



Hubertus Wald Tumorzentrum
Universitäres Cancer Center Hamburg

Ein Kompetenznetzwerk des UKE



Liquid biopsy: tracing of tumor cell dissemination in cancer patients at the single cell level

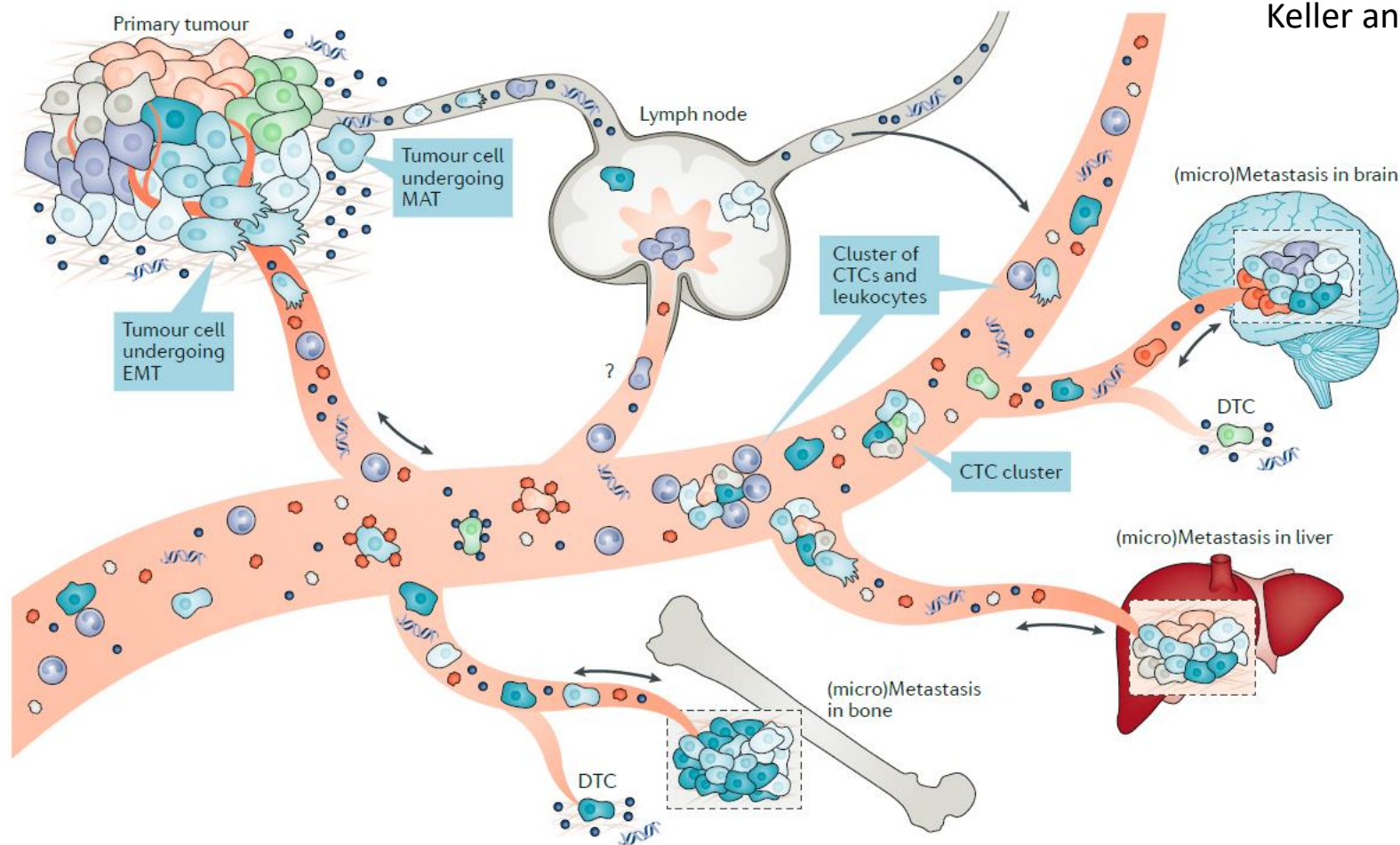
Klaus Pantel, MD, PhD

Institut für Tumorbilogie, UKE, University Cancer Center Hamburg (UCCH)



Liquid Biopsy*: Comprehensive assessment of circulating blood biomarkers

Keller and Pantel, Nature Reviews Cancer, 2019



*Pantel & Alix-Panabieres, *Trends Mol. Med.* 2010, 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, *Nature Rev. Cancer* 2014 & *Cancer Discovery*, 2016; Bardelli & Pantel, *Cancer Cell* 2017; Hayes & Pantel, *Nature Rev. Clin. Oncol.* 2018, Poudineh, Pantel, Kelley et al, *Nature Biomed. Engineering* 2018; Pantel & Alix-Panabieres, *Nature Rev. Clin. Oncol.* 2019

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

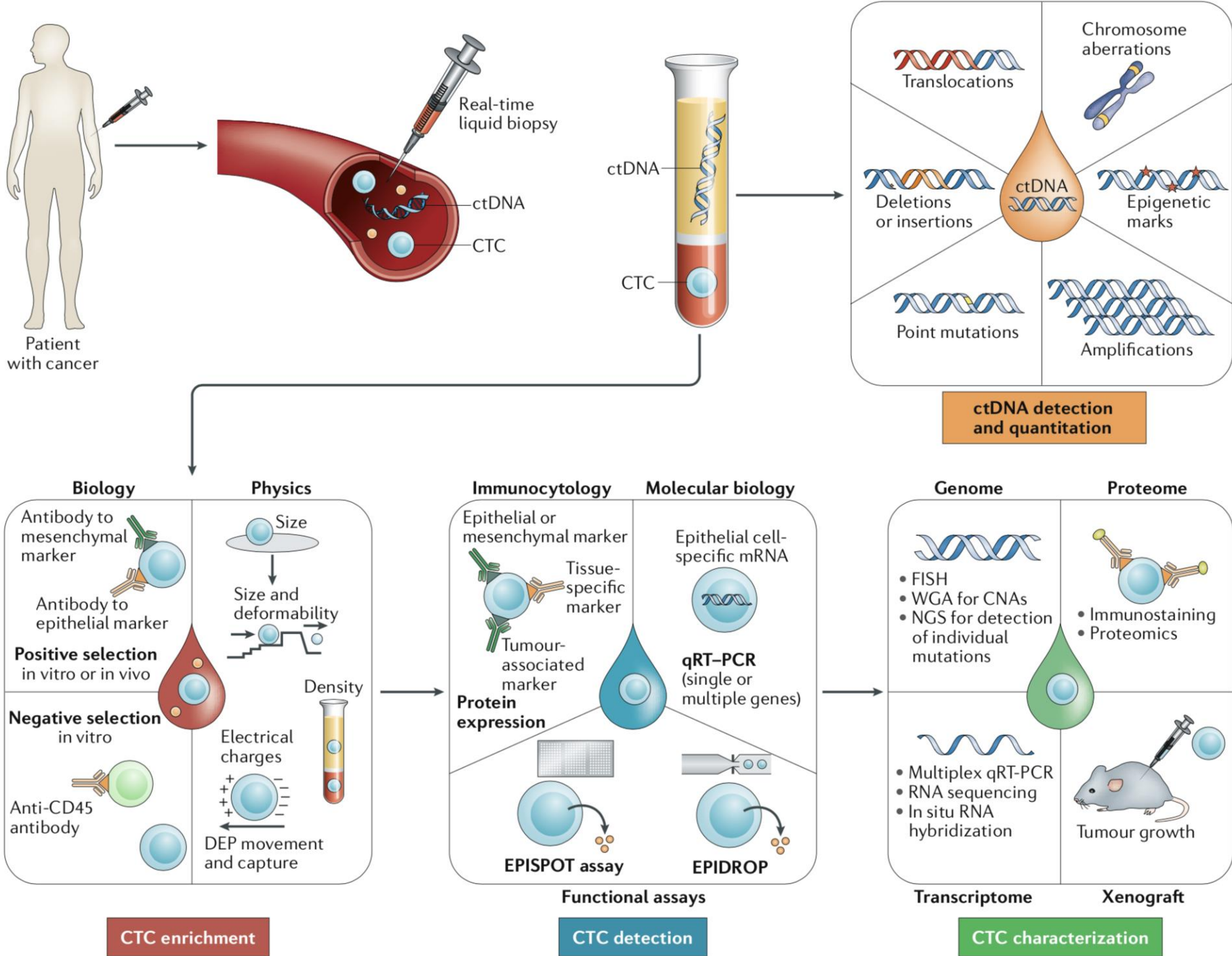
Rationale: Tissue biopsies are invasive and some locations are difficult to access (e.g., lung or brain)

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 feasible in clinical practise

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

ctDNA
&
CTC
TECHNOLOGIES

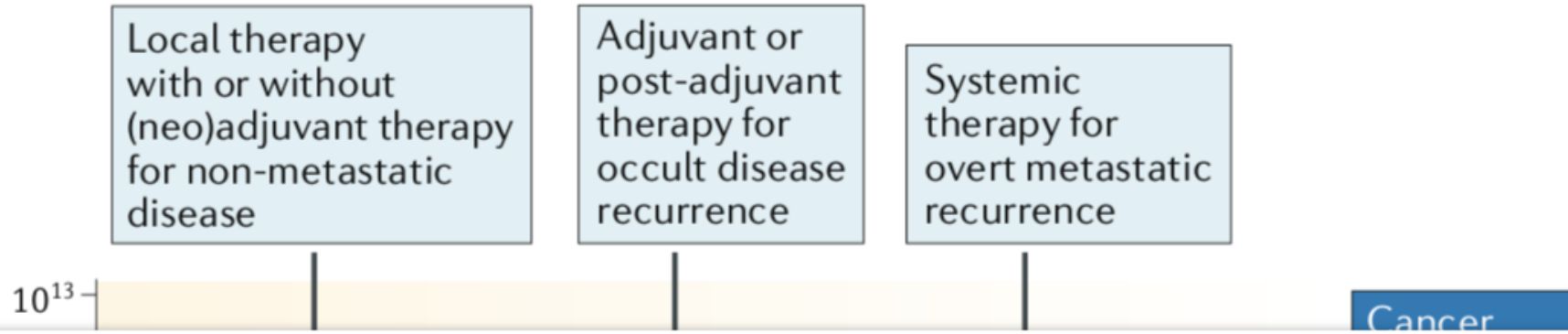


Chun, Pantel et al BJU Int. 2006

Schwarzenbach, Hoon, Pantel, Nature Cancer Rev. 2011

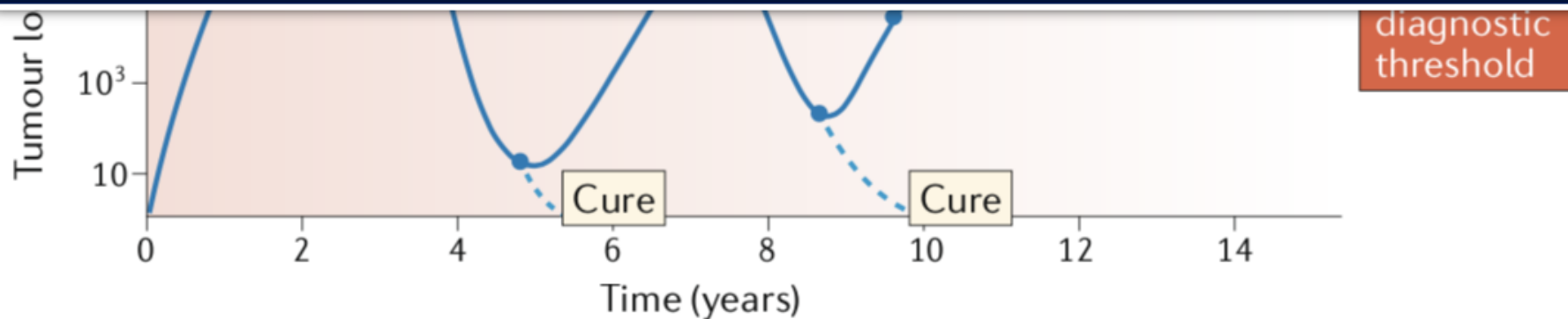
Pantel & Alix-Panabières Nat Rev Clin Oncol 2019

Clinical applications: Monitor tumour burden in cancer patients



Bone marrow is a reservoir for disseminating dormant tumor cells

(Pantel et al, Cancer Res. 1991, JNCI 1993 & Lancet 1996; Braun, Pantel et al, NEJM, 2000 & 2005; Köllermann, Pantel et al., J. Clin. Oncol. 2008; Werner, Pantel, Wikman et al, Cancer Discovery 2015)

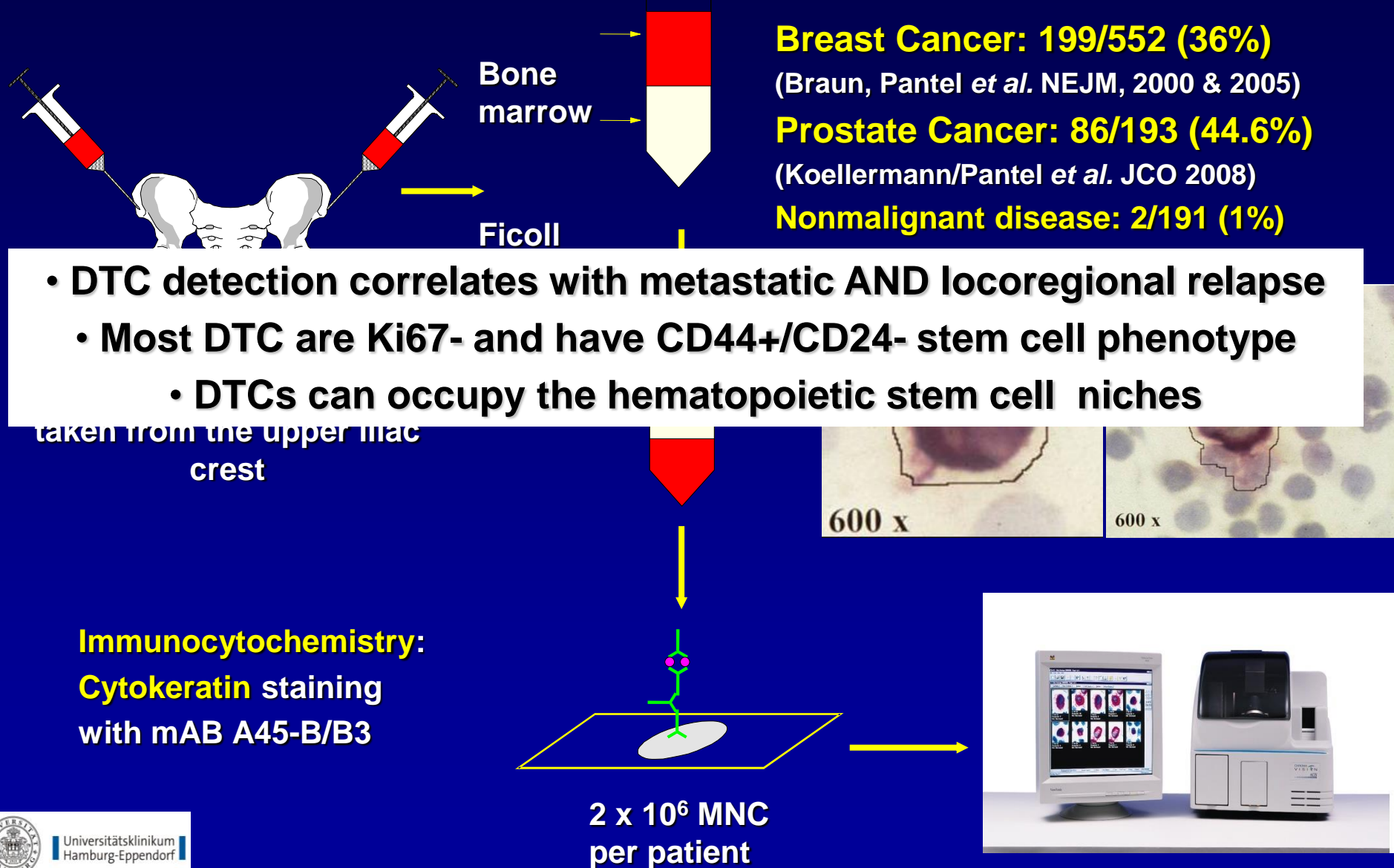


nature
REVIEWS CLINICAL
ONCOLOGY

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

Klaus Pantel^{1*} and Catherine Alix-Panabières²

DTCs in bone marrow of cancer patients



BM is a homing organ and putative reservoir for DTC derived from various primary sites

Tumor type	Detection rate (%)
Breast Cancer	20-40
Prostate cancer*	20-30
Lung cancer (NSCLC)	40-60
Gastric cancer	35-60
Esophageal cancer	30-40
Colorectal cancer	20-30
Pancreatic cancer	10-20
Head and neck cancer	20-30

Tumor cell dissemination and cancer dormancy

Cancer cells **disseminate early** into the bone marrow and pose a **risk** for subsequent **relapse** (Braun/Pantel et al., NEJM, 2005; Köllermann/Pantel et al., J Clin Oncol., 2008)

VCAM1, **Jagged-1** and tumor-induced **osteoclast miRNAs** as regulators of progression from DTCs to bone metastases (Lu/Pantel/Kang et al Cancer Cell 2011; Ell/Pantel/Kang et al, Cancer Cell 2013; Bednarz-Knoll, Kang Pantel et al, Clin Chem 2016)

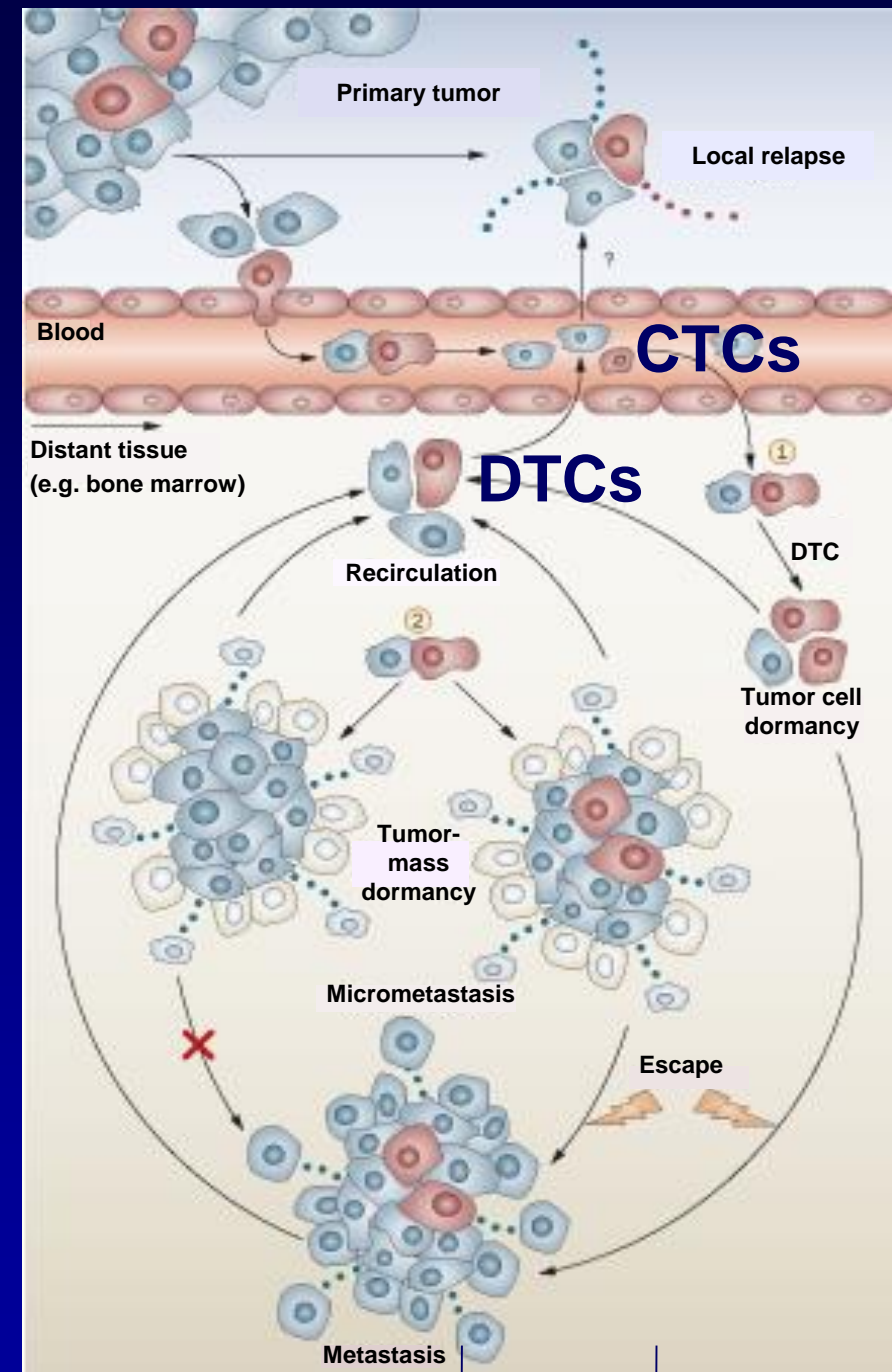
Therapeutic antibody targeting tumor- and osteoblastic niche-derived jagged1 sensitizes bone metastasis to chemotherapy (Zheng, Tupitsyn, Pantel, Kang et al, Cancer Cell 2017)

Metabolic adaptation of DTCs is important for survival (LeBleu, Pantel, Kalluri et al, Nature Cell Biol. 2014)

RAI2 as new **metastasis-suppressor** (Werner, Wikman, Wilmanns, Pantel et al, Cancer Discovery 2015)

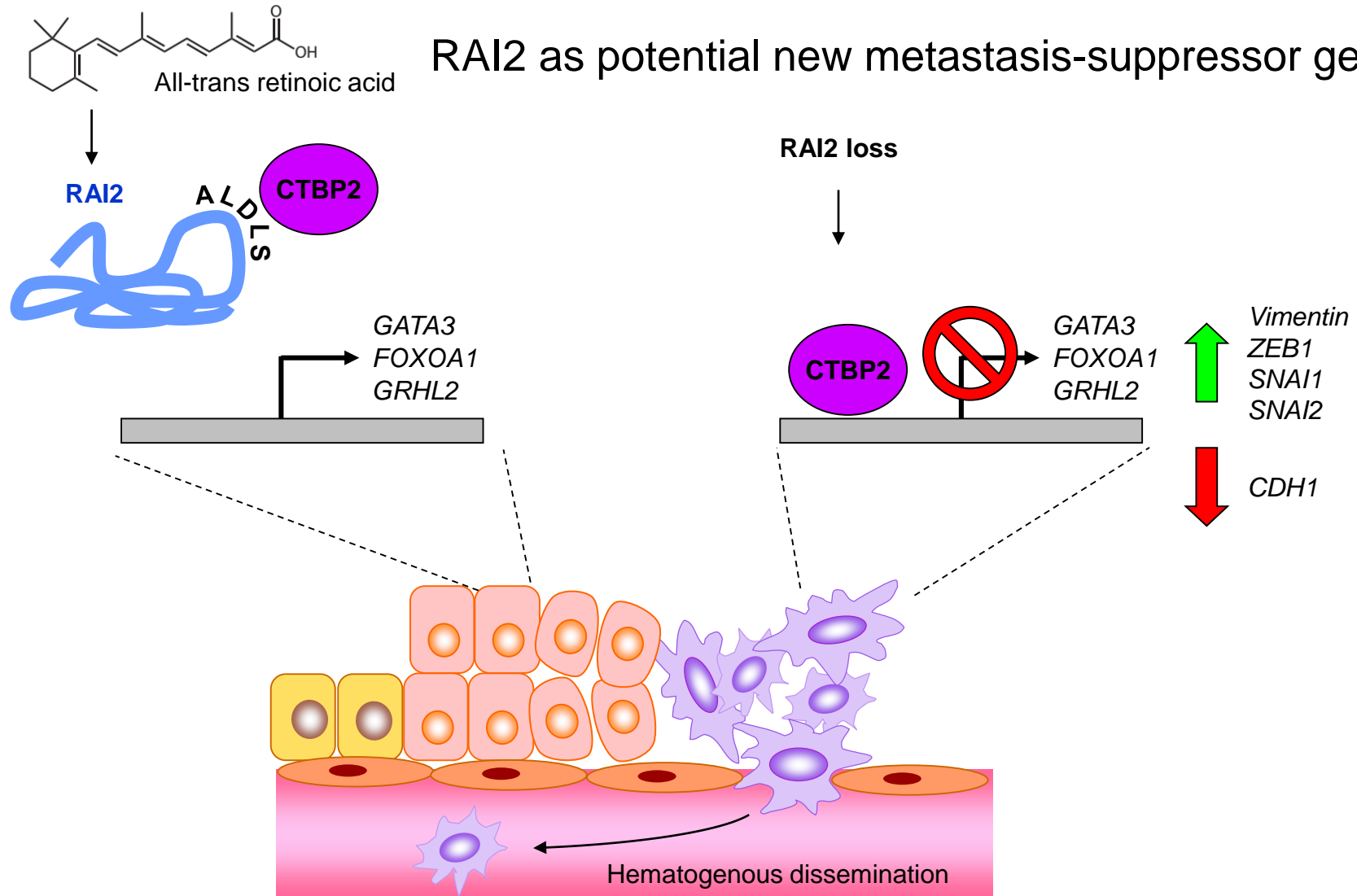
DTCs persist in the **bone marrow** through sustained activation of the **unfolded protein response** (Bartkowiak, Pantel et al., Cancer Res. 2015)

Exosome-mediated homing of metastatic cells to specific distant sites (Hoshino, Pantel, Bissell, Peinado, Lyden et al., Nature, 2015)



