Liquid Biopsy – Prostate Cancer

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<table>
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<tr>
<th>Definition:</th>
<th>Liquid Biopsy* - Analysis of tumor cells (CTCs) or their products (e.g., DNA, miRNA, extracellular vesicles) in blood or other body fluids</th>
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<td>Rationale:</td>
<td>Tissue biopsies are invasive and some locations are difficult to access (e.g., lung or brain)</td>
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<td>Single biopsy can miss relevant tumor clones due to intra-patient tumor heterogeneity</td>
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<td>Sequential tissue biopsies in individual patients for real-time monitoring of therapy response are less feasible in clinical practise</td>
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<td>Vision:</td>
<td>Comprehensive and real-time tumor information by the analyses of blood (or other body fluids)</td>
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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 still 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

CTCs: Cristofanilli, Hayes et al., NEJM 2004
ctDNA: Rosenfeld, Caldas et al., NEJM 2013
Clinical Trials - CTC and ctDNA in ClinicalTrial.Gov

Number of Clinical Trials

- **Completed**
  - CTC: ~100
  - ctDNA: ~10

- **Active**
  - CTC: ~350
  - ctDNA: ~150

Legend:
- CTC
- ctDNA
Anoikis resistance
Epithelial-to-mesenchymal transition
Invasion/Intravasation ability (single CTCs and/or clusters)

Liquid phase of tumor progression

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

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

The surrogate discriminates low-risk from high-risk patients

Scher et al, J Clin Oncol 2015
Decline in Circulating Tumor Cell Count and Treatment Outcome in Advanced Prostate Cancer


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

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 determined using Cox regression with CTC response as the categorical variable and stable disease as the reference.
Liquid Biopsy Concept for Metastatic Patients

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

CTC characterization

(DNA, RNA, proteins)

- Therapeutic targets

- Resistance mechanisms
Clinical outcomes of prostate cancer patients starting treatment with abiraterone or enzalutamide according to CTC & ARv7 status

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

PSMA on CTCs as predictive future biomarker?
PSMA-Spiegel nimmt mit Progressions- und Metastasierungsgrad zu

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

Genomic Characterization of single CTC

CTC detection

CTC isolation

WGA +
- Mutation analysis
- CGH (conv./array)
- NextGen Sequencing
Automated individual CTC sorting with DEPArray™

Whole genome amplification & NGS

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

Anna Babayan¹, Malik Alawi¹,², Michael Gormley¹, Volkmar Müller¹, Harriet Wikman¹, Ryan P. McMullin¹, Denis A. Smirnov¹, Weimin Li¹, Maria Geffken¹, Klaus Pantel¹, Simon A. Joosse¹

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³Heinrich-Pette-Institute, Leibniz-Institute for Experimental Virology (HPI), Hamburg, Germany
Cell-free ctDNA/miRNA and exosomes as Blood-Based Biomarkers


Cancer-ID is a project funded by the Innovative Medicines Initiative Joint Undertaking (IMI JU).
Liquid Biopsies, What We Do Not Know (Yet)

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http://dx.doi.org/10.1016/j.ccell.2017.01.002

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

FISH: BRCA1 CEP17

Immunocytochemistry

CK

Vimentin

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 87/107 patients (81%)
5 CTCs in 21.5%
Candidates for adjuvant therapy?

Kuske et al, Nature Scientific Reports, 2016
New approach: *In vivo* capture of CTC

**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. Martin¹,²,⁴, Robin L. Anderson³,⁴, Kailash Narayan¹,⁴,⁵ and Michael P. MacManus¹,⁴
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 radioresistant

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
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
diagnosis
cancer
diagnosis
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
Validation of Liquid Biopsy in Lung and Breast Cancer (CTCs, ctDNA, cfmiRNA & exosomes)

40 EU Partners (Academic institutions, non-profit organisations & companies)
Cancer-ID philosophy

Development of technologies for blood-based companion diagnostics ideally up to proof of clinical utility supporting regulatory approval.
Key Aim: Combined Analysis of CTCs and cfDNA

1. **Patient Blood**
   - Additional purification
   - cfDNA extraction
   - Quantification and QC

2. **Separation of the blood fractions**
   - Plasma fraction
   - MNC fraction
   - Cell Enrichment

3. **Molecular Analysis**
   - (gDNA, cfDNA, and RNA)
   - Identification and quantification of CTCs staining with AB cocktail, fluorescent microscopy
   - Single-Cell Recovery using micromanipulator/DEP-Array

4. **Quality Control (QC)**
Exosomes: Biogenesis, release, structure and uptake

Current challenge: Detection of tumor-derived exosomes

Meng, Pantel, Schwarzenbach et al., Oncotarget 2016: Exosomes in early detection of ovarian cancer
Clinical Applications of Circulating Tumor Cells and Circulating Tumor DNA as Liquid Biopsy

Catherine Alix-Panabères1,2 and Klaus Pantel³

CTCs, ctDNA and exosomes provide complementary information for liquid biopsy
Micrometastasis Research Network at UCCH/UKE

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Klinik und Poliklinik für Neurochirurgie
Klinik und Poliklinik für Urologie/Martiniklinik
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