A New Paradigm in Cancer Support: maintrac™ Cancer Cell Testing

Saturday 24 March 2018

Holiday Inn London Regent's Park

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The Academy of Nutritional Medicine (AONM)
www.aonm.org/maintrac/

Agenda

- Cancer-free .... ?
- Detection
- Validation
- Comparison with other methods
- Chemosensitivity testing
- Cytotoxicity of natural agents

"Cancer-free" – ?

- Malignant tumours are detectable when they have reached a size of about 1 cm
- The first therapy is usually complete surgical removal of the tumour
- Patients are often declared cancer free soon afterwards; more cautious advice is to wait for 5 years relapse-free before such assurances
  are given

How do metastases develop?

- **However, cells can break away from tumours during tumour growth**
- It is these cells that are responsible for **distant metastases** even after complete resection of the original tumour
- Such metastases occur in 25 - 50% of cases after "successful" surgery, most frequently in vital organs, e.g. liver, lungs, bone marrow

Solid-tumour metastases

Example: Breast cancer

100 breast cancer patients

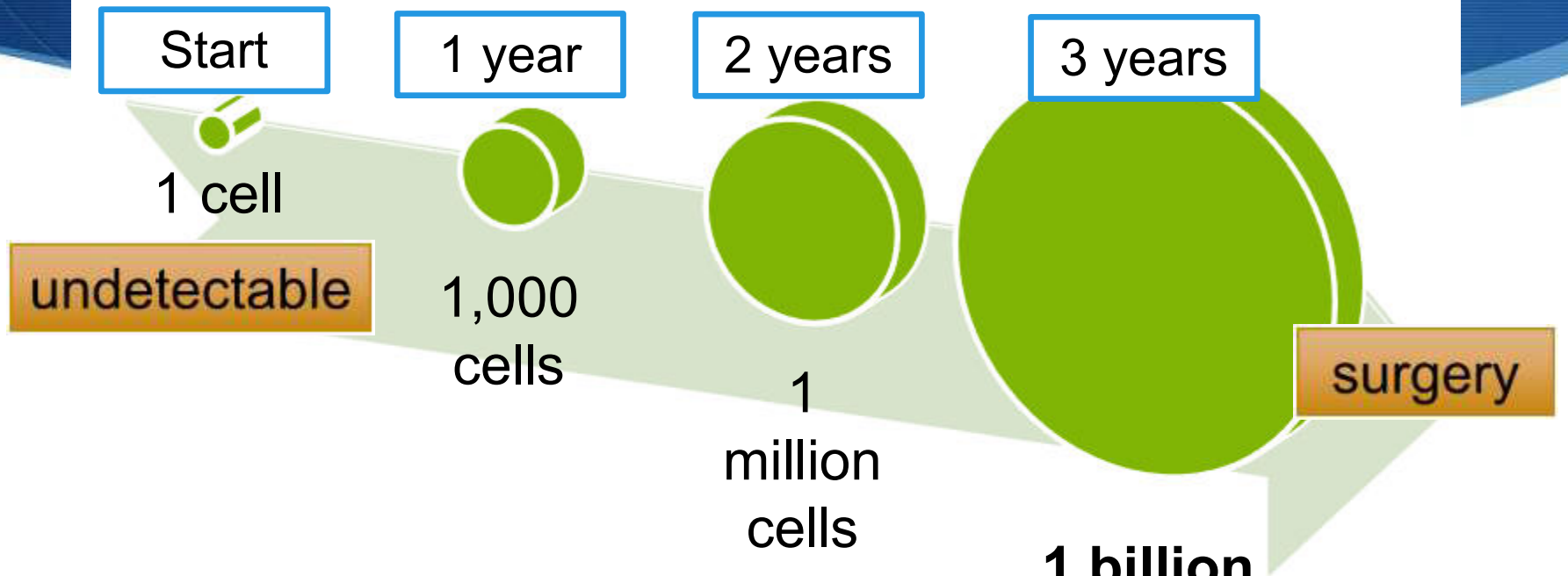
10 with primary metastases

90 without metastases

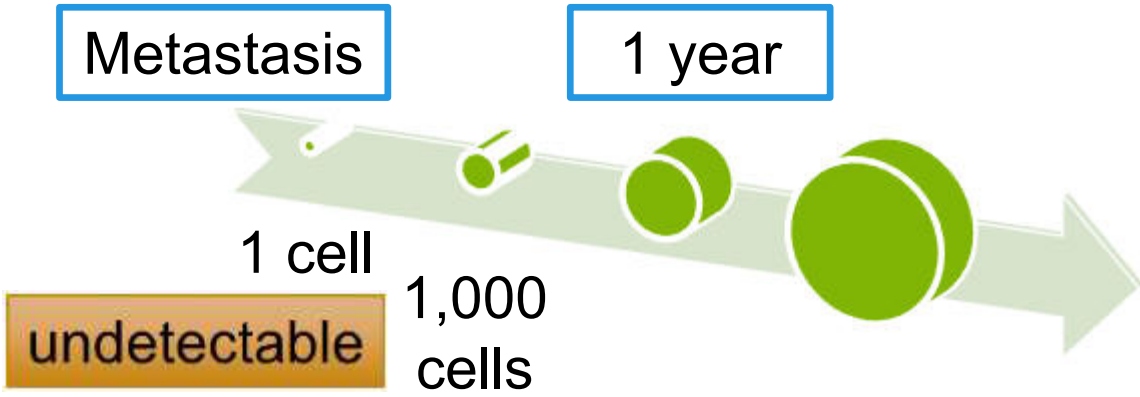
22 (25%) develop metastases during the following 1 - 5 years

Others may develop metastases up to 30 years later

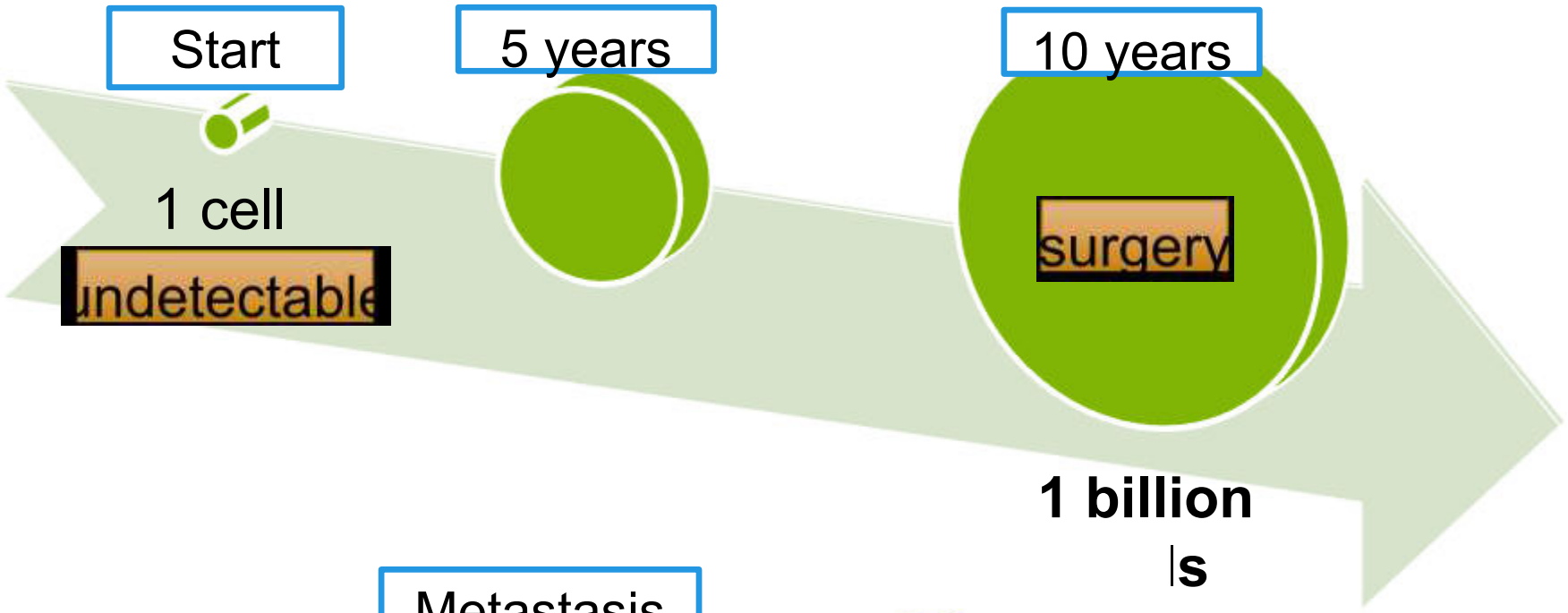
Development of metastases



- Fast-growing tumour; Doubling time: 36 days



Development of metastases in solid tumours



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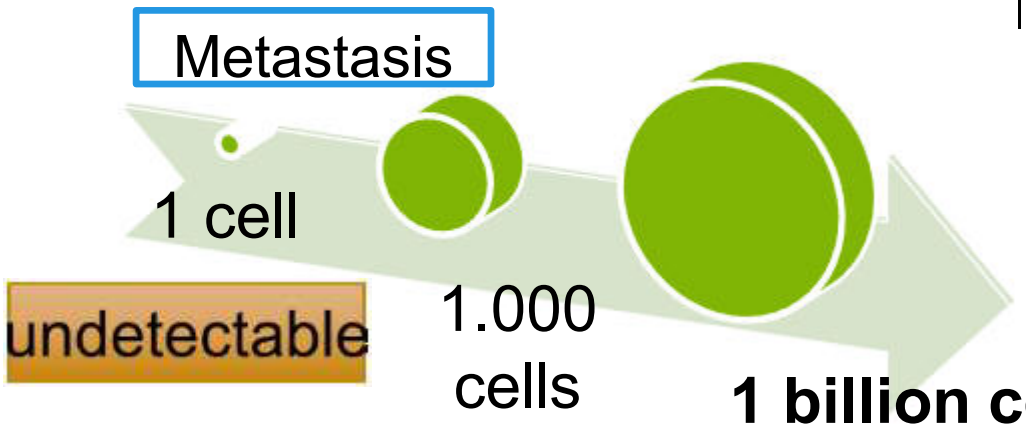

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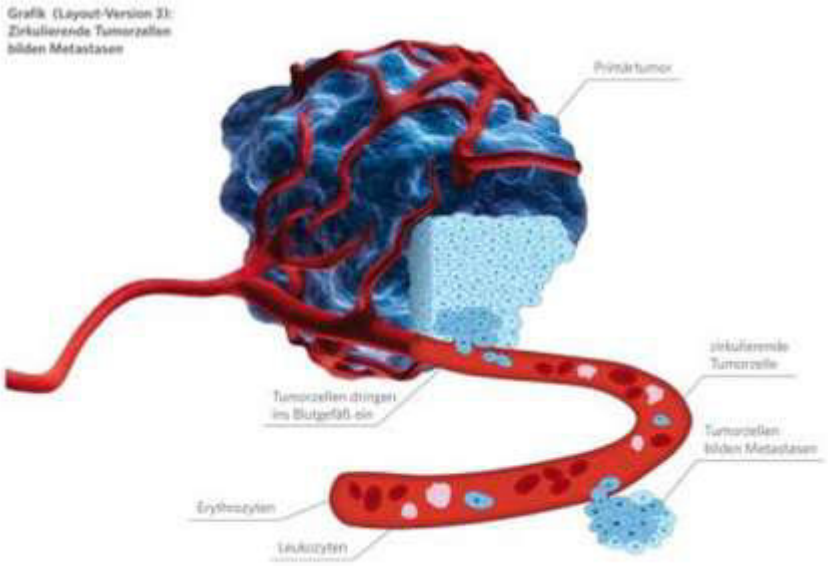
Circulating tumour cells from solid tumours



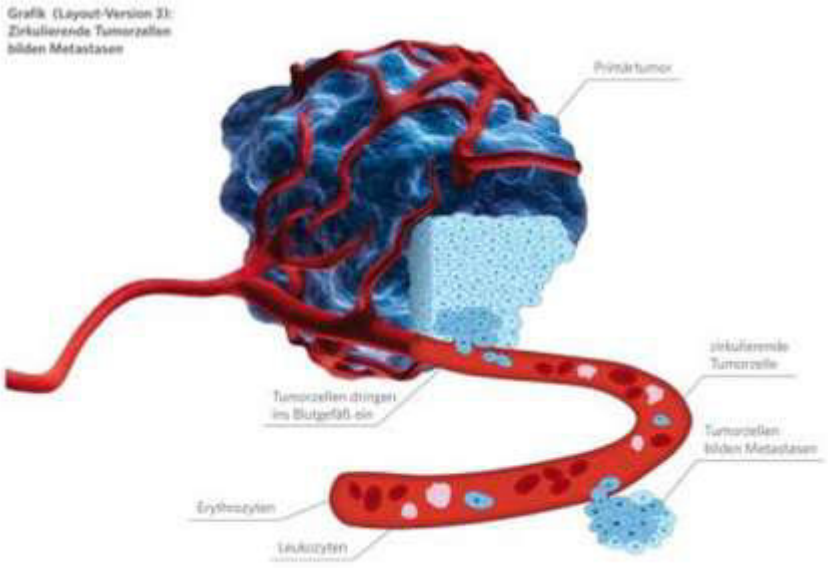
- Carcinomas are of **epithelial origin**
- Carcinomas **disseminate** epithelial cells

⇒ **CETCs** (circulating epithelial tumour cells)

Y. Shiozawa, A M Havens, K J Pienta and R S Taichman, Leukemia (2008) 22, 941–950

Arbeitsgemeinschaft TZB

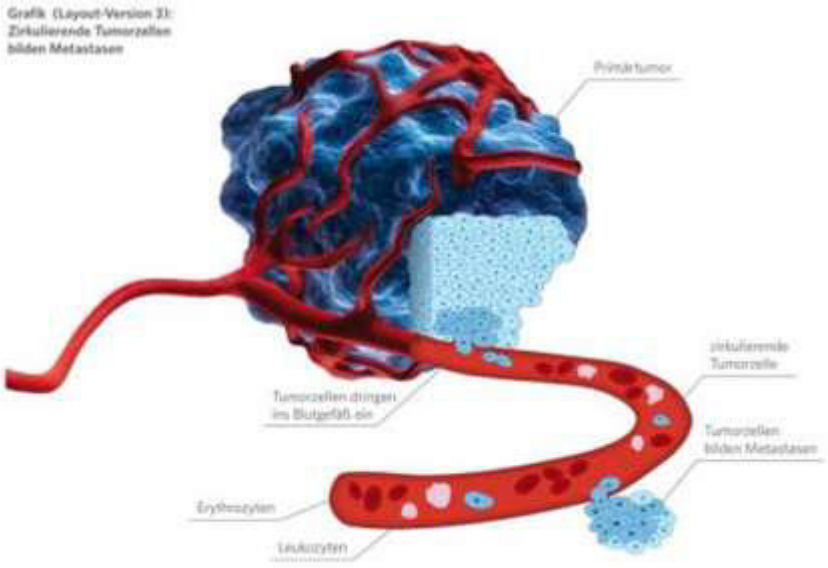
Circulating tumour cells from solid tumours



- Vascularisation begins when the tumour has reached a size of about 1mm (1 million cells)
- Together with the uptake of nutrition by the tumour, debris and cells are shed into the circulation
- Seeding starts from the time of vascularisation

Arbeitsgemeinschaft TZB

Circulating tumour cells from solid tumours



- Even if 99.9% of the shed cells die, the number of cells remaining in the circulation over time adds up to several million cells
- Debris can also comprise DNA from dying cells

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Methodology

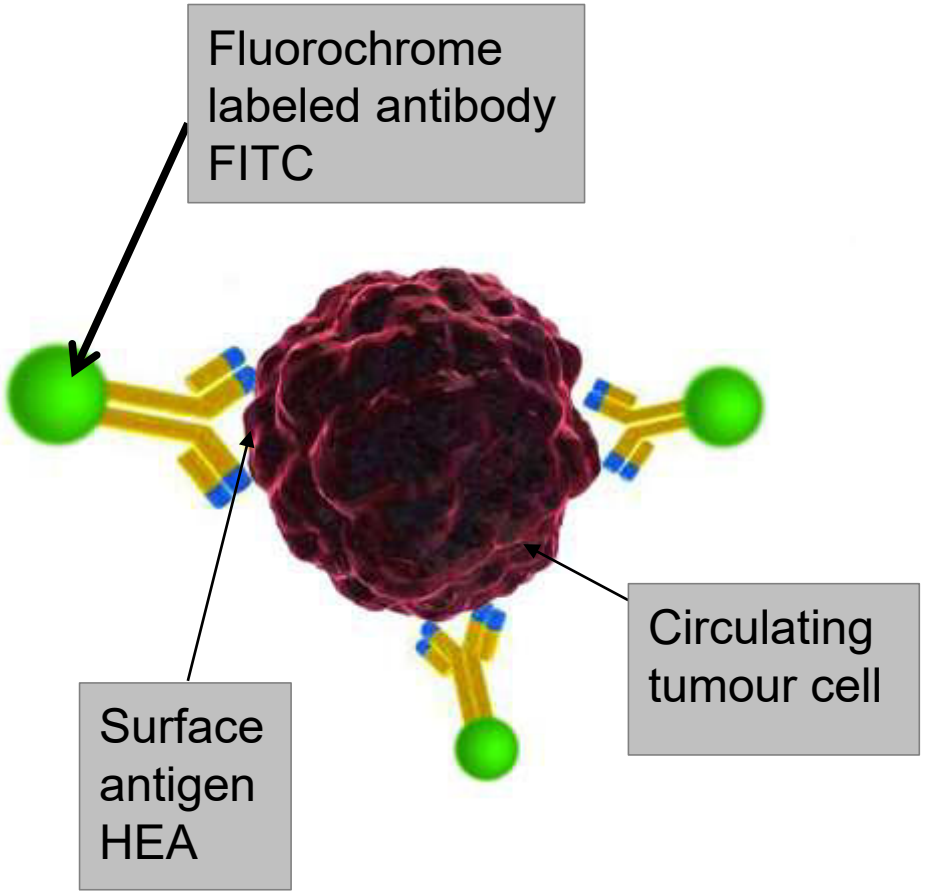
Liquid biopsy technique

Maintrac **liquid biopsy** cell staining allows **quantitative** detection of live circulating tumour cells

NO fixation.

NO isolation.

NO enrichment.



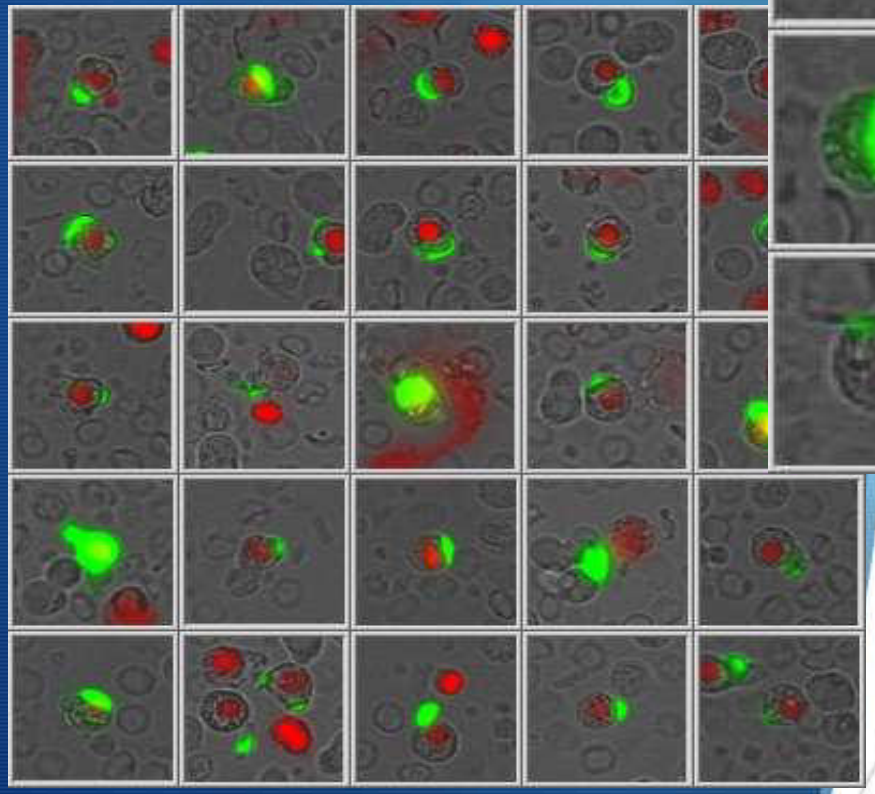
Testing

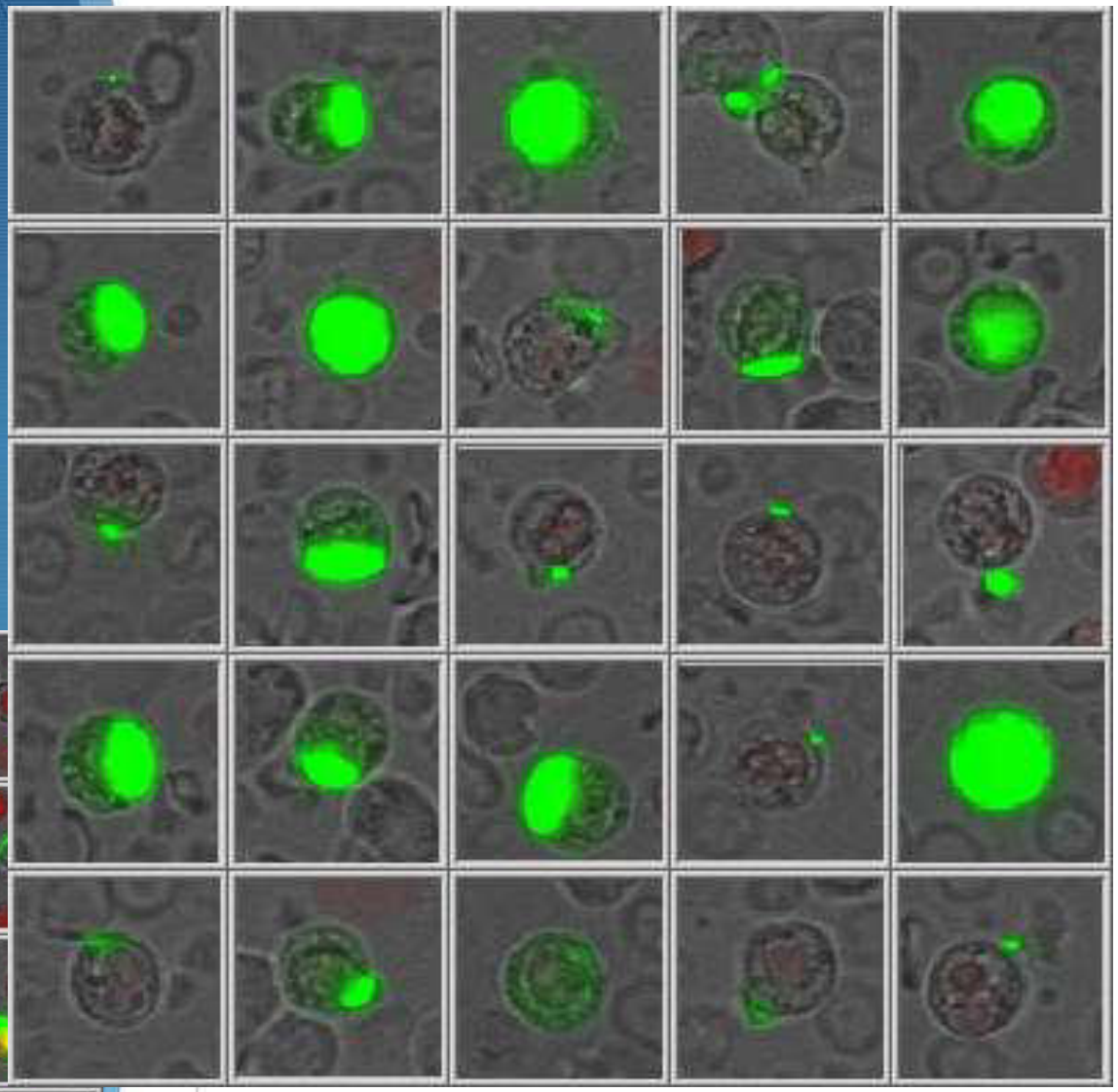
Microscope-based semi-automated image evaluation

Recording of

- All solid tumours
- **Not** for lymphoma or leukaemia

Heterogeneity in cells from one patient





Red-stained nucleus
= dead cell

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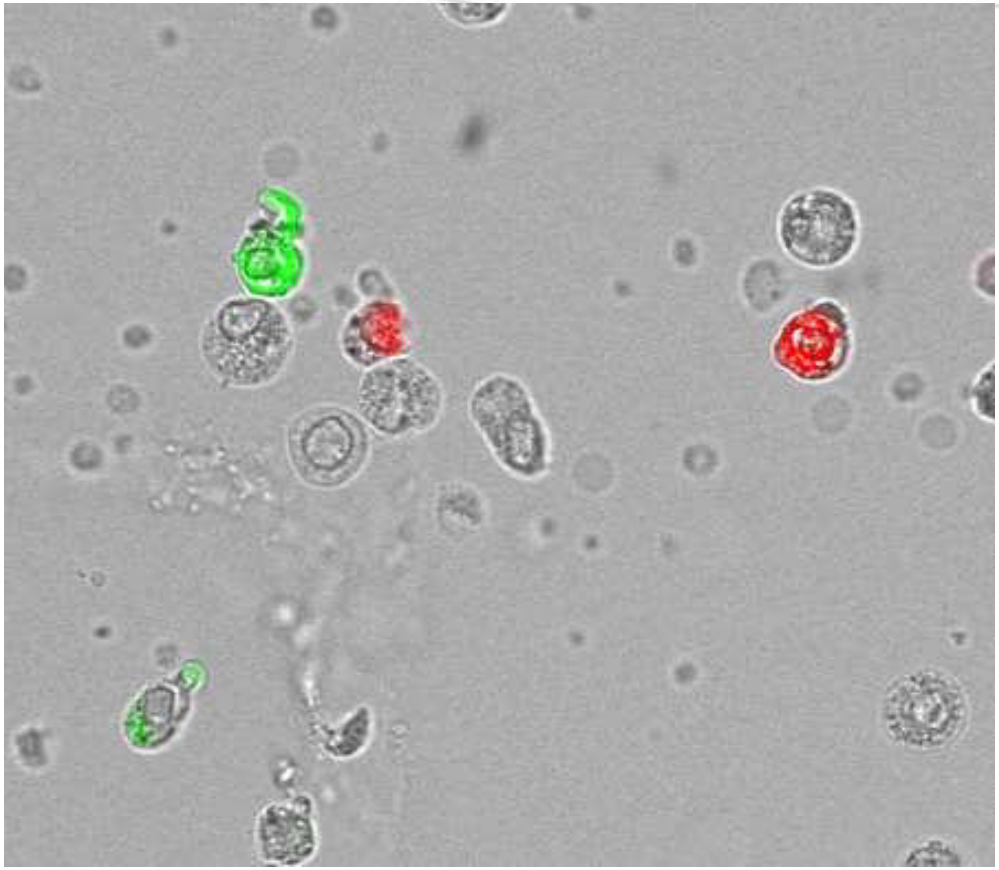
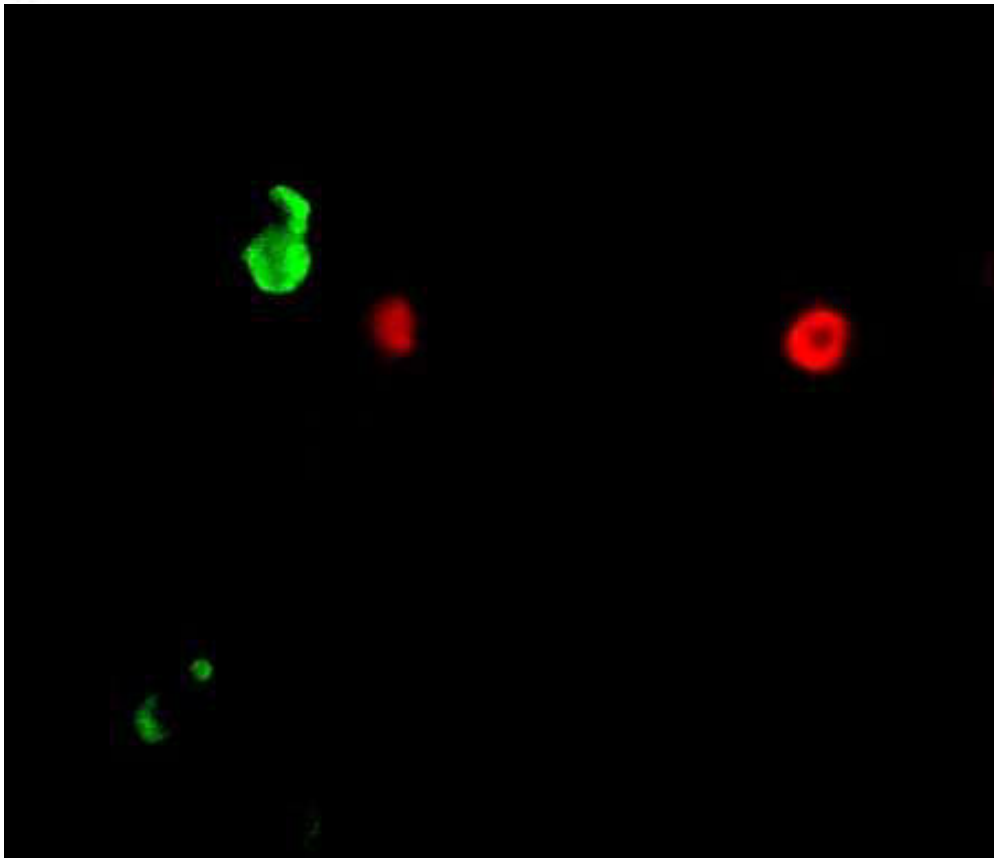
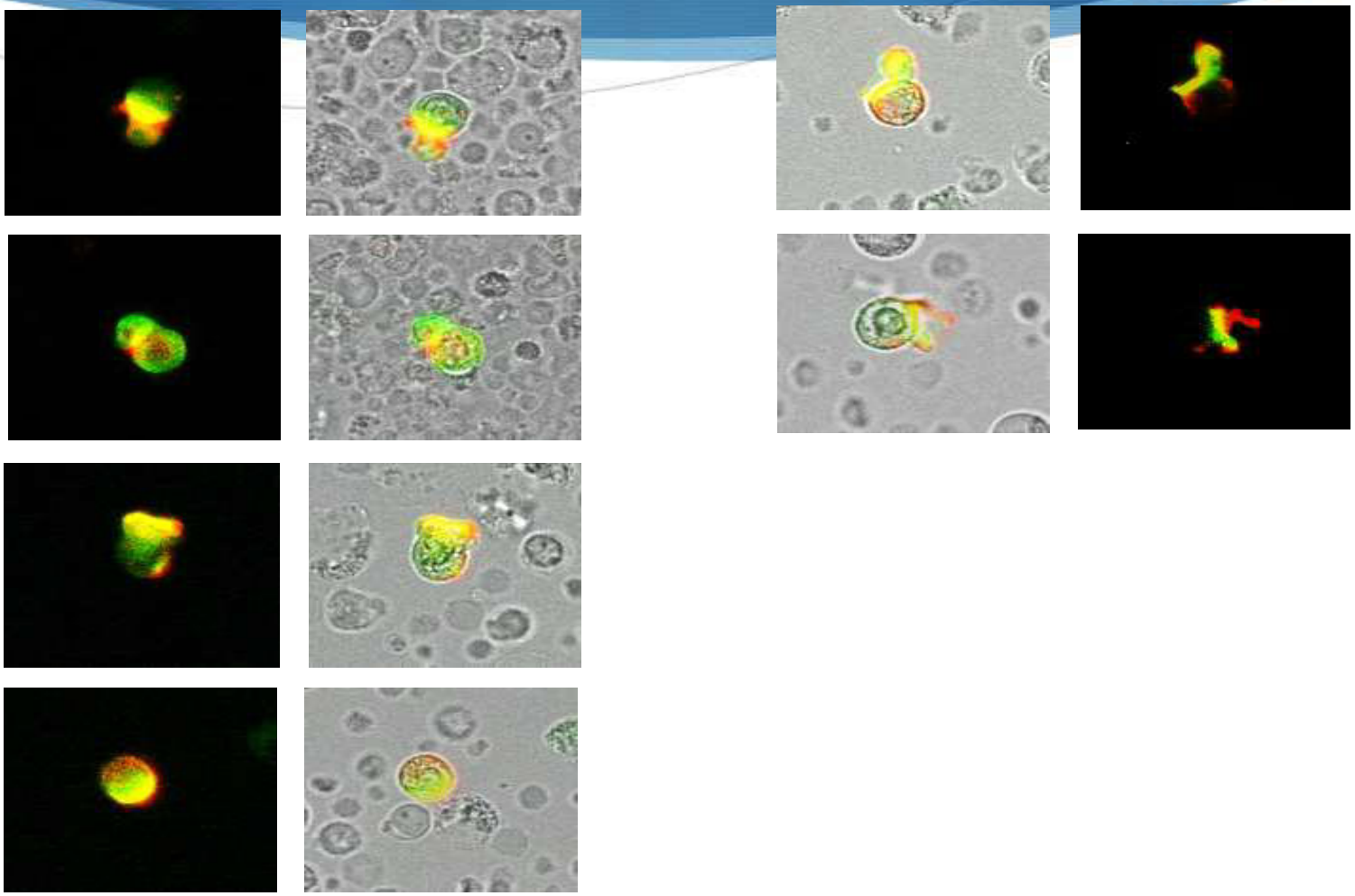
Validation

Counterstaining CD45/EpCAM

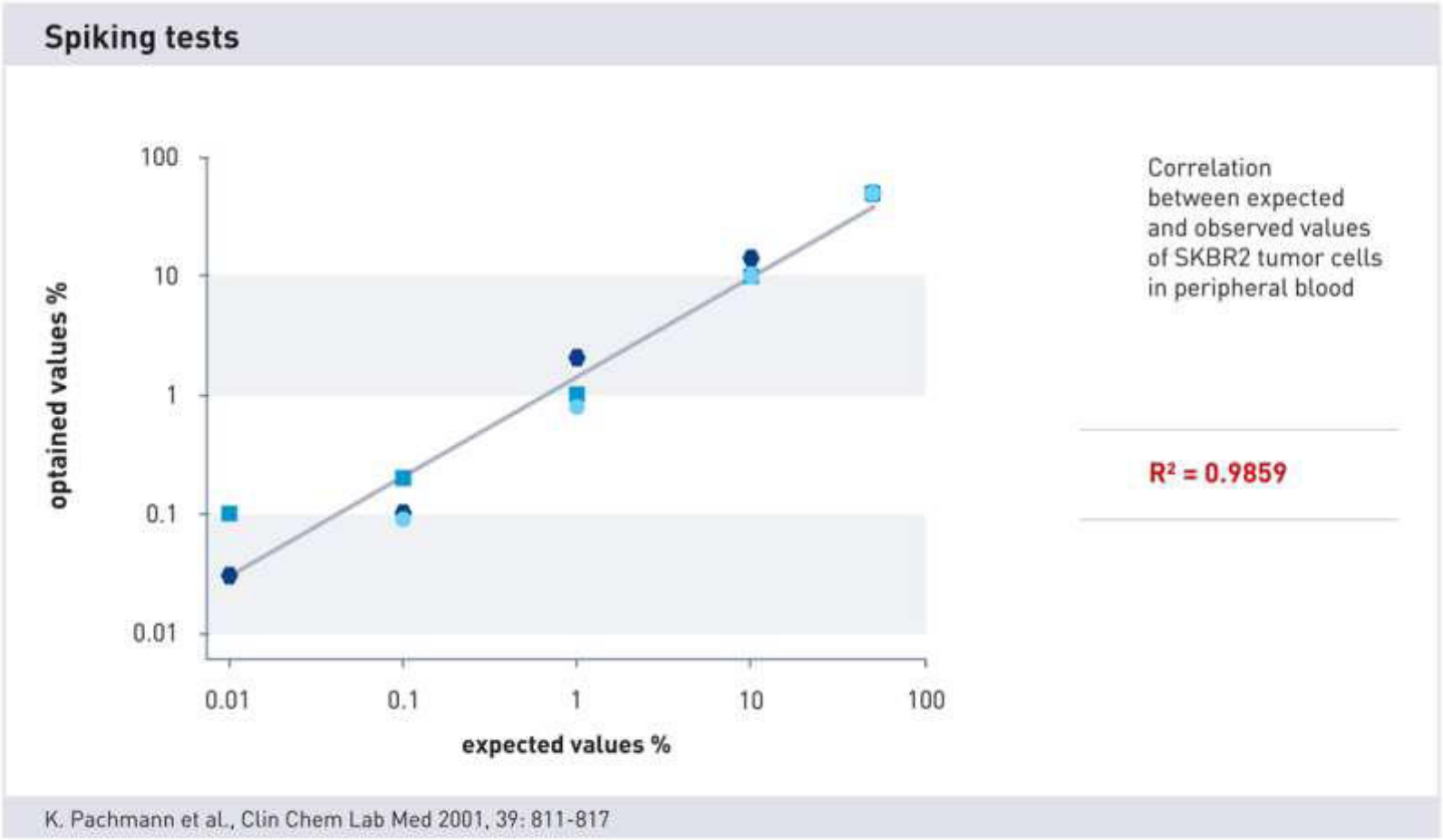




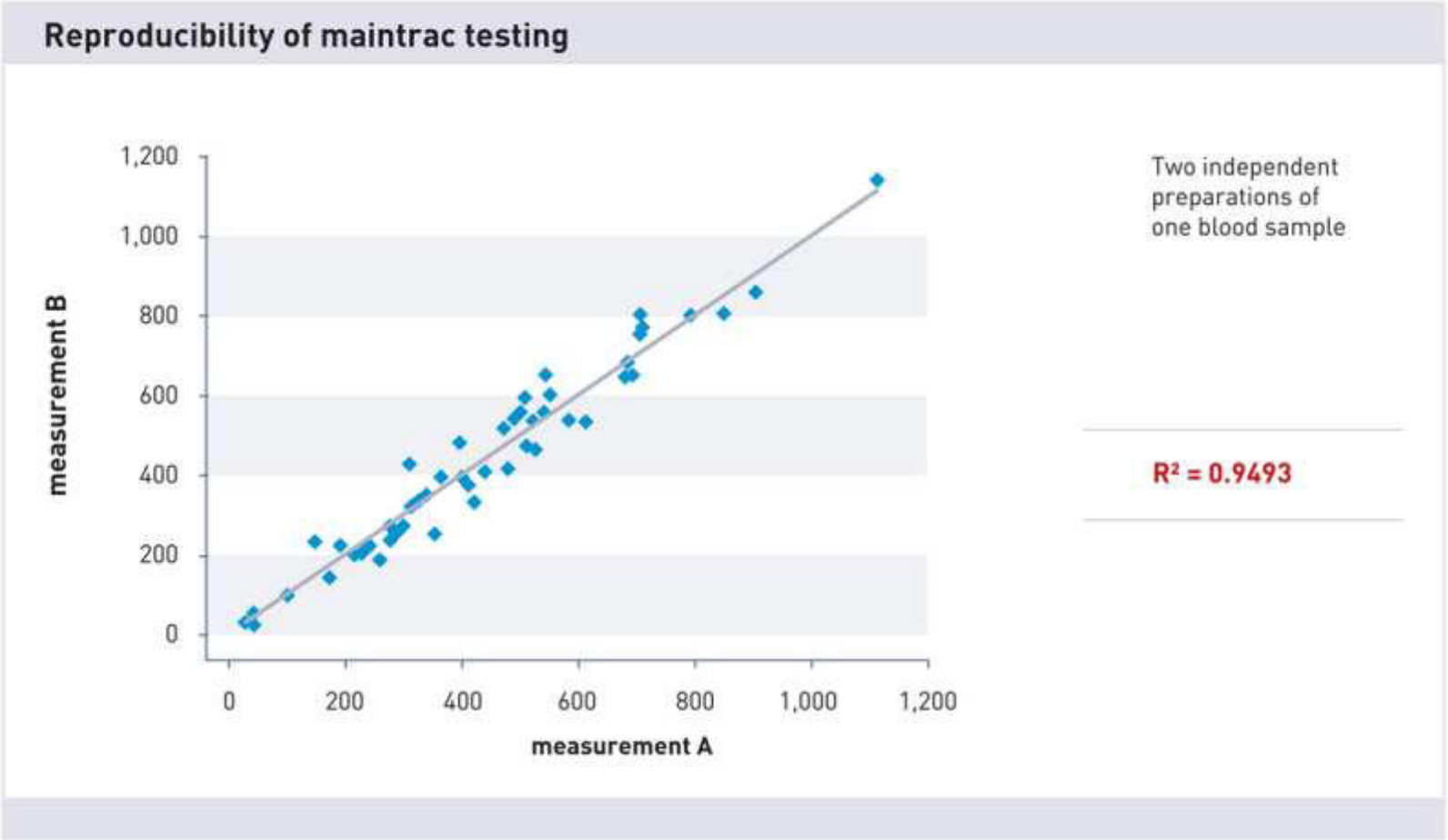
Counterstaining Cytokeratin/EpCAM



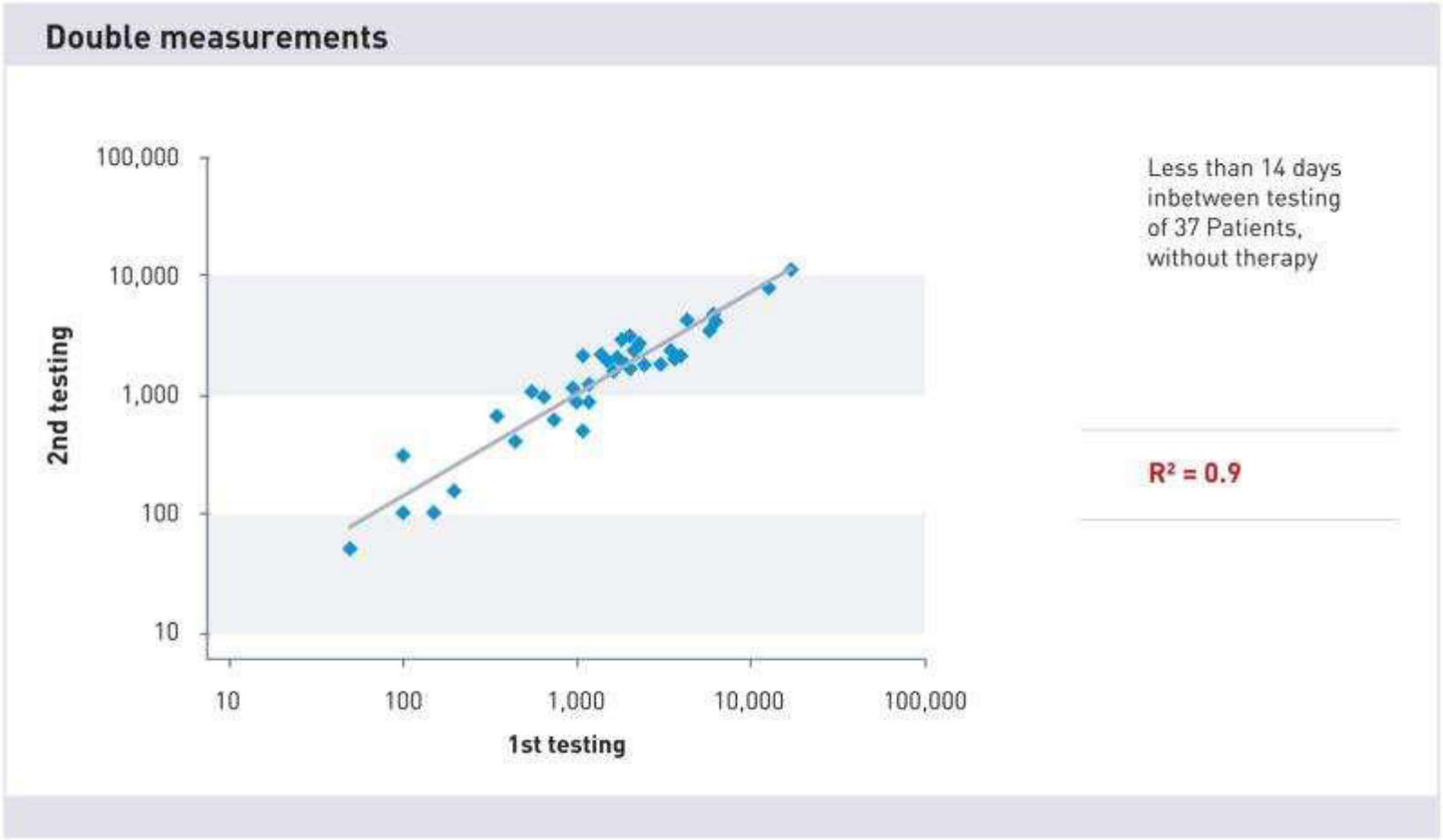
Spiking tests



Duplicate analyses from one blood sample in 80 patients



Two analyses from the same patient less than 2 weeks apart



Comparison with other methods

How frequent are tumour cells in blood?

Cellular components	Per ml of blood
Erythrocytes	4.5 - 5.5 billion
Leukocytes	4 - 11 million
Neutrophils	2.5 - 7.5 million
Eosinophils	40,000 - 400,000
Basophils	10,000 - 100,000
Lymphocytes	1.5 - 3.5 million
Monocytes	200,000 - 800,000
Thrombocytes	300 million
Circulating tumour cells	10 – 100,000

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Other CTC technologies

Technique	Problems	Disadvantage
Magnetic bead enrichment (e.g. Cellsearch)	Is EpCam expression sufficent for enrichment?	- Cell loss - Low antigen expression
Microfiltration (e.g. ISET)	Are all circulating tumour cells larger than blood cells?	- Cell loss - Small tumour cells not found
Negative depletion (e.g. RGCC)	Are all circulating tumour cells CD45 negative?	- Cell loss - False negative
Adhesion to micropoles	Technical problems?	

Molecular CTC expression analyses

Method	Problems
Enrichment via Ficoll	Red blood cell lysis can compromise PCR amplification, but most of the tumour cells are lost via the Ficoll technique
None of the enrichment methods is able to maintain **pure cell populations**	mRNA expression of other cells may distort the results
cDNA	The mRNA in the cells has to be translated into complementary DNA. This is not uniformly possible across the entire genome
RT-PCR	RT-PCR (reverse transcription polymerase chain reaction) varies for different gene segments

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CETC comparison with ctDNA

Technique	Problems
Isolation from plasma	DNA derived from destroyed cells.
Derived from dead cells	Stability of tumour DNA
Mutation analysis	Additional mutations due to DNA degradation

Fully accredited laboratory

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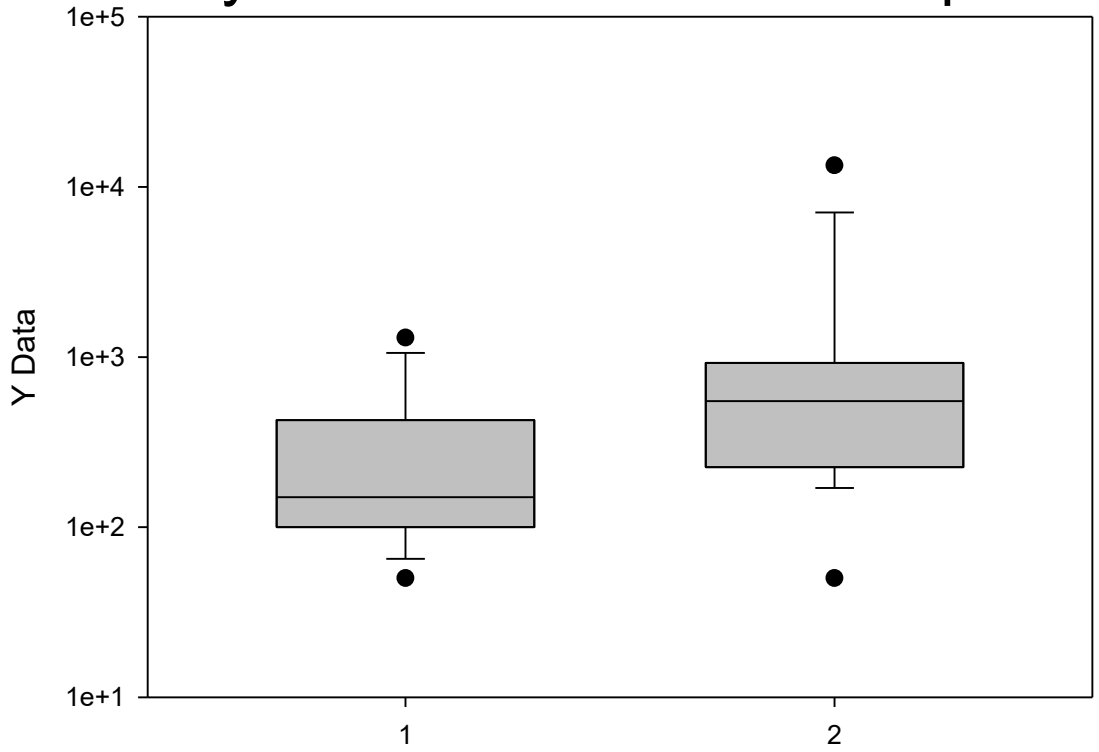
Screening
Screening individuals at risk

- Patients must be aware of the problematic issues
- Increasing numbers of circulating suspect cells over time might trigger additional tests (imaging)
- Only when sufficiently discussed with a caring physician

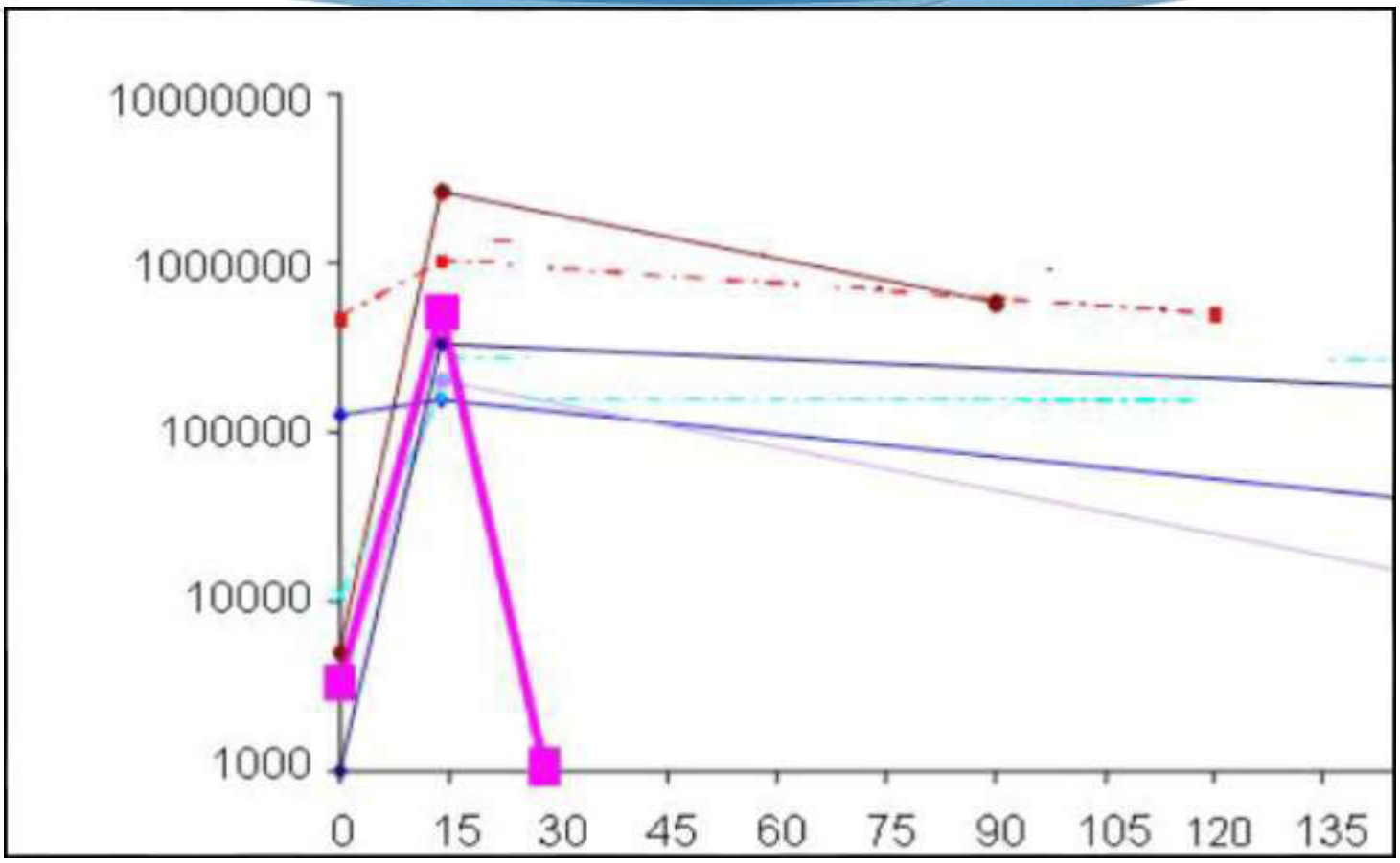
Screening individuals at risk

Male individuals above 65 years of age with repeatedly detected high numbers of circulating epithelial cells have a higher probability of detection of low risk prostate cancer



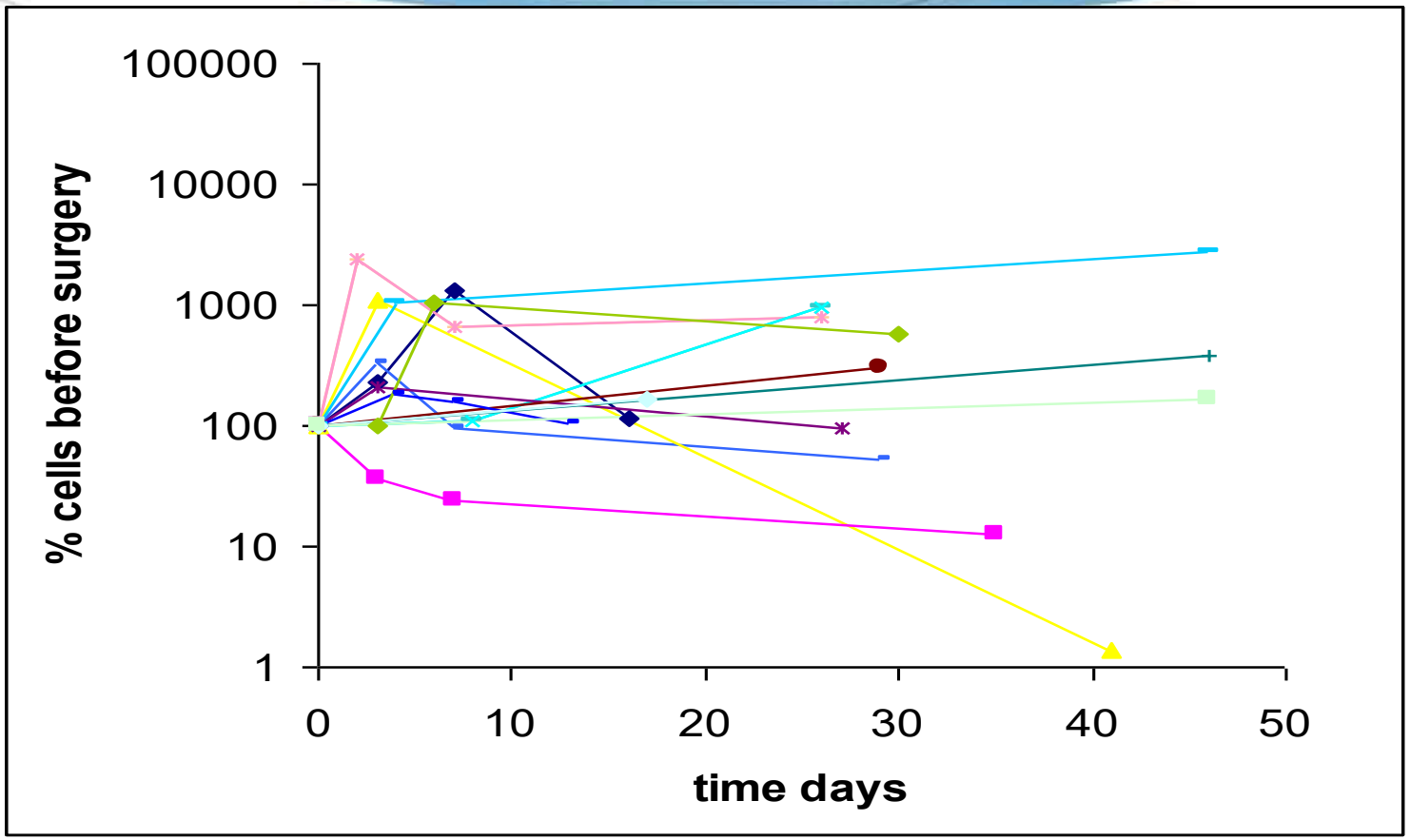
Monitoring therapy using circulating tumour cells

Patterns of CETCs before and after surgery (lung)

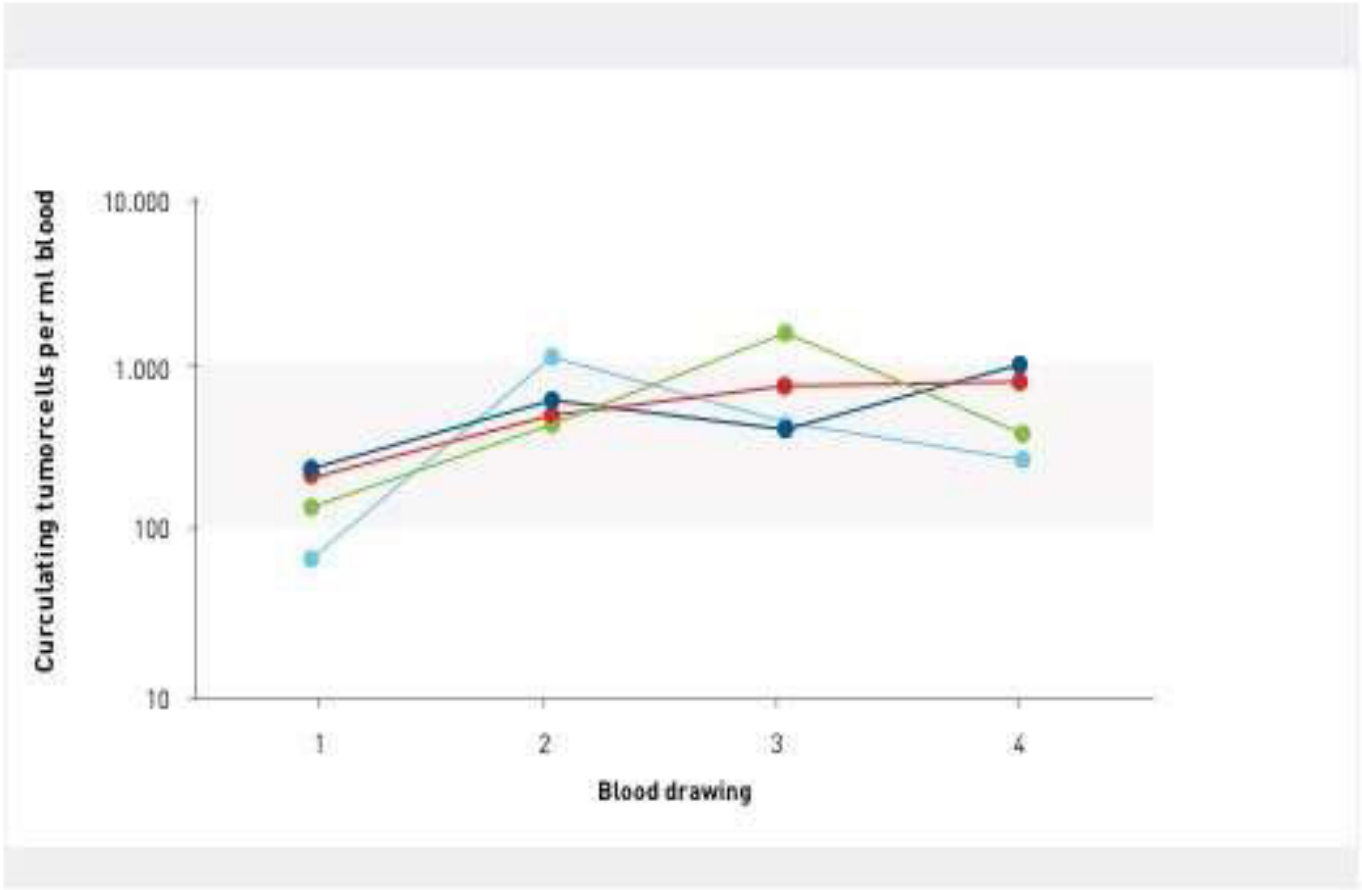


A. Rolle et al, World Journal surgical Oncology 2005, 3:18

Patterns of CETCs before and after surgery (breast)



Patterns of CETCs before and after surgery (colon)



before 4days 15days 6 weeks

UICC-Stadium I UICC-Stadium II UICC-Stadium III UICC-Stadium IV

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Neoadjuvant treatment

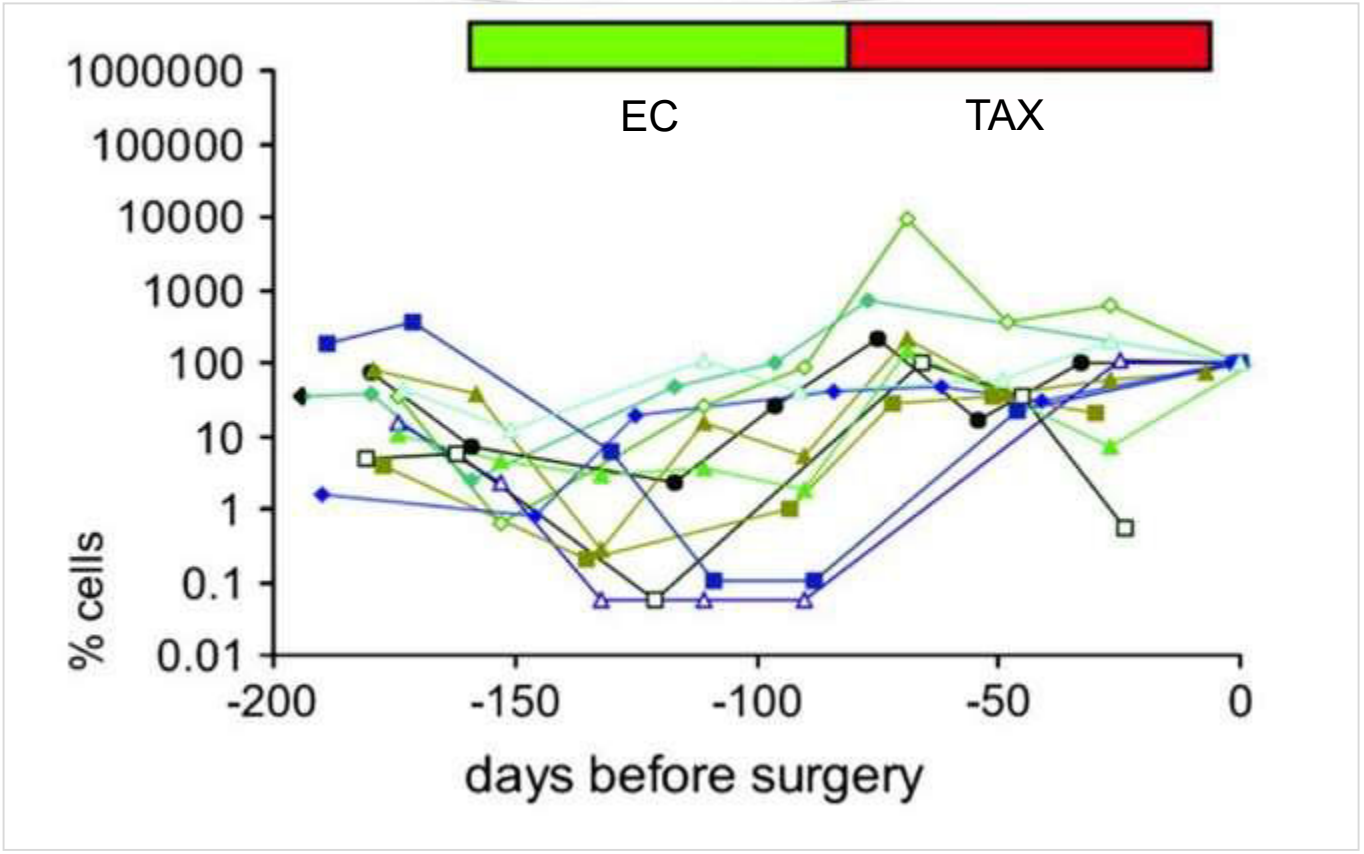
Neoadjuvant treatment: Background

- Neoadjuvant treatment was initially used in inoperable tumours to reduce the size of the tumour to make it operable
- It was hypothesized that overall survival would be improved via neoadjuvant chemotherapy by simultaneously eliminating minimal residual disease

Neoadjuvant treatment: Background

- Increase in complete eradication of the tumour from the tumour bed (pathologic complete response – pCR) using different combination therapies was assumed to improve outcome
- However, improvements in pCR were not associated with similar improvements in overall survival (OS), suggesting that neoadjuvant chemotherapy outcomes are not an appropriate surrogate for
long-term outcome

Neoadjuvant treatment

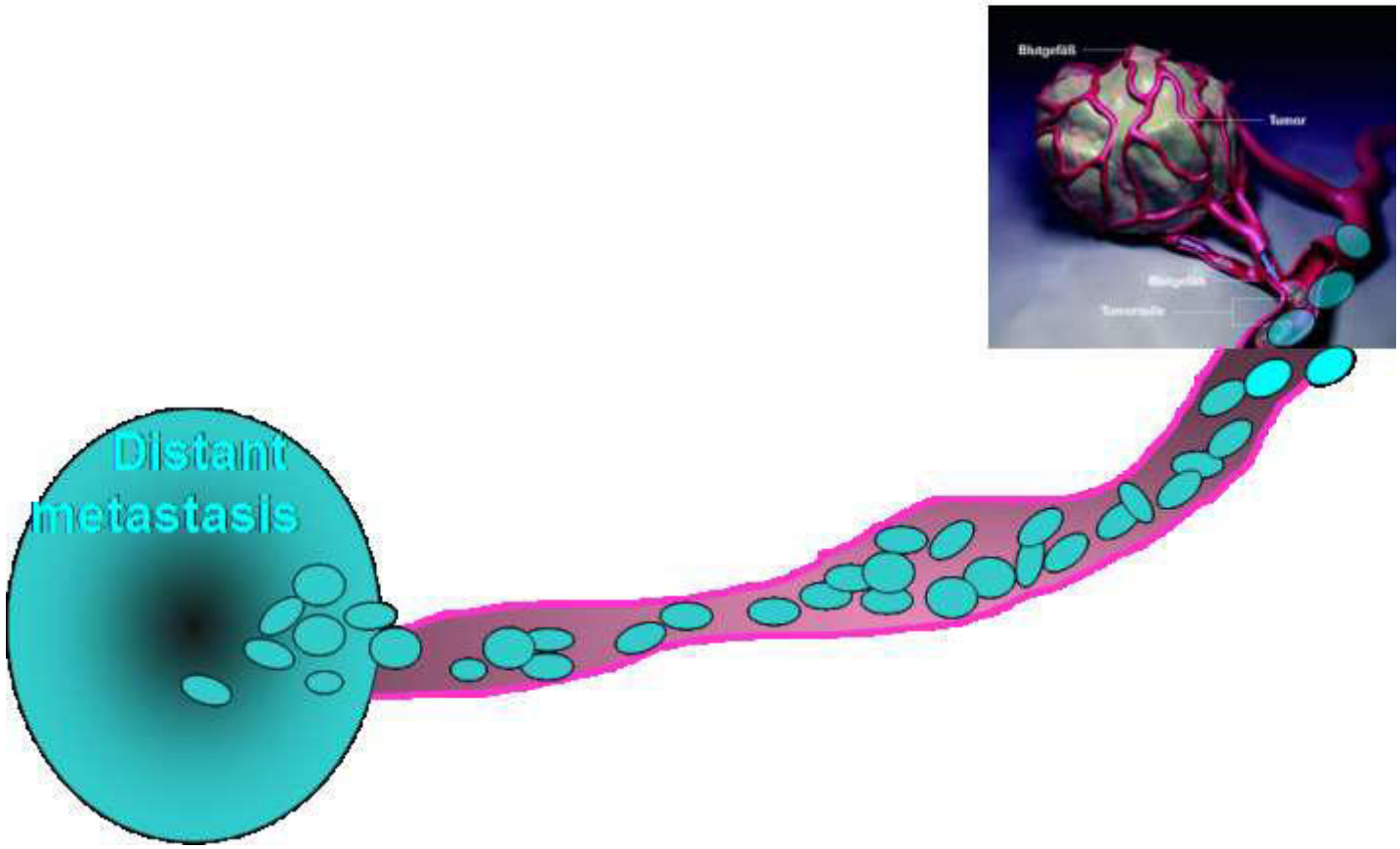


NB: At the end of neoadjuvant therapy almost all patients experience increasing CECT levels

This can be due to release of cells in addition to cell death during tumour shrinkage

O. Camara et al, Ann Oncol 2007 18:1484-92

Neoadjuvant chemotherapy shrinks the tumour, seeding cells into blood

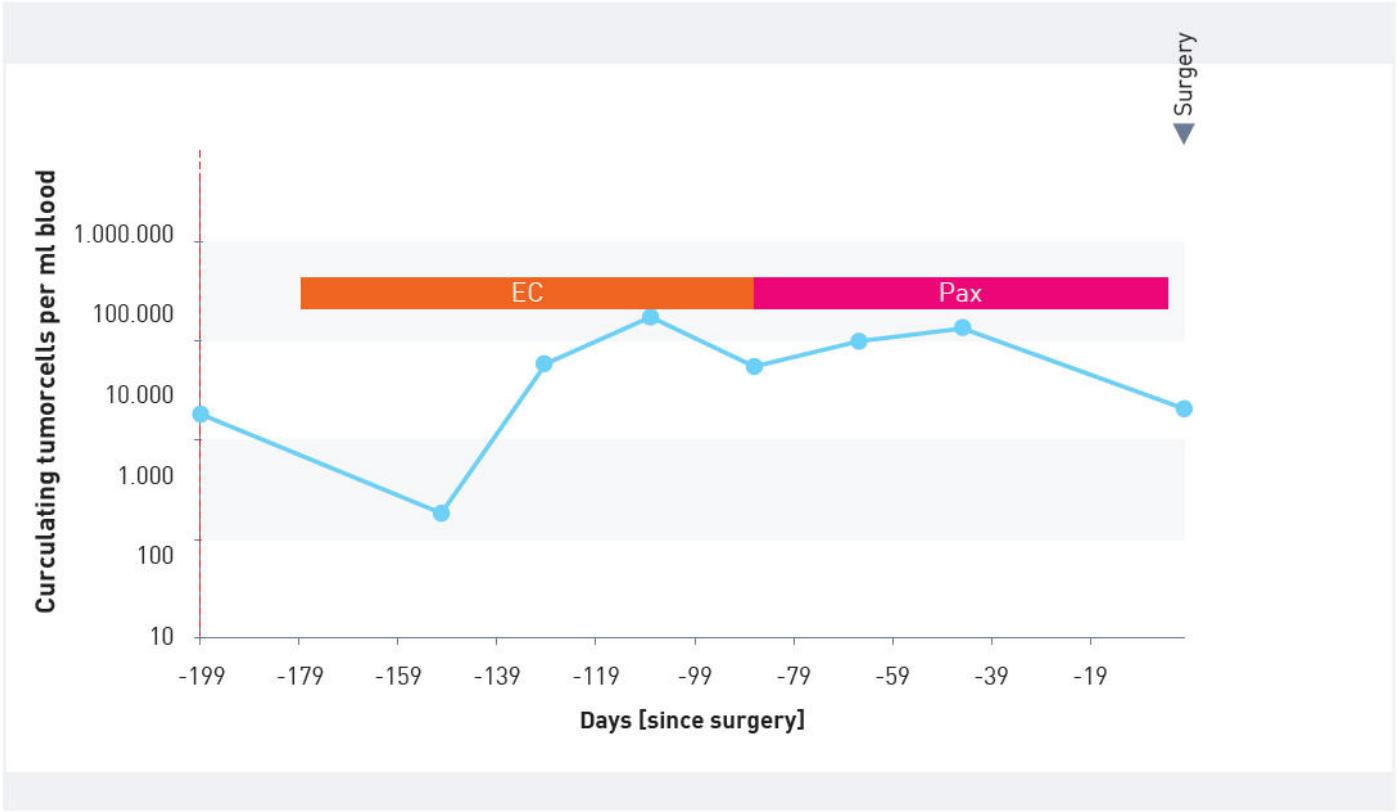


Neoadjuvant treatment

Increasing CETC levels at the end of therapy

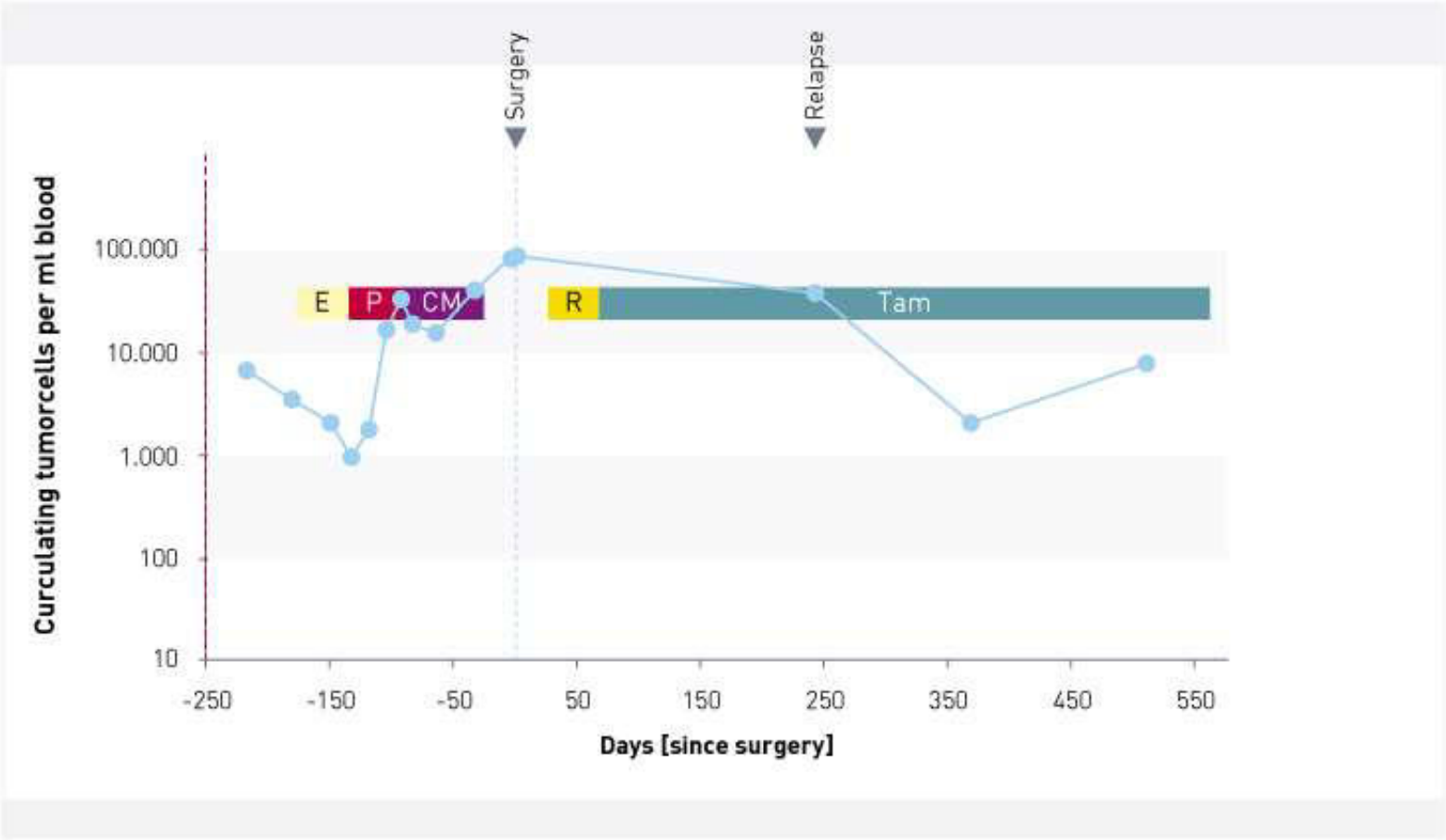
Neoadjuvant treatment

Decreasing numbers of cells at the end of therapy



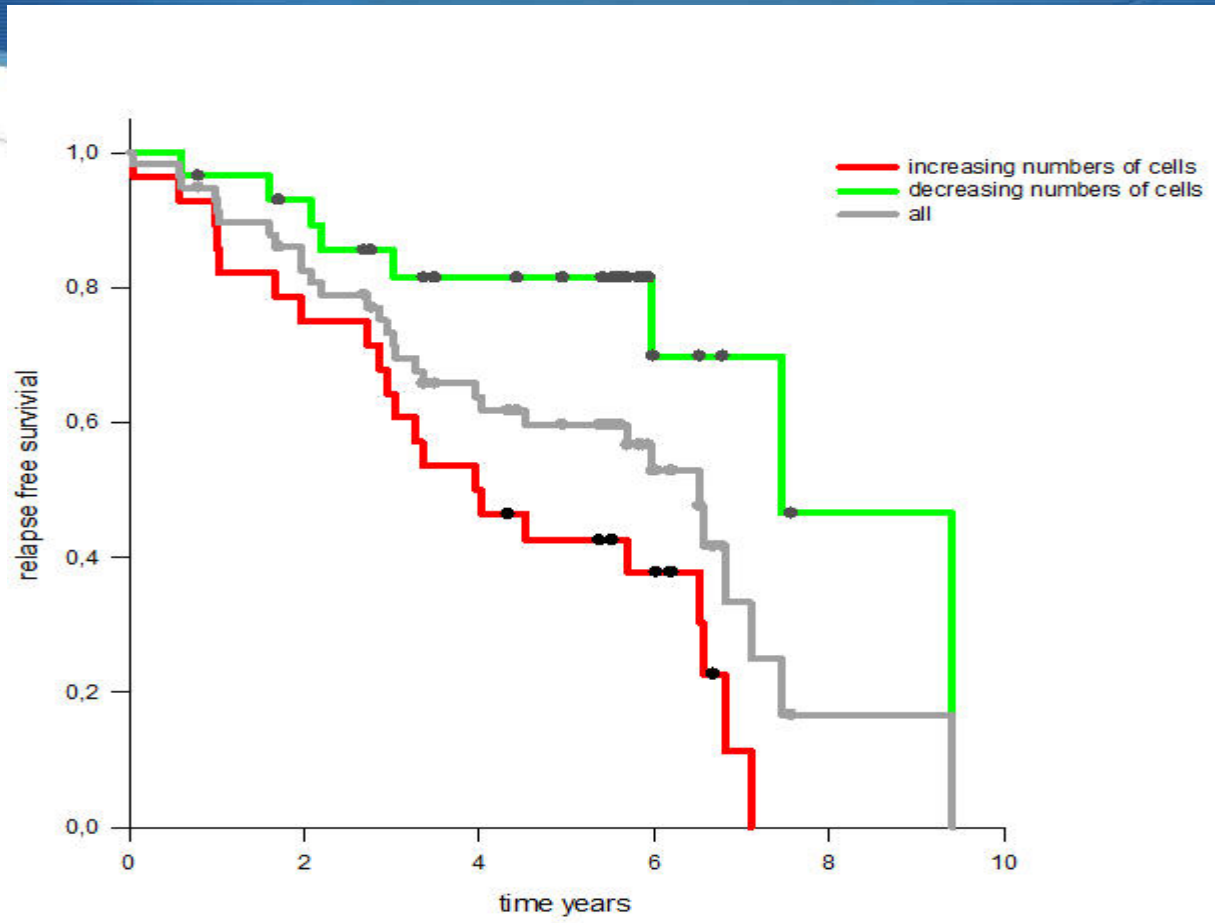
Neoadjuvant treatment

Typical course of disease



Kaplan-Meyer survival results

relevance of circulating tumour cells during neoadjuvant therapy



total number of patients = 59
patients with increasing numbers of cells = 28; relapses = 21
patients with decreasing numbers of cells =30; relapses = 8

P Value =0,006

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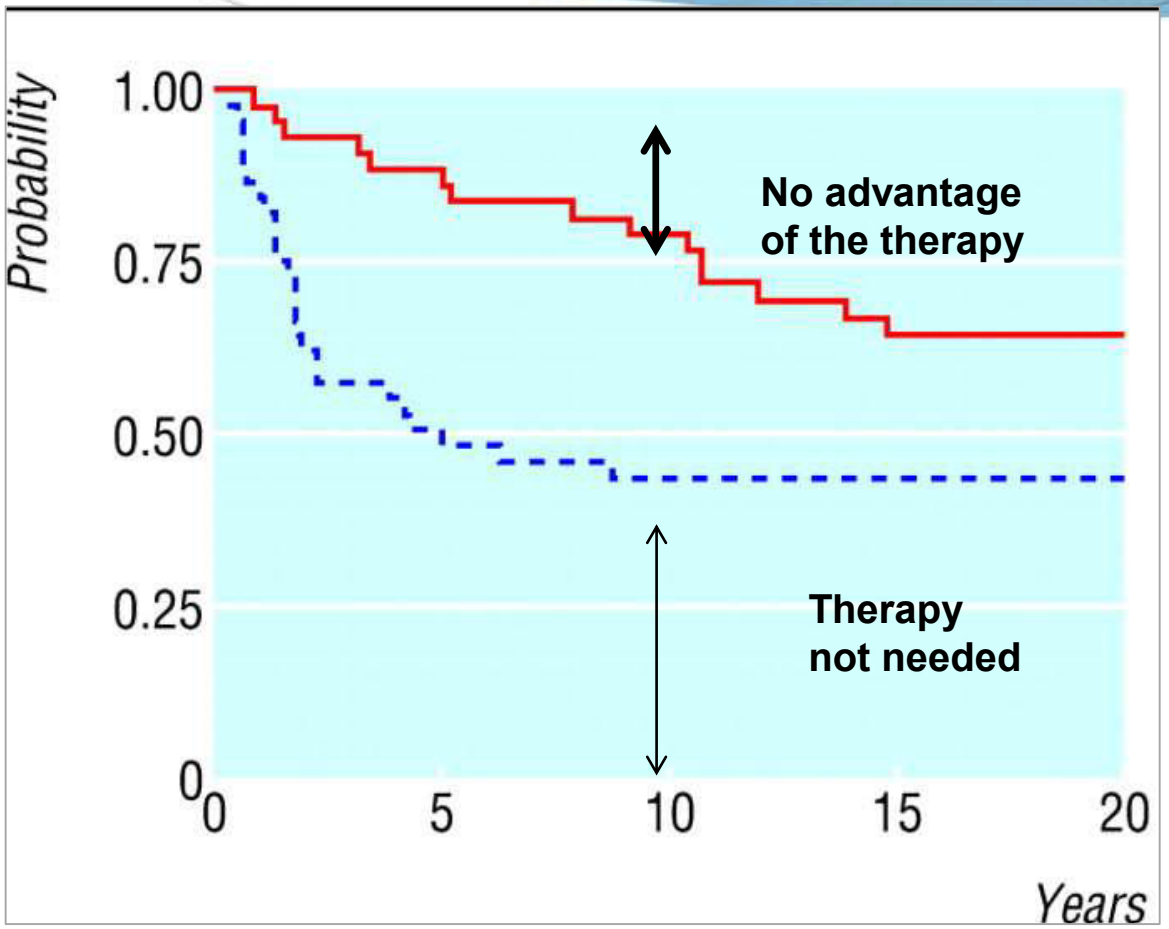
Adjuvant treatment using allopathic agents

Adjuvant treatment: Background

- **Systemic adjuvant therapy was established to eliminate the cells remaining in the body after surgery**
- We **count the changes** in numbers of these cells in **response to therapy**

30 years of adjuvant CMF* therapy



- Relapse-free survival
- Lymph node negative, ER-negative patients

* Cyclophosphamide, methotrexate and fluorouracil

G. Bonnadonna et al, BMJ 2005; 330:217

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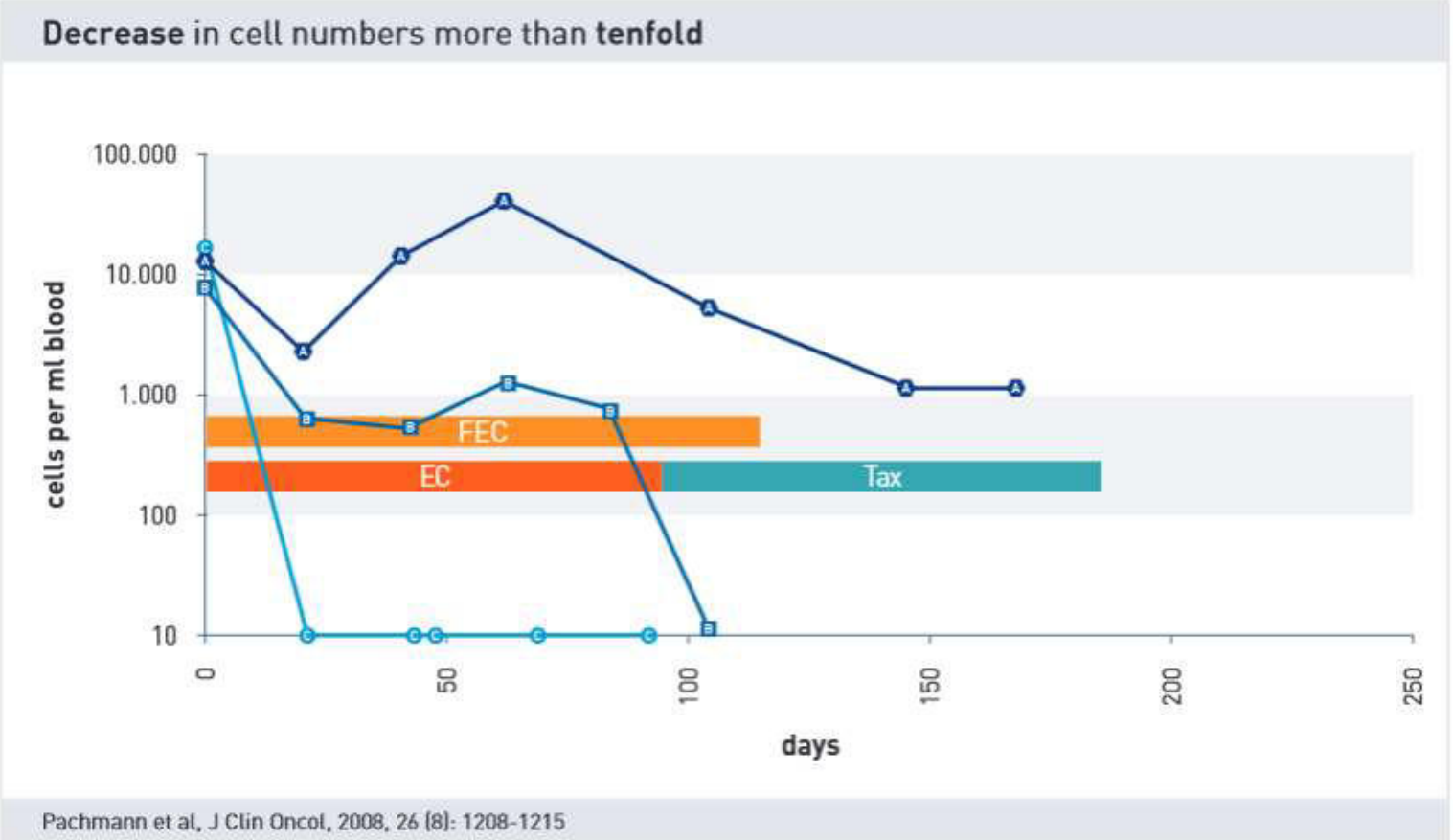
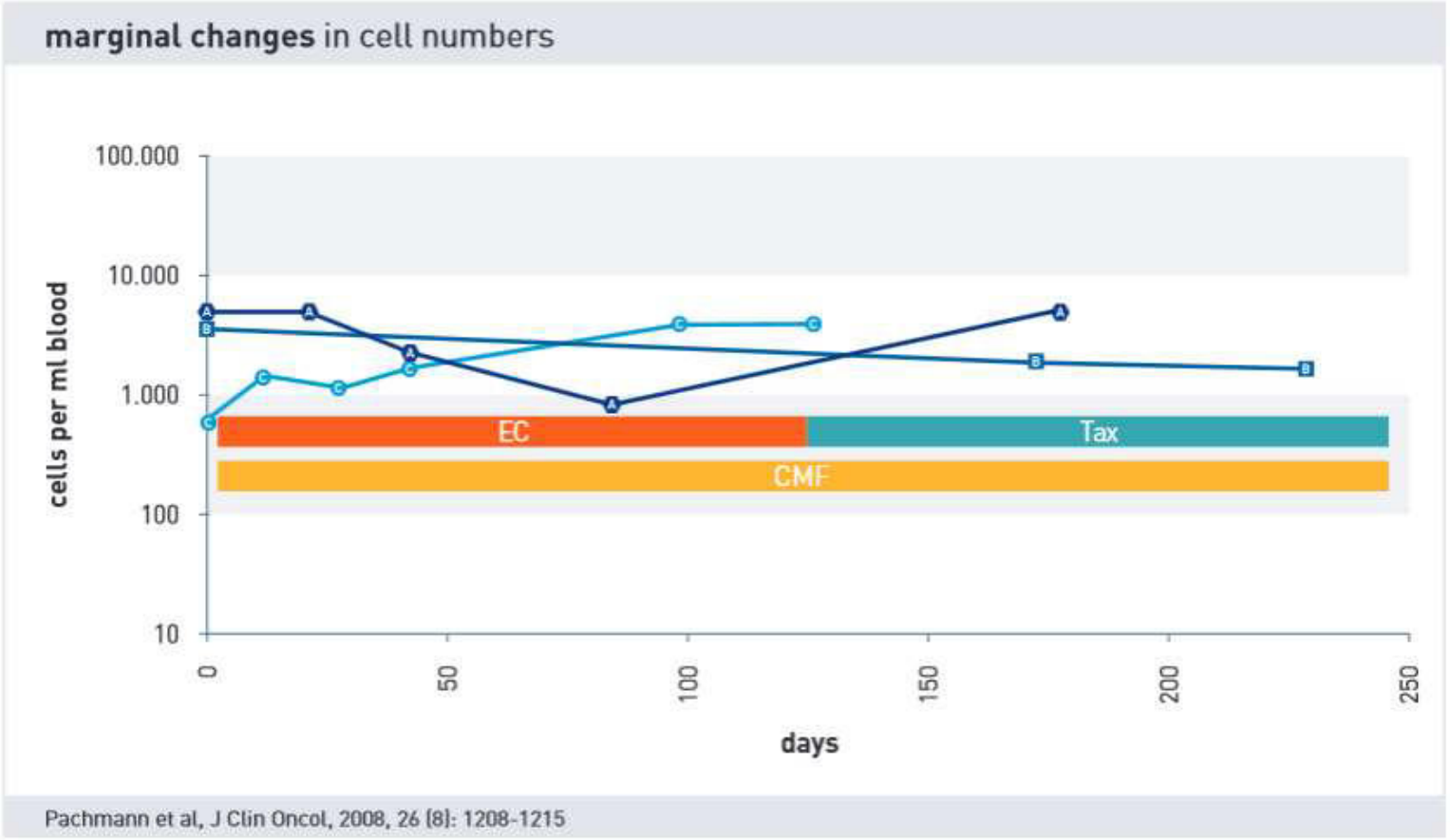
Adjuvant treatment

Decreasing cell numbers



Adjuvant treatment
marginal change in cell numbers

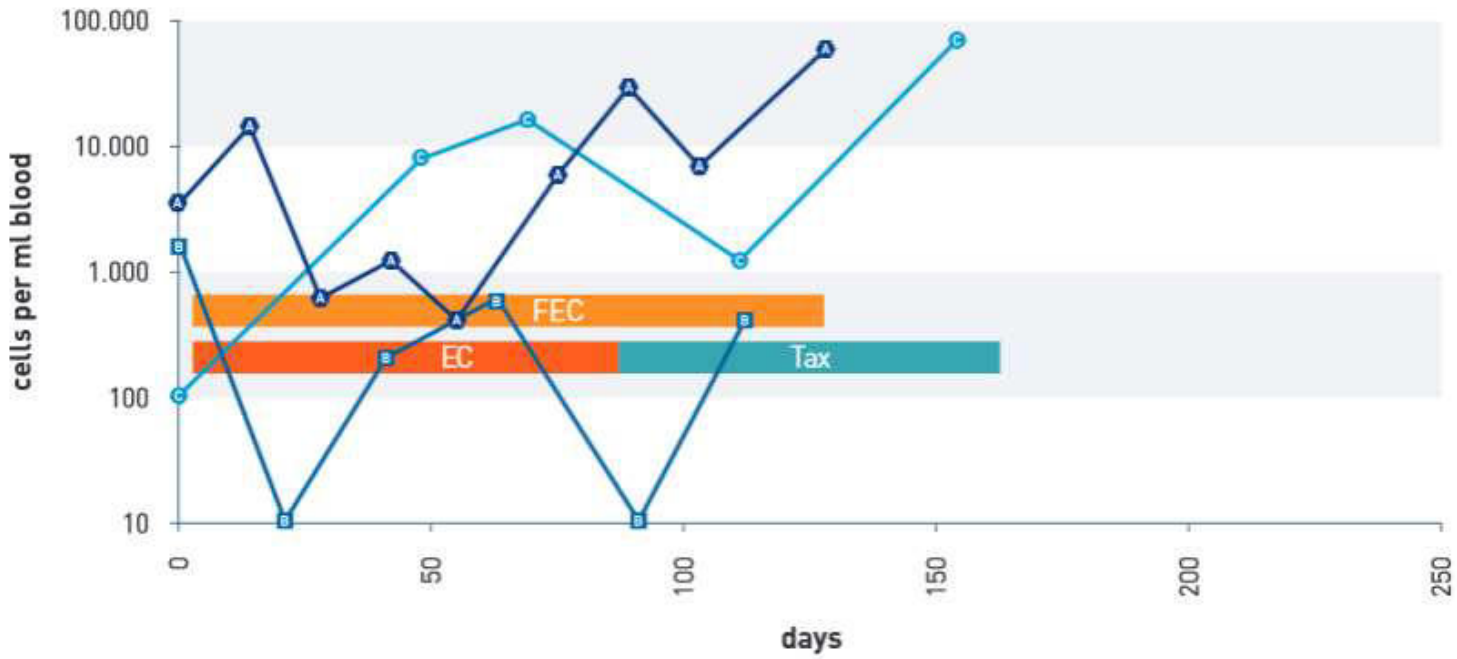


Pachmann et al, J Clin Oncol, 2008, 26 (8): 1208-1215

Adjuvant treatment

Increasing cell numbers

Increase in cell numbers more than **tenfold**



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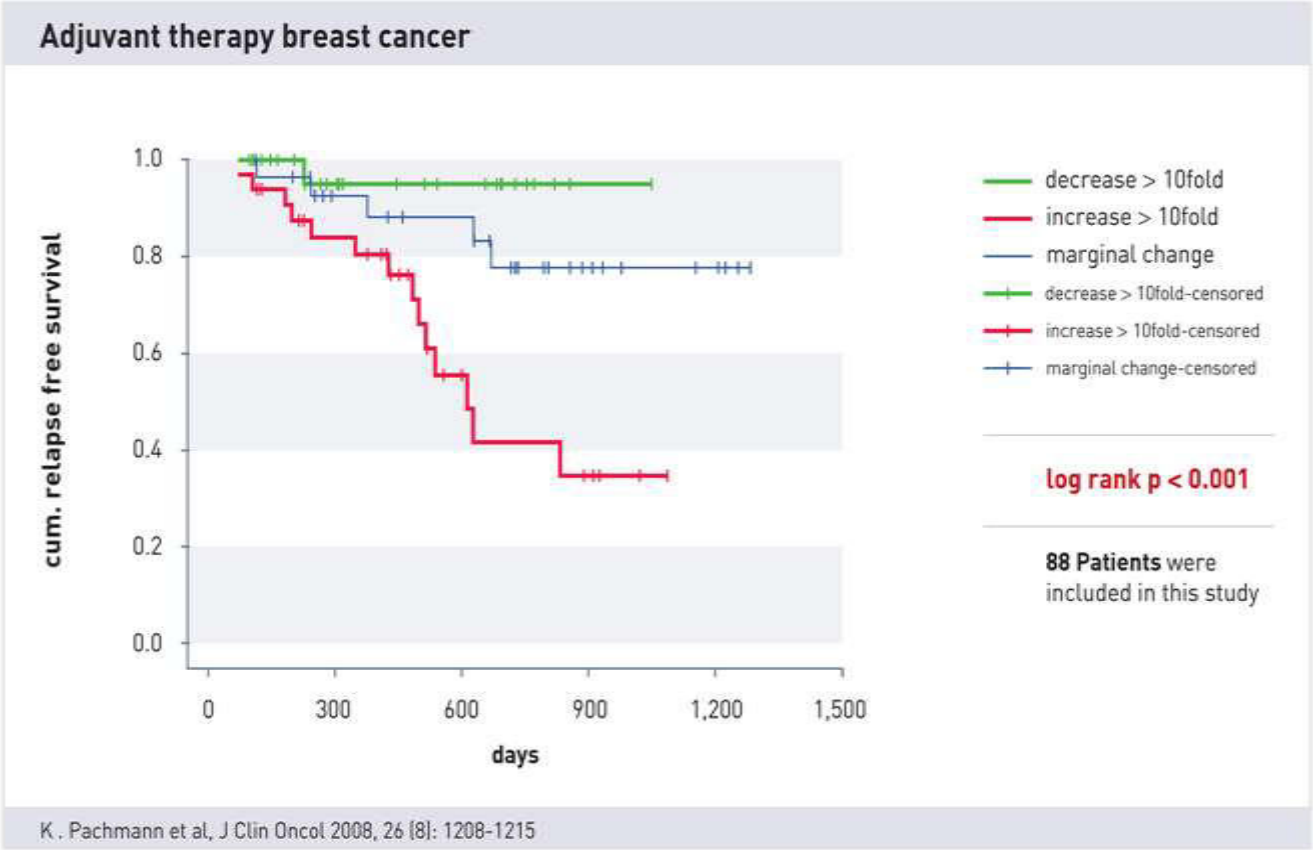
Adjuvant treatment



Increasing cell numbers correlate highly significantly with a **poor prognosis**

K . Pachmann, et al. J Clin Oncol 2008, 26 (8): 1208-1215

Chemo-sensitivity

J Cancer Therapy 2013, 4:597-605

Chemosensitivity Testing of Circulating Epithelial tumour Cells (CETC) in Vitro: Correlation with in Vivo Sensitivity and Clinical Outcome.

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[1], Matthias Rengsberger[3], Ingo B. Runnebaum[4], Ulrich Pachmann[2], Katharina Pachmann[2,5*]

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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.

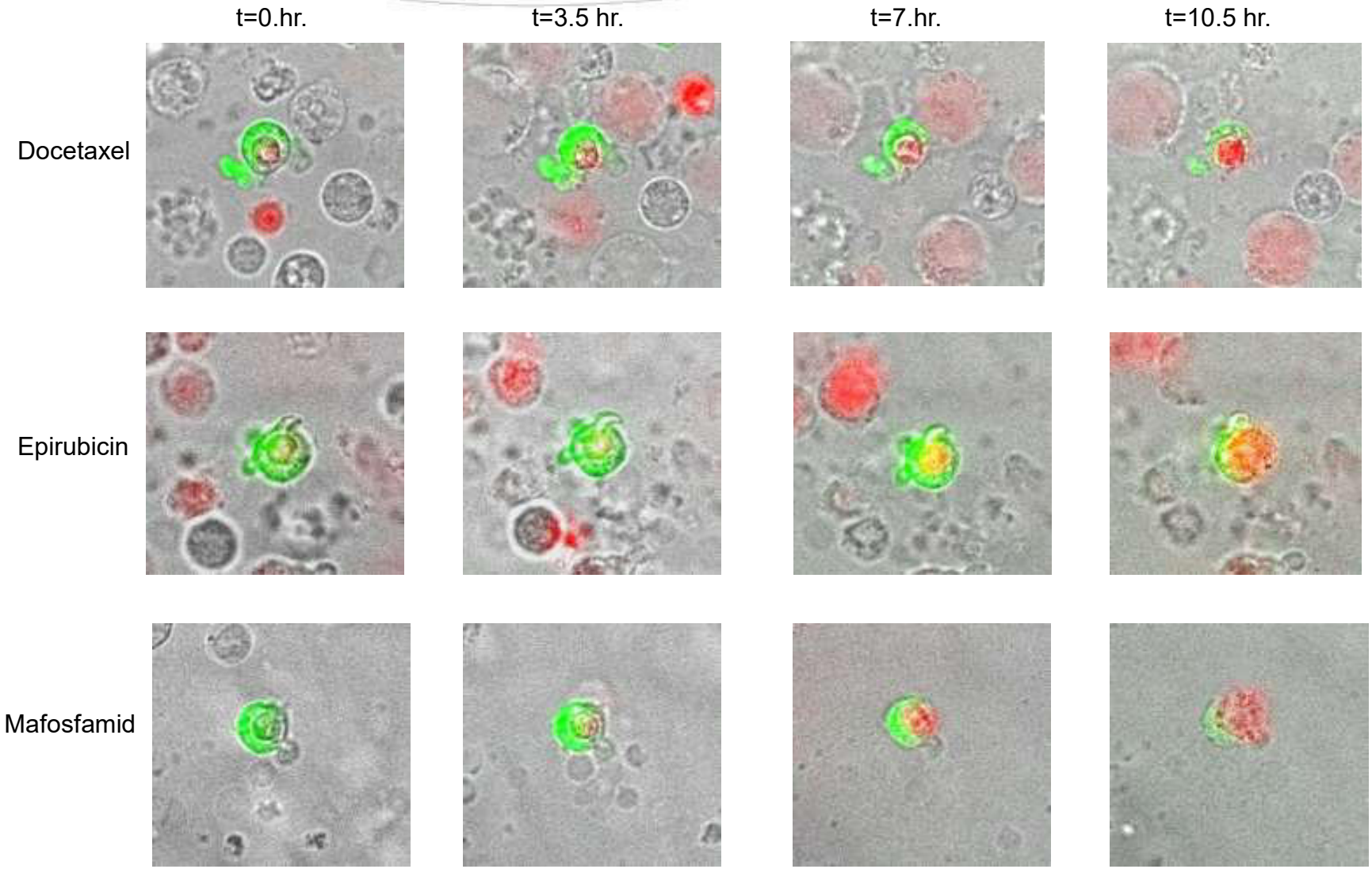
Keywords: Circulating Epithelial Tumor Cells; Chemosensitivity Testing; Breast Cancer; Ovarian Cancer

1. Introduction

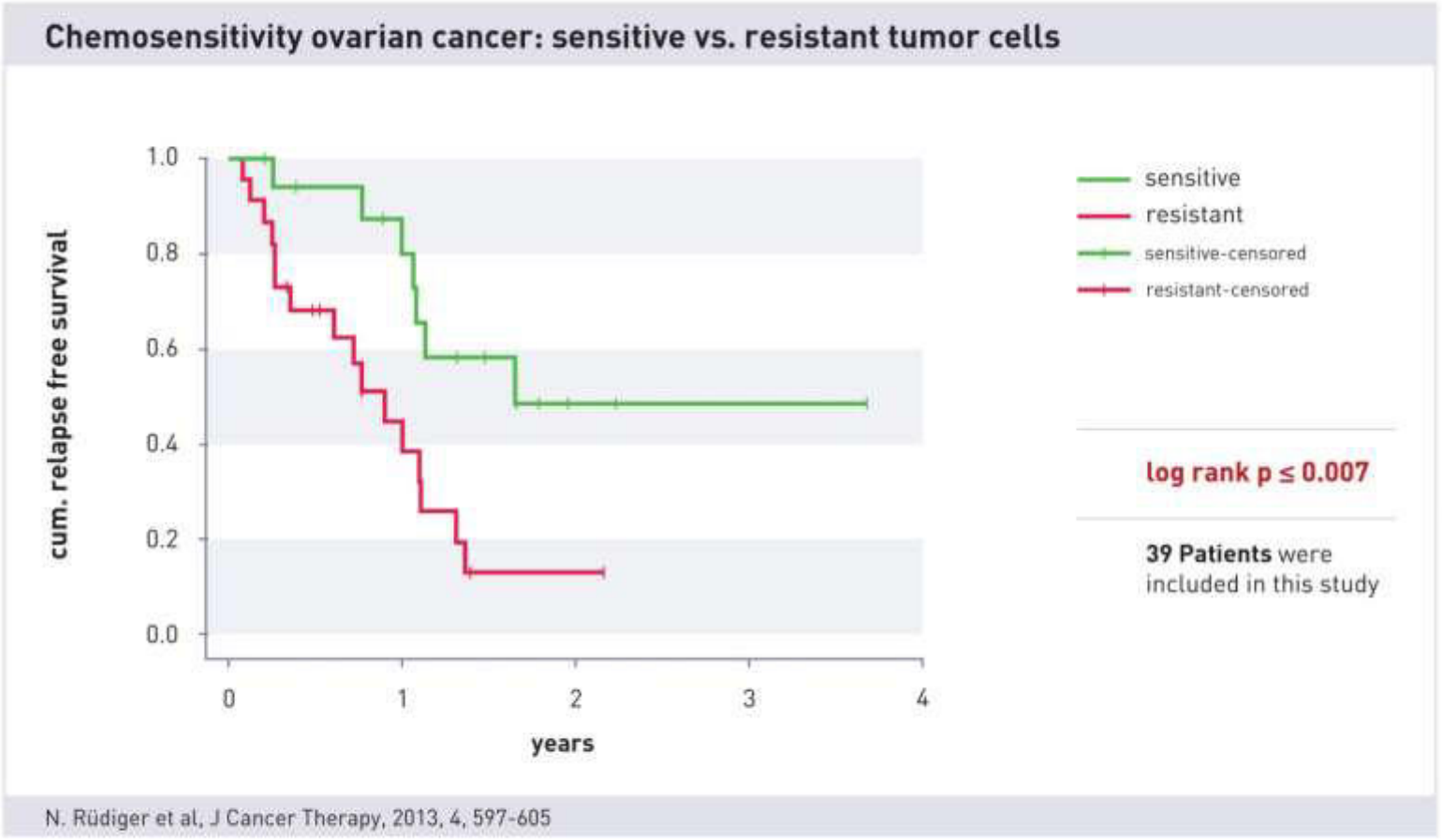
For patients diagnosed with a malignant tumor, cure is presumably only possible if the tumor is completely eradicated. Initially, the main aim is to eliminate the primary tumor, the major tumor burden, preferentially by surgery. However, most cancer patients do not die from their primary tumor but from 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.

Cell decay

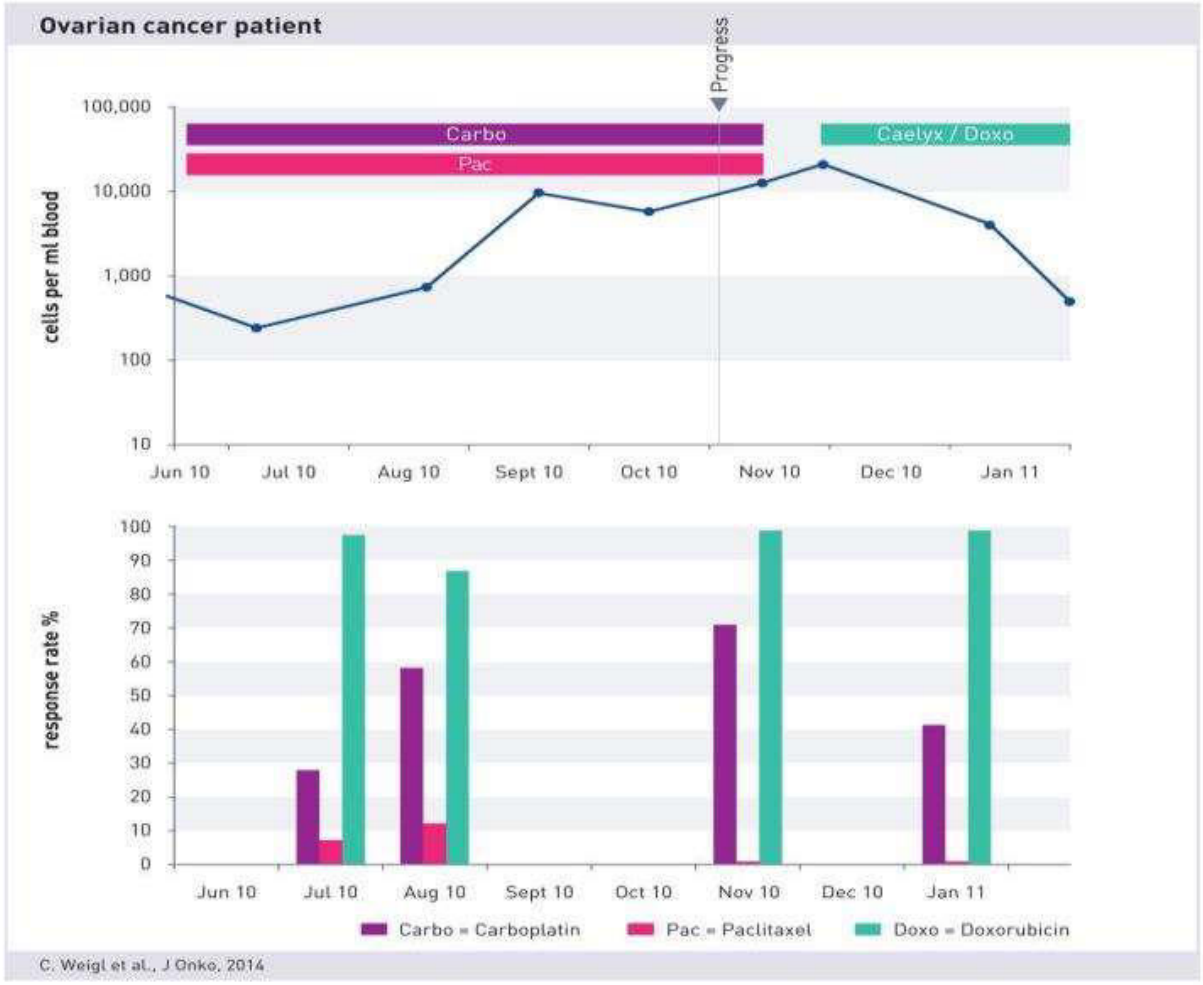


Pilot Study: Relapse-free survival of patients with ovarian carcinoma patients with sensitive vs. resistant CETCs



Case report: Ovarian carcinoma

Resistance to guideline drugs with progress, sensitivity to second-line drug



Case report breast cancer

Increasing resistance to drugs

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Adjuvant therapy using natural agents

No other lab has comparable flexibility

- Test natural agents for their cytotoxicity against your patient's own cancer cells
- Send in your own selection of agents (small sample required)
- And/or select from our list of suggestions
- Test the same agent as an infusion and an oral supplement – often very different results
- Test mixtures in one formula – you choose the combination

Natural agents suggested by maintrac

- ○ Helixor A ; M ; P

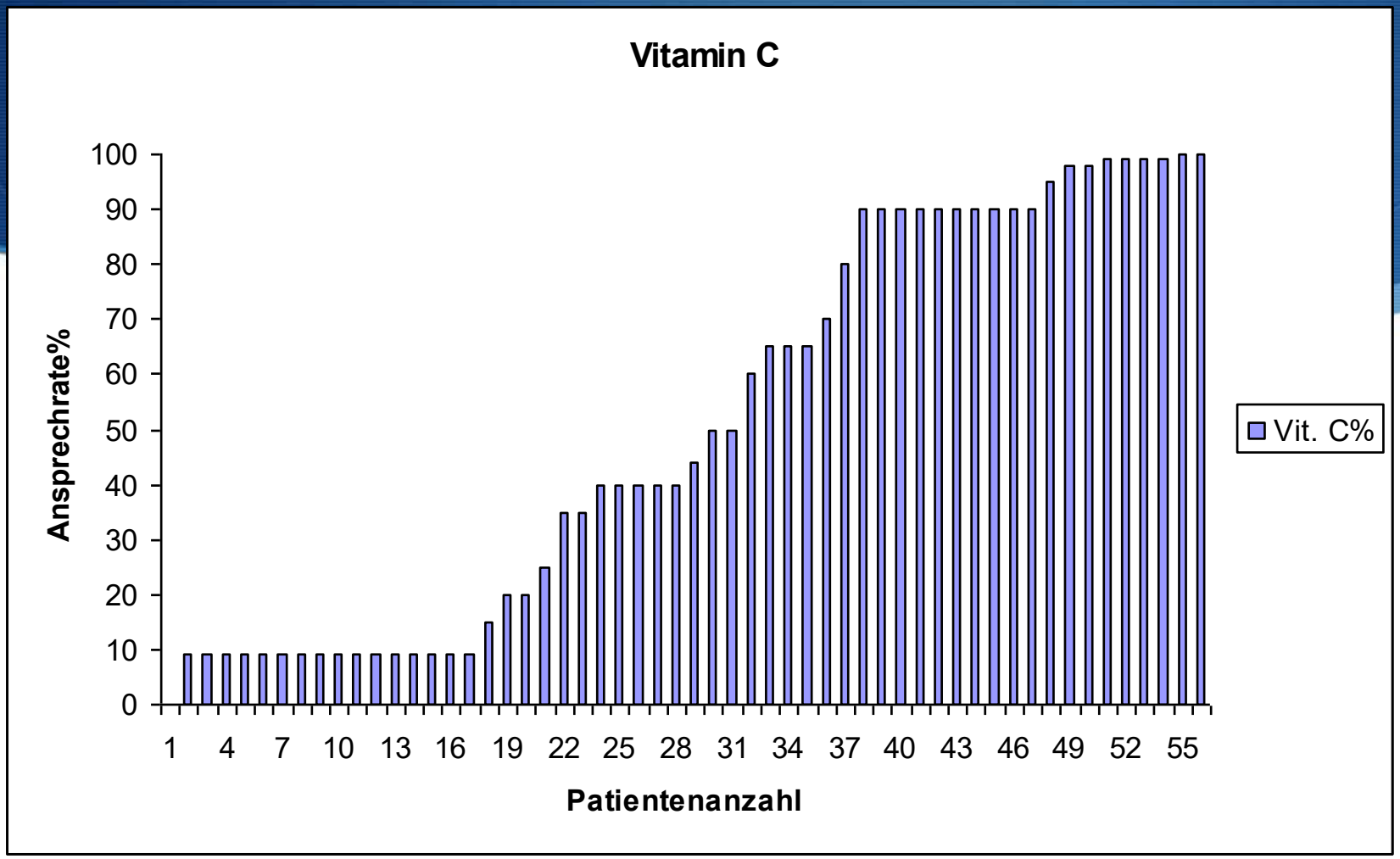
Please name manufacturer:

daily dose

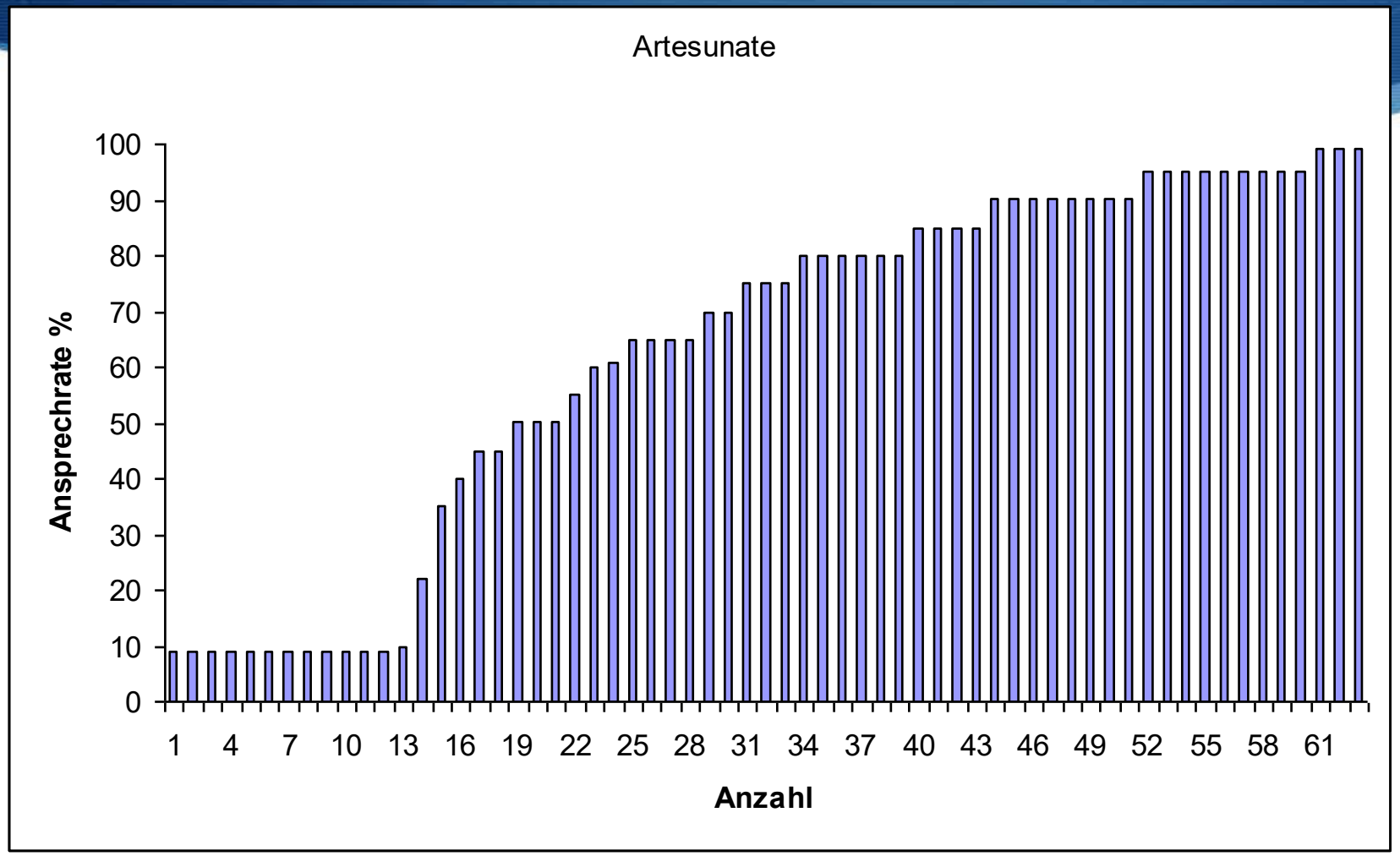
- ○ Vitamin C
- ○ Graviola
- ○ Iscador M; Q; U; P
- ○ DCA (Dichloracetat)
- ○ Amygdalin
- ○ Sulforaphan
- ○ Hypericin
- ○ Curcumin
- ○ Artesunat

- ○ Further substances:

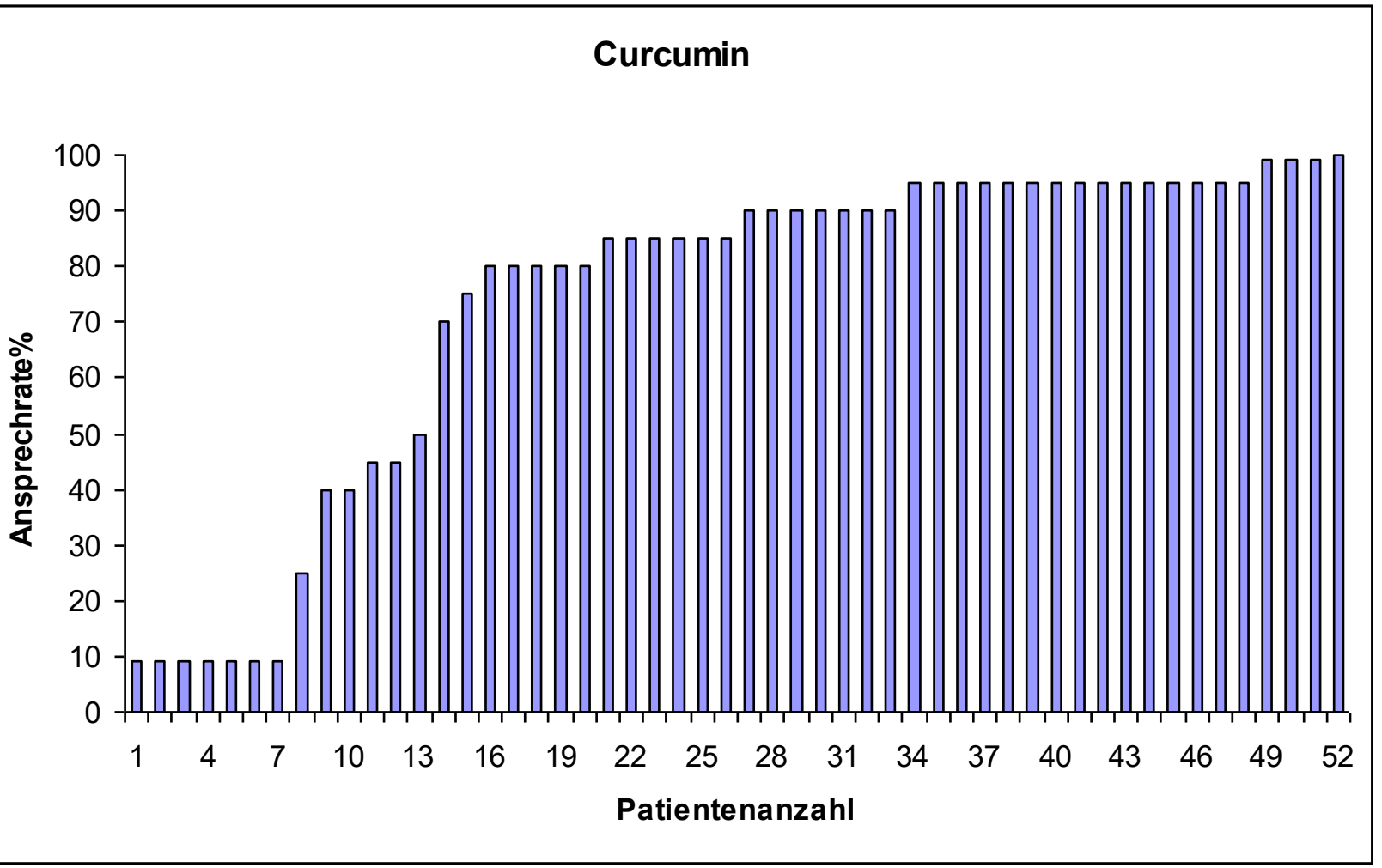
- ○ Combination testing:



Patients total: 56		
Sensitivity > 50%	25 Patients	45%
Sensitivity < 50%	31 Patients	55%



Patients total: 63		
Sensitivity > 50%	42 Patients	67%
Sensitivity < 50%	21 Patients	33%



Patients total: 52		
Sensitivity > 50%	39 Patients	75%
Sensitivity < 50%	13 Patients	25%

Dr. med. Ulrich Pachmann
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Email: mail@laborpachmann.de

DAkkS Deutsche Akkreditierungsstelle D-ML-13345-01-00

Labor Dr. med. Ulrich Pachmann, Kontumazstraße 2, 95448 Bayreuth

Therapist

mail:

Bayreuth, 14.03.2017

Your patient:
Born:

Your request from: 08.03.2017
Our Lab number: T731890

Report on diagnostic findings on Circulating Tumor Cells (MAINTRAC)

Dear Dr.

Many thanks for sending your examination request regarding the detection of circulating tumor cells. After Therapy.

Diagnosis:
Colon Cancer, Initial diagnosis: 08/15

- 1.Therapy: Mexico, Oasis of Hope 3 visits
  Therapy: B17, Prosanalin, Xeloda, Curcumin
- 10/15-07/16: DCA, Vitamin C
- until: 10/16: Ozone, Boswellia, Hyperhtermia
- 11/16: Surgery (Removal of remaining tumor 5mm)

The automated microfluorimetric image analysis of the **epithelial cell adhesion molecule (EpCAM)**-positive cells with visual control (MAINTRAC) from **1 ml EDTA blood** resulted in following findings (detection limit is at 10 cells/ml):

	Number of potential tumor cells			
Examination parameter	In the sample (1ml)	In circulation (5l) (in millions)	In addit. examination: % of EpCAM-pos. cells	Cell fragments
EpCAM	**500**	**2,5**		numerous

in-vitro-vitality reduction in relation to concentration and time (in%) with eutherapeutic concentrations of				
Vitamin C	**70**	DCA	**60**	The ideal is a reduction by 100% in short-term cell culture
Amygdalin	**70**	Curcuma*	**40**	
Artesunat	**95**	Prosanalin*	**85**	
Boswellia*	**60**			

*provided by the patient

TZB Arbeitsgemeinschaft

Prioritisation of natural agents suggested by the results

The automated microfluorimetric image analysis of the **epithelial cell adhesion molecule (EpCAM)**-positive cells with visual control (MAINTRAC) from **1 ml EDTA blood** resulted in following findings (detection limit is at 10 cells/ml):

	Number of potential tumor cells			
Examination parameter	In the sample (1ml)	In circulation (5l) (in millions)	In addit examination: % of EpCAM-pos cells	Cell fragments
EpCAM	**500**	**2,5**		numerous

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Vitamin C	**70**	DCA	**60**	The ideal is a reduction by 100% in short-term cell culture
Amygdalin	**70**	Curcuma*	**40**	
Artesunat	**95**	Prosanalin*	**85**	
Boswellia*	**60**			

*provided by the patient

Curcumin and artemisia better than chemotherapeutic agents for this PX

Diagnosis:
Lung Cancer, initial diagnosis: 26.06.2017
TNM: T4 N3 M1b, Stage IV
- no Surgery
- no Radiation therapy
- post Complementary therapy
- no current therapy
- Medication: Herbal supplements

The automated microfluorimetric image analysis of the **epithelial cell adhesion molecule (EpCAM)**-positive cells with visual control (MAINTRAC) from **1 ml EDTA blood** resulted in following findings (detection limit is at 10 cells/ml):

	Number of potential tumor cells			
Examination parameter	In the sample (1ml)	In circulation (5l) (in millions)	In addit. examination: % of EpCAM-pos. cells	Cell fragments
EpCAM	**150**	**0,75**		numerous

in-vitro-vitality reduction in relation to concentration and time (in%) with eutherapeutic concentrations of				
Avastin	**20**	Alimta	**60**	The ideal is a reduction by 100% in short-term cell culture
Cisplatin	**65**	Vitamin C	**40**	
Curcumin	**90**	Artemisia	**80**	

The material for examination could be thoroughly evaluated.
Under Therapy with herbal supplements we found only **a slightly increased number of live, potentially malignant tumor cells circulating in the blood.**
In addition, there were numerous specific cell fragments detected.
Specific cell fragments occur, for example, after chemotherapy or radiation, or as part of an immune response and indicate damaged cells.

Combination of curcumin and hypericin come out at 85% in this case

The automated microfluorimetric image analysis of the **epithelial cell adhesion molecule (EpCAM)**-positive cells with visual control (MAINTRAC) from **1 ml EDTA blood** resulted in following findings (detection limit is at 10 cells/ml):

	Number of potential tumor cells			
Examination parameter	In the sample (1ml)	In circulation (5l) (in millions)	In addit. examination: % of EpCAM-pos. cells	Cell fragments
EpCAM	**450**	**2,25**		numerous

in-vitro-vitality reduction in relation to concentration and time (in%) with eutherapeutic concentrations of			
Curcumin/ Hypericin	**85**		The ideal is a reduction by 100% in short-term cell culture

The material for examination could be thoroughly evaluated.
After the recent surgery we found **a slightly to moderately increased number of live, potentially malignant tumor cells circulating in the blood.**
In addition, there were numerous specific cell fragments detected.
Specific cell fragments occur, for example, as part of an immune response and indicate damaged cells.

In vitro vitality reduction occurred at **Curcumin/Hypericin.**

In connection with a detected tumor the cells are most probably cells from this tumor.

The current cell numbers present a basic value, only an increase in cell numbers is relevant for disease progress.

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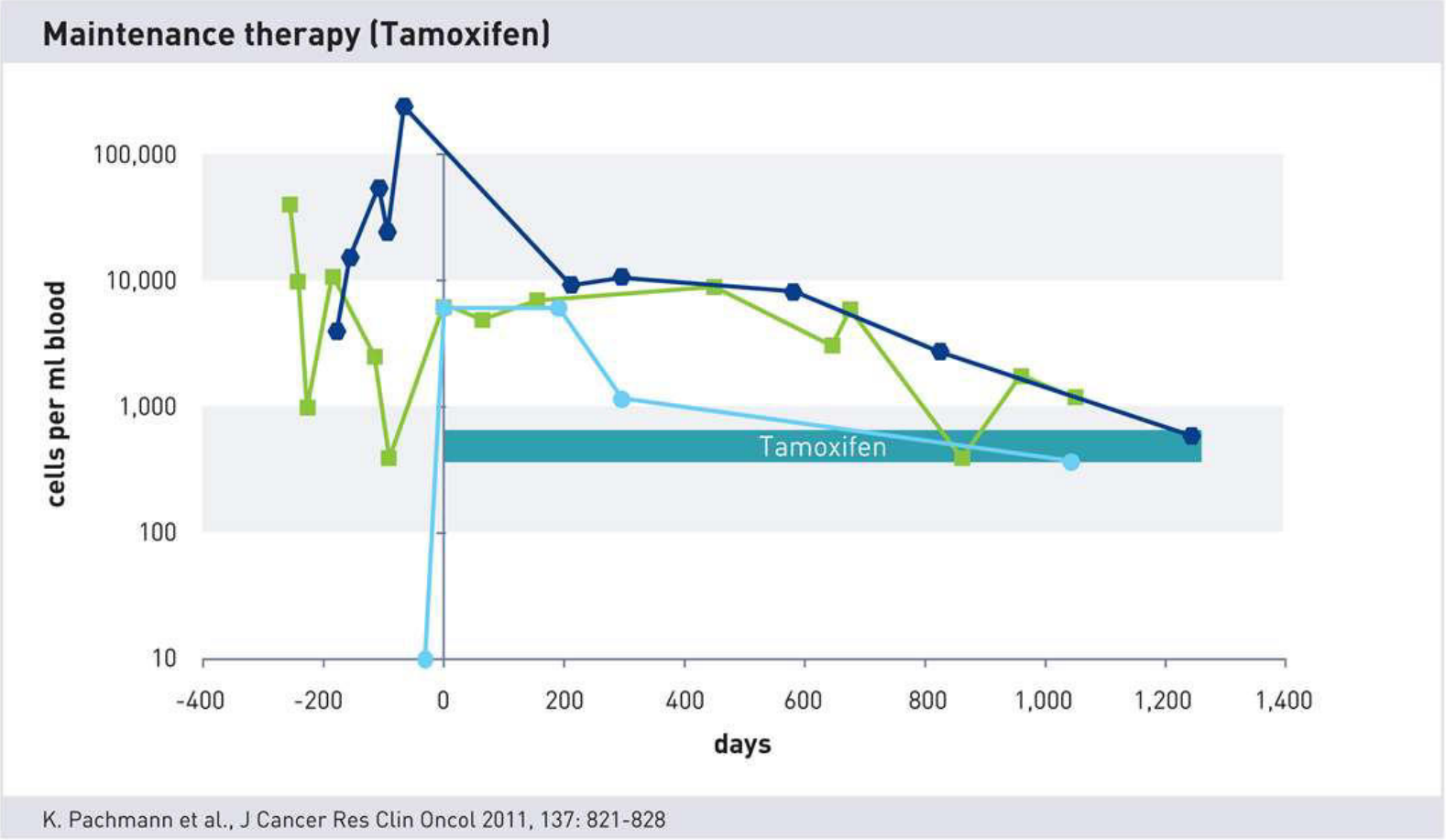
Results now available in three levels of concentration

in-vitro-vitality reduction in relation to concentration and time (in%) with eutherapeutic concentrations of The ideal is a reduction by 100% in short-term cell culture					
Quercetin 0,1-fold	**85**	Quercetin 1-fold	**90**	Quercetin 10-fold	**99**
Vitamin C 30g 0,1-fold	**55**	Vitamin C 30g 1-fold	**75**	Vitamin C 30g 10-fold	**90**
Artesmisinin 250mg 0,1-fold	**25**	Artesmisinin 250mg 1-fold	**90**	Artesmisinin 250mg 10-fold	**98**
Curcumin 450mg 0,1-fold	**n.a.**	Curcumin 450mg 1-fold	**90**	Curcumin 450mg 10-fold	**n.a.**

Maintenance therapy

Endocrine therapy breast cancer

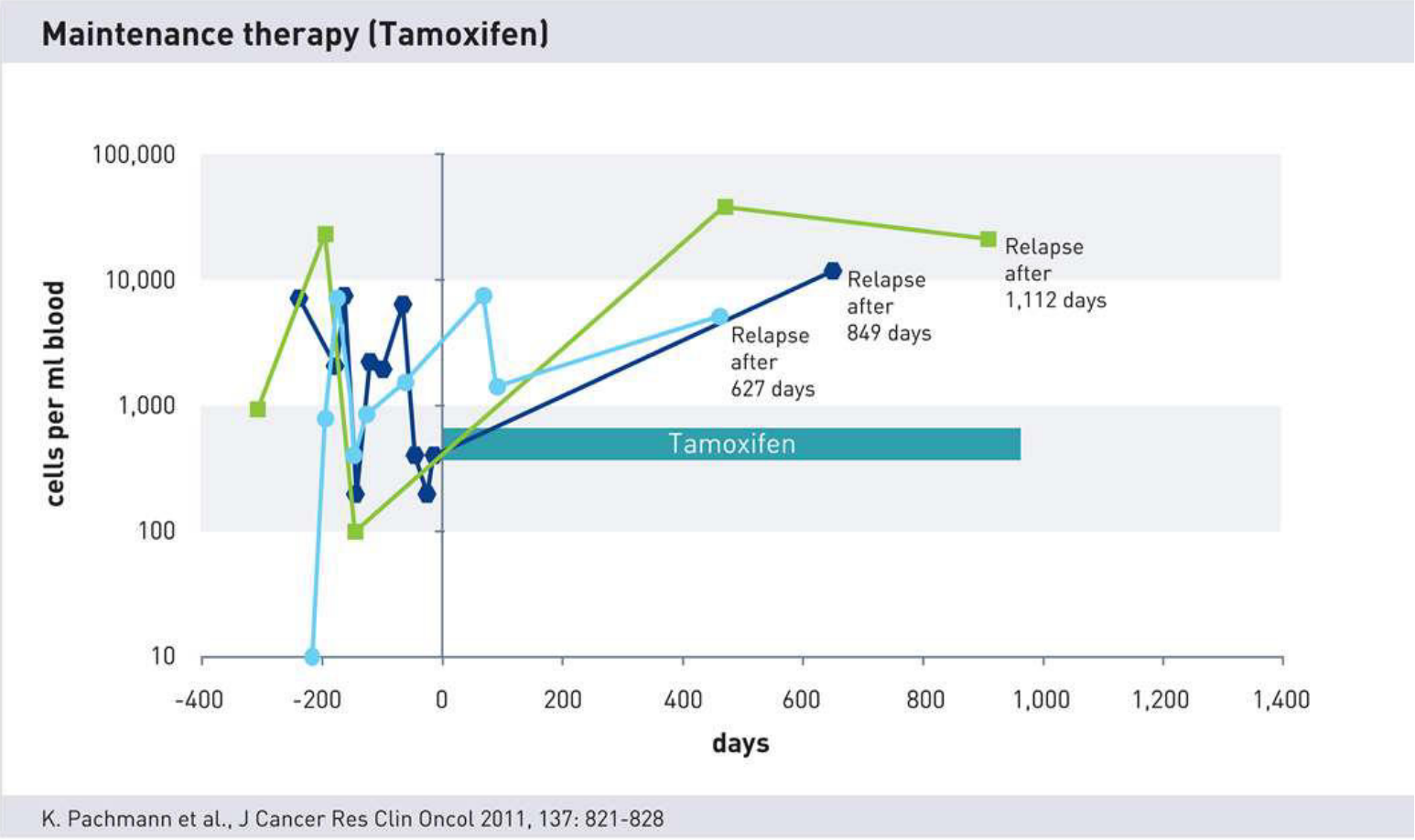
Decreasing numbers of cells



TZB

Endocrine therapy breast cancer

Increasing numbers of cells

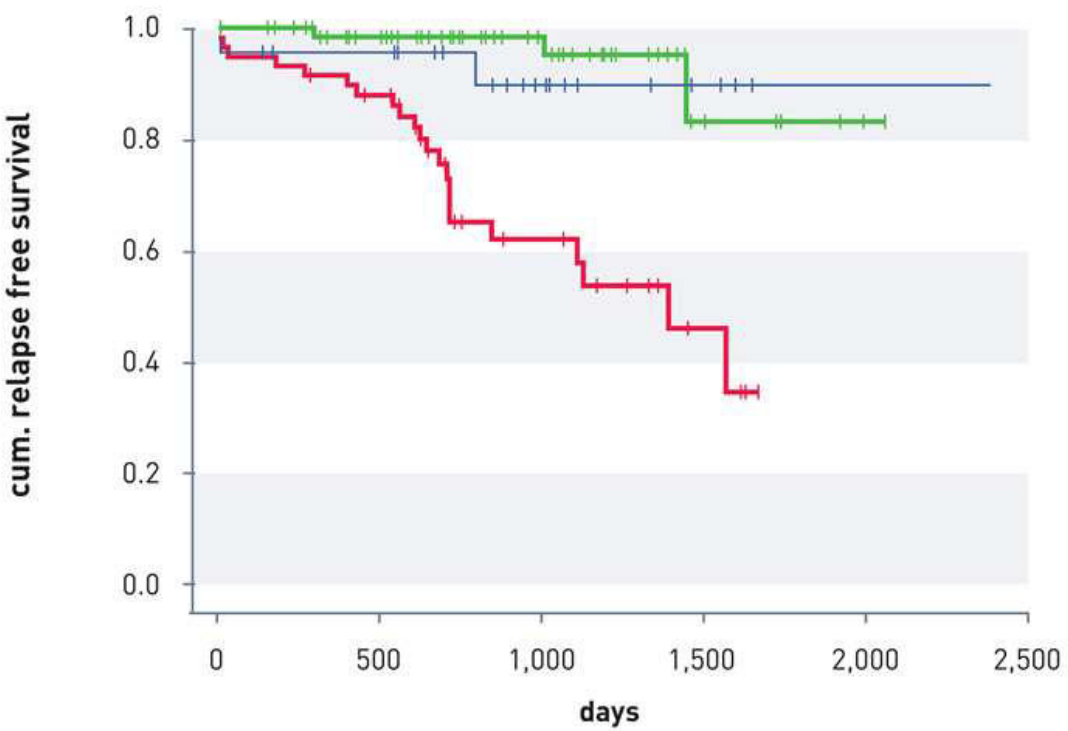


A repeated increase during therapy with Tamoxifen is highly significantly correlated with relapse

Endocrine therapy breