and Extravasation of CTCs



Permissive flow forces allow stable intravascular arrest of CTCs.

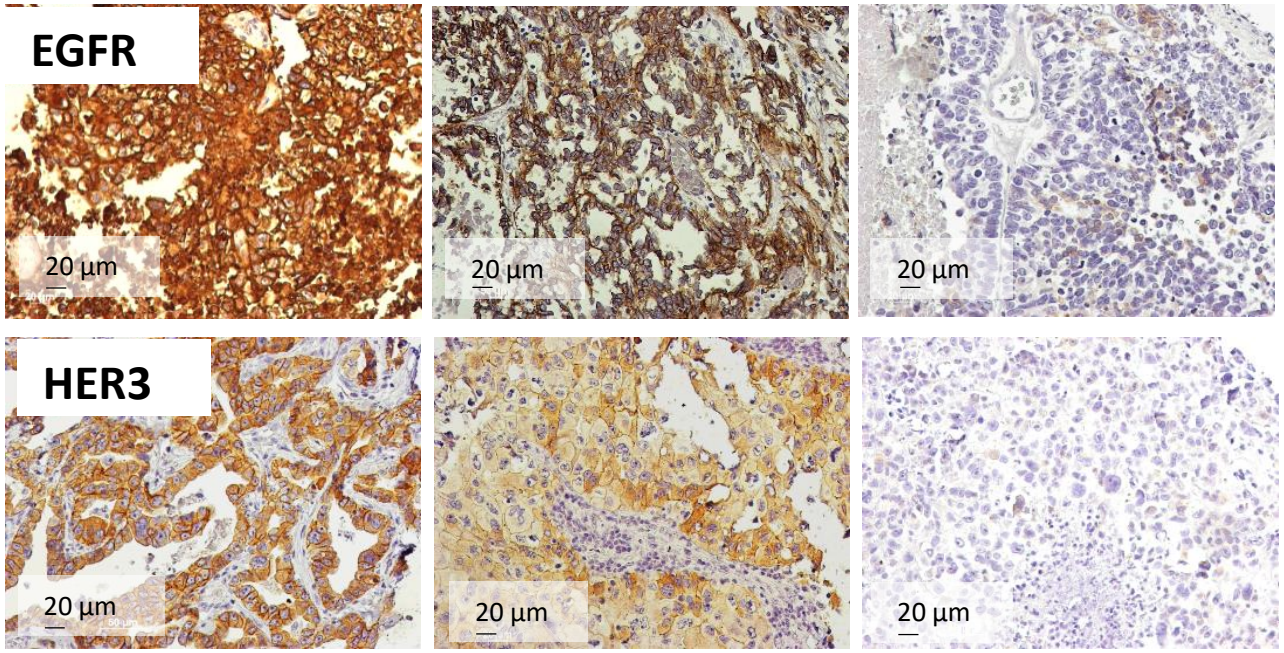
Flow forces drive endothelial remodeling around arrested tumor cells, favoring extravasation preceding metastatic outgrowth.

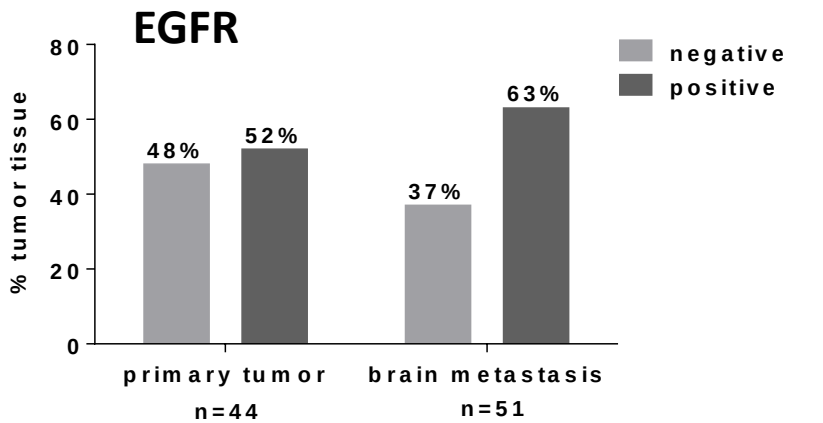
Clinical relevance for brain metastasis

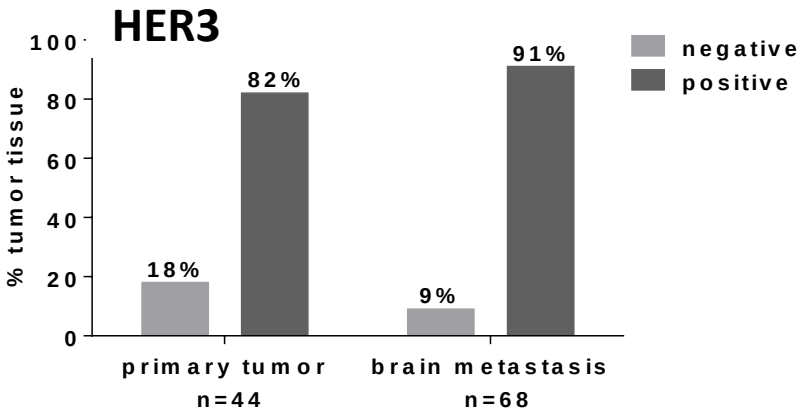
Follain *et al. Developmental Cell* 2018

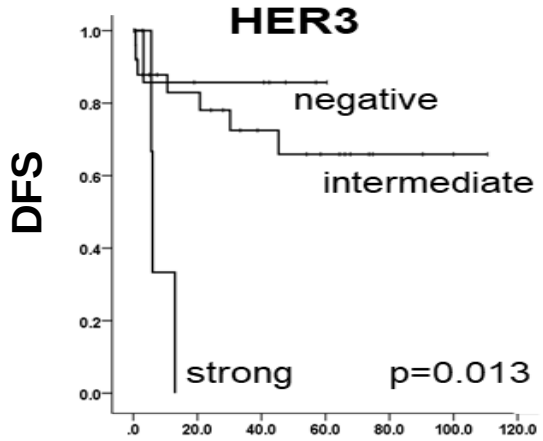
How to improve sensitivity of CTC detection in patients with brain metastases (BM)?

HER3/ EGFR based CTC isolation







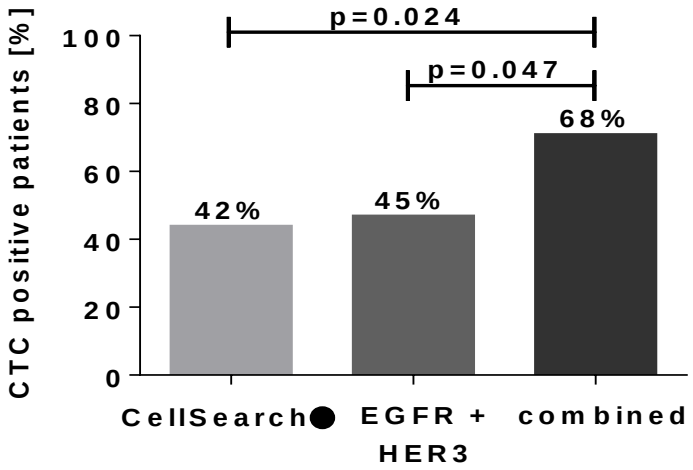


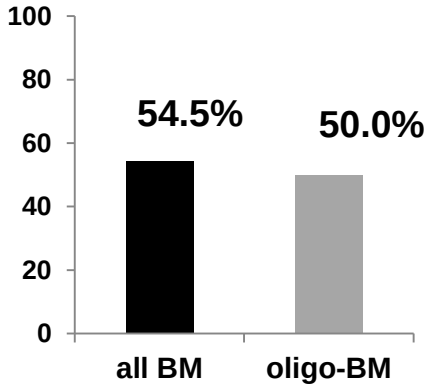
⇒ **CTC isolation based on EGFR and HER3 expression**

