Institute for Tumor Biology
Klaus Pantel

“Liquid Biopsy”: Current Status and Future Perspective
CTC as Liquid Biopsy for metastatic cells

Metastasis evolve many years after primary tumor resection and can harbor unique genomic alterations.

Biopsy of metastases is an invasive and sometimes dangerous procedure.

Can the molecular characterization of CTCs reveal representative information on metastatic cells located at different sites?

Detection of CTC in the peripheral blood

September 2013:

> 400 registered clinical trials with CTC as biomarkers
2013: > 50 different CTC assays!

The technical challenge:
Finding one tumor cell in $10^6$ – $10^8$ normal blood cells

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

Insertion into patient’s vein at the doctor’s office
30 minutes exposure time in a vein

Decision ← Result ← Diagnostics
- cytology
- PCR, etc.

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

Real-time RT-PCR

Cytokeratins as standard CTC markers
BUT differential expression of individual CKs
(Joosse/Pantel et al., Clin Cancer Res 2012)

> 13,000 publications on CTC in PubMed 2013

Immunocytochemistry

EPISPOT assay

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

nucleic acids

mRNA

DNA

intra-cytoplasmic proteins

secreted proteins by VIABLE cells

Tumor cell
Design of robust automated systems for reproducible CTC detection
CellSearch™ System (FDA-cleared)

Enrichment of CTC with anti-EpCAM ferro fluids

Cristofanilli et al., NEJM, 2004
Riethdorf et al., CCR, 2007 & 2010
DeBono et al, CCR, 2008
Cohen et al, JCO, 2008
Krebs et al, JCO, 2012

CellSave™ Preservative Tube
CellSearch™ Epithelial Cell Kit
MagNest™
CellTracks™ autoprep system
CellTracks® Analyzer II w/ Linux operating system
CellSearch™ System: Images of Tumor Cells

<table>
<thead>
<tr>
<th>Cytoplasm</th>
<th>Nucleus</th>
<th>Cell Membrane</th>
<th>Composite</th>
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</thead>
<tbody>
<tr>
<td>CK-PE pos</td>
<td>DAPI pos</td>
<td>CD45-APC neg</td>
<td>Tumor Cell</td>
</tr>
</tbody>
</table>

Leukocyte nucleus

CD45+ Membrane

Leukocyte

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

Breast Cancer
Christofanilli, NEJM, 2004

Colorectal Cancer
Cohen, JCO, 2008

Prostate Cancer
De Bono, Clin Can Res, 2008

FDA clearance
Detection of CTC in early stage cancer patients (low CTC counts):
Is the ability to release cancer cells into the circulation relevant for the development of distant metastases?
### Multivariate Analysis for DFS for different CTC cut-offs

<table>
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<tr>
<th>Variable</th>
<th>Hazard Ratio adjusted for treatment</th>
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<tbody>
<tr>
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<td>0 vs. ≥ 1</td>
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<tr>
<td>CTCs in blood pos/neg</td>
<td>1.878 *</td>
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<tr>
<td>Hormone receptor status pos/neg</td>
<td>2.073 *</td>
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<tr>
<td>Lymph Node Involvement pos/neg</td>
<td>1.698 *</td>
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<tr>
<td>Grading</td>
<td>2.961 *</td>
</tr>
<tr>
<td>G1 vs. G2-3</td>
<td>1.629 *</td>
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<tr>
<td>Tumor size</td>
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<tr>
<td>T1 vs. T2-4</td>
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</table>

* *P < 0.05

Rack, Janni et al, unpublished
Meta-Analysis of 49 studies comprising 6815 breast cancer patients

Progression-free survival

CTC detection: ICC & RT-PCR

Overall survival

TNM 2010: CTC in new cM0(i+) Classification

### Distant Metastases (M)

- **M0**: No clinical or radiographic evidence of distant metastases.
- **cM0(i+)**: No clinical or radiographic evidence of distant metastases, but deposits of molecularly or microscopically detected tumor cells in circulating blood, bone marrow, or other nonregional nodal tissue that are no larger than 0.2 mm in a patient without symptoms or signs of metastases.
- **M1**: Distant detectable metastases as determined by classic clinical and radiographic means and/or histologically proven larger than 0.2 mm.
Challenge of CTC detection:
Epithelial-Mesenchymal Transition (EMT) of carcinoma cells
Tumor cell dissemination, plasticity and EMT
(Bednarz-Knoll et al CMR 2012; Kang & Pantel, Cancer Cell 2013)

Dormancy > 10 years

Early diagnostic of progression: CTC isolation

Late diagnostics of progression: standard imaging methods

Early diagnostic of relapse: CTC isolation
Epithelial-Mesenchymal Plasticity of CTC

Epithelial-Mesenchymal transition

Epithelial phenotype

EpCAM, CK

Mesenchymal-Epithelial transition

Vimentin

<table>
<thead>
<tr>
<th>Epithelial phenotype</th>
<th>Epithelial phenotype with minor mesenchymal features</th>
<th>Semi-mesenchymal phenotype</th>
<th>Mesenchymal phenotype</th>
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<tbody>
<tr>
<td>Epithelial markers strongly expressed</td>
<td>Epithelial markers moderately expressed</td>
<td>Epithelial markers weakly expressed</td>
<td>No epithelial markers</td>
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<tr>
<td>No mesenchymal markers</td>
<td>Mesenchymal markers weakly expressed</td>
<td>Mesenchymal markers moderately expressed</td>
<td>Mesenchymal markers strongly expressed</td>
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<tr>
<td>Detection by standard CTC technology</td>
<td>Detection by standard CTC technology</td>
<td>Limited detection by standard CTC technology</td>
<td>No detection by standard CTC technology</td>
</tr>
</tbody>
</table>

Bednarz-Knoll, Alix-Panabières & Pantel Cancer & Met Rev 2012
Expression profile of CTCs in breast cancer

Direct link between EMT and gain of stem cell properties and chemotherapy resistance (Mani/Weinberg, et al., Cell, 2008;)

Yu et al, Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. Science, Febr. 2013

Yokobori, Mimori, Pantel, Mori et al. Plastin-3 as new CTC marker not downregulated during EMT, Cancer Res. Febr. 2013

Molecular Characterization of CTC for therapeutic targets

(„real-time liquid biopsy“)
Detection of therapeutic targets on CTC: HER2 oncogene in breast cancer

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

<table>
<thead>
<tr>
<th>B</th>
<th>Composite</th>
<th>CK</th>
<th>DAPI</th>
<th>CD45</th>
<th>HER2</th>
<th>C</th>
<th>CB11</th>
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Discordance between HER2 status of primary tumor and CTC

Riethdorf/Pantel et al., *Clinical Cancer Res* 2010 - Fehm/Pantel et al., *Breast Cancer Res Treat* 2010
Heterogeneity of ER status in CTCs of breast cancer patients with ER-positive primary tumors

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

ER-negative CTCs may survive endocrine therapy
Genomic Characterization of single CTC

CTC detection

CTC isolation

WGA +
- Mutation analysis
- CGH (conv./array)
- NextGen Sequencing
### Detection of mutations in genes relevant for resistance of targeted therapies (eg, EGFR inhibition)

<table>
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<tr>
<th>Patient</th>
<th>No. Of CTCs</th>
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<th>KRAS</th>
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<td>81</td>
<td>8</td>
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<td>68</td>
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</table>

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

Heitzer, Riethdorf, Pantel, Speicher et al, Cancer Res. 2013
Tumor-associated circulating cell-free nucleic acids in blood

Correlation CTC & Circulating Tumor DNA:

Correlation CTC & Circulating microRNA:

BUT: ctDNA is released from apoptotic/necrotic cells

Aims of Research on Circulating Tumor Cells

• Estimation of the risk for metastatic relapse or metastatic progression (prognostic information)

• Stratification & real-time monitoring of therapies

• Identification of therapeutic targets and resistance mechanisms (biological therapies)

• Understanding the biology of metastatic development
Metastasis Models

early dissemination

early stage cancer

advanced stage cancer

DTCs

genetic progression

± Dormancy

no metastasis

overt metastasis

DTCs

overt metastasis

Pantel et al., Nature Rev Cancer 2008
Cancer Dormancy: Research questions

- Do all cancer patients have dormant tumor cells?
- Can host factors induce or break dormancy? Stress? Inflammation?
- Are there preferred reservoirs of dormant cells (e.g., bone marrow)?
- Does the immune system play a role in dormancy?
- What is the effect of current therapies on dormant cells or dormancy?
- What signaling pathways or events reactivate dormant cells?
- Do dormant cells have properties of cancer stem cells?
- How does genetic background affect dormancy?

Uhr & Pantel PNAS 2011; Kang & Pantel, Cancer Cell 2013
Metastasis Biology

Tumor Cell Dissemination: Emerging Biological Insights from Animal Models and Cancer Patients

Yibin Kang* and Klaus Pantele...
Grant Support:

- Sabine Riethdorf/Christin Gasch
- Heidi Schwarzenbach
- Harriet Wikman/Michaela Wrange
- Katharina Effenberger
- Simon Joosse, Anna Babayan
- Kai Bartkowiak, Natalia Bednarz-Koll

DFG
BMBF
EU / ERC
Dt. Krebshilfe
Sander-Stiftung
Roggenbuck-Stiftung
Micrometastasis Research Network at UCCH/UKE

Institut für Anatomie II
Institut für Klinische Chemie
Institut für Rechtsmedizin
Labor für Strahlenbiologie und -onkologie
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I. Medizinische Klinik und Poliklinik
Klinik und Poliklinik für Neurochirurgie
Klinik und Poliklinik für Mund-, Kiefer- und Gesichts-chirurgie
Klinik und Poliklinik für Urologie
EU-Consortium-DISMAL

Start: November 2005    Coordinator: Klaus Pantel

Free University of Amsterdam Medical Center (The Netherlands)

University Medical Center Hamburg-Eppendorf (Germany)

Imperial College London (United Kingdom), Radium Hospital Oslo, (Norway)

German Cancer Research Center, (Germany)

SME 1 Applied Imaging, (United Kingdom)

University of Graz (Austria)

University of Utrecht (The Netherlands)

Lapeyronie Hospital, Montpellier, (France)

SME 2 TILL Photonics (Germany)

SME 3 Agendia, (The Netherlands)

Leiden University Medical Center, (The Netherlands),

ERC Advanced Investigator Grant „DISSECT“ (2011-2016)

9th International Symposium on Minimal Residual Cancer
September 24-27, 2013
Pullman Paris Bercy, France

Organizers
Jean-Yves Pierga
MD, PhD, Institut Curie
Paris Descartes University, France

Catherine Alix-Panabières
Ph.D, University Medical Centre Montpellier,
UM1, Montpellier, France

Klaus Pantel
MD, PhD, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany

www.ismrc2013.com