THE ROLE OF CIRCULATING TUMOUR CELLS (CTCS) IN CANCER MANAGEMENT

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Chairman, Institute for Tumour Biology
AIMS OF RESEARCH ON CTCS & CTDNA

- Screening & early detection of cancer
- 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
TUMOUR CELL DISSEMINATION AND CANCER DORMANCY

- Escape from dormancy (bone marrow):
  - VCAM1 promotes osteoclast differentiation & activation & attracts osteoclast progenitors (Lu/Pantel/Kang et al. Cancer Cell 2011)
  - Tumour-induced osteoclast miRNA changes as regulators and biomarkers of osteolytic bone metastasis (Ell/Pantel/Kang et al., Cancer Cell 2013)
  - Metabolic adaptation of DTCs is important for survival (LeBleu, Pantel, Kalluri et al., Nature Cell Biol. 2014)

Dormancy
> 10 years

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

Biopsy of metastases is an invasive and sometimes dangerous procedure.

The technical challenge:
Finding one tumour cell in $10^6 – 10^8$ normal blood cells from different sites.
CTC ENRICHMENT STRATEGIES

Biological properties
Protein expression

**Positive selection**
- anti-E markers Ab (E.g., EpCAM)
- anti-M markers Ab (E.g., N-Cadherin)
- anti-E/M markers Ab

**Ex vivo**
- CellSearch® system
- MagSweeper™
- EPHESSIA CTC-chip
- CTC-chip
- Velcro-like device

**In vivo**
- CellCollector®
- Photoacoustic nanodetector

**Negative selection**
- Anti-CD45

Physical properties
Label-free strategies

**Label-free strategies**
- CTC-iChip

**CTC ENRICHMENT STRATEGIES**
Images courtesy of Universitätsklinikum Hamburg Eppendorf
**APPRAOCHES FOR CTC DETECTION**

**Immunocytological technologies**
- Anti-E/M markers Ab
  (E.g., CK, Vimentin, E/N-Cadherin)
- Anti-tissue-specific markers Ab
  (E.g., PSA, Mammaglobin, MAGE)
- Anti-tumour associated markers Ab
  (E.g., HER2, EGFR)

**Molecular technologies**
- RT-qPCR
  (single/multiple genes)

**Functional assays**
- EPISPOT
  (E.g., CK19, HER2, EGFR, VEGF, PSA)
- Invasion assay

**In vitro Cell Culture**
- Cell culture
- Secreted protein
  - Ab1
  - Ab2
- Immunospots

**RNA-based Technologies**

**Xenotransplantation models (CDx)**
- Viable CTC with stem-cell properties
  - Immunospots
  - Metastases

Images courtesy of Universitätsklinikum Hamburg Eppendorf
CELLSEARCH™ SYSTEM (FDA-CLEARED)

Enrichment of CTC with anti-EpCAM ferro fluids: Captures tumour cells with very low EpCAM expression
CELLSEARCH™ SYSTEM: IMAGES OF TUMOUR CELLS

Cytoplasm     Nucleus     Cell Membrane Composite

CK-PE pos | DAPI pos | CD45-APC neg | Tumour cell

Images courtesy of Universitätsklinikum Hamburg Eppendorf
PROGNOSTIC VALUE OF CTC COUNTS FOR SURVIVAL
In cancer patients with advanced disease

Breast Cancer
Christofanilli, NEJM, 2004

No. at Risk
<5 CTC 90 90 88 87 85 80 80 77 67 59 50 40 40 34 28 24 18 9 2 2 1 0
≥5 CTC 87 63 73 68 62 57 52 49 40 33 24 18 9 2 2 1 0

Probability of Overall Survival (%)
Weeks from Baseline
<3 CTC
0 10 20 30 40 50 60 70 80 90 100
0 5 10 15 20 25 30 35 40 45 50

Colorectal Cancer
Cohen, JCO, 2008

No. of patients at risk
<3 CTC 595 266 276 252 227 180 154 157 126 70 23
≥3 CTC 102 68 66 69 36 24 13 12 7 4 1

Probability of Overall Survival (%)
Time From Baseline Blood Draw (months)
<3 CTC
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30

Prostate Cancer
De Bono, Clin Can Res, 2008

No. of patients at risk
<3 CTC 95 82 79 77 77 71 58 47 35 23 7 2 0 0 0 0
≥3 CTC 125 110 100 82 80 71 59 47 35 23 11 8 4 1 0 0

Probability of Survival
Time from Baseline Blood Draw (Months)
<3 CTC
0 20 40 60 80 100

3) Reprinted from Clin Can Res, 2008, 14(19):6302-9, de Bono JS, et al., Circulating Tumor Cells Predict Survival Benefit from Treatment in Metastatic Castration-Resistant Prostate Cancer, with permission from AACR.

→ FDA approval
CTCS BEFORE INITIATION OF THERAPY IN 7.5 ML OF BLOOD – FDA-APPROVAL

Reprinted from Clin Can Res, 2008;14(19):6302-9, de Bono JS, et al., Circulating Tumor Cells Predict Survival Benefit from Treatment in Metastatic Castration-Resistant Prostate Cancer, with permission from AACR.
PROGNOSTIC VALUE OF CTC COUNTS FOR SURVIVAL
In cancer patients with advanced disease

Published Ahead of Print on March 10, 2014 as 10.1200/JCO.2013.51.7417
The latest version is at http://jco.ascopubs.org/cgi/doi/10.1200/JCO.2013.51.7417

Circulating Tumor Cell Counts Are Prognostic of Overall Survival in SWOG S0421: A Phase III Trial of Docetaxel With or Without Atrasentan for Metastatic Castration-Resistant Prostate Cancer
Rising CTC at 3 weeks associated with worse OS – helpful for redirection and optimization of therapy
CLINICAL RELEVANCE OF CTCs

Can changes in CTC counts predict the efficacy of therapeutic interventions (e.g., chemotherapy, hormonal therapy)?
17 CENTRES PROVIDED DATA FOR 1944 ELIGIBLE PATIENTS

From 20 studies: Meta-analysis on raw data

Clinical validity of circulating tumour cells in patients with metastatic breast cancer: a pooled analysis of individual patient data
### CTCS VS. CONVENTIONAL TUMOUR MARKERS

(Progression-free survival, p values) in metastatic breast cancer patients receiving chemotherapy

<table>
<thead>
<tr>
<th>Model used as reference</th>
<th>Baseline</th>
<th>3-5 weeks</th>
<th>6-8 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CTCBL</td>
<td>CA15-3BL</td>
<td>CEABL</td>
</tr>
<tr>
<td>N patients</td>
<td>1193</td>
<td>914</td>
<td>593</td>
</tr>
<tr>
<td>CP</td>
<td>6 E-10</td>
<td>.10</td>
<td>.04</td>
</tr>
<tr>
<td>CP + CTCBL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP + CTCBL + CTC3-5</td>
<td>.26</td>
<td>.41</td>
<td></td>
</tr>
<tr>
<td>CP + CTCBL + CTC6-8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IMPACT OF CTCS & LDH LEVEL ON SURVIVAL

In prostate cancer patients treated with abiraterone

The surrogate discriminates low-risk from high-risk patients

Reprinted with permission © 2015 American Society of Clinical Oncology. All rights reserved. Scher HI, et al., J Clin Oncol 2015; 33(12):1348-55
CTCS IN EARLY STAGE CANCER PATIENTS CHALLENGE

Very low number of CTCs
# Prognostic Impact of CTC in Breast Cancer Patients Without Overt Metastases

## Multivariate Analysis for DFS for Different CTC Cut-offs

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR Adjusted for Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 vs ≥ 1</td>
</tr>
<tr>
<td>CTCs in blood</td>
<td></td>
</tr>
<tr>
<td>Pos/neg</td>
<td>1.878*</td>
</tr>
<tr>
<td>Hormone receptor status</td>
<td></td>
</tr>
<tr>
<td>Pos/neg</td>
<td>2.073*</td>
</tr>
<tr>
<td>Lymph node status</td>
<td></td>
</tr>
<tr>
<td>Pos/neg</td>
<td>1.698*</td>
</tr>
<tr>
<td>Grading</td>
<td></td>
</tr>
<tr>
<td>G1 vs G2–3</td>
<td>2.961*</td>
</tr>
<tr>
<td>Tumour size</td>
<td></td>
</tr>
<tr>
<td>T1 vsT2–4</td>
<td>1.629*</td>
</tr>
</tbody>
</table>

*p<0.05

PROGNOSTIC VALUE OF CTC IN URINARY BLADDER CANCER

Survival outcomes: Independent prognostic factor

Median follow-up: 18 months

DFS HR: 4.6

CSS HR: 5.2

ERA-NET TRANSCAN: CTC-SCAN PROJECT

High-risk Prostate Cancer (stage M0)
Partners: Germany, France, Greece, Poland, Austria

Coordinator: Klaus Pantel, Hamburg
NEW APPROACH: IN VIVO CAPTURE OF CTC

Proof of principle data in breast, prostate, colon and lung cancer

1. Image courtesy of GILUPI Nanomedizin GmbH. Available at: http://www.gilupi.com/
### Distant Metastases (M)

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>No clinical or radiographic evidence of distant metastases</td>
</tr>
<tr>
<td>cM0(i+)</td>
<td>No clinical or radiographic evidence of distant metastases, but deposits of molecularly or microscopically detected tumour 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</td>
</tr>
<tr>
<td>M1</td>
<td>Distant detectable metastases as determined by classic clinical and radiographic means and/or histologically proven larger than 0.2 mm</td>
</tr>
</tbody>
</table>
HAEMATOGENEOUS SPREAD OF PRIMARY BRAIN TUMOURS:
Detection of CTCs in glioma patients (~20%)

Translational relevance
Glioma patients with CTCs may not be used as transplant donors
CTCs may serve as liquid biopsy

GFAP stain
EGFR amplification

Single Cell CGH

From Müller C, et al., The role of circulating tumor cells (CTCs) in cancer management. Sci transl Med; 2014, 6:247ra101. Reprinted with permission from AAAS.
MOLECULAR CHARACTERISATION OF CTC

Therapeutic targets
Resistance mechanisms
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 tumours and HER2-positive CTC

Reprinted from Clinical Cancer Res. Copyright 2010, 16(9): 2634-2645, Riethdorf S, et al., Detection and HER2 Expression of Circulating Tumor Cells: Prospective Monitoring in Breast Cancer Patients Treated in the Neoadjuvant GeparQuattro Trial, with permission from AACR.
HETEROGENEITY OF ER STATUS IN CTCS OF BREAST CANCER PATIENTS

With ER-positive primary tumours

ER-negative CTCs may survive endocrine therapy

Babayan A, Hannemann J, Spötter J, Müller V, Pantel K, Joosse SA (2013). Heterogeneity of Estrogen Receptor Expression in Circulating Tumor Cells from Metastatic Breast Cancer Patients. PLoS ONE 8(9): e75038. © 2013 Babayan et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License.
GENOMIC CHARACTERISATION OF SINGLE CTC

CTC detection

CTC isolation

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

Images courtesy of Prof Klaus Pantel and from Riethdorf S, et al., Clin Cancer Res 2007;13:920–8
GENOMIC PROFILES (CNVS) OF ER+ AND ER- CTCS
In breast cancer patients determined by NGS

Images courtesy of Prof Klaus Pantel.
AR-V7 IN CTCS OF METASTATIC PROSTATE CANCER PATIENTS

Association with resistance to enzalutamide and abiraterone

AR-V7 IN CTCS OF METASTATIC PROSTATE CANCER PATIENTS

Association with resistance to enzalutamide and abiraterone

Deep targeted sequencing revealed that 17 of 20 “private CTC mutations” were also present in subclones of the primary tumour and metastases

Reprinted from Cancer Research. Copyright 2013, 73(10): 2965-75, Heitzer E, et al., Complex Tumor Genomes Inferred from Single Circulating Tumor Cells by Array-CGH and Next-Generation Sequencing, with permission from AACR.
FUNCTIONAL STUDIES ON CTCS
FUNCTIONAL ANALYSES OF CTCS IN XENOGRRAFT ASSAYS AND CELL LINES

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 Scharpf, Frederik Marmé, Hans Peter Sinn, Klaus Pantel, Wilko Weichert & Andreas Trumpp
NUCLEIC ACIDS (DNA, RNA) AS BLOOD-BASED BIOMARKERS
In cancer patients
Release by dying cells
Active secretion by viable cells

COMPARISON OF PLASMA DNA CONCENTRATIONS IN PATIENTS
With localised M0 (n=69) and M1 (n=12)

Reprinted from Clinical Cancer Research. Copyright 2009, 15(3), 1032-1038, Schwarzenbach H, Cell-free Tumor DNA in Blood Plasma As a Marker for Circulating Tumor Cells in Prostate Cancer, with permission from AACR.
TMPRSS-ERG-ASSOCIATED 3 MB DELETION ON CHROMOSOME 21

And mapping of the breakpoint on ctDNA in prostate cancer

Heitzer E, et al., Genome Medicine 2013;5:30. This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 2.0) (http://creativecommons.org/licenses/by/2.0)
How can the analysis of DNA fragments released from apoptotic/necrotic cells reveal important information on resistant tumour cells surviving therapy?

COMPARATIVE ANALYSIS OF CTCs AND ctDNA IN BREAST CANCER

1. Progressive disease with increasing liver metastases and ascites – no chemoT

2. Excessive numbers of CTCs (~50,000/7.5 ml) in three blood samples; each with multiple homogeneous copy number changes and mutations in CTCs

3. However, very low concentration of ctDNA fragments at each measurement

ctDNA levels may not always reflect disease progression in cancer patients

CTCs analyses are not restricted to dying cancer cells and provide complementary information

Heidary M, et al., Breast Cancer Research 2014;16:421. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0) (http://creativecommons.org/licenses/by/4.0)
Therapy sensitive tumour cells: undergo apoptosis and release ctDNA

Therapy resistant tumour cells: do not undergo apoptosis and can disseminate through the blood

Top: Tumour consists of heterogeneous clones that are sensitive or resistant to cytotoxic therapies. Bottom: Cytotoxic therapies kill sensitive tumour cells, leading to the release of ctDNA from these dying cells into the circulation, while CTCs are derived from resistant clones.
<table>
<thead>
<tr>
<th>Status</th>
<th>Pre-neoplasm Subclinical</th>
<th>Primary (-) CTCs and/or DTCs</th>
<th>Primary (+) CTCs and/or DTCs</th>
<th>Dormancy</th>
<th>Oligometastases</th>
<th>Systemic metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focus</td>
<td>Management of primary tumor</td>
<td>Prevention of metastasis</td>
<td>Treatment of metastasis</td>
<td></td>
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</tr>
<tr>
<td>Challenge</td>
<td>Early detection and prevention Identify high-risk patients</td>
<td>Prevent local and distant relapse Drug resistance of DTCs</td>
<td>Early detection of relapse Heterogeneity and drug resistance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New tools</td>
<td>Diagnostic markers</td>
<td>Prognostic markers</td>
<td>Profiling of primary tumor, metastases, CTCs and/or DTCs for accurate targeting Biomarkers and imaging technologies for disease monitoring Biomarkers for therapeutic efficacy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Possible treatment strategies</td>
<td>Prophylactic treatment Vaccination</td>
<td>Surgery, radiotherapy (+) Systemic therapy</td>
<td>Targeted therapy against driver oncogenes and their pathways tailored by genetic makeup of tumor cells</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Long-term adjuvant treatment (for high-risk patients): Metronomic chemotherapy and anti-angiogenesis Targeting common driver oncogenes and pathways Immunotherapy Targeting dormancy-related survival and CSC signaling and niche components</td>
<td>Systemic therapy Immunotherapy Stromal-targeting treatments Palliative radiation and/or surgery</td>
<td>Surgery stereotactic radiotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Possible new targets</td>
<td>DTC and/or CTC survival pathways; stem cell features; tumor-stroma crosstalk and niche factors Activation of metastasis-suppressive signaling</td>
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</table>
CTCs and ctDNA provide complementary information as liquid biopsy.
CENTER OF EXPERIMENTAL MEDICINE INSTITUTE OF TUMOUR BIOLOGY - KLAUS PANTEL

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