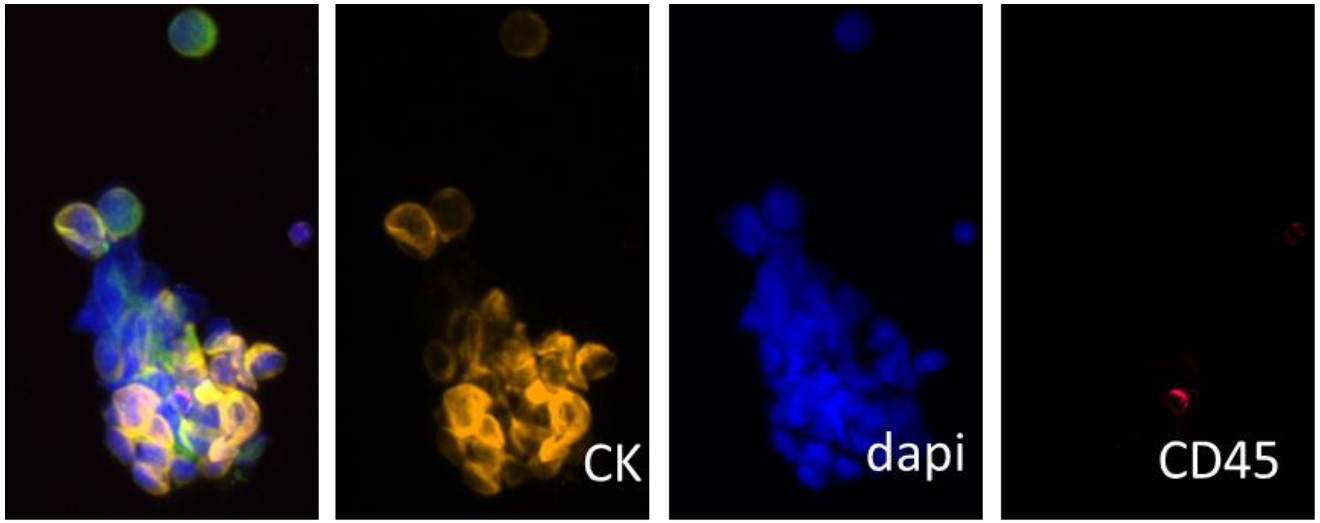
Scharpenseel et al. Sci Rep 2019

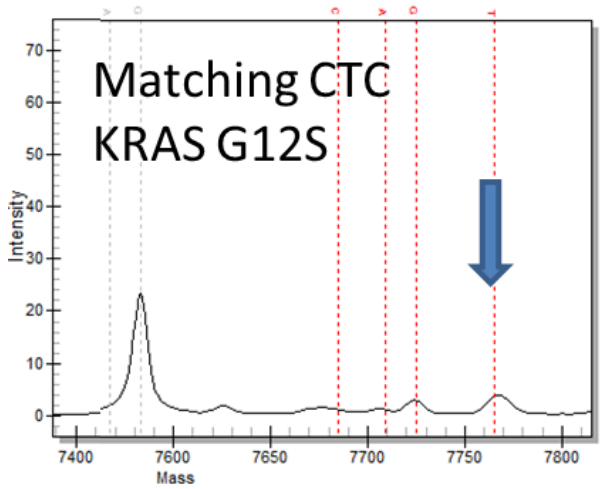
HER3/ EGFR based CTC isolation

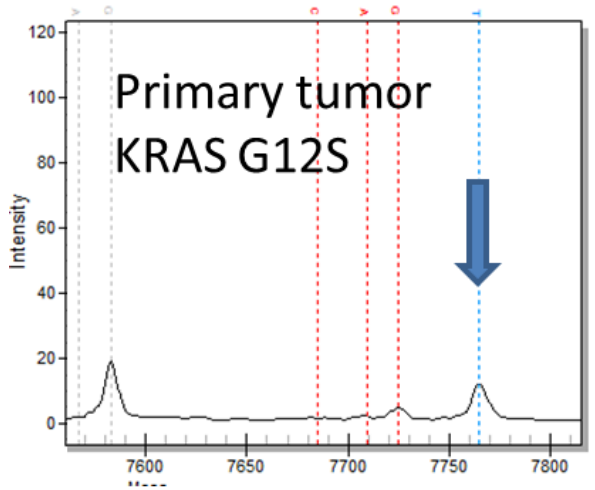
n= 50 mNSCLC, concordance 17%







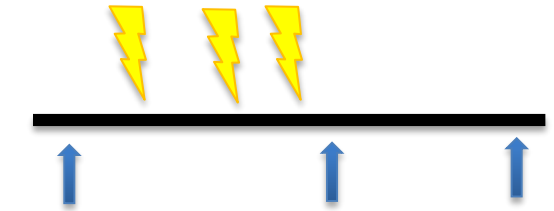


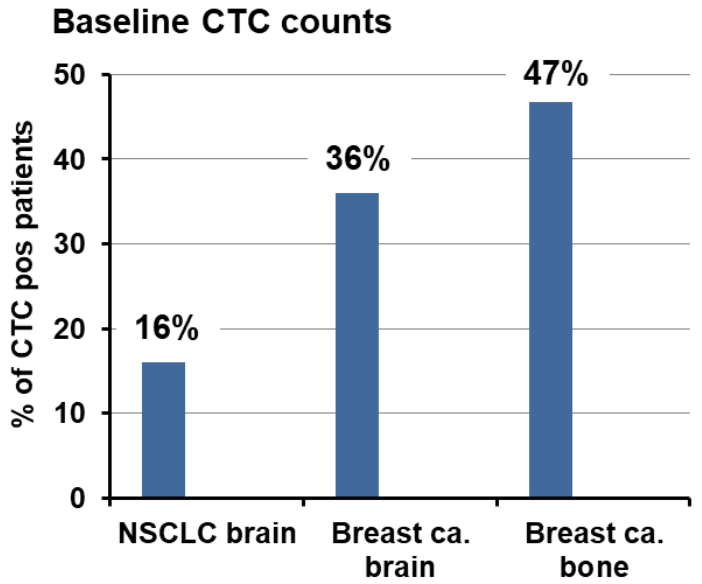


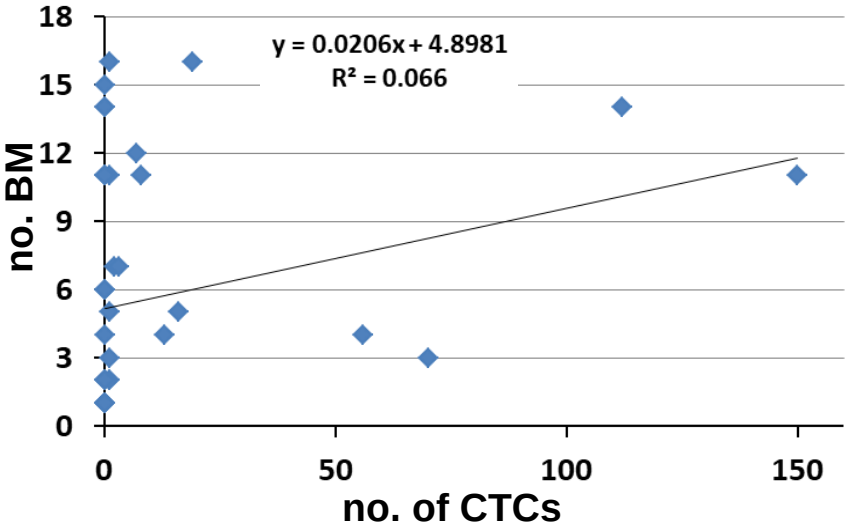
Scharpenseel et al. Sci Reports 2019

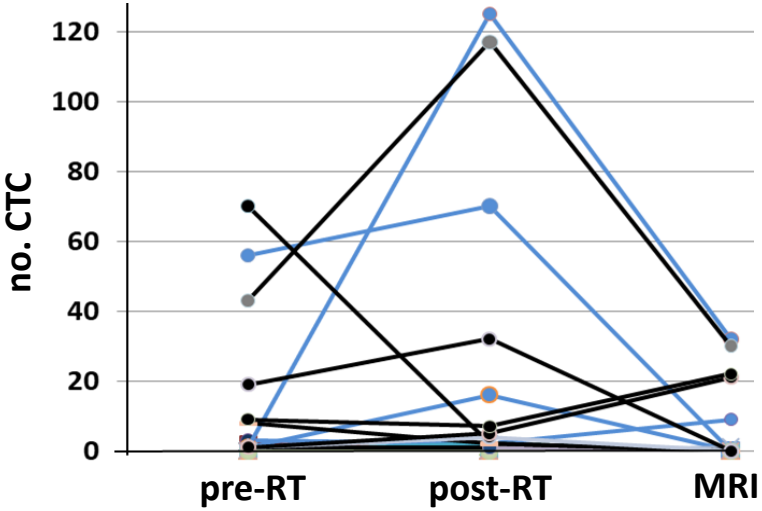
Detection of CTC during radiotherapy of brain metastases

Radiotherapy (WBRT/STRS):
e.g. 1x 23.0 Gy or 10x 3.0 Gy



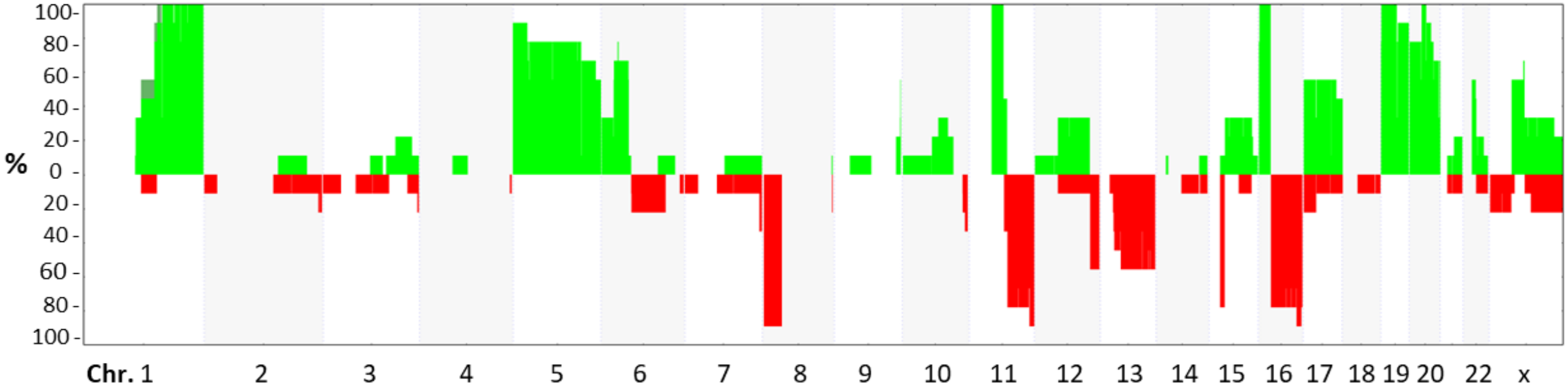






High Clonality of CTCs in Blood of Breast Cancer Patients with Brain Metastases

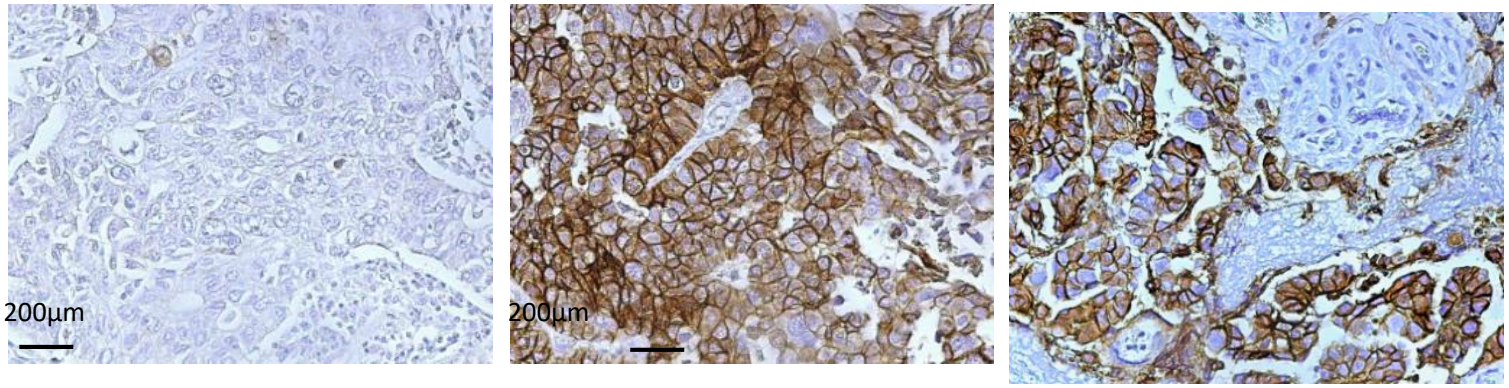
NGS-based exome sequencing of single CTCs isolated from blood

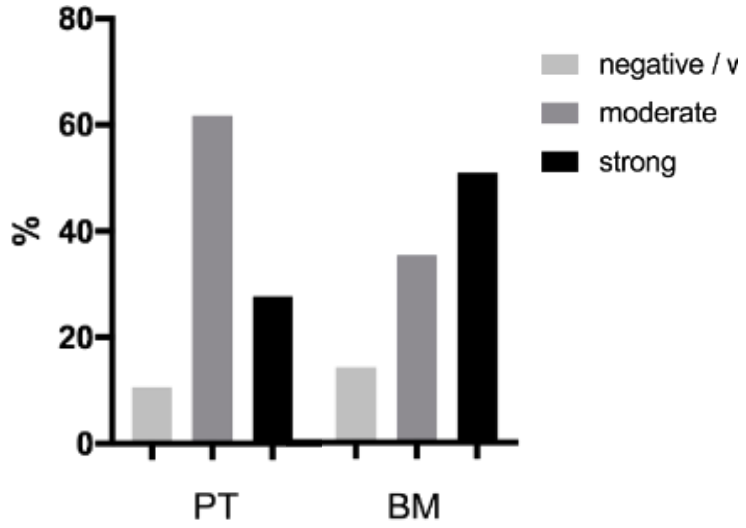


Riebensahm, Pantel, Wikman et al., BCR 2019

Role of ALCAM in NSCLC brain metastases

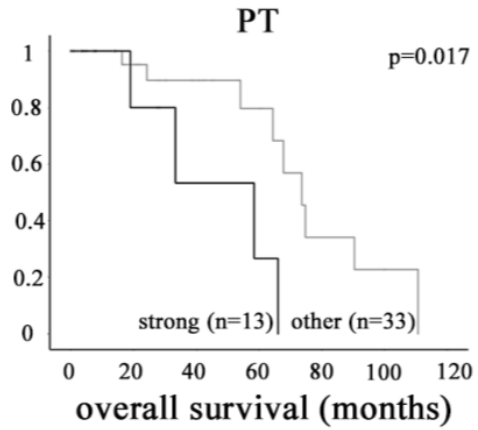
primary tumor | brain met 1 | brain met 2

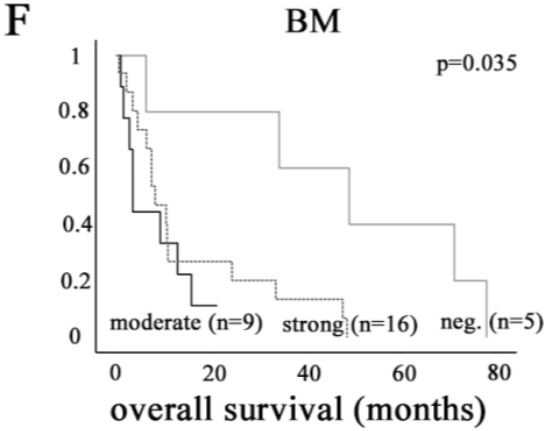




=> 30% of patients: *de novo* ALCAM expression in matched brain metastases

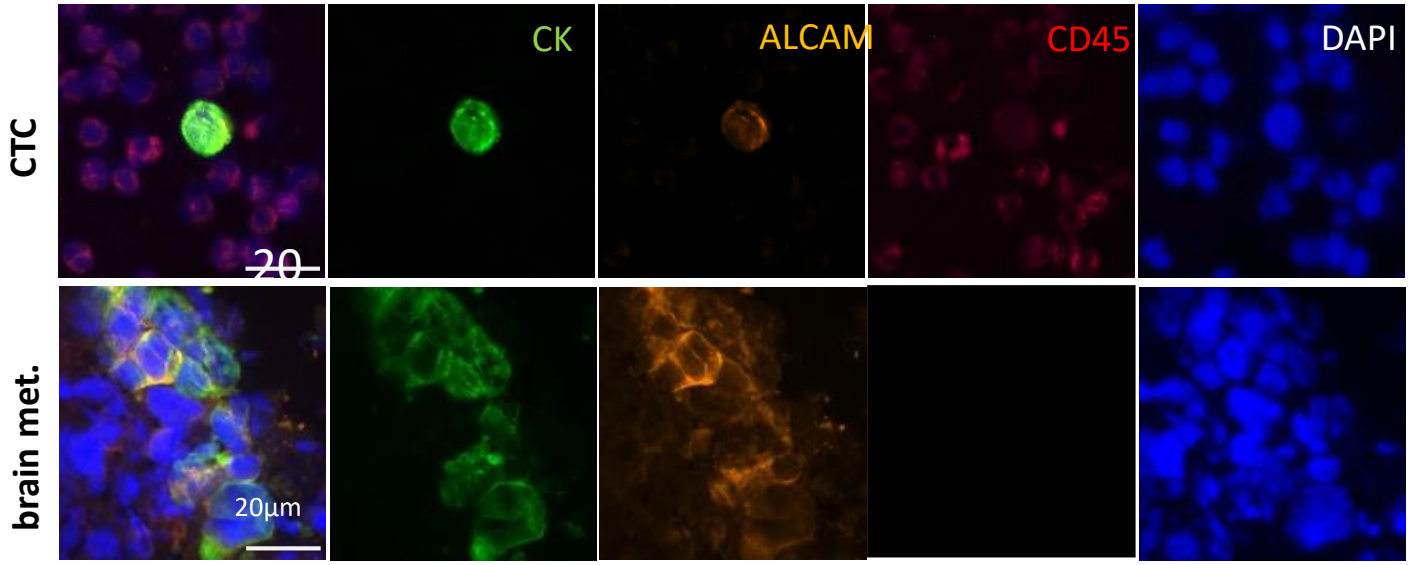
=> High ALCAM expression is associated with worse prognosis





Münsterberg, Pantel, Wikman et al., Neuro-Oncology, 2020

ALCAM expression on CTCs in NSCLC brain metastases



	CTC	BM
Pat. 1	neg	neg
Pat. 2	pos	pos
Pat. 3	weak	weak
Pat. 4	pos	pos

=> ALCAM expression on CTCs isolated from patients blood correlated with ALCAM expression on matched brain metastases

Münsterberg, Pantel, Wikman et al., Neuro-Oncology, 2020

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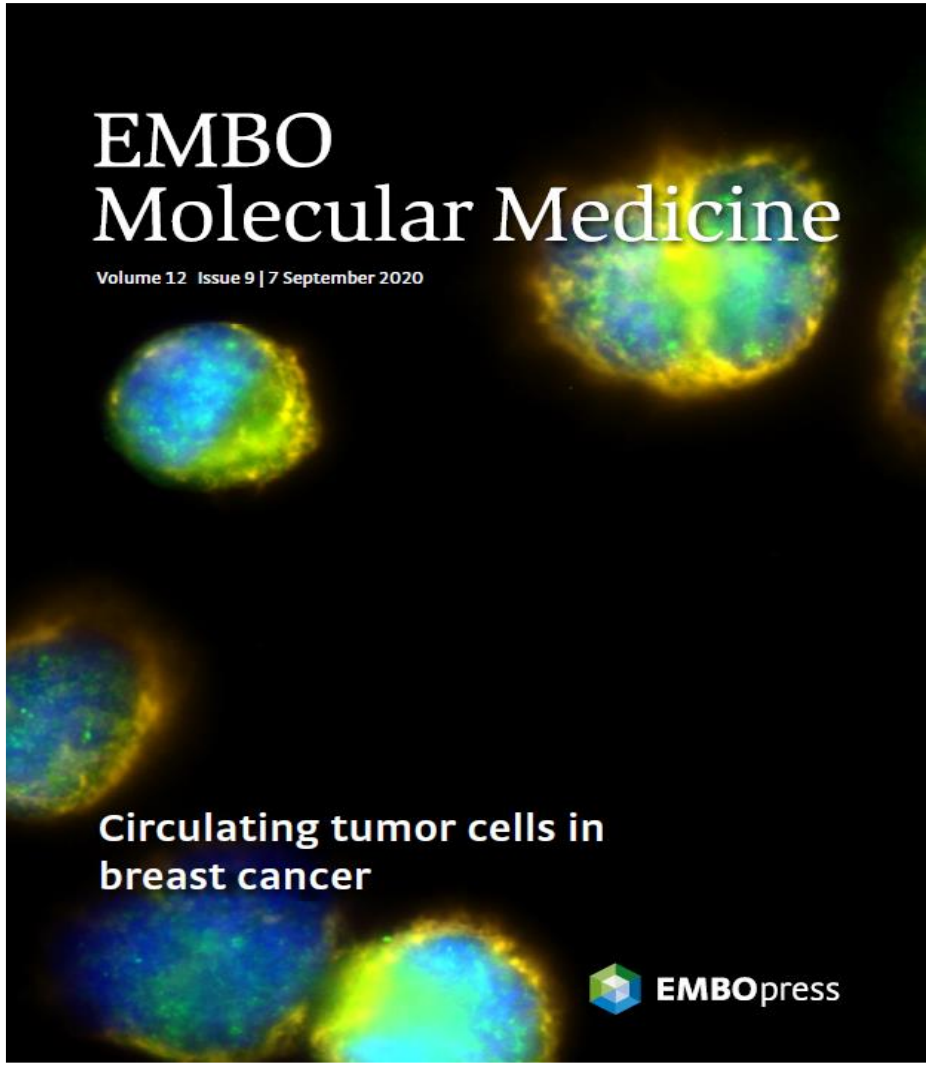
Establisment of experimental models:

- Understanding the biology of CTCs

- Drug screening



Koch C,…,Pantel K: EMBO Mol Med; Sep 7th, 2020

Article

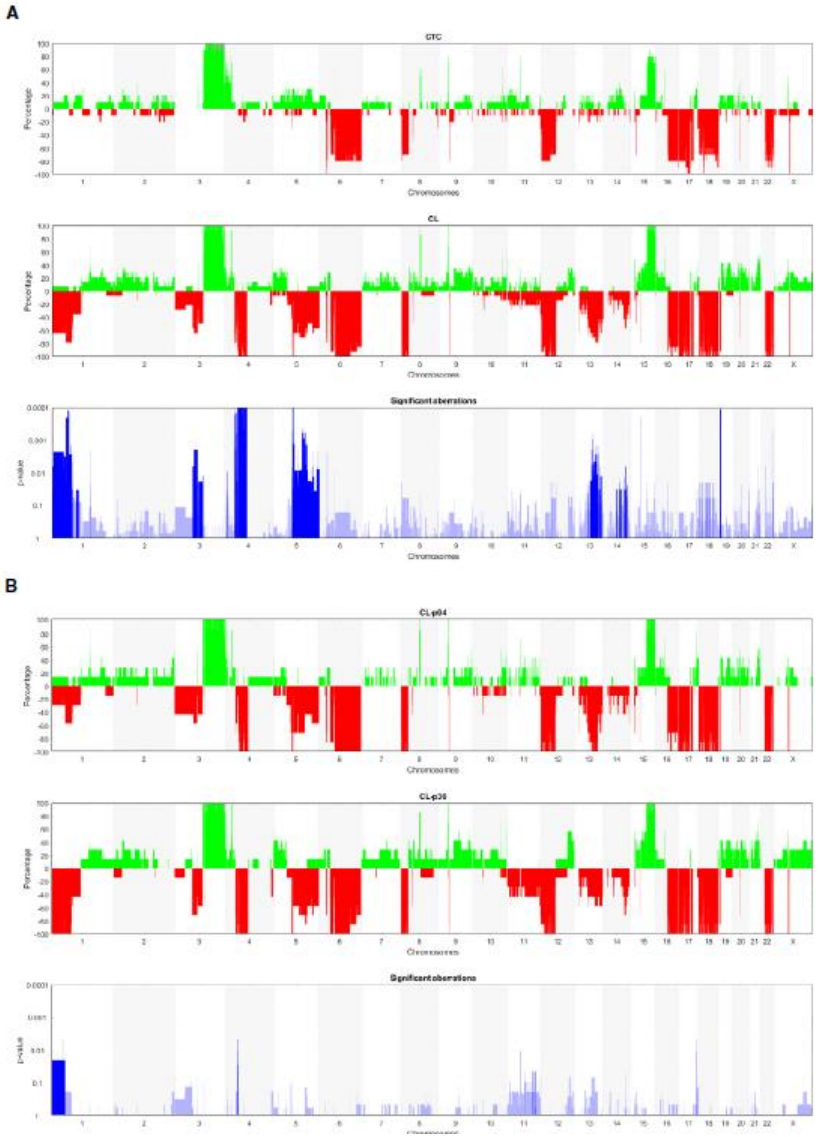
SOURCE DATA | TRANSPARENT PROCESS | OPEN ACCESS

EMBO Molecular Medicine

Characterization of circulating breast cancer cells with tumorigenic and metastatic capacity

Claudia Koch[1,†], Andra Kuske[1,†], Simon A Joosse[1], Gökhan Yigit[2], George Sflomos[3], Sonja Thaler[4], Daniel J Smit[5], Stefan Werner[1], Kerstin Borgmann[6], Sebastian Gärtner[1], Parinaz Mossahebi Mohammadi[1], Laura Battista[3], Laure Cayrefourcq[7,8], Janine Altmüller[9], Gabriela Salinas-Riester[10], Kaamini Raithatha[10], Ame Zibat[2], Yvonne Goy[6], Leonie Ott[1], Kai Bartkowiak[1], Tuan Zea Tan[11], Qing Zhou[12], Michael R Speicher[12], Volkmar Müller[13], Tobias M Gorges[1], Manfred Jücker[5], Jean-Paul Thiery[14], Cathrin Brisken[3,15,‡], Sabine Riethdorf[1,‡], Catherine Alix-Panabières[7,8,‡] & Klaus Pantel[1,*]

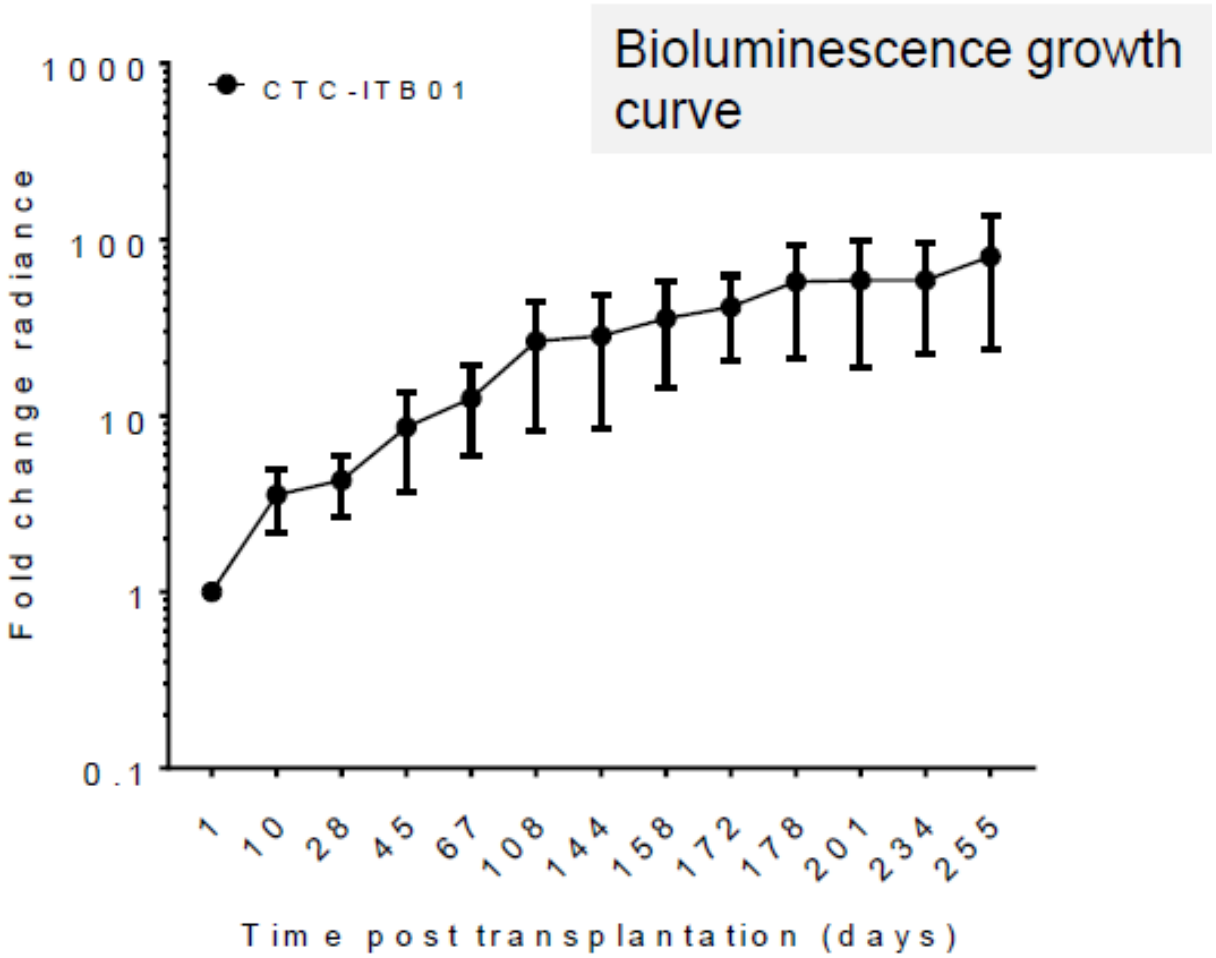
Genomic profiling (CNA) of CTC line and primary CTCs

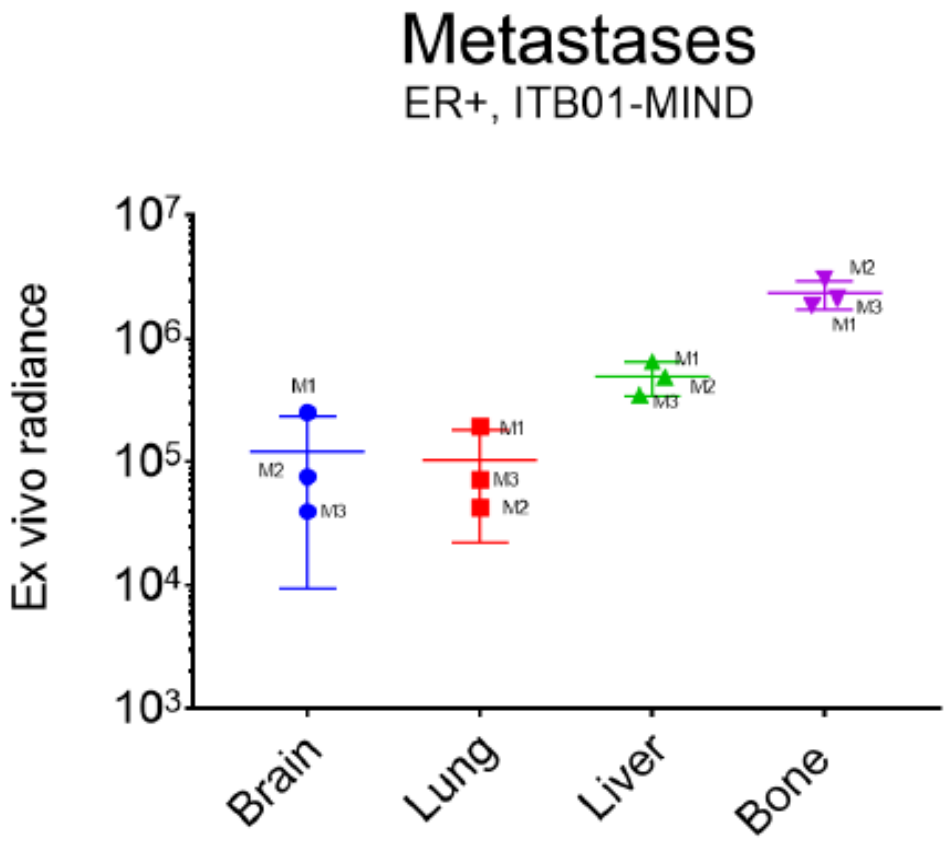


Common aberrations of CTC-ITB-01 cells and primary CTCs

CNA profile of CTC-ITB-01 remains stable during culture

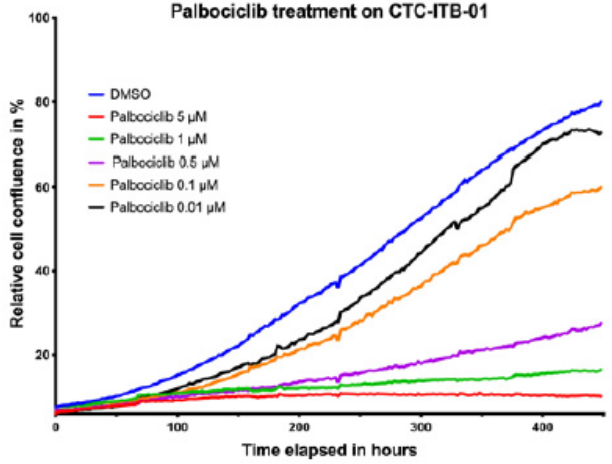
In vivo growth and metastasis of a new human ER+ CTC line after intra-mammilar injection into immunodeficient mice



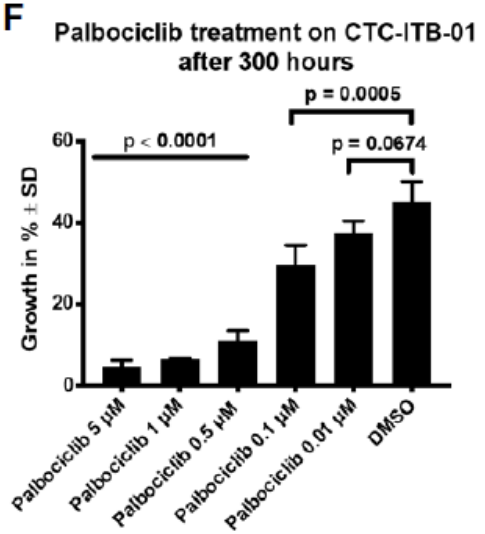


Koch, Riethdorf, Alix-Panabieres, Pantel et al, EMBO Mol. Med. 2020

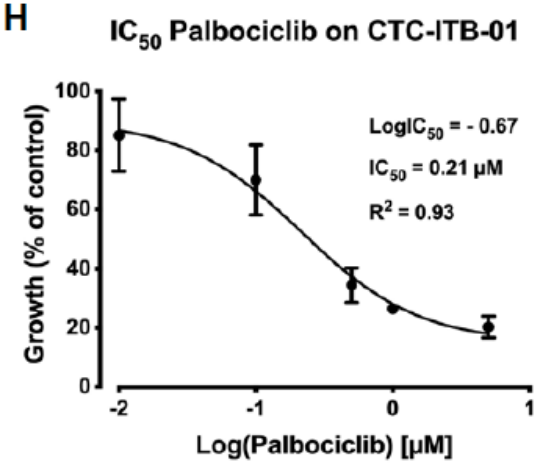
D


Palbociclib treatment on CTC-ITB-01
Relative cell confluence in %
Time elapsed in hours
DMSO
Palbociclib 5 µM
Palbociclib 1 µM
Palbociclib 0.5 µM
Palbociclib 0.1 µM
Palbociclib 0.01 µM

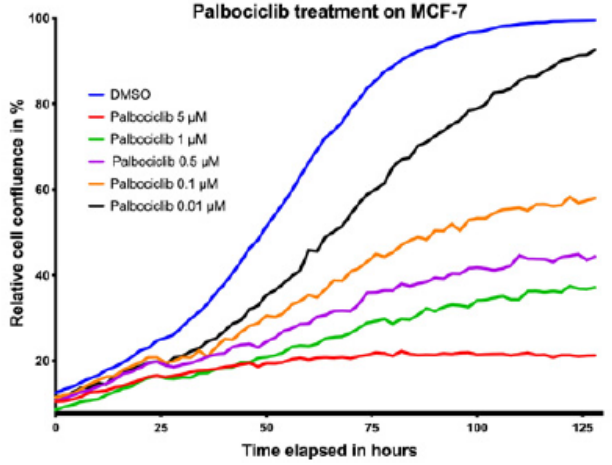
F


Palbociclib treatment on CTC-ITB-01 after 300 hours
p < 0.0001
p = 0.0005
p = 0.0674
Growth in % ± SD
Palbociclib 5 µM
Palbociclib 1 µM
Palbociclib 0.5 µM
Palbociclib 0.1 µM
Palbociclib 0.01 µM
DMSO

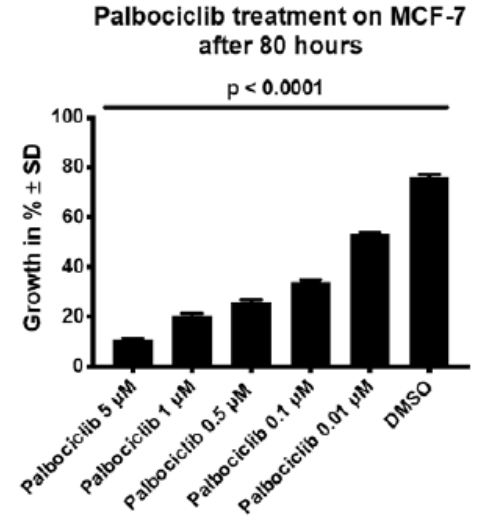
H


IC50 Palbociclib on CTC-ITB-01
LogIC50 = - 0.67
IC50 = 0.21 µM
R2 = 0.93
Growth (% of control)
Log(Palbociclib) [µM]

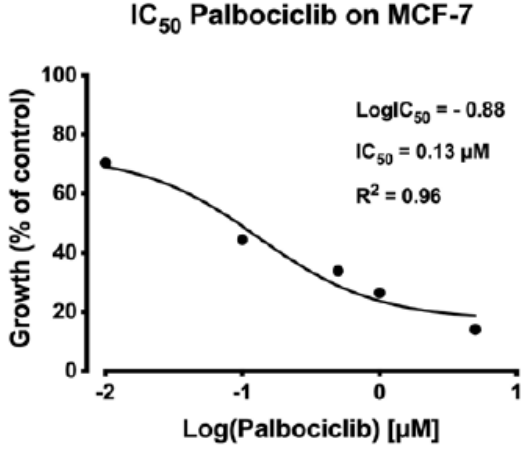
E

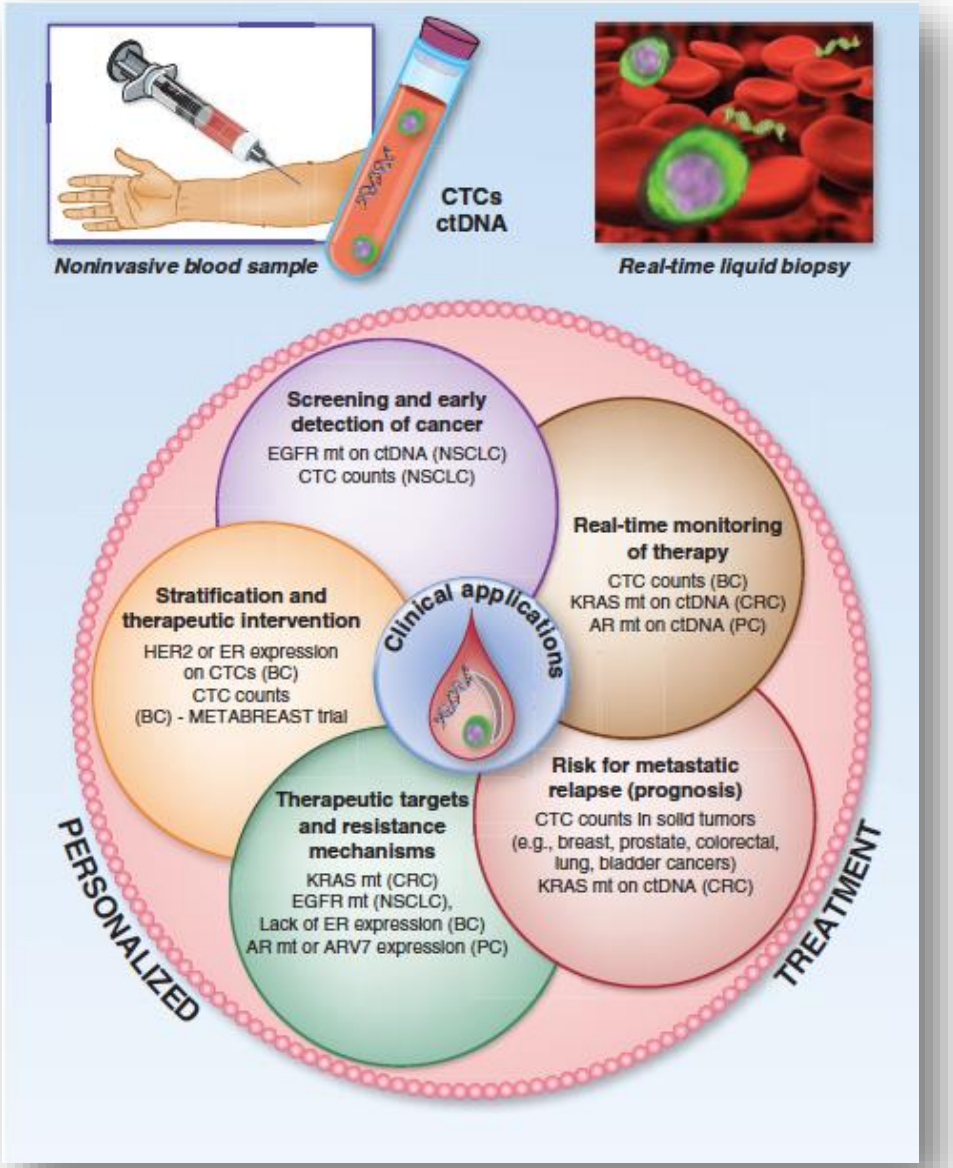

Palbociclib treatment on MCF-7
Relative cell confluence in %
Time elapsed in hours
DMSO
Palbociclib 5 µM
Palbociclib 1 µM
Palbociclib 0.5 µM
Palbociclib 0.1 µM
Palbociclib 0.01 µM

G


Palbociclib treatment on MCF-7 after 80 hours
p < 0.0001
Growth in % ± SD
Palbociclib 5 µM
Palbociclib 1 µM
Palbociclib 0.5 µM
Palbociclib 0.1 µM
Palbociclib 0.01 µM
DMSO

I


IC50 Palbociclib on MCF-7
LogIC50 = - 0.88
IC50 = 0.13 µM
R2 = 0.96
Growth (% of control)
Log(Palbociclib) [µM]



Conclusions:

Liquid biopsy can provide clinically relevant information

Assays need to be validated and harmonized (QA)

Interventional clinical studies are required to demonstrate clinical utility of liquid biopsy

Liquid Biopsy Research Network at UKE (since 1999, > 300 publications)

THE EUROPEAN LIQUID BIOPSY SOCIETY

UKE HAMBURG

Institutes

- Immunologie
- Klin. Chemie Laboratoriumsmedizin
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- Neuropathologie
- Rechtsmedizin
- Transfusionsmedizin
- Anatomie und Experimentelle Morphologie
- Biochemie und Molekulare Zellbiologie
- Experimentelle Herz-Kreislaufforschung
- Experimentelle Pharmakologie und Toxikologie
- Medizinische Biometrie und Epidemiologie
- Osteologie und Biomechanik
- Tumorbiologie
- Medizinische Systembiologie
- Neuroimmunologie und Multiple Sklerose
- Arbeitsmedizin

Clinics

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- Hals-, Nasen- und Ohrenheilkunde
- Allge.-, Viszeral- und Thoraxchirurgie
- Osteologie und Biomechanik
- Unfall-, Hand- und Wiederherstellungs-chirurgie
- Viszerale Transplantations-chirurgie
- Knochenmarktrans-plantation (Med II)
- Stammzelltrans-plantationschirugie
- Dermatologie und Venerologie
- Gynäkologie
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- Urologie
- Gastroentero-logie (Med II)
- Interdisziplinäre Endoskopie
- Onkologie (Med II)
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- Neurologie
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- Strahlentherapie
- Zahnärtzliche Prothetik

Center of Experimental Medicine

Institute of Tumor Biology - THE TEAM !

Funding:

ERC Advanced Investigator Grants „DISSECT“ & “INJURMET”

ERC PoC Grant „CTCapture“

EU/IMI, EU TRANSCAN

DFG, BMBF

Deutsche Krebshilfe

(Mildred-Scheel-Nachwuchszentrum)

THE EUROPEAN LIQUID BIOPSY SOCIETY

Major Symposium at AACR Annual Meeting 2022, April 8-13, 2022, New Orleans, USA

Liquid biopsy: From Discovery to Clinical Application

Tuesday, April 12, 2022, 12:30 pm - 2:00 pm.

Chairperson: Klaus Pantel, University Medical Center Hamburg-Eppendorf, Germany

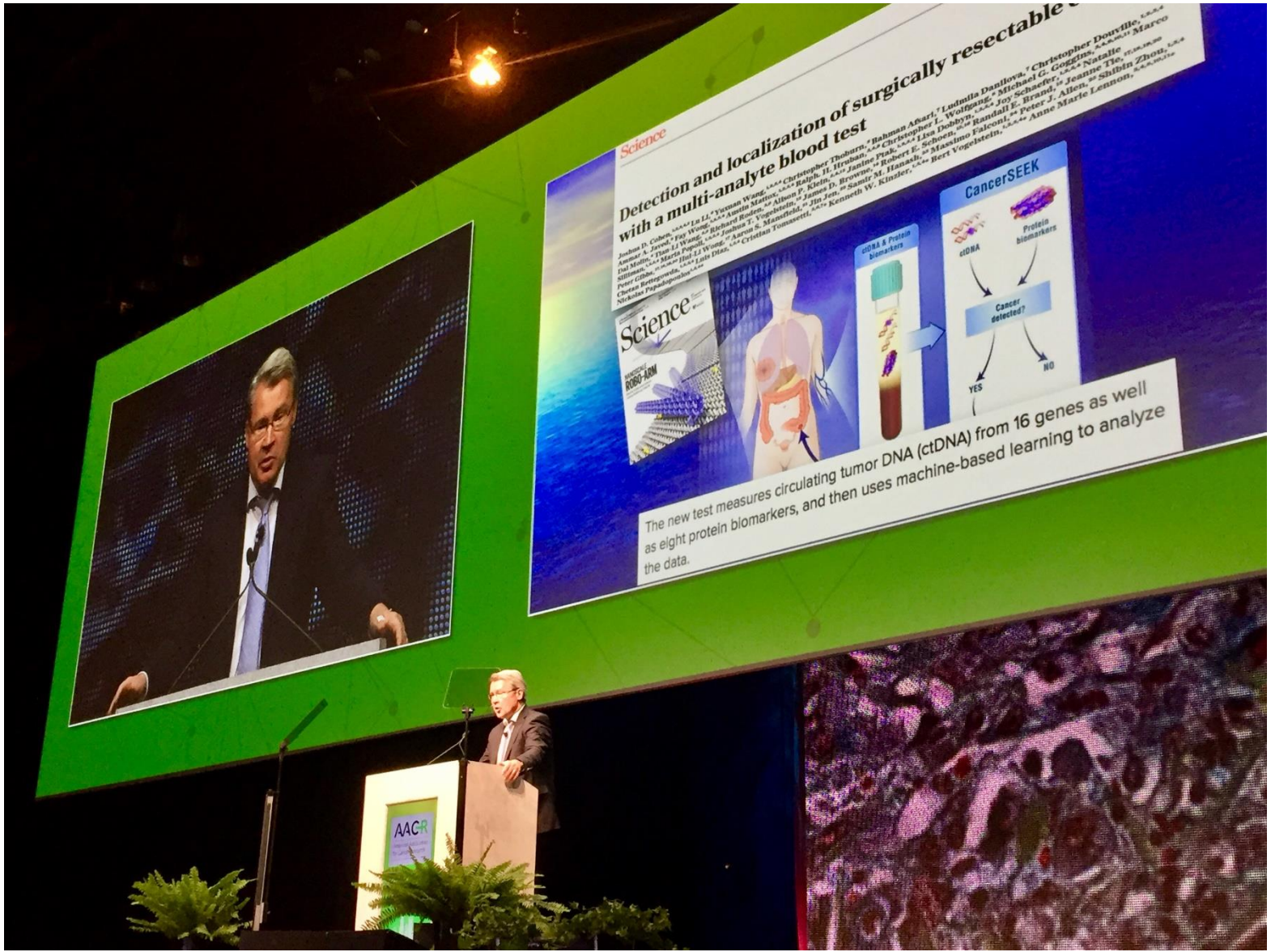
Speakers:

Klaus Pantel: Opportunities and challenges of liquid biopsy research

Catherine Alix-Panabières, University Medical Center of Montpellier, France: Biology and clinical relevance of circulating tumor cells for precision medicine

***Victor* E. Velculescu,** John Hopkins University School of Medicine, Baltimore, USA: Clinical relevance of cell-free DNA fragmentomes for cancer detection and monitoring

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



THE EUROPEAN LIQUID BIOPSY SOCIETY

Gordon Research Conferences

frontiers of science

Date and Location:

June 19-June 24, 2022

Mount Holyoke College, 50 College Street, South Hadley, MA, United States

Organizers:

Chair: *Klaus Pantel, University Medical Center Hamburg-Eppendorf, Germany*

Vice Chair: Shana Kelley, University Toronto, Canada

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