Editorial: Esposito & Kang, Cancer Discovery, 2015

RAI2 as potential new metastasis-suppressor gene

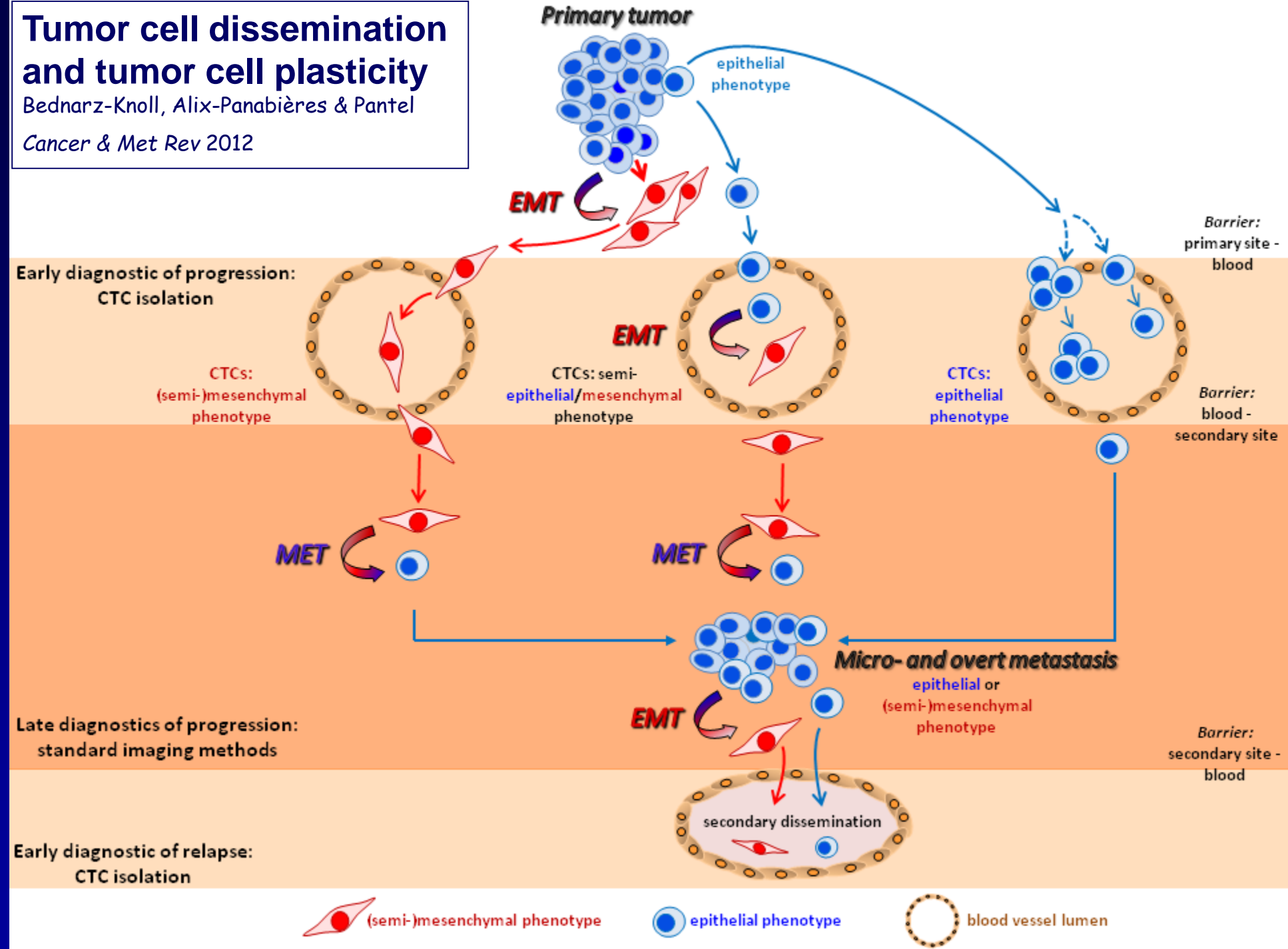


Werner, Wikman, Pantel et al, Cancer Discovery, 2015

Tumor cell dissemination and tumor cell plasticity

Bednarz-Knoll, Alix-Panabières & Pantel

Cancer & Met Rev 2012



- **Breast Cancer:** Rack, Pantel, Janni *et al. JNCI* 2014; Janni *et al. Clin Cancer Res* 2016; Riethdorf, Pantel *et al., Clin Cancer Res.*, 2017; Xenidis, Lianidou, Mavroudis *et al., JCO* 2006
- **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; Garrel, Alix-Panabieres *et al., Clin. Chem.* 2019 (Editorial: Pantel, *Clin. Chem.* 2019)

CTCs can be used as enrichment tool to study a high risk population

cM0(i+), AJCC Cancer Staging Manual 2018 for breast cancer

- **Pancreatic Cancer:** Effenberger, Bockhorn, Pantel *et al. Clin Cancer Res* 2018
- **Merkel Cell Cancer:** Riethdorf, Pantel *et al., Clin. Chem.* 2018
- **Melanoma:** Wiltfang, Roeck, Pantel *et al, Cancers*, 2019; Cayrefourcq *et al, Clin Chem* 2019

Published OnlineFirst July 5, 2017; DOI: 10.1158/1078-0432.CCR-17-0255

Cancer Therapy: Clinical

Clinical
Cancer
Research

Prognostic Impact of Circulating Tumor Cells for Breast Cancer Patients Treated in the Neoadjuvant "Geparquattro" Trial

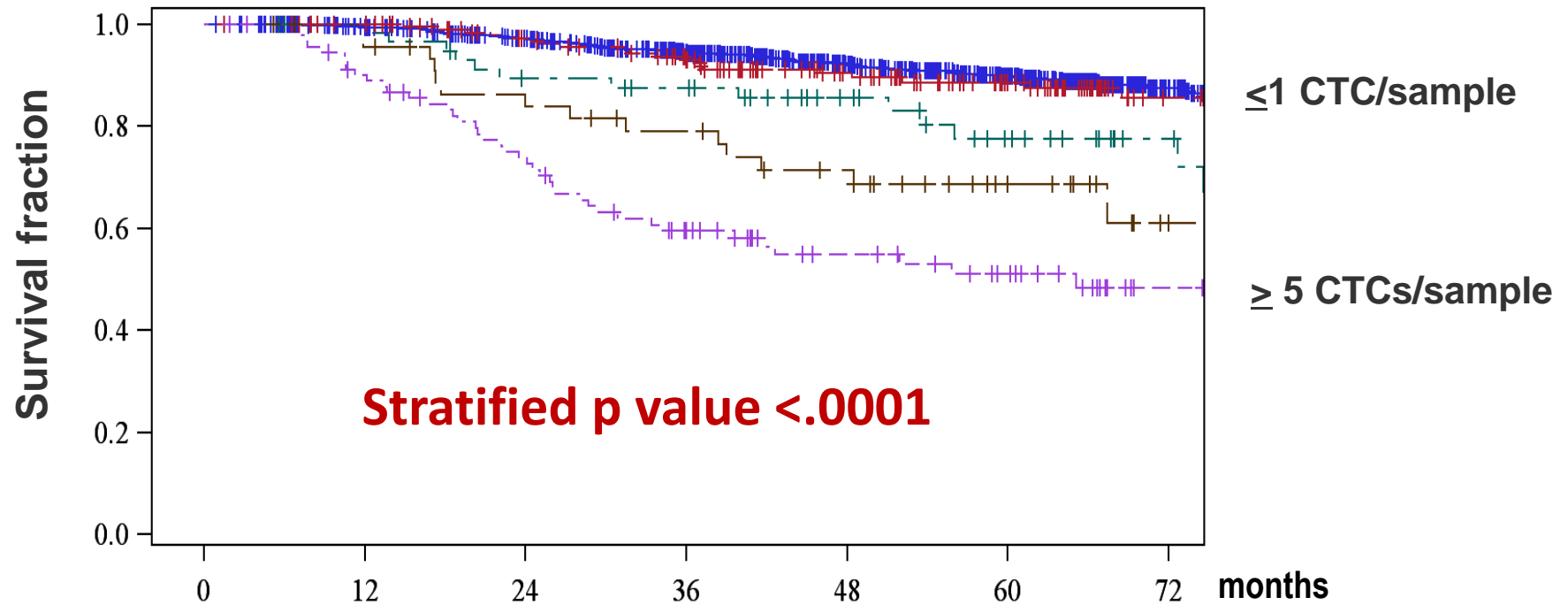


CTCs reflect the potential of the tumor to disseminate

Frank Holms¹, Hans Tesch¹, Christian Schell¹, Günter von Minckwitz¹,
Michael Untch¹, and Klaus Pantel¹

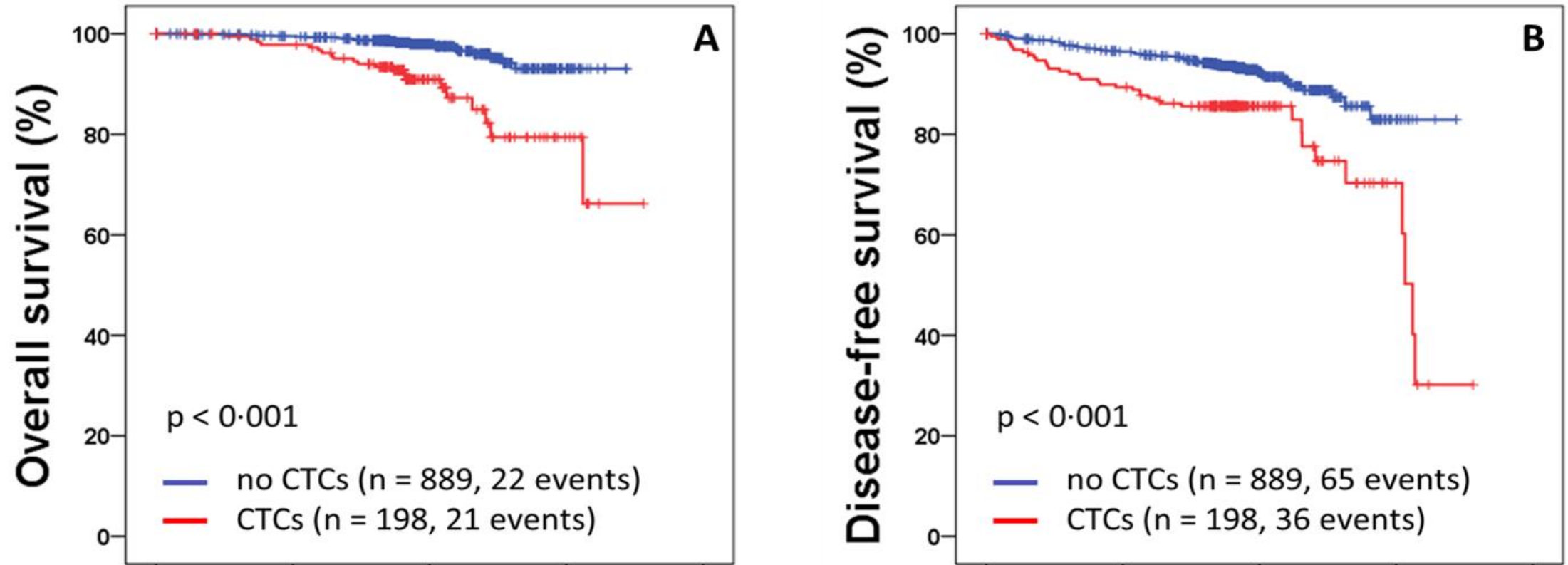
CTC counts provide prognostic information independent from pathological complete response (pCR) of the primary tumor

Validated by an international meta-analysis of 2,156 individual patients 21 studies & 16 centers (Bidard, Pantel et al., *JNCI* 2018)



	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]

**Detection of Minimal Residual Disease
(Post-surgical surveillance)**



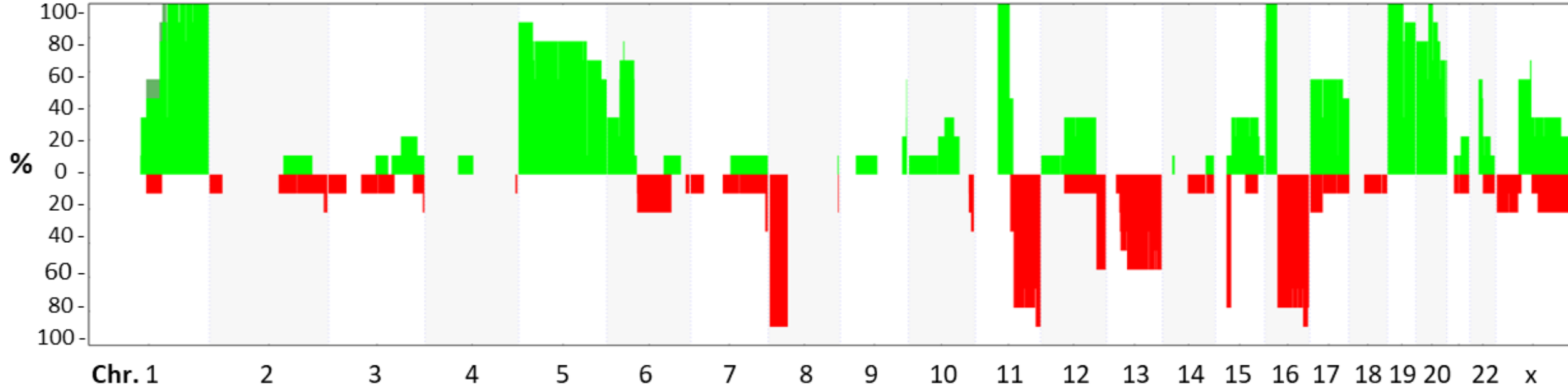
Need for „Post-Adjuvant“ Clinical Trials
(Pantel & Hayes, *Nature Rev. Clin. Oncol.* 2018)

Figure 2 CTCs detected 2 years after adjuvant chemotherapy

Identification of the Tissue Origin of CTCs

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

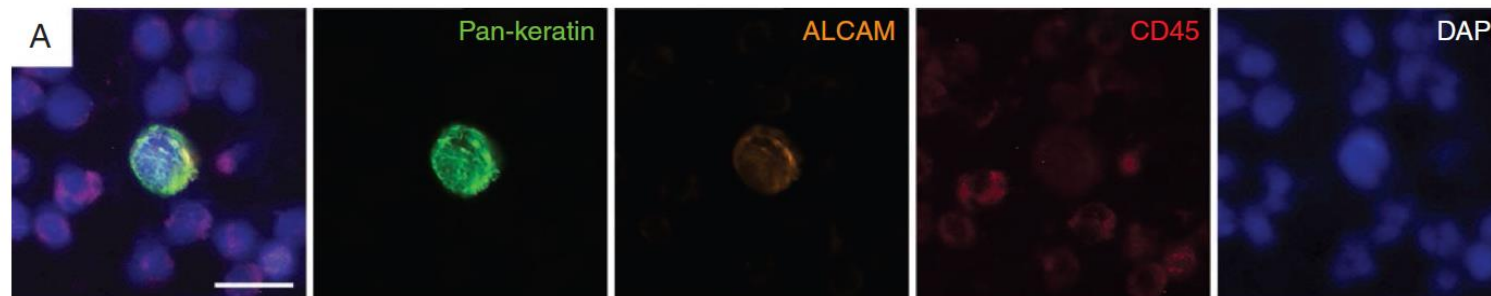
NGS-based exome sequencing of single CTCs isolated from blood



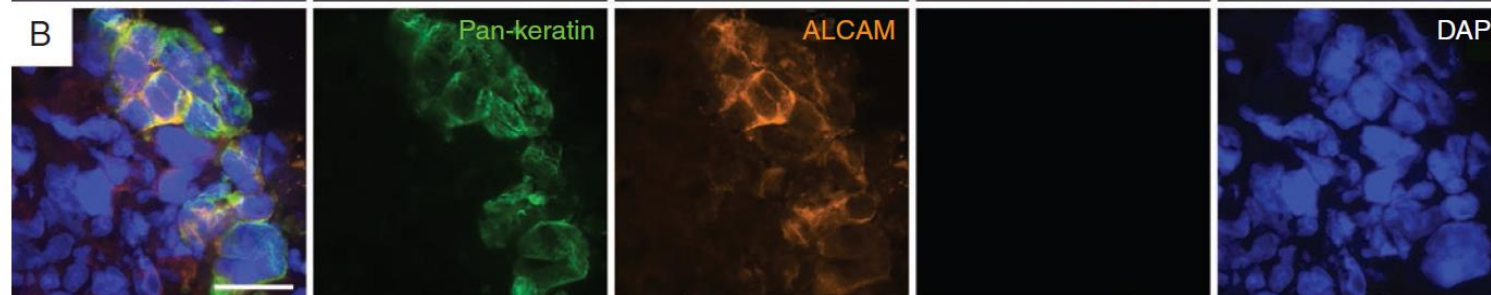
ALCAM contributes to brain metastasis formation in non-small-cell lung cancer through interaction with the vascular endothelium

Justine Münsterberg,[†] Desirée Loreth,^{†,⊙} Laura Brylka, Stefan Werner, Jana Karbanova, Monja Gandrass, Svenja Schneegans,[⊙] Katharina Besler, Fabienne Hamester, José Ramon Robador, Alexander Thomas Bauer, Stefan Werner Schneider, Michaela Wrage, Katrin Lamszus, Jakob Matschke, Yogesh Vashist, Güntac Uzunoglu, Stefan Steurer,[⊙] Andrea Kristina Horst, Leticia Oliveira-Ferrer,[⊙] Markus Glatzel, Thorsten Schinke, Denis Corbeil, Klaus Pantel,[⊙] Cecile Maire, and Harriet Wikman,[⊙]

CTCs

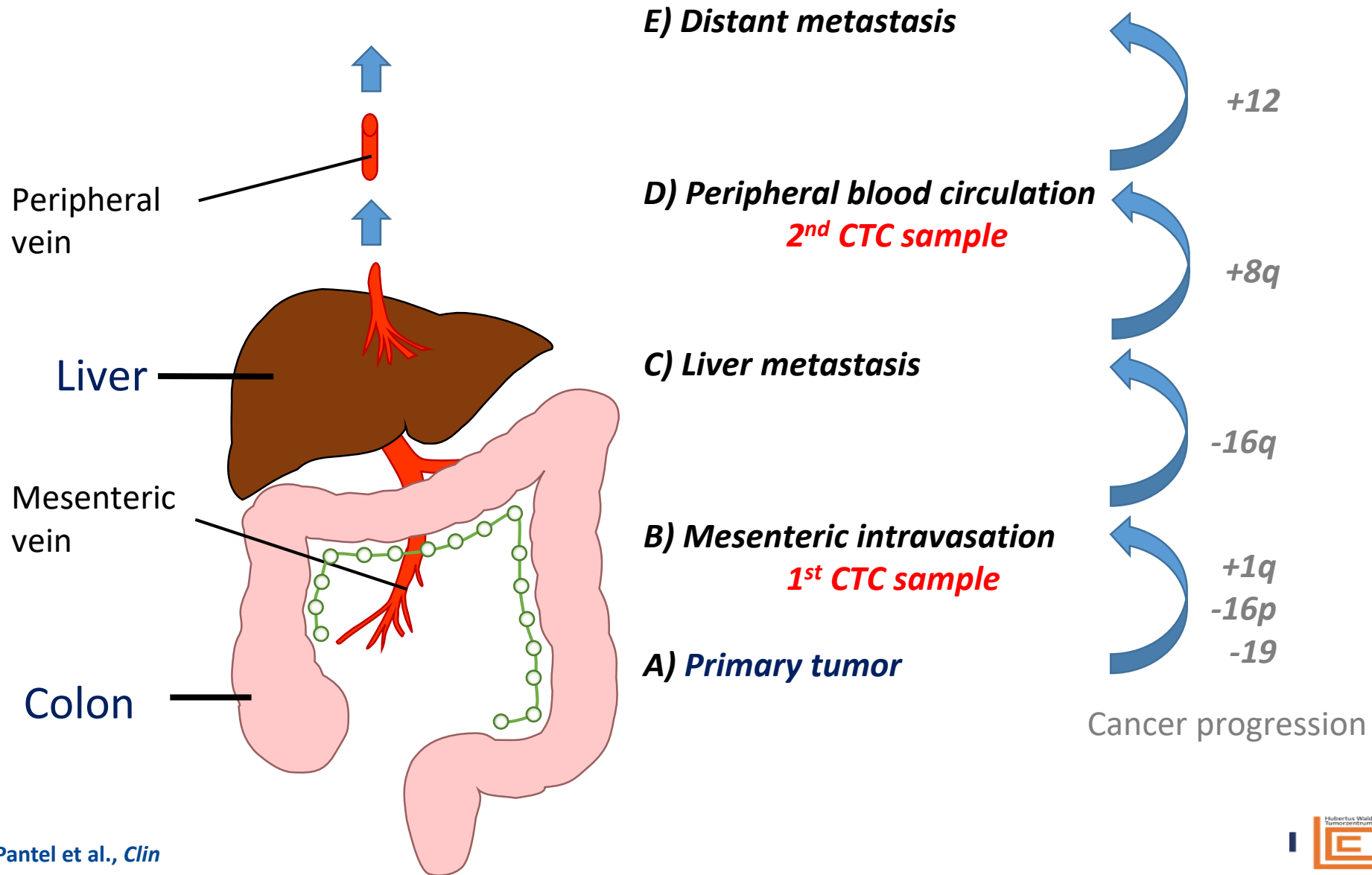


Brain
metastases

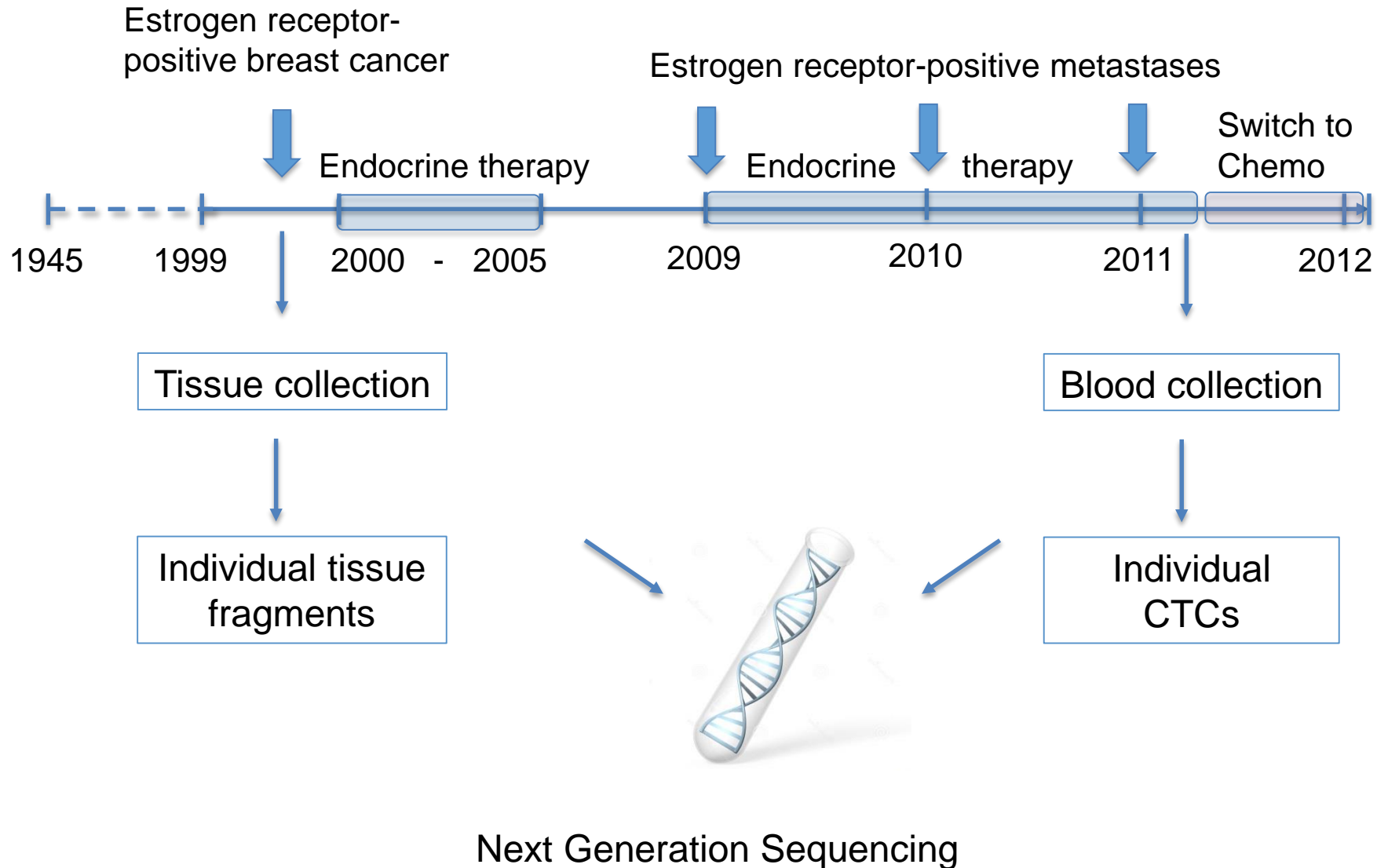


Assessment of tumor evolution from primary to metastatic disease

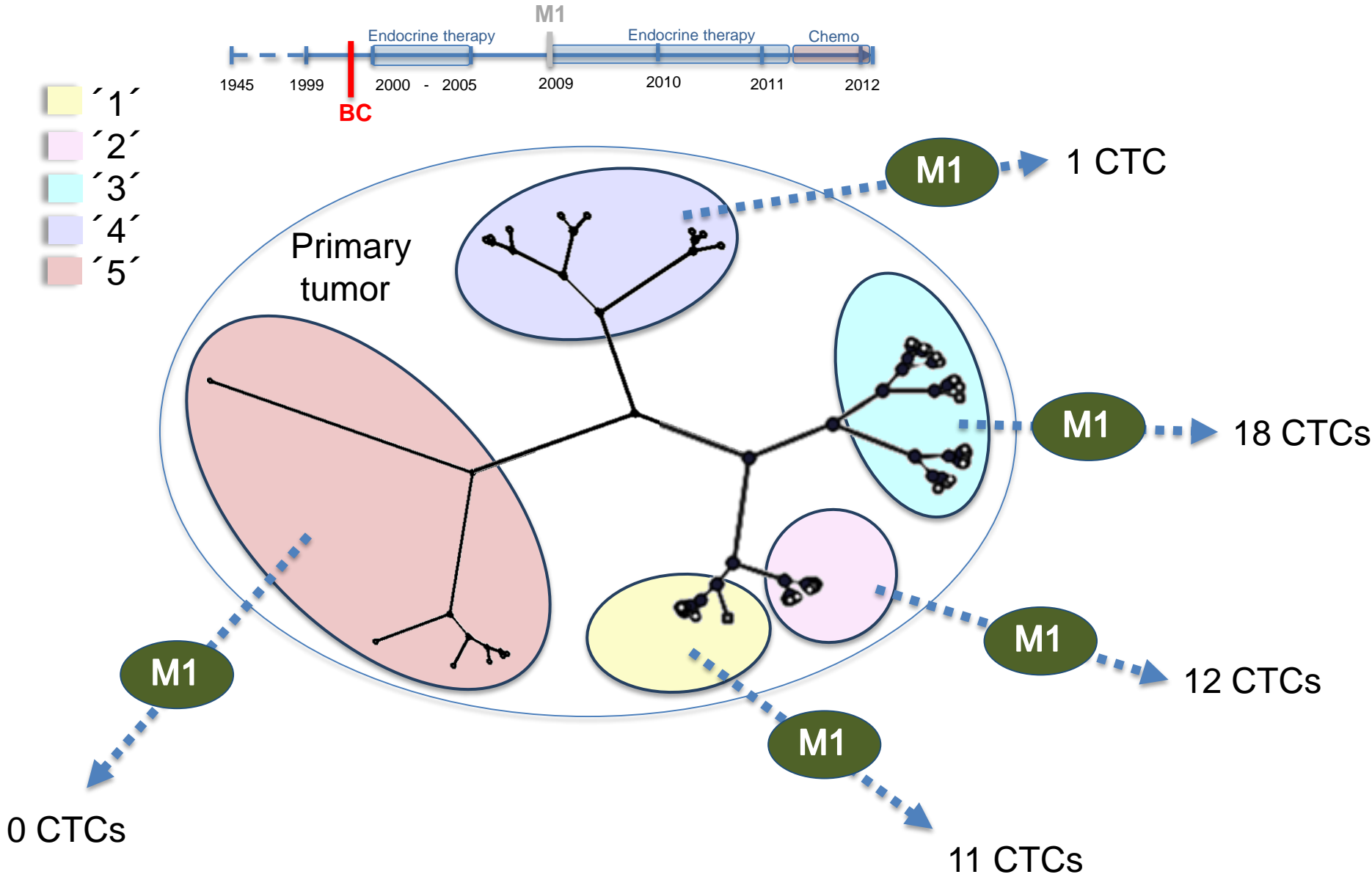
Chromosomal aberrations in the primary tumor associated with sequential steps of the metastatic cascade in colorectal cancer patients



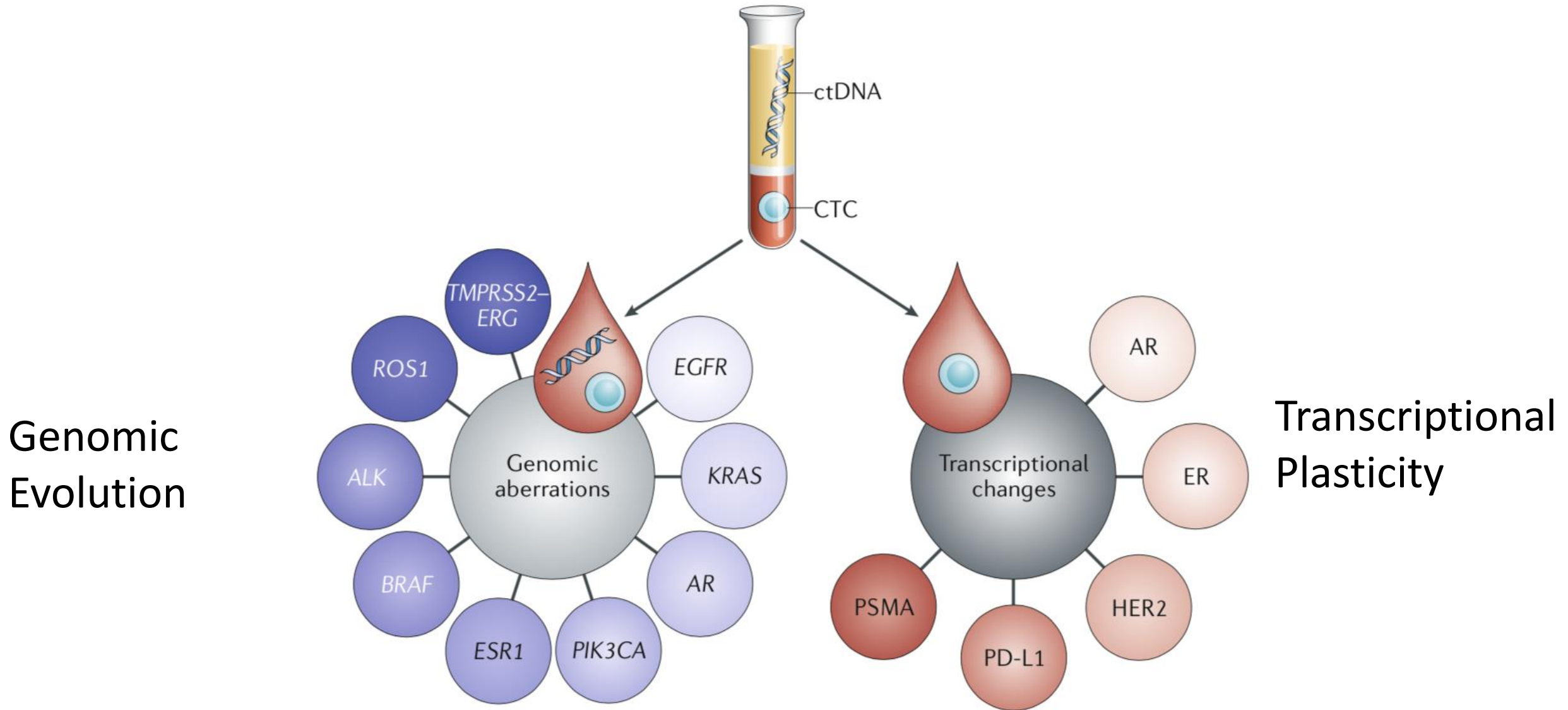
Clonal Evolution Analysis: CTCs Captured at Diagnosis of Metastasis vs. Primary Tumor Resected 10 Years Earlier



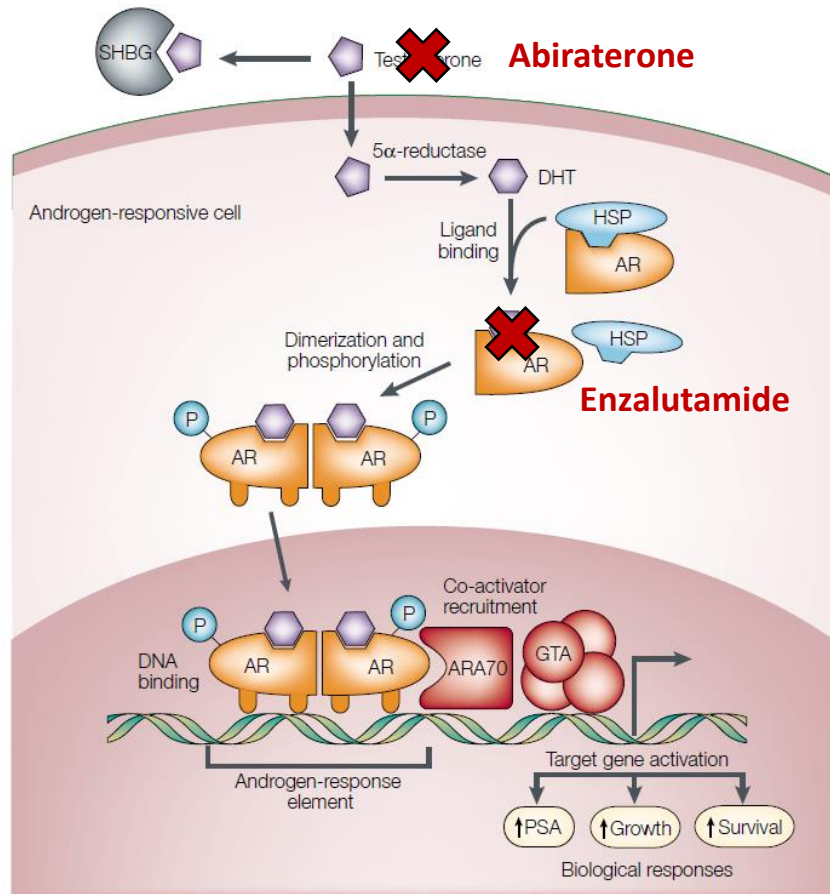
Genomic Classification of Metastases-derived CTCs vs. Primary Tumor



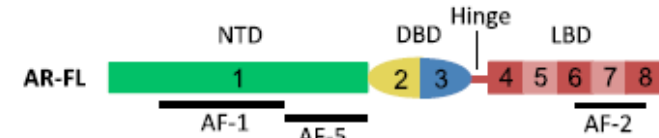
Liquid Biopsy: Therapeutic targets and resistance mechanisms



AR variant 7 (ARv7): Reactivation of the AR pathway in prostate cancer



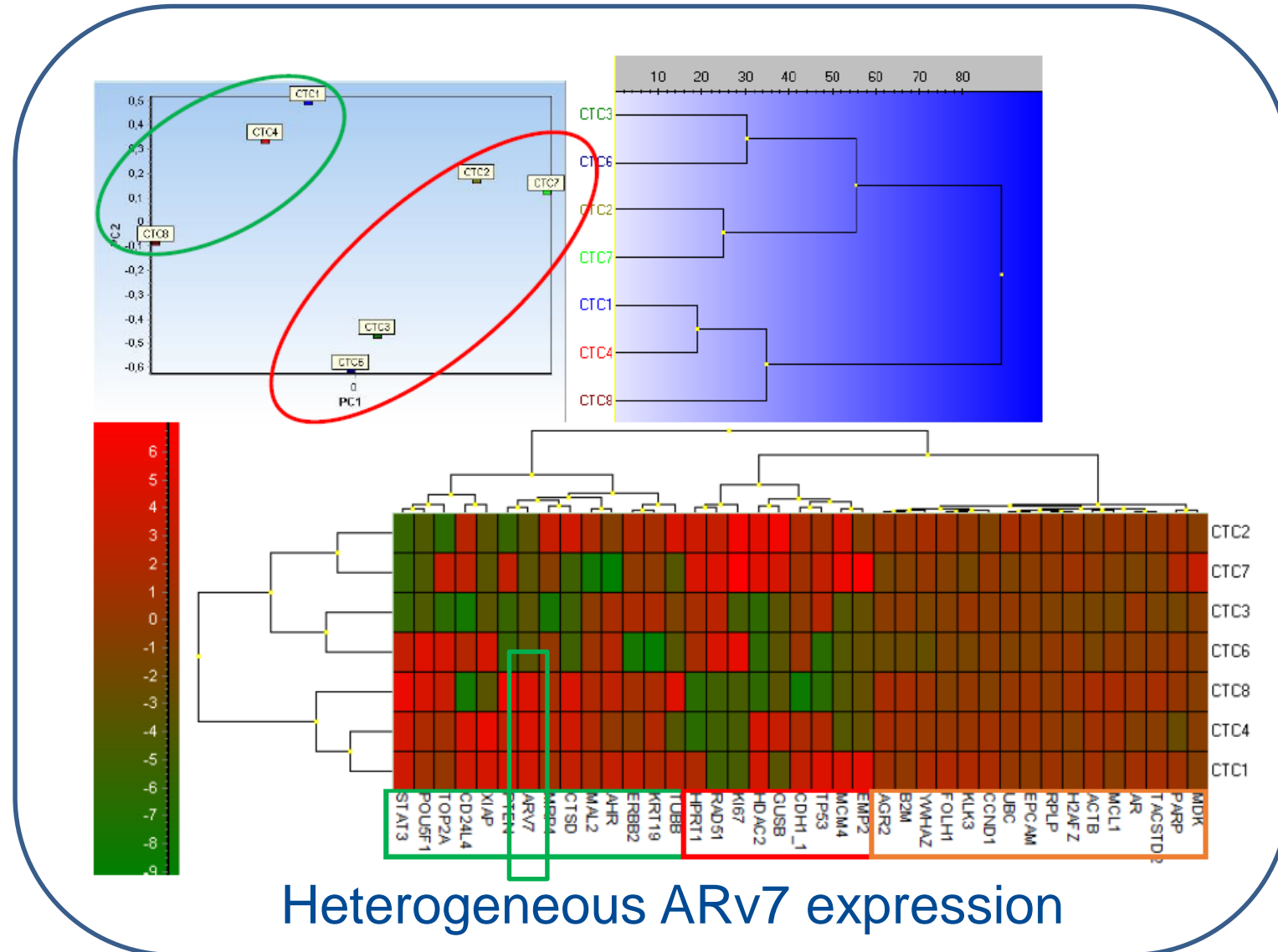
Feldman (2001)



Clinical relevance:

ARV7 expression in CTCs from CRPC patients is associated with resistance towards enzalutamide and abiraterone (Antonarakis, Scher et al)

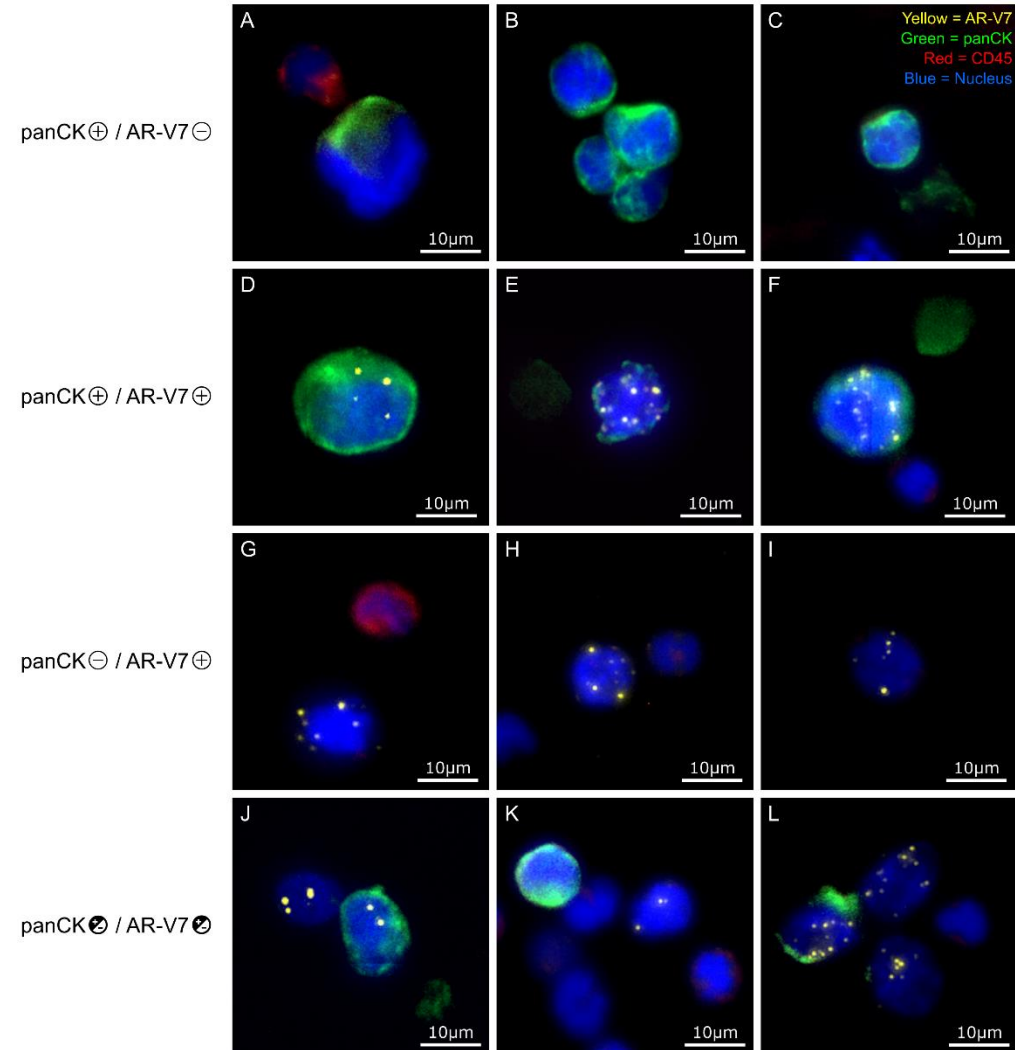
Multiplex mRNA Profiling of Single CTCs Captured from a Prostate Cancer Patient



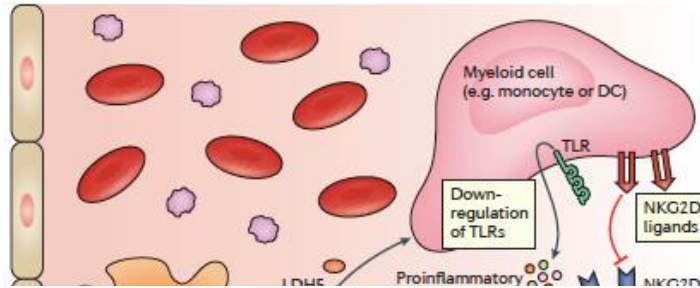
In situ ARV7 transcript detection in CTCs from prostate cancer patients

17 CRPC patients (19 samples):

- 89% of samples: CTC-positive (range 1-158 CTCs / 7.5ml blood)
- 79% of samples showed *ARV7* positivity (1-30 rolling circle transcripts/ CTC)
- 3 distinct CTC types:
 - CK+/ARV7+
 - CK+/ARV7-
 - CK-/ARV7+



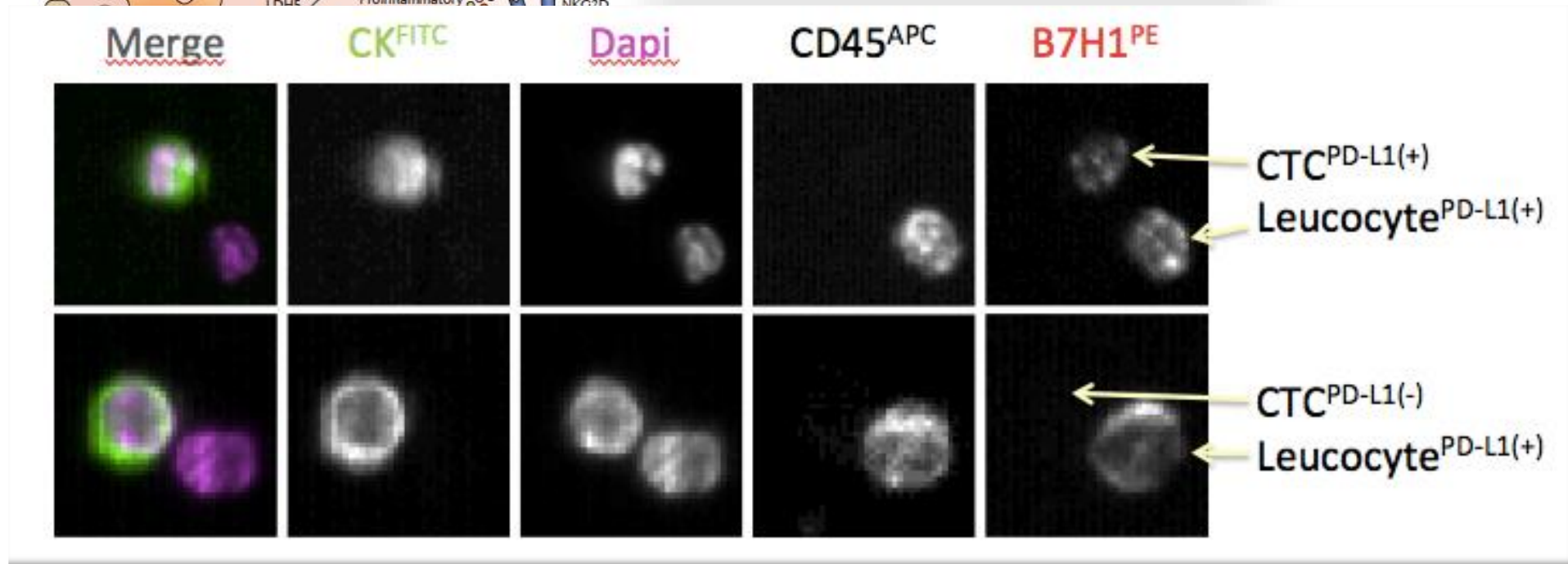
Combined CTC & ctDNA analysis



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

Malte Mohme^{1,2}, Sabine Riethdorf¹ and Klaus Pantel¹

nature
REVIEWS
CLINICAL
ONCOLOGY



Mazel, Pantel, Alix-Panabieres et al, Mol. Oncol. 2015

Review: Hofman, Heeke, Alix-Panabiers, Pantel, Ann. Oncol. 2019

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

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

nature
REVIEWS

CLINICAL ONCOLOGY

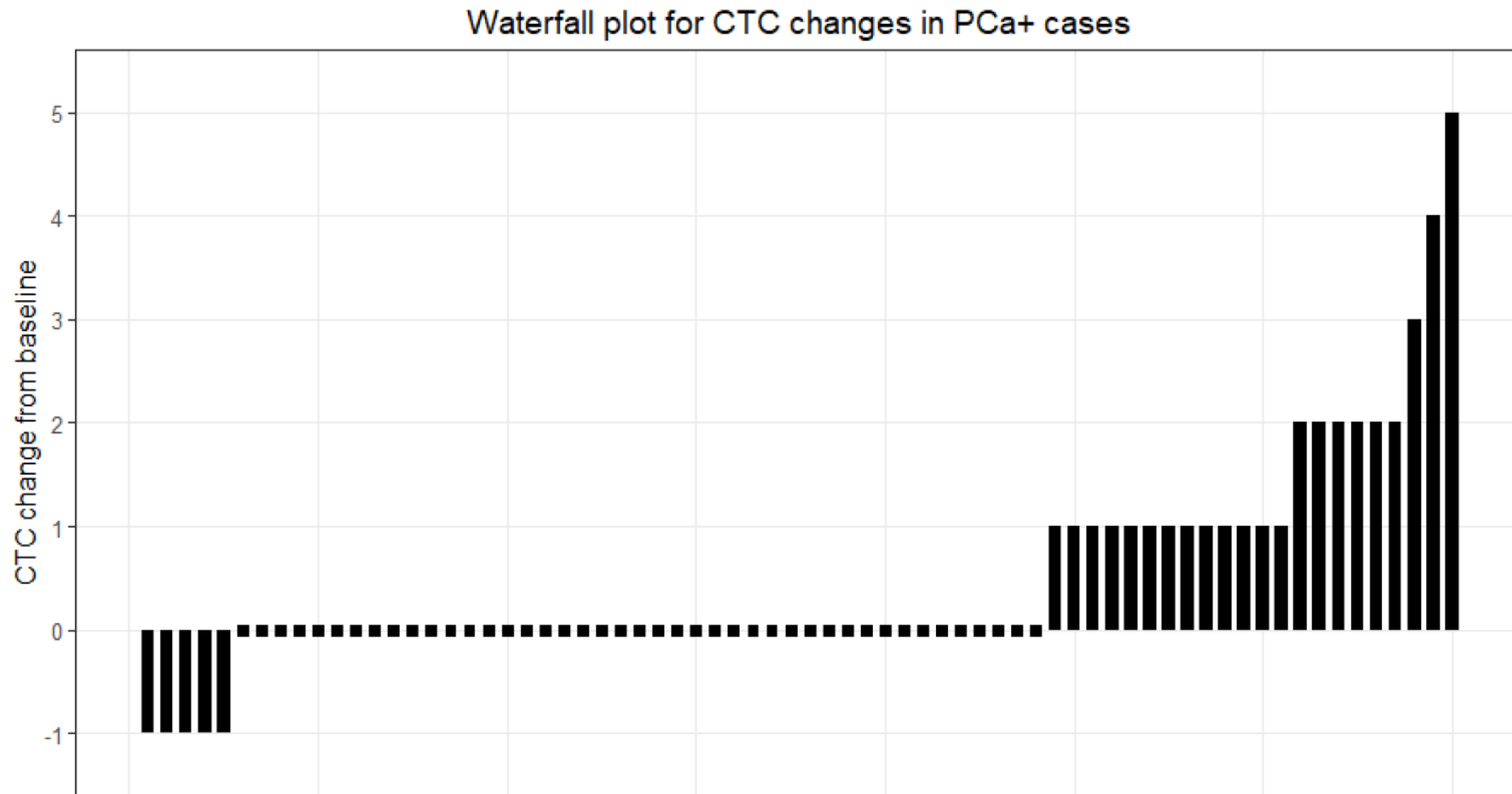


REVIEWS

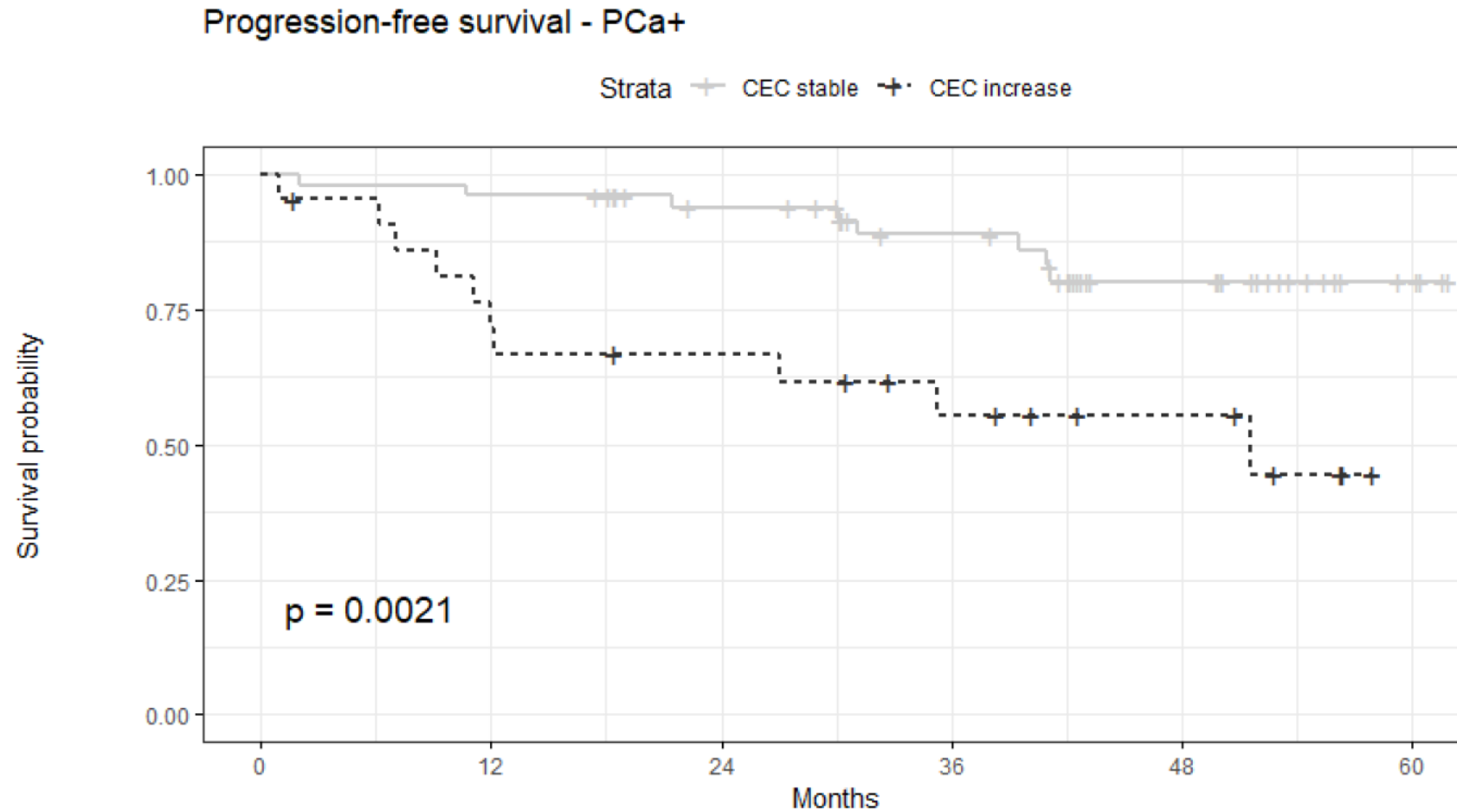
Does the mobilization of circulating tumour cells during cancer therapy cause metastasis?

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 subsequently diagnosed with prostate cancer



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



Early Detection of Cancer

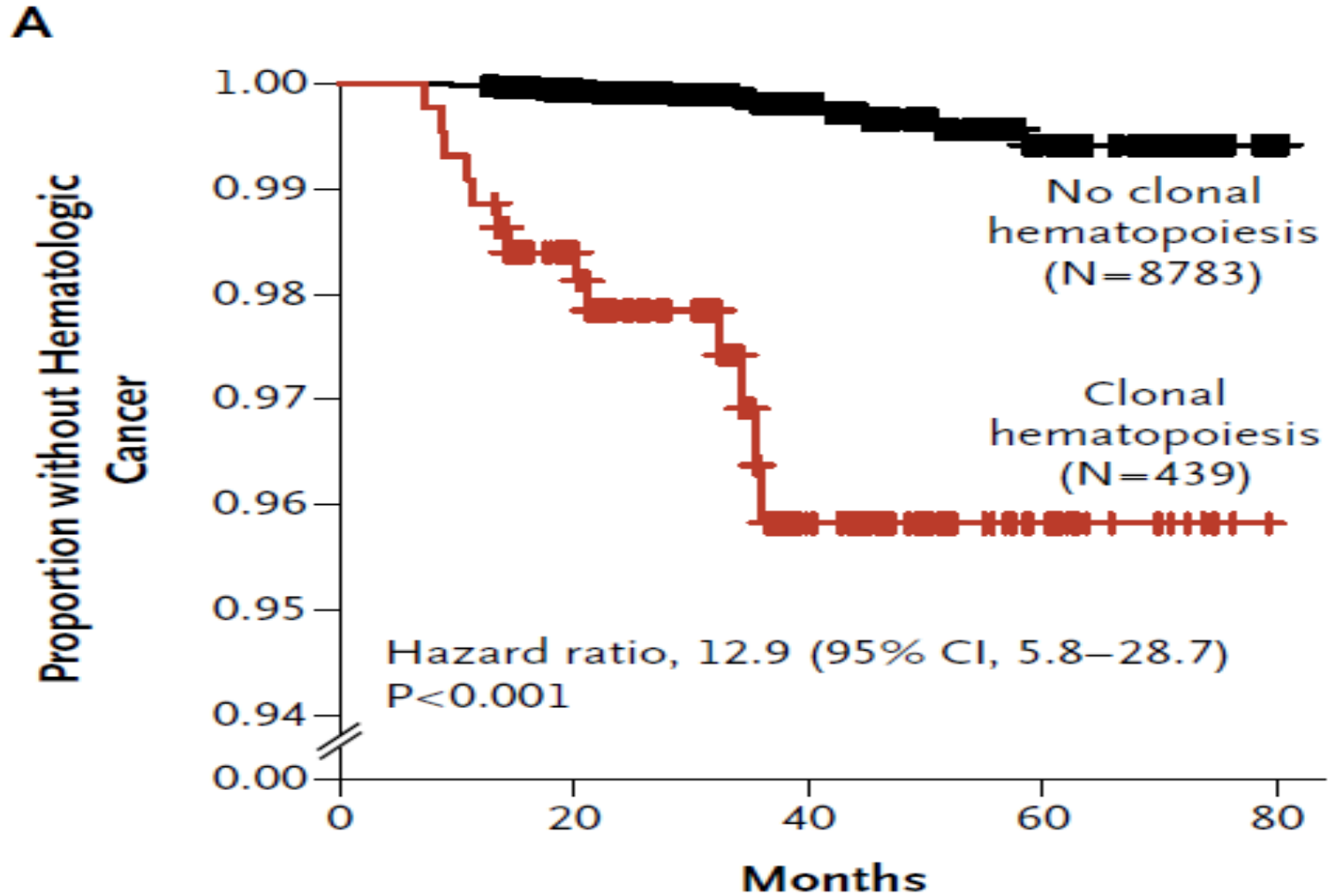
Aim: Detection of EARLY stages (stage I/pT1)

Sensitivity: False-negatives?

**Specificity: False-positives? (1% = 1/100,
screening of 10 Mio = 100.000)**

**Control group: Age-matched healthy & confounding
non-cancer diseases**

Background of cancer-associated mutations in non-cancer control patients (CHIP)



Cite as: J. D. Cohen *et al.*, *Science*
10.1126/science.aar3247 (2018).

Detection and localization of surgically resectable cancers with a multi-analyte blood test

Joshua D. Cohen,^{1,2,3,4,5} Lu Li,⁶ Yuxuan Wang,^{1,2,3,4} Christopher Thoburn,³ Bahman Afsari,⁷ Ludmila Danilova,⁷ Christopher Douville,^{1,2,3,4} Ammar A. Javed,⁸ Fay Wong,^{1,2,3,4} Austin Mattox,^{1,2,3,4} Ralph H. Hruban,^{3,4,9} Christopher L. Wolfgang,⁸ Michael G. Goggins,^{3,4,9,10,11} Marco Dal Molin,⁴ Tian-Li Wang,^{3,9} Richard Roden,^{3,9} Alison P. Klein,^{3,4,12} Janine Ptak,^{1,2,3,4} Lisa Dobbyn,^{1,2,3,4} Joy Schaefer,^{1,2,3,4} Natalie Silliman,^{1,2,3,4} Maria Popoli,^{1,2,3,4} Joshua T. Vogelstein,¹³ James D. Browne,¹⁴ Robert E. Schoen,^{15,16} Randall E. Brand,¹⁵ Jeanne Tle,^{17,18,19,20} Peter Gibbs,^{17,18,19,20} Hui-Li Wong,¹⁷ Aaron S. Mansfield,²¹ Jin Jen,²² Samir M. Hanash,²³ Massimo Falconi,²⁴ Peter J. Allen,²⁵ Shubin Zhou,^{1,3,4} Chetan Bettegowda,^{1,2,3,4} Luis Diaz,^{1,3,4} Cristian Tomasetti,^{3,6,7*} Kenneth W. Kinzler,^{1,3,4*} Bert Vogelstein,^{1,2,3,4*} Anne Marie Lennon,^{3,4,8,10,11*} Nickolas Papadopoulos^{1,3,4*}

- High specificity and sensitivity for ovarian cancer but low sensitivity for breast cancer (33%)
- Lower sensitivity for detection of early stages

The new test measures circulating tumor DNA (ctDNA) from 16 genes as well as eight protein biomarkers, and then uses machine-based learning to analyze the data.

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

Start: January 2018, Focus: Detection of Lung Cancer

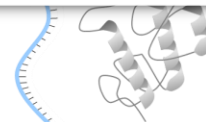
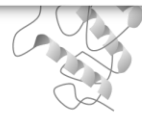
Coordinator: Tom Würdinger, Amsterdam
Deputy Coordinator: Klaus Pantel, Hamburg

**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

, Wuerdinger *et al.*, *Cancer*
2015 & 2017



DNA, RNA, proteins

DNA, RNA

RNA, proteins

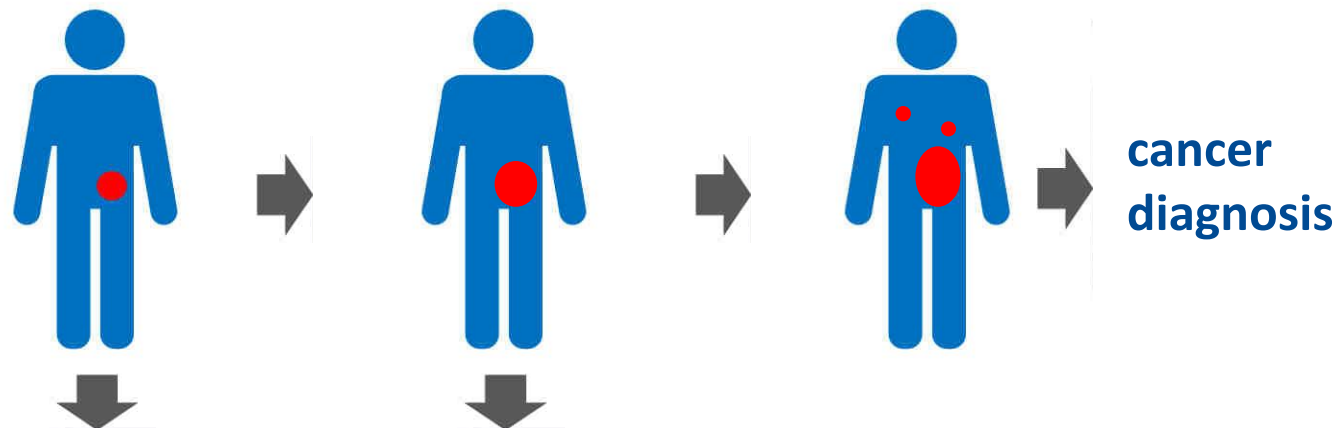
RNA

Joose & Pantel, *Cancer Cell* 2015

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



pre-diagnostic samples / datasets

Functional CTC Models

CTC xenografts (CDX)

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

2013

nature
biotechnology

CTC cell lines

AACR Award 2017

Tumor and Stem Cell Biology

Cancer
Research

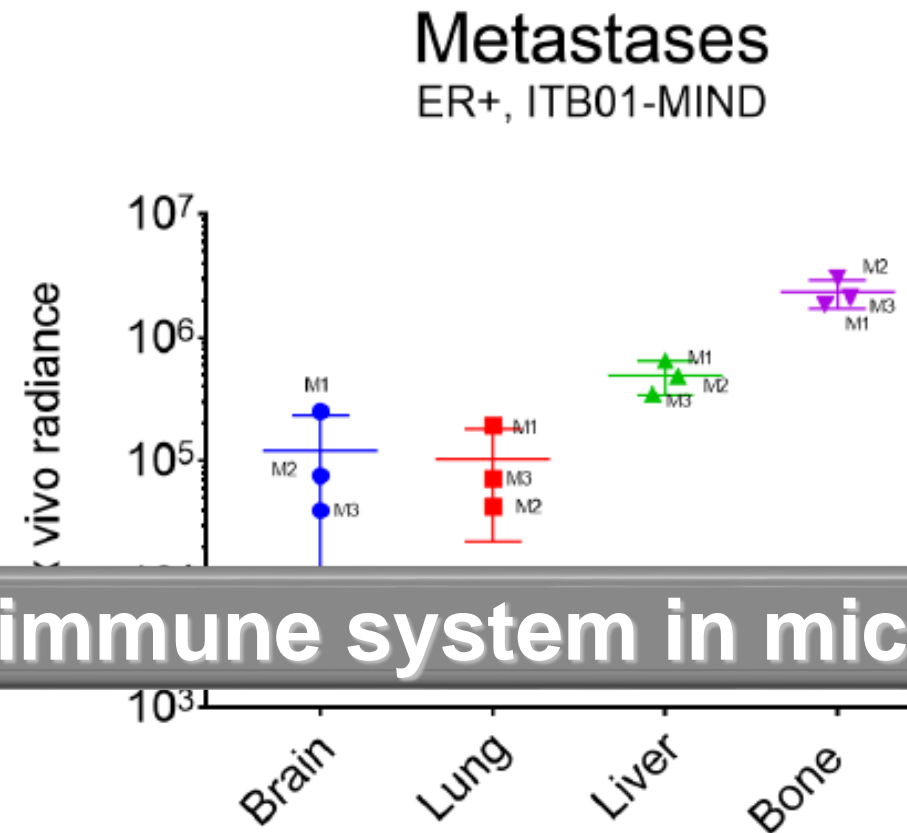
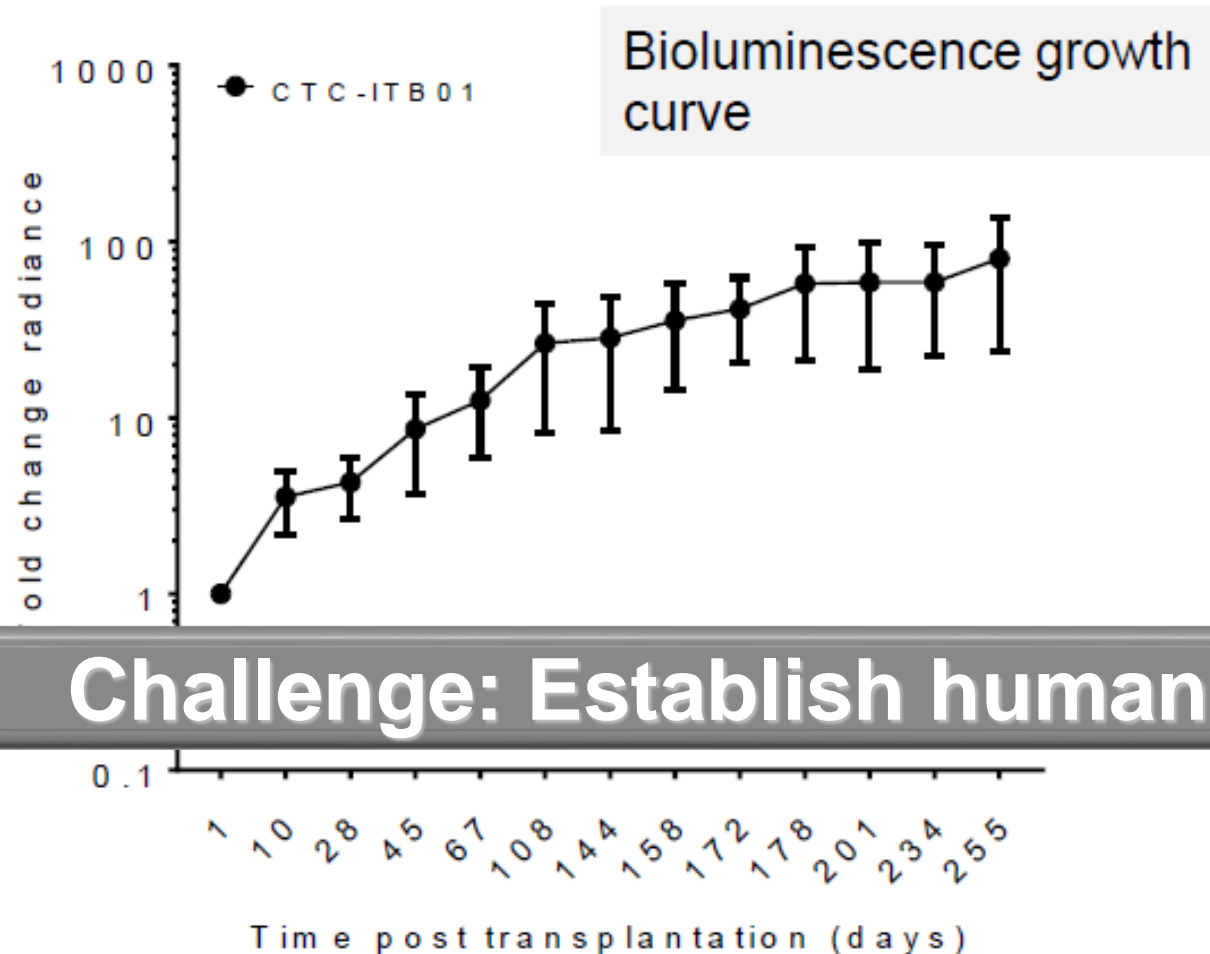
Establishment and Characterization of a Cell Line from Human Circulating Colon Cancer Cells

Laure Cayrefourcq^{1,2}, Thibault Mazard³, Simon Joosse⁴, Jérôme Solassol⁵, Jeanne Ramos⁶, Eric Assenat³, Udo Schumacher⁷, Valérie Costes⁶, Thierry Maudelonde^{2,5}, Klaus Pantel⁴, and Catherine Alix-Panabières^{1,2}

Autologous CTC lines established at different time points during therapy

(Soler et al., Sci Rep, 2018)

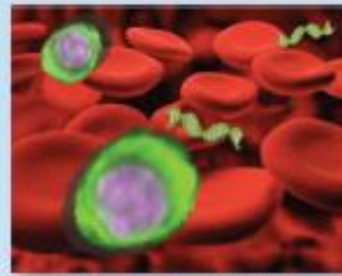
In vivo growth and metastasis of a new ER+ CTC line after intramammary injection into immunodeficient mice



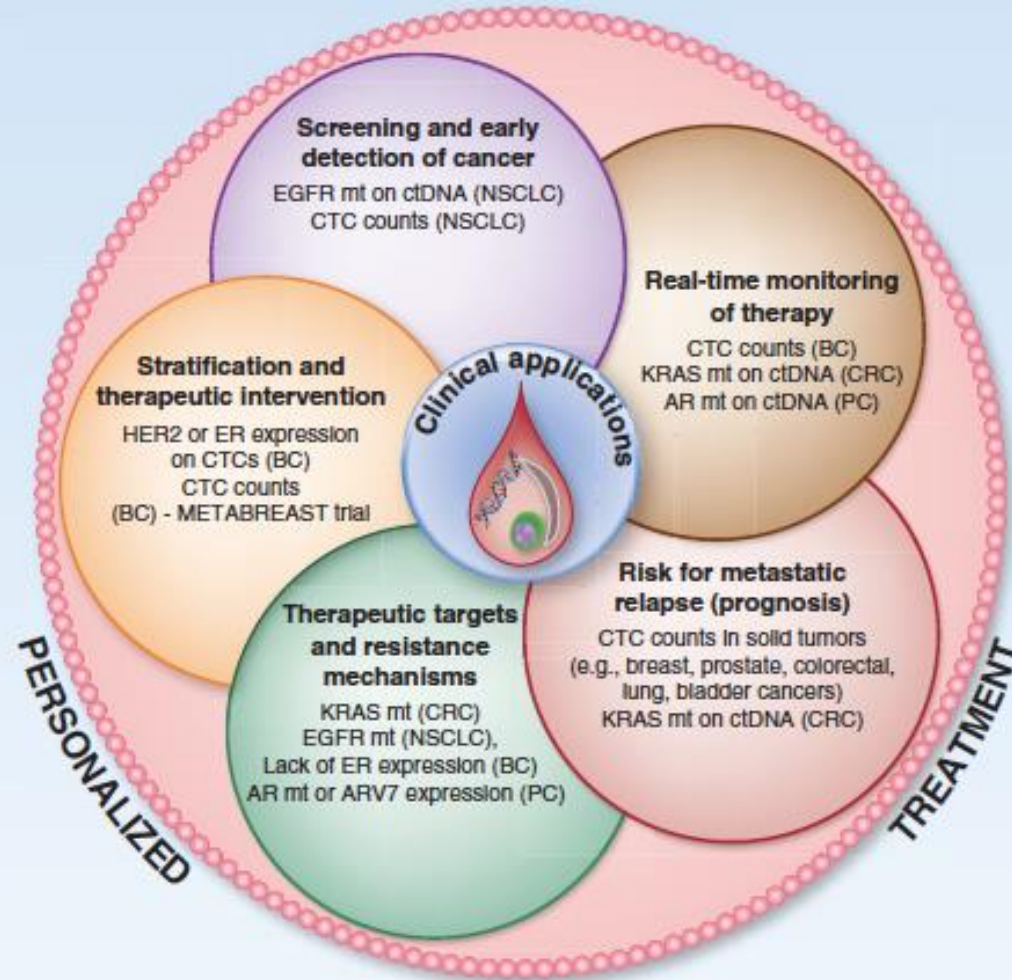
Challenge: Establish human immune system in mice



Noninvasive blood sample



Real-time liquid biopsy



Conclusions:

CTCs & ctDNA provide complementary information for liquid biopsy

Assays need to be validated by independent expert groups
(Ratner, Nature Biotech 2018)

Interventional clinical studies are required to demonstrate clinical utility of liquid biopsy
(Merker et al, JCO 2018)
!Therapy must work!

**Translation from Publication to
Clinical Routine**

CANCER-ID EU Consortium 2015-2019

Scientific Management: Klaus Pantel, UKE
Coordination: Thomas Schlange, BAYER



Clinical 32 partners:



**Blood-based Diagnostic in Lung and Breast Cancer
(CTCs, ctDNA & cfmiRNA)**

**40 EU Partners (Academic institutions, non-profit
organisations & companies)**

Non-profit
organizations



THE EUROPEAN
LIQUID BIOPSY
SOCIETY



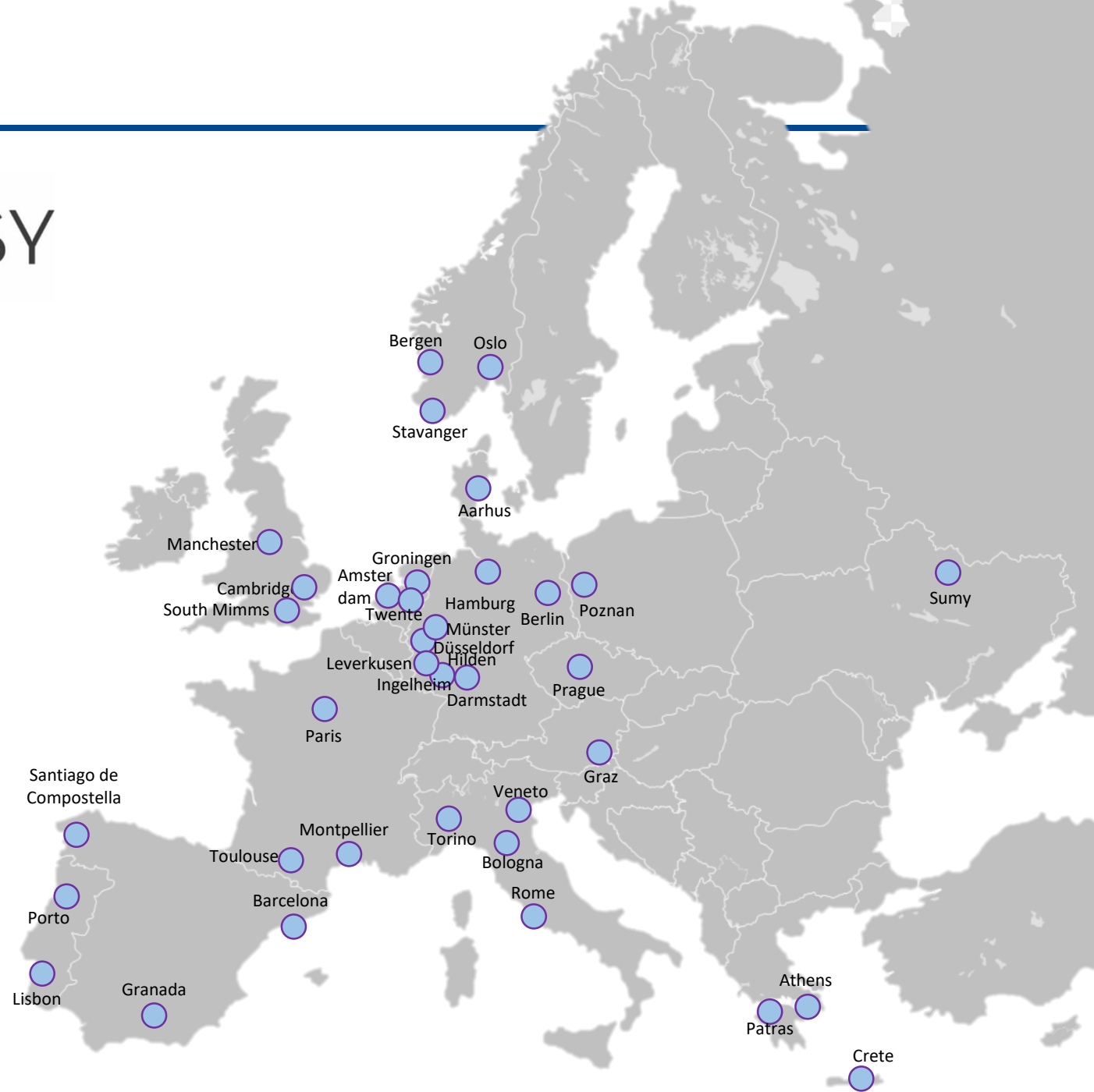
THE EUROPEAN
LIQUID BIOPSY
SOCIETY

Participants from Europe:
40 Institutions from
Academia (26)
& companies (14)

Kickoff Meeting:
3 May 2019, UKE

Coordinator: K. Pantel
Lead institution: UKE

Aims:
Support translational liquid
biopsy research



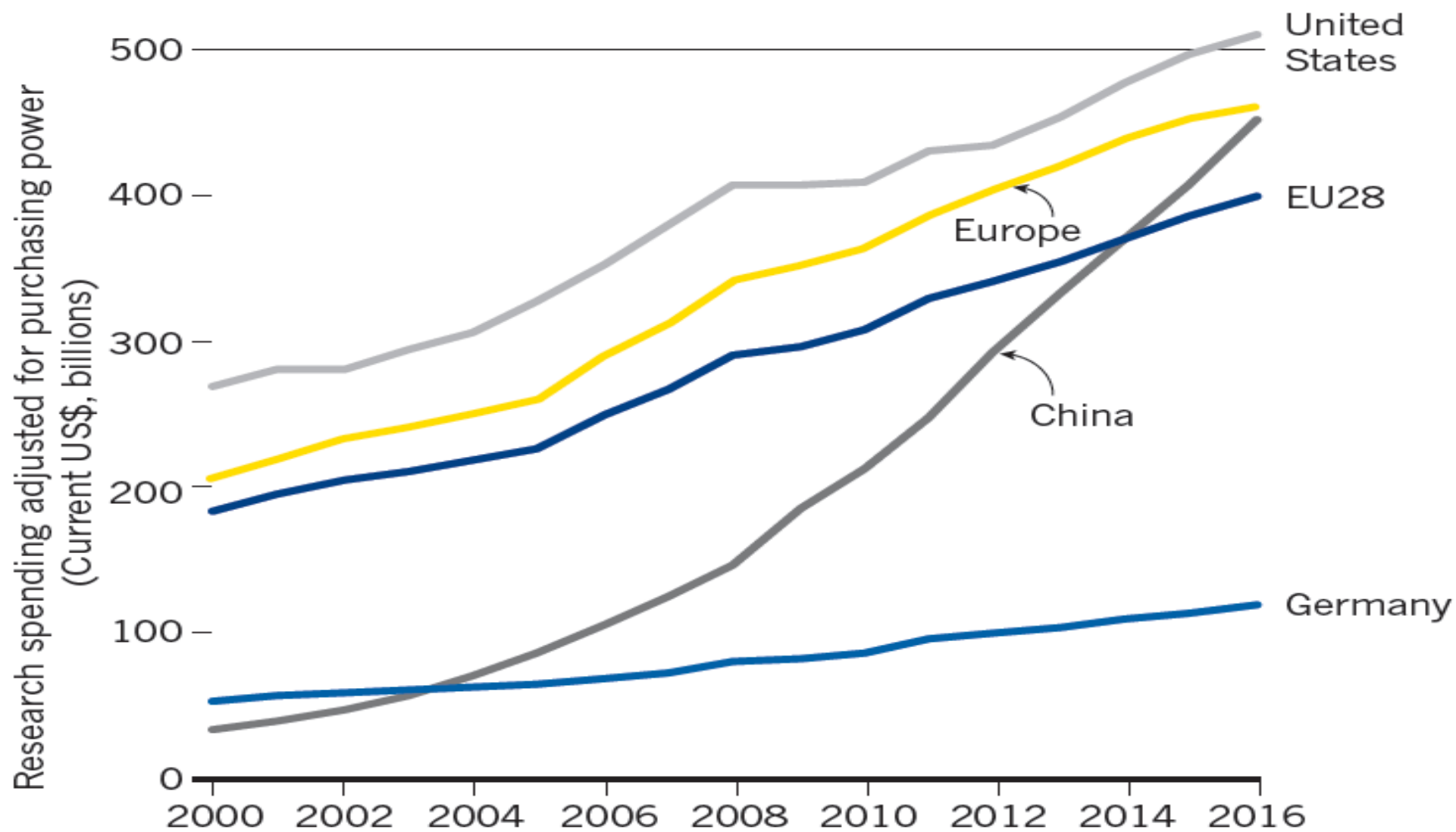
ELBS is Founding
Member of the
**International Liquid
Biopsy Alliance**
coordinated by
Foundation of the
National Institute of
Health (NIH), USA



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* for medical scientists
- Disseminate the knowledge about *liquid biopsy* to the medical community through regular symposia, publications and press releases
- Increase visibility of Europe as leading hub for *liquid biopsy* research
- Outreach to non-EU networks of *liquid biopsy* research (USA, Asia, Australia)

Ausgaben für Wissenschafts- und Entwicklungsprojekte (R&D)



THE FUTURE OF SCIENCE IN EUROPE

23 MAY 2019 | VOL 569 | NATURE | 469



Top 200 healthcare institutions in biomedical sciences:

UKE: No 4 in Europe (No. 2 in Germany, No. 31 world wide)

Publication record with researchers in other countries (2015 to 2018)

UKE: No. 3 world wide

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Immunologie	Klin. Chemie Laboratoriumsmedizin	Med. mikrobiologie, Virologie, Hygiene	Dermatologie und Venerologie	Hals-, Nasen- und Ohrenheilkunde	Allge., Viszeral- und Thoraxchirurgie	Osteologie und Biomechanik
Neuropathologie	Rechtsmedizin	Transfusionsmedizin	Unfall-, Hand- und Wiederherstellungs- chirurgie	Viszerale Transplantations- chirurgie	Knochenmarktrans- plantation (Med II)	Stammzelltrans- plantationschirurgie
Anatomie und Experimentelle Morphologie	Biochemie und Molekulare Zellbiologie	Experimentelle Herz- Kreislaufforschung	Dermatologie und Venerologie	Gynäkologie	Martini-Klinik	Urologie
Experimentelle Pharmakologie und Toxikologie	Medizinische Biometrie und Epidemiologie	Osteologie und Biomechanik	Gastroentero-logie (Med II)	Interdisziplinäre Endoskopie	Onkologie (Med II)	Pneumologie (Med II)
Tumorbiologie	Medizinische Systembiologie	Neuroimmunologie und Multiple Sklerose	Pädiatrische Hämatologie und Onkologie	Neurochirurgie	Neurologie	Neuroradiolo-gische Diagnostik und Intervention
Arbeitsmedizin			Strahlentherapie	Zahnärztliche Prothetik		

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



Funding:

Post-Doc & PhD student positions available at UKE (pantel@uke.de)



EU/IMI, EU TRANSCAN



DFG, BMBF



Deutsche Krebshilfe
(Mildred-Scheel-Nachwuchszentrum)

