GENOSTICS IN COLLABORATION WITH MAINTRAC PRESENT

MONITORING YOUR CANCER PATIENT CTC'S

WHEN, WHAT AND WHY?

● LIVE
WEBINAR

Prof. Katharina Pachmann

Director of Science

Bayreuth, Germany

Dr. Joachim Fluhrer

Medical Practitioner

Sydney, Australia

TUESDAY 19TH

OCT

6PM
(AEST)

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CETC/CTCs as a monitoring tool

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When, What and Why ?

Liquid biopsy on **vital** circulating tumor cells

Monitoring your cancer patient

- Tumour heterogeneity that enables malignant progression by evolutionary selection is a major challenge
- Tumour heterogeneity is also a major cause of emerging resistance during cancer treatment
- Circulating markers and especially circulating tumour cells (CETC/CTCs) give us an insight into these issues
- Disease progression and increased risk of disease recurrence is at the heart of early diagnosis and treatment
  - To improve Quality of Life
  - To improve Overall Outcome
  - To improve cost effectiveness of treatment

Review Article

Liquid biopsy for cancer screening, patient stratification and monitoring

Graham Brock, Elena Castellanos-Rizaldos, Lan Hu, Christine Coticchia, Johan Skog

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Abstract: Molecular characterization of a patient's tumor to guide treatment decisions is increasingly being applied in clinical care and can have a significant impact on disease outcome. These molecular analyses, including mutation characterization, are typically performed on tissue acquired through a biopsy at diagnosis. However, tumors are highly heterogeneous and sampling in its entirety is challenging. Furthermore, tumors evolve over time and can alter their molecular genotype, making clinical decisions based on historical biopsy data suboptimal. Personalized medicine for cancer patients aims to tailor the best treatment options for the individual at diagnosis and during treatment. To fully enable personalized medicine it is desirable to have an easily accessible, minimally invasive way to determine and follow the molecular makeup of a patient's tumor longitudinally. One such approach is through a liquid biopsy, where the genetic makeup of the tumor can be assessed through a biofluid sample. Liquid biopsies have the potential to help clinicians screen for disease, stratify patients to the best treatment and monitor treatment response and resistance mechanisms in the tumor. A liquid biopsy can be used for molecular characterization of the tumor and its non-invasive nature allows repeat sampling to monitor genetic changes over time without the need for a tissue biopsy. This review will summarize three approaches in the liquid biopsy field: circulating tumor cells (CTCs), cell free DNA (cfDNA) and exosomes. We also outline some of the analytical challenges encountered using liquid biopsy techniques to detect rare mutations in a background of wild-type sequences.

Keywords: Liquid biopsy; exosome; circulating tumor cell (CTC); cell free DNA (cfDNA); nucleic acids

Circulating "Biomarkers"

- **Whole Cells**
- Cell Fragments
- Circulating Tumour DNA
- Free DNA
- RNA, various
- Proteins
- Exosomes
- Immune Markers
- Lipids
- Specific Cancer Markers

The Role of Circulating Biomarkers in the Early Diagnosis of Ovarian Cancer
By Ece Gumusoglu and Tuba Gunel 2018 DOI: 10.5772/intechopen.75484

Friend, Stiebing. Profiling Circulating Tumor Cells and cfDNA in….
British JoCancer 2021

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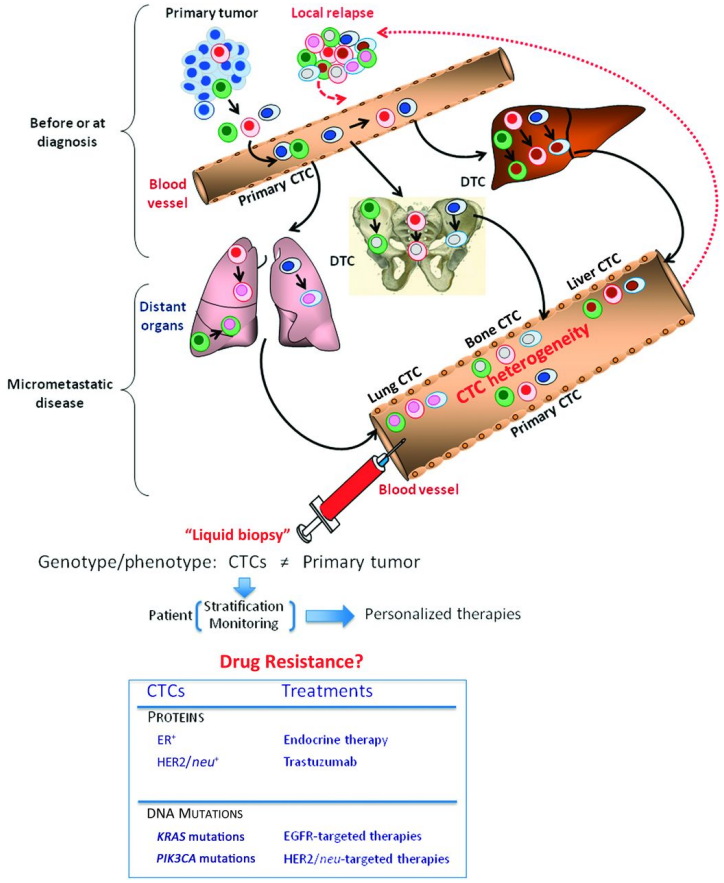
Circulating "Biomarkers"

1. Circulating Biomarkers as **diagnostics** in cancer
2. Circulating Biomarkers as **prognosticator**
3. Circulating Biomarker**s as predictive** marker
4. Circulating Biomarkers as an assessment for **treatment outcomes**

- **Jones R, Brown J, Circulating Biomarkers in Cancer Care. What possible use? Practical Laboratory Medicine April 2017**

Issues about "Whole Tumour Cells"

- "Needle in the haystack"
- Identification
  - Surface markers
  - Size
  - Microscopic live and dead cells
- Isolation
  - Enrichment methods
  - Filtration
  - Others
- Non-enrichment methods



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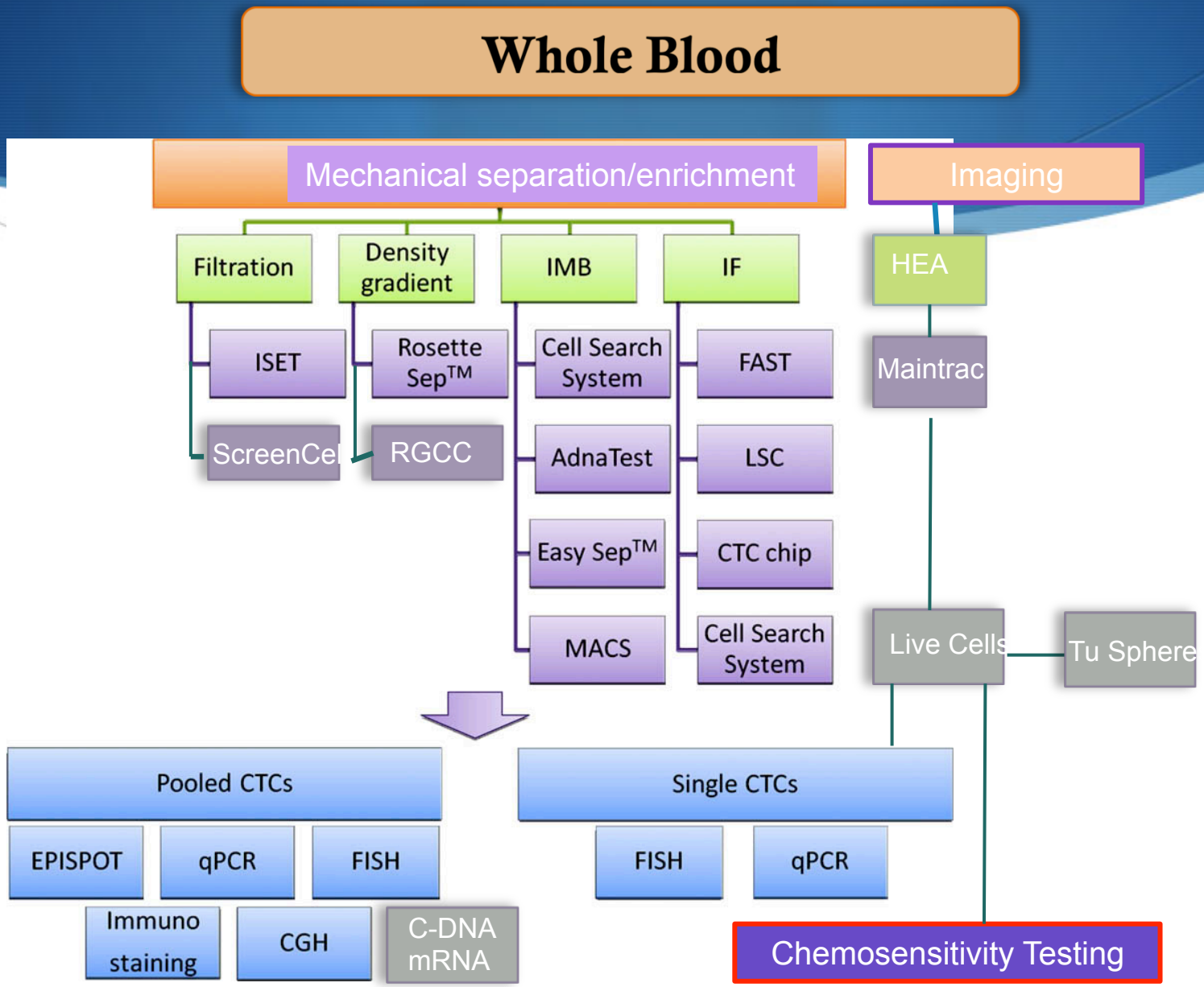
CTC Isolation



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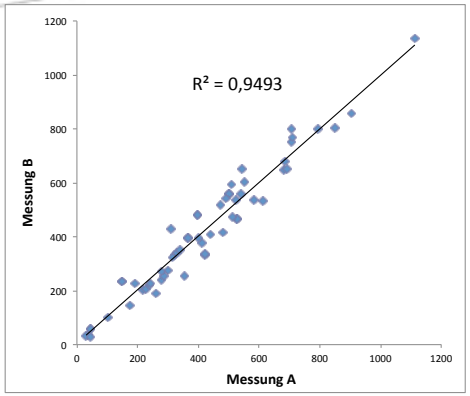
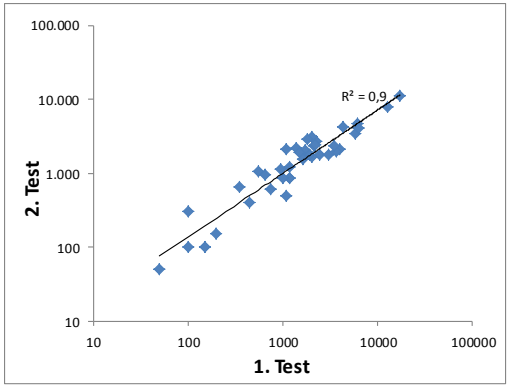
Issues about "Whole and Live Tumour Cells"

- Sensitivity
- Specificity





Clin Chem Lab Med 2005;43(6):617–627

- Correlation with Outcomes
  - maintrac®
  - Recently completed data analysis of 15 year data collection

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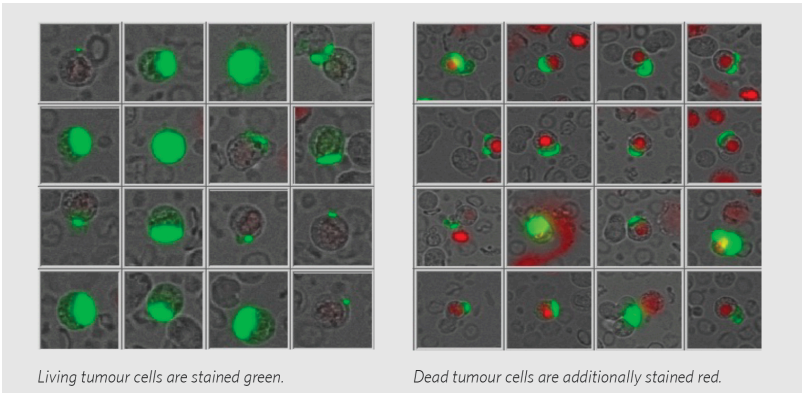
- Maintrac is an imaging analysis from peripheral blood and has demonstrated high sensitivity in detecting and enumerating CETC/CTCs. Results of preclinical and clinical studies have been presented in high impact journals and also at national and international cancer conferences, including ASCO, ESMO, SABM



Living tumour cells are stained green. *Dead tumour cells are additionally stained red.*

WHEN and WHAT and WHY maintrac® tests

- Scenarios in the continuum of the cancer journey
  - 1. pre- and post-surgical treatment for primary tumours
  - 2. long term monitoring and DRiP test
  - 3. end of treatment cycle
  - 4. first diagnosis of metastatic disease
  - 5. monitoring and treatment selection
  - 6. How and when to use cell surface markers
  - 7. How and when to use chemosensitivity testing
  - 8. How and when to use maintrac® testing in advanced metastatic disease and compromised patients

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Test Selection

- 1. Pre- and post- surgical treatment for primary tumours
  - Test
    - CTC count
  - Times and frequencies
    - Before surgery as a baseline
    - After surgery (2-4 weeks, depending on extent of surgery)

- Theil G et al. Review. Position of Circulating Tumour Cells in the Clinical Routine in Prostate Cancer and Breast Cancer Patients. Cancers 2020,12,3782

- Lab Pachmann: In House Data

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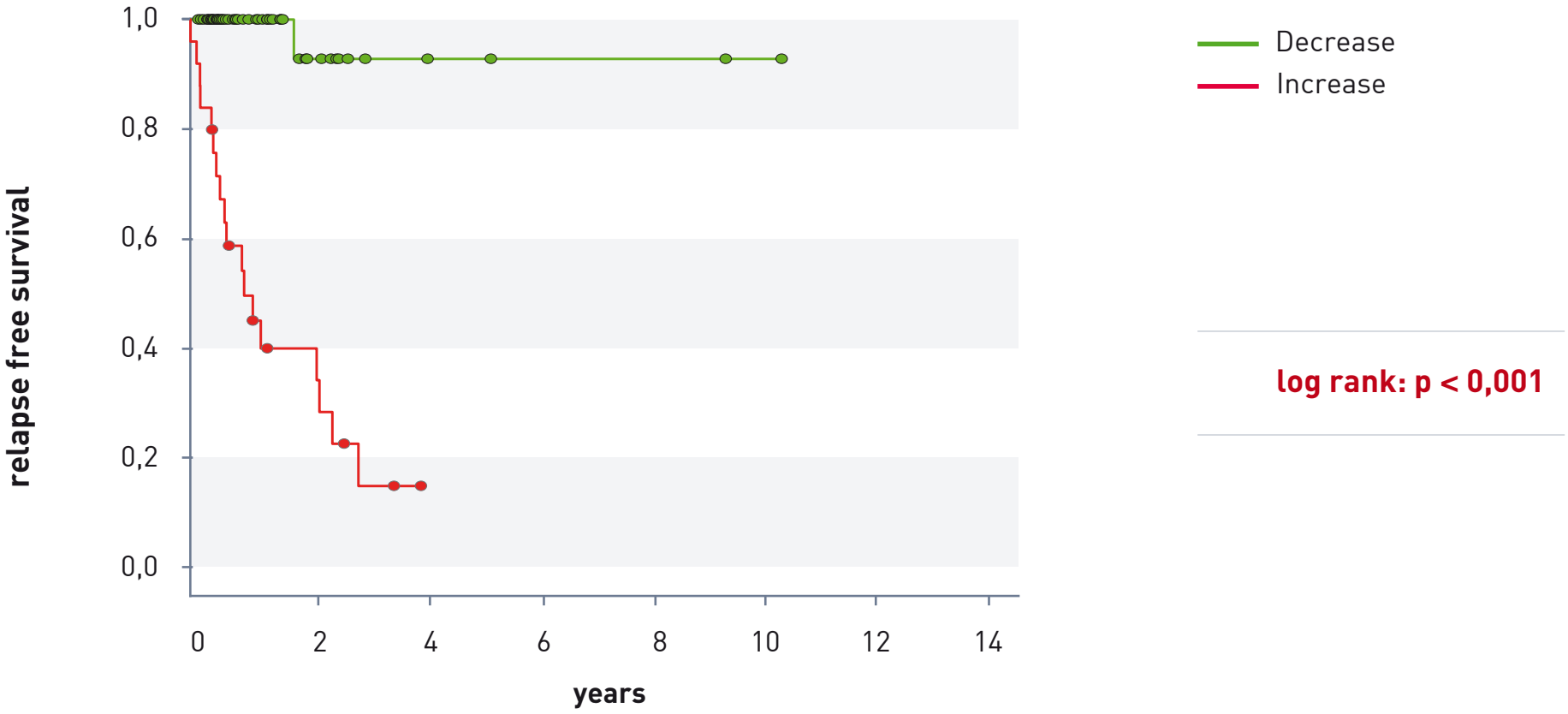
Test Selection

- 2. Long term monitoring and DRiP
  - Test
    - CTC count
  - Times and frequencies
    - 3 monthly for first 2 years
    - 6 monthly if count is low and stable

- Theil G et al. Review. Position of Circulating Tumour Cells in the Clinical Routine in Prostate Cancer and Breast Cancer Patients. Cancers 2020,12,3782

- Lab Pachmann: In House Data

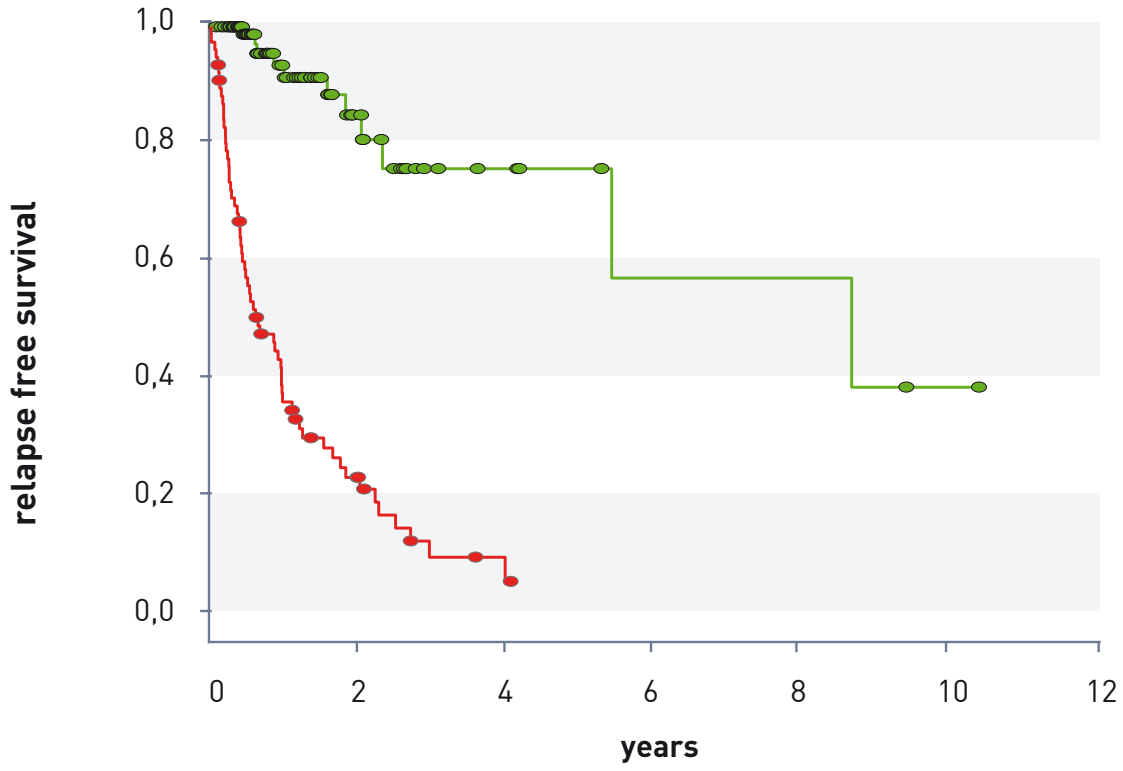
Colon Cancer – non metastatic relapse free survival



rezidivfreies Überleben

Zeit in Jahren

Colon Cancer – all stages at diagnosis

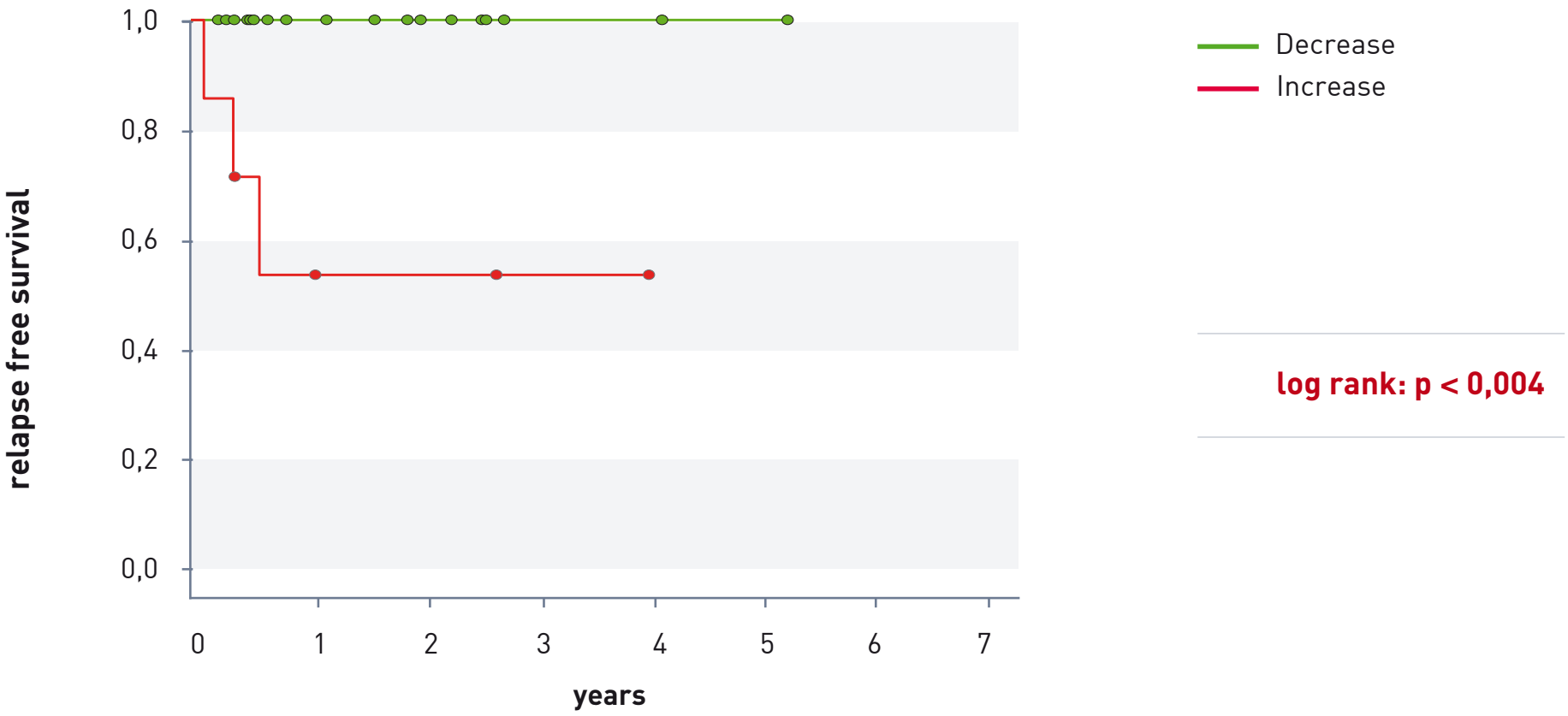


Decrease
Increase

log rank: p < 0,001

69 Pt 93Pt abnehmende Zellzahlen rezidivfreies Überleben

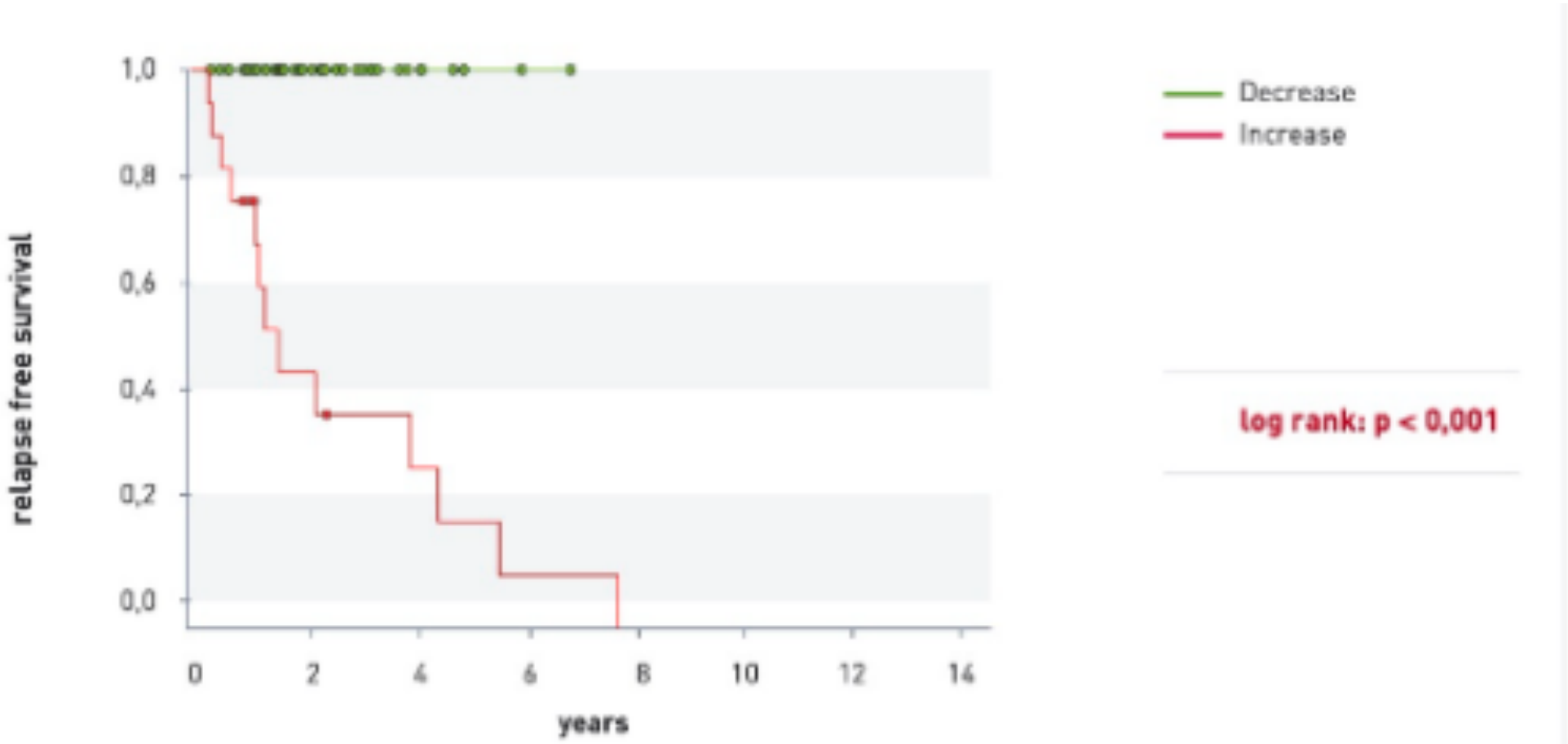
Colon Cancer non-metastatic at diagnosis biological therapies



BC on SERM therapy

increase in CTCs in 38 %
decrease in CTCs in 62 %

decrease in CTCs = no recurrence during 15 years
increase in CTCs = median survival time 1.58 years



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Test Selection

- 3. Monitoring at the end of treatment cycle. Example ER+ Breast Cancer after 5 years of Treatment
  - Test
    - CTC count
  - Time
    - Before stop or switch to other therapies
    - 4 weeks after stop or switch to other therapies

- JCancerResClinOncol 2010: PachmannK,CamaraO,KohlhaseA, RabensteinC, et al Assessing the efficacy of targeted therapy using circulating epithelial tumour cells (CETC): the example of SERM therapy monitoring as a unique tool to individualise therapy

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Test Selection

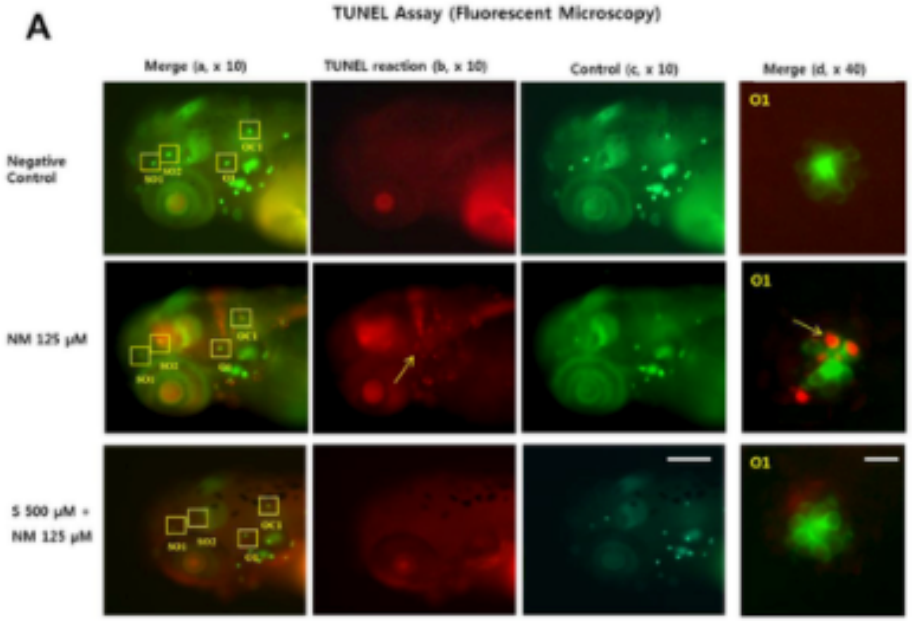
- 4. First diagnosis of metastatic disease development
  - CTC count
  - Plus
    - Activity markers
      - Ki67, TUNEL assay, Sphere development
    - Chemosensitivity
      - Cytotoxicity assay to proposed and other treatments

- Chemosensitivity Testing of CETC in Vitro. Correlation to in vivo. J of CancerTherapy 2013

TUNEL Assay

- TUNEL
  - Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling
- Detecting Apoptotic Cells
  - Undergoing DNA degradation during the late stages of apoptosis

- Detection of apoptosis by TUNEL Assay
  Kyrylkova K et al. Methods Mol Biol 2012



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Test Selection

- 5. Monitoring and treatment selection
  - Test
    - CTC count
    - Cell surface and activity markers
      - ER, AR, PSA, pd-L1, ki67 etc
  - Time
    - 3 monthly and/or at the end of a treatment cycle

- 2008. Monitoring CTCs in Adjuvant Chemotherapy in BC. Indicator for risk of early relapse. J Clin Oncol 2008.pdf

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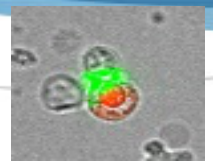
Test Selection

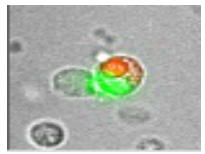
- 6. Cell surface markers
- Test
  - Receptors
    - Estrogen, Androgen and others
  - Proteins
    - EGFR, pd-L1, PSA and others
  - Sugars
  - Coagulation and Inflammatory markers
- Time
  - Dependent on clinical circumstances

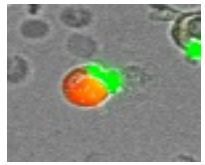
DOWNSTREAM ANALYSIS

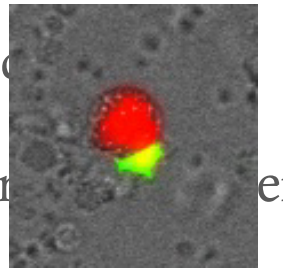
IHC, FISH, PCR, qPCR, Mutation Analysis

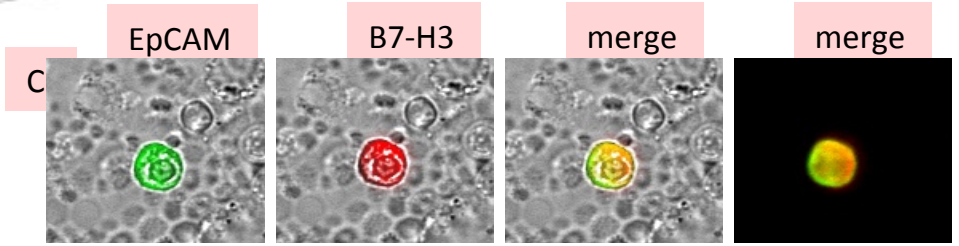
- ER, PR, AR,
- Her2/Neu
- IGFR
- PSA
- PSMA
- c-Kit
- ...pc
- Ster ers
- Activation Markers, ki67

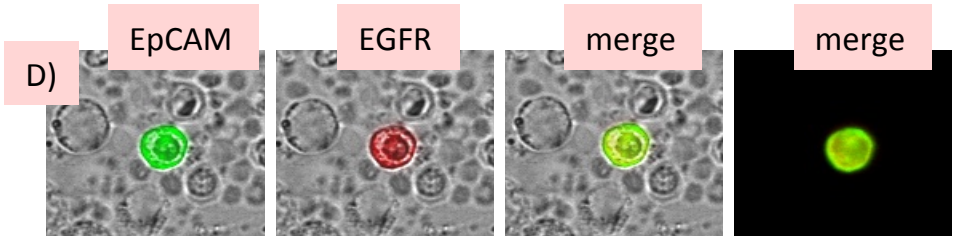


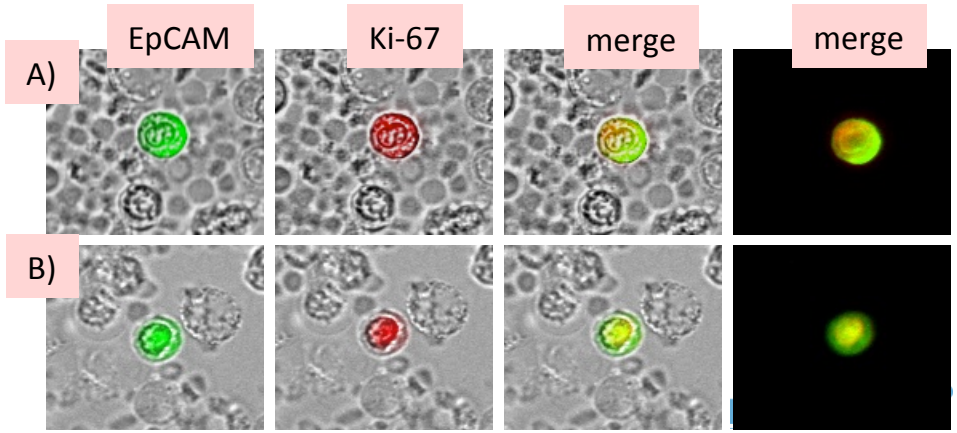












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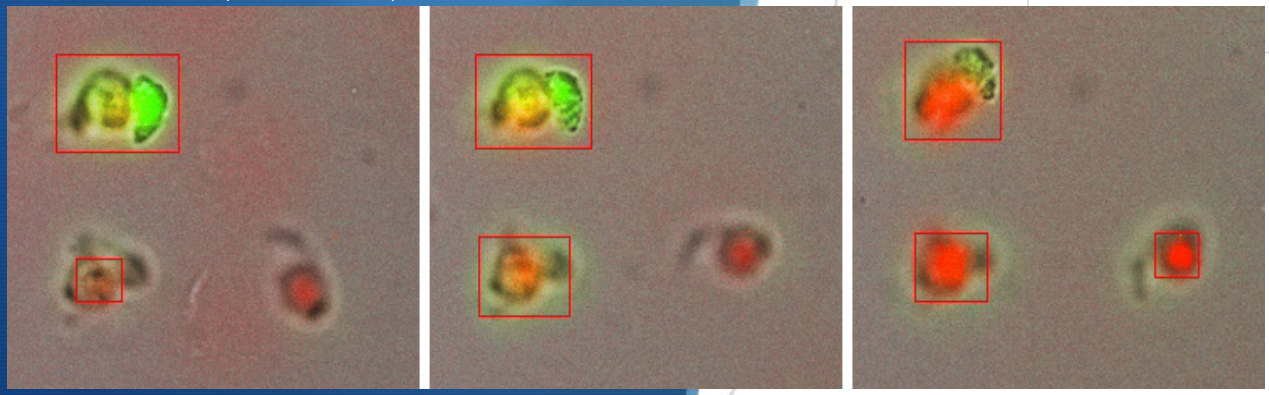
Test Selection

- 7. How and when to use chemosensitivity test
  - Discussion of test methods
  - Time
    - Pre treatment
    - 2-6 weeks post individual cytotoxic treatment
  - 2013. Chemosensitivity Testing of CTCs. Journal of Cancer Therapy 2013

Chemo-sensitivity

J Cancer Therapy 2013, 4:597-605

Chemosensitivity Testing of Circulating Epithelial Tumo Cells (CETC) in Vitro:



Journal of Cancer Therapy, 2013, 4, 597-605
doi:10.4236/jct.2013.42077 Published Online April 2013 (http://www.scirp.org/journal/jct)

Scientific Research

Chemosensitivity Testing of Circulating Epithelial Tumor Cells (CETC) *in Vitro*: Correlation to *in Vivo* Sensitivity and Clinical Outcome

Nadine Rüdiger[1], Ernst-Ludwig Stein[2], Erika Schill[2], Gabriele Spitz[2], Carola Rabenstein[2], Martina Stauch[3], Matthias Rengsberger[4], Ingo B. Runnebaum[4], Ulrich Pachmann[2], Katharina Pachmann[1,2*]

[1]Clinic for Internal Medicine II, University Hospital, Friedrich Schiller University, Jena, Germany; [2]Transfusionsmedizinisches Zentrum, Bayreuth, Germany; [3]Onkologische Schwerpunktpraxis, Kronach, Germany; [4] Women's Hospital, University Hospital, Friedrich Schiller University, Jena, Germany.
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ABSTRACT

Background: Chemotherapy is a mainstay of tumor therapy, however, it is predominantly applied according to empirically developed recommendations derived from statistical relapse rates occurring years after the treatment in the adjuvant situation and from progression-free interval data in the metastatic situation, without any possibility of individually determining the efficacy in the adjuvant situation and with loss of time and quality of life in the metastatic situation if the drugs chosen are not effective. Here, we present a method to determine the efficiency of chemotherapeutic drugs using tumor cells circulating in blood as the part of the tumor actually available in the patient's body for chemosensitivity testing. Methodology/Principal Findings: After only red blood cell lysis, omitting any enrichment (analogous to other blood cell enumeration methods, including rare CD34 cells), the white cells comprising the circulating epithelial tumor cells (CETC) are exposed to the drugs in question in different concentrations and for different periods of time. Staining with a fluorescence-labeled anti-epithelial antibody detects both vital and dying tumor cells, distinguishing vital from dying cells through membrane permeability and nuclear staining with propidium iodide. Increasing percentages of dying tumor cells are observed dependent on time and concentration. The sensitivity can vary during therapy and was correlated with decrease or increase in CETC and clinical outcome. Conclusions/Significance: Thus, we are able to show that chemosensitivity testing of circulating tumor cells provides real-time information about the sensitivity of the tumor present in the patient, even at different times during therapy, and correlates with treatment success.

Keywords: Circulating Epithelial Tumor Cells; Chemosensitivity Testing; Breast Cancer; Ovarian Cancer

1. Introduction

For patients diagnosed with a malignant tumor, cure is presumably only possible if the tumor is completely eradicated. Initially, the main aim is to eliminate the primary tumor, the major tumor burden, preferentially by surgery. However, most cancer patients do not die from their primary tumor but from distant metastases, developing some years after the removal of the primary tumor. During tumor growth, cells from the tumor are disseminated continuously via lymph vessels or directly into blood [1]. These cells are assumed to be the source of metastasis formation. Patients with affected lymph nodes have a less favorable chance of disease-free survival than patients without lymph-node-positive disease, indicating that cells detached from the tumor were able to settle and grow in foreign tissue. Therefore, as the second pillar of tumor therapy, chemotherapy has evolved and is applied after surgery as adjuvant chemotherapy, e.g. in breast and ovarian cancer, to eliminate such early disseminated cells, when no detectable tumor is present. Such therapies have been shown to avert metastasis formation and ultimately save lives in breast cancer patients [2]. In the adjuvant situation, these therapies have been developed in clinical trials using the statistical improvement of relapse-free survival as a measure. This cannot, however, predict for the individual patient whether the

*Corresponding author.

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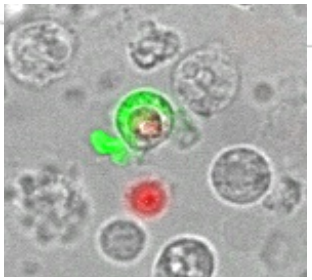
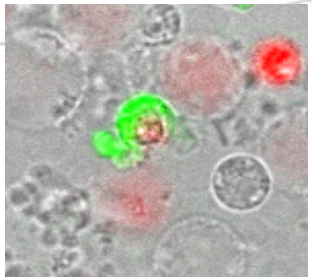
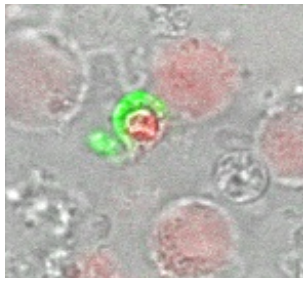
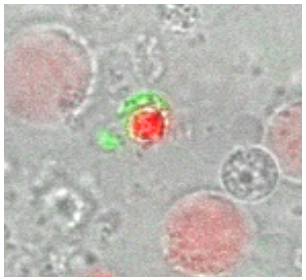
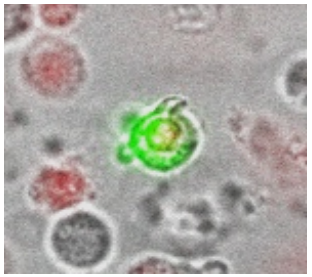
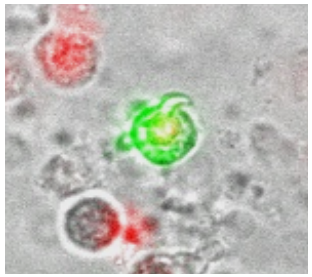
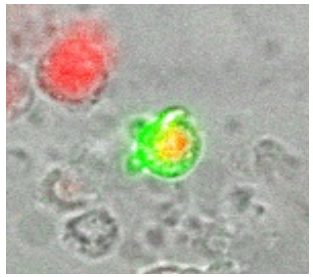
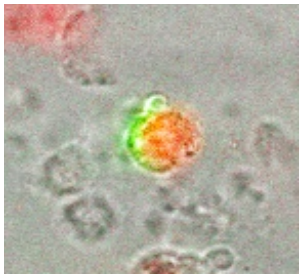
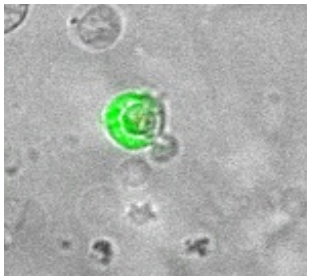
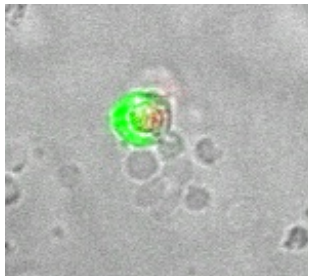
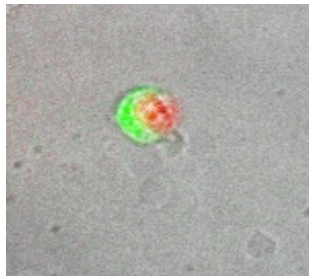
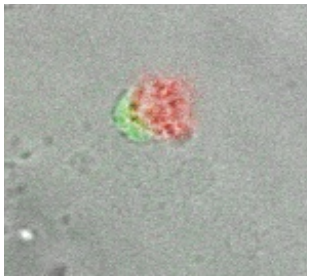
Cell decay of CTCs over time in the presence of a drug

	t=1 hr.	t=3,5 hrs.	t=7 hrs.	t=10,5 hrs.
Docetaxel				
Epirubicin				
Mafosfamid				

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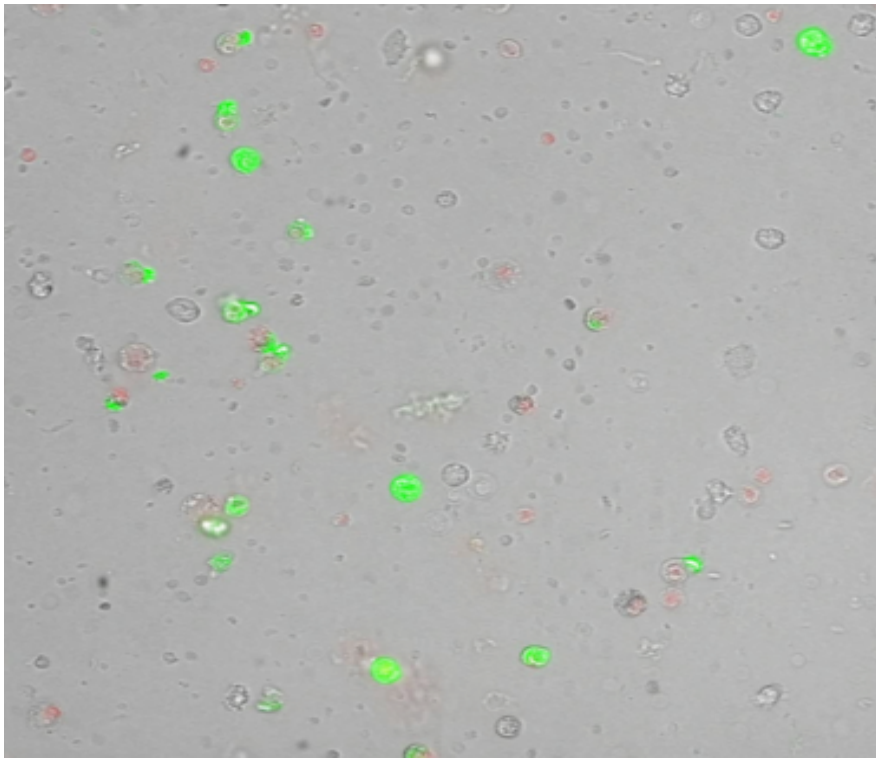
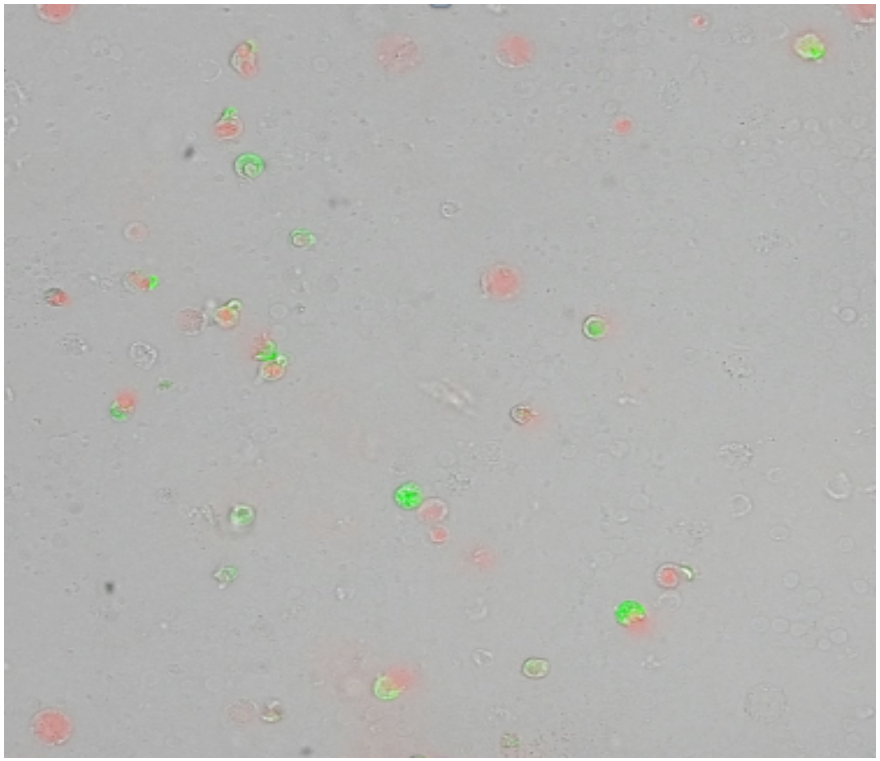
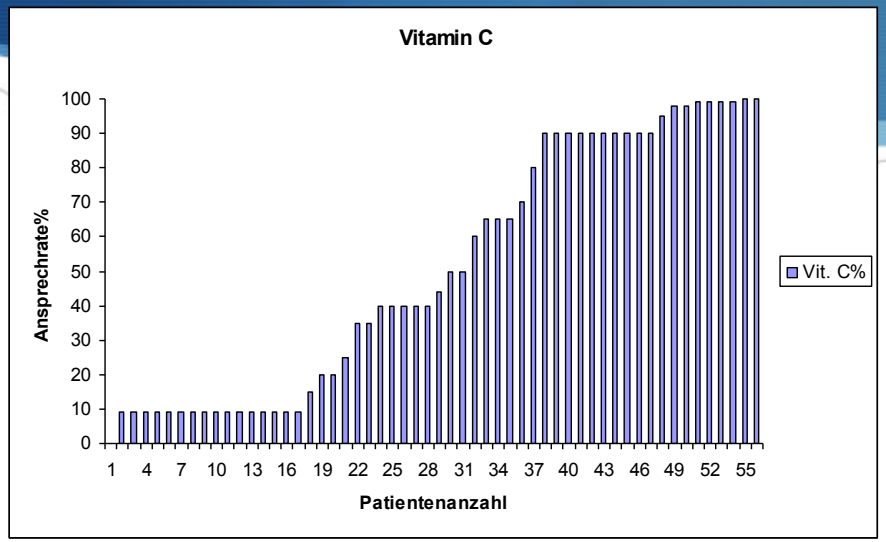
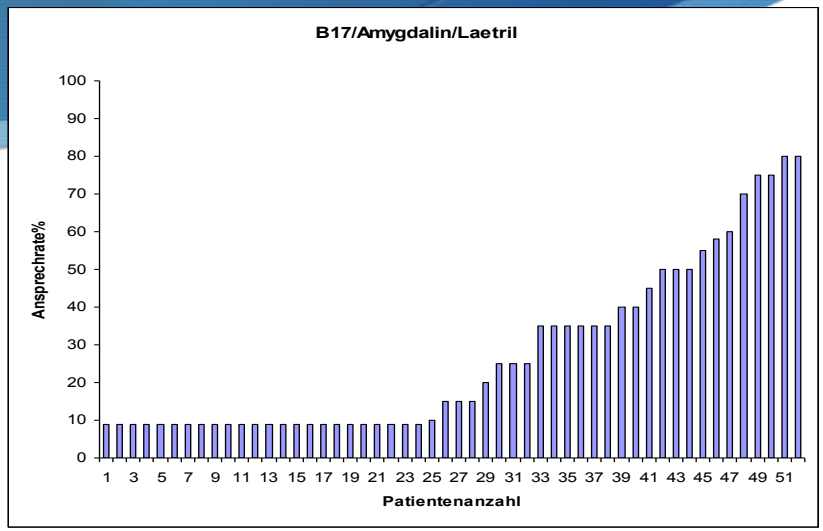
Pre and post

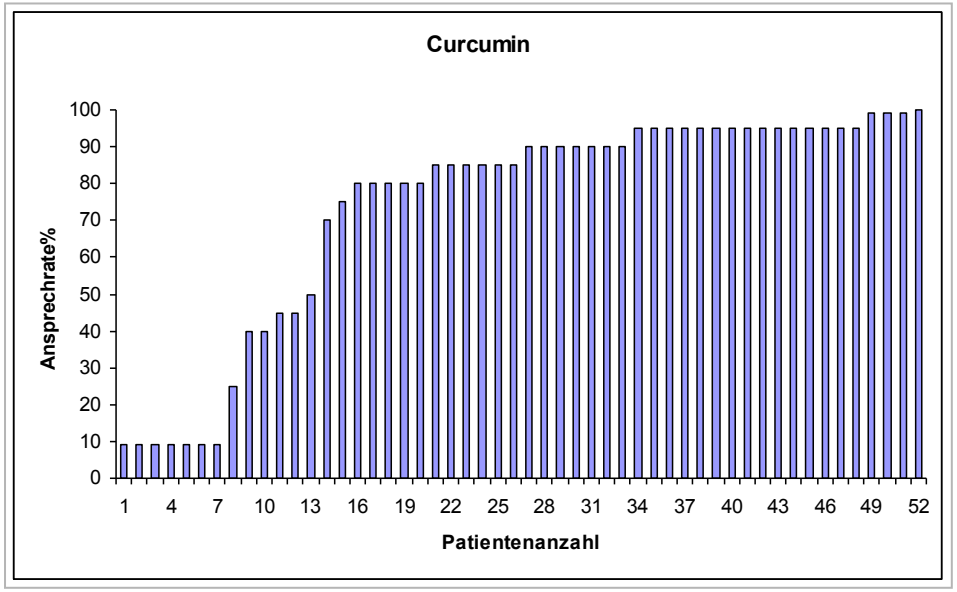




Cytotoxicity Assays (Chemosensitivity) To IV BOTANICAL MEDICINES







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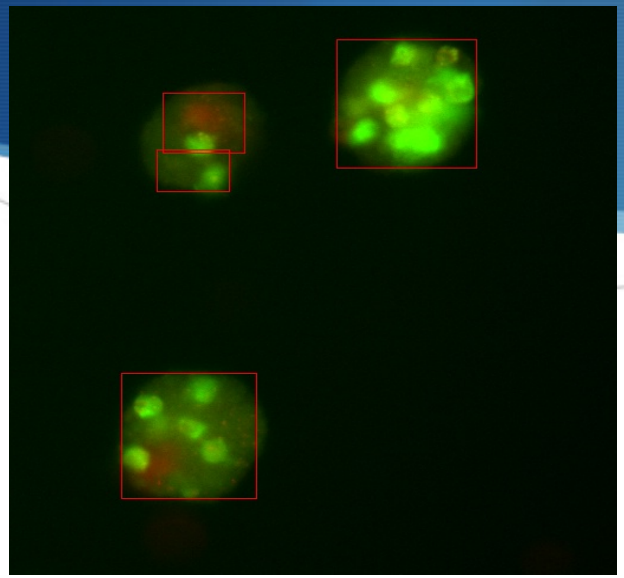
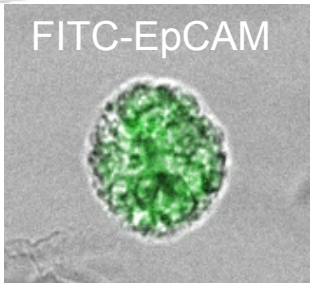
Test Selection

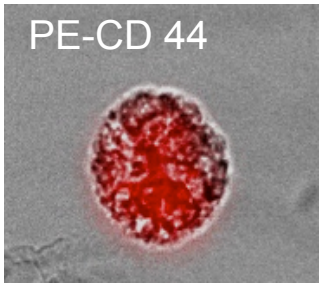
- 7. maintrac® CETC/CTC testing in advanced metastatic disease
  - Resistance development
  - Seeding and recruiting of cells
  - De-differentiation
- Test Selection
  - CTC count
  - Tumour Spheres,
  - Chemosensitivities on CTCs and Tumour Spheres

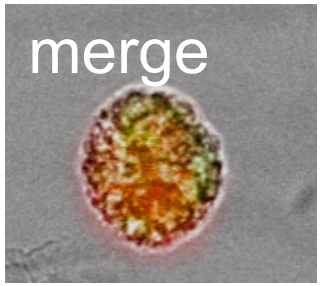
EpCAM expression in tumor spheres

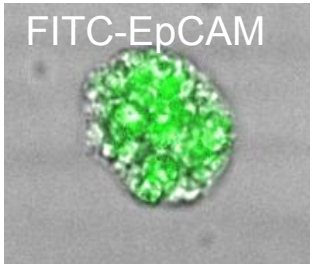


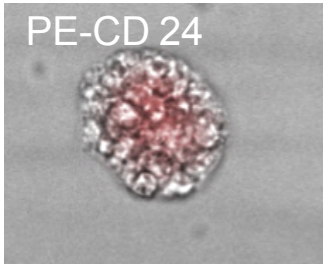
Stem cell marker expression in tumor spheres

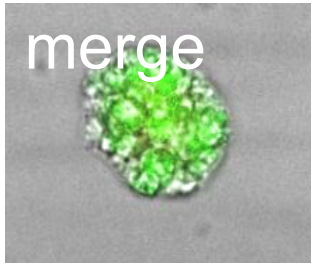


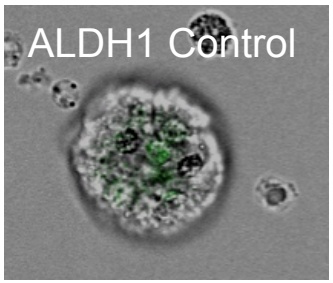


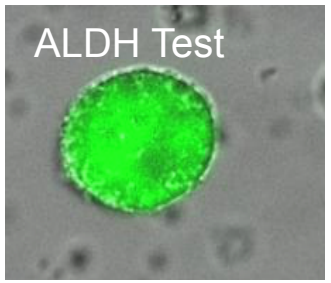












Maintrac®

- **Highlights of maintrac CTC technology are**
- **Monitoring:** the success of maintenance therapy in breast cancer and course of the disease can be monitored in real-time during therapy applied every three to six months.
  Increasing cell counts are early indicators for relapse and need for change of medication;
- **Treatment decision**: supporting a decision on continuation of the maintenance/hormone therapy in breast cancer at the end of the therapy, if cell counts are increasing, then restart therapy;
- **High sensitivity**: CTCs are also detectable in primary cancer patients not only metastasized patients;
- **Clinical proof**: with more than 600 patients analysed in 13 clinical trials with focus on breast cancer. More than 280 patients were monitored during maintenance therapy (Tamoxifen, Trastuzumab) and after the end of the therapy. Increasing cells can predict very early the failure of the therapy, so that therapy can be either changed or restarted;
- **Therapy optimization**: Characterization of CTC to stratify therapy even when biopsy is not possible
- **Experience:** up to now more than 80.000 (clinical samples analysed from all over the world;
- **High Throughput**: scale-up capacities are available through development of a high throughput maintrac-AUTO CTC analyser using 96 well plates, will achieve more than 400 samples per day; a benchtop maintrac-Auto CTC analyser is projected;
- **Scientific Credibility**: accepted scientific reputation acknowledged in several peer reviewed journals. Several prizes including poster prizes (DGHO 2013), oral presentation awards (COSA 2014) prove the scientific excellence.

maintrac®

WHEN and WHAT and WHY maintrac® tests

- Scenarios in the continuum of the cancer journey
  - 1. pre- and post-surgical treatment for primary tumours
  - 2. long term monitoring and DRiP test
  - 3. end of treatment cycle
  - 4. first diagnosis of metastatic disease
  - 5. monitoring and treatment selection
  - 6. How and when to use cell surface markers
  - 7. How and when to use chemosensitivity testing
  - 8. How and when to use maintrac® testing in advanced metastatic disease and compromised patients

GENOSTICS IN COLLABORATION WITH MAINTRAC PRESENT

MONITORING YOUR CANCER PATIENT CTC'S

WHEN, WHAT AND WHY?

● LIVE
WEBINAR

Prof. Katharina Pachmann

Director of Science

Bayreuth, Germany

Dr. Joachim Fluhrer

Medical Practitioner

Sydney, Australia

TUESDAY 19TH

OCT

6PM
(AEST)

maintrac®