DEPARTMENT OF LABORATORY DIAGNOSTICS, FNKV, PRAGUE

presents

CIRCULATING TUMOR CELLS IN SOLID TUMORS
Liquid biopsy

- Liquid biopsy – a noninvasive blood test detecting circulating tumor cells (CTCs) and tumor DNA fragments (cfDNA - ctDNA) shed to the circulation from a primary tumor or metastasis

**Will the promise of liquid biopsies be a clinical reality?** It is hard for me to not to be excited about the benefits they can offer to patients...

Giuseppe Curigliano
**Affiliation:** European Institute of Oncology, Milan, Italy
Circulating Tumor Cells (CTCs)

- Diagnostic
- Prognostic
- Predictive
- Therapy monitoring
Circulating Tumor Cells (CTCs)

- Cost-effective way to tailor the therapy
- Increased therapy efficiency
- Limited drug side-effects
- New druggable biomarkers detection
- CTCs = immediate real-time information on tumor development
What is the problem now?
CTC clinical studies

ClinicalTrials.gov
A service of the U.S. National Institutes of Health

Find Studies  About Clinical Studies  Submit Studies  Resources  About This Site

Home > Find Studies > Search Results

868 studies found for: circulating tumor cells

Found 868 studies with search of: circulating tumor cells

Recognized Terms and Synonyms:
circulating tumor cells: 405 studies
Scientific Rationale – Update June 2016
The NCCN Clinical Practice Guidelines on Breast Cancer (Version 2.2016) notes: “The clinical use of circulating tumor cells (CTC) in metastatic breast cancer is not yet included in these guidelines for disease assessment and monitoring. Patients with persistently increased CTC after 3 weeks of first-line chemotherapy have a poor progression free survival (PFS) and overall survival (OS). In spite of its prognostic ability, CTC count has failed to show a predictive value.”
Why predictive value of CTCs could not be shown so far?
• MetaCell® platform = making CTC separation simple
• getting viable CTCs to propagate – no sample amount limitation for downstream application !!!!!
MetaCell® platform

....a tube size-based exclusion CTC
1. CYTOMORPHOLOGY

A. Without cultivation
B. After in vitro culture

2. GENE expression testing

A. TUMOR ASSOCIATED GENES
B. CHEMOREZISTANCE ASSOC.GENES
CTC identification process

Viable Nuclear Stain (NucBlue™) + vital cytoplasm stain

Cytomorphological evaluation (Live)

DNA Mutational analysis
Methylation analysis

RNA Multimarker Gene expression profiling

High variability and multiple marker combinations

Proliferation
Plasticity
Circulating tumor cell – a microscopic view of a captured cells on a separation membrane

Cell size > 20 μm

Nuclear size > 10 μm

Nucleoli

Nucleolus size

Morphology of a nuclear membrane

Pores in separation membrane

8μm

Separation membrane
Captured cells on a separation membrane –

**CYTOPATHOLOGY CRITERIA (vital fl.stain)**

1. Cell size
2. Nuclear size
3. Prominent nucleoli
4. Cells making 2D sheets - epithelial like
5. Irregular nuclear membrane
6. Nuclear / cytoplasmatic ratio
7. Forming of 3D sheets
8. Invading the membrane
9. Proliferation
Cell size > 20 μm

Nuclear size > 10 μm

Prominent nucleoli
How to standardize the testing outcomes?

Blood withdrawal (EDTA) → CTC isolation → Automatization of IHC analysis (Ventana) = Standardized outcomes of cytopathological testing for all tumor types
Clear cell carcinoma CTC
CTCs – ovarian cancer (BRCA 1- mutated)
CTC – *in vitro* culture of ovarian cancer
1. CYTOMORPHOLOGY

A. Without cultivation
B. After in vitro culture

2. GENE expression testing

A. TUMOR ASSOCIATED GENES
B. CHEMOREZISTANCE ASSOC.GENES
C. RNA in vivo detection
Gene expression analysis

Gene expression analysis of CTC-enriched fraction based on qPCR

<table>
<thead>
<tr>
<th>Tumor as genes</th>
<th>T</th>
<th>↑</th>
<th>Tumor as genes</th>
<th>T</th>
<th>↑</th>
<th>Chemoresistance</th>
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T: Genes tested by qPCR

↑: Genes with relatively elevated gene expression tested by qPCR

!: Consider therapy change

RNA is isolated
Gene expression analysis - Dx.

- Whole blood
- CTC fraction - after separation process
- CTC fraction after separation and in vitro culture process
- Peripheral blood
- Tumor blood
- Primary tumor / Metastases / DTC

Positive control of tumor associated genes expression
Fig. Cluster analysis of gene expression data for all CTC- membrane fractions (PK- SK) is clearly identifying CTC- positive and CTC negative group of patients with ovarian cancer (higher expression is shown in red). (Published data)
RNA in vivo detection - SmartFlare™
(an alternative to qPCR testing)
Import via endocytosis
Export via exosomes
RNA - EpCAM in vivo / CTCs isolated from a patient with CRC
RNA - EpCAM in vivo / CTCs isolated from a patient with CRC
1. Do CTCs reflect tumor heterogeneity? 

2. Chemoresistance testing on the CTC-level?
Do CTCs reflect tumor heterogeneity?
Does the CTC-count matter?

Clinical studies (based on FDA-approved Cellsearch protocol) →
CTC-count matters!!!

Patients with elevated CTC-numbers are doing worse (OS, DFS)
Could the treatment be predicted by CTC - count?
CTC- character matters ...
What if only EpCAM + cells are captured ... ??
What if only some cell populations are eradicated by administered chemotherapy ...?
What if only some cell populations are eradicated by administered chemotherapy ... ?
What cells do survive the treatment?
They may change and give the origin to new dangerous populations..
Specification of chemoresistance on the CTC level

Multi-drug resistance (MDR) pumps

Multi-drug resistance (MDR) - modulators

Anticancer drugs

The modulation of ABC transporter-mediated multidrug resistance in cancer: A review of the past decade
Rishil J. Kathawala, Pranav Gupta, Charles R. Ashby Jr.*, Zhe-Sheng Chen*
Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, St. John’s University, Queens, NY, USA
STUDY 2014-2016: Breast cancer treatment response monitoring in neoadjuvant setting by means of CTCs molecular analysis.

Oncology clinic, VFN, Prague
Dept. Laboratory diagnostics, FNKV, Prague

Dr. Zuzana Bielčíková
Ing. Anna Jakabová
NEOADJUVANT CHEMOTHERAPY  SURGERY  ADJUVANT TR.

ANTHRACYCLINES  ANTHRACYCLINES

CTC test  CTC test  CTC test  CTC test

CTC CHEMORESISTANCE  CTC CHEMORESISTANCE  CTC CHEMORESISTANCE

MPR1  MPR1  MPR2  MPR1  MPR2

CTC amount  Time
NEOADJUVAT CHEMOTHERAPY | SURGERY | ADJUVANT TR.

ANTHRACYCLINES | TAXANES

CTC test | CTC test | CTC test | CTC test

CTC CHEMORESISTANCE | CTC CHEMORESISTANCE

MPR1 | MPR1 | MPR2
What if the chemosensitivity test is done before chemotherapy start?
BC NEO- STUDY

- 20 BC patients / undergoing neoadjuvant treatment were tested within the study protocol
- 7/20 BC patients were having triple negative histology
- Several blood withdrawals were taken and analyzed (min. 4)
- The clinical response has been monitored in parallel with CTC examinations
- The patients were treated according the standard guidelines
## CTC - character matters ...

<table>
<thead>
<tr>
<th>Date</th>
<th>Timing of CTC test</th>
<th>MRP1</th>
<th>MRP2</th>
<th>MRP4</th>
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Relative gene expression elevation of the genes associated with chemoresistance in CTC enriched samples

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<th>Triple negative</th>
<th>Others</th>
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<td>MRP5</td>
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Triple negative

Others
STUDY 2013-2016: CTC in GIT tumors

Dept. Laboratory Diagnostics, FNKV, Prague

Ing. Michael Pinkas
Elevated gene expression of tumor associated genes in CTC – fractions in CRC patients.
Elevated genes expression of chemoresistance associated genes in CTC – fractions in CRC patients.
Timing of CTC – testing during oncological treatment

- Confirmation for tumors of unknown origin based on CTC qPCR / Immunohistochemistry
- Additional diag. Information

- CTC before surgery – prognostic information
- CTC after surgery – predictive information for ongoing oncological treatment
- STAGING ?
- Mutational analysis based on DNA from CTC for targeted therapy (eg. KRAS, HER2)

- Treatment monitoring
- Early relapses detection
- Chemoresistance testing during applied therapy (adjuvant, neoadjuvant, palliative)

- Identification of new target molecules on separated CTC

Wanted information CTC presence or and / CTC chemoresistance
Acknowledgement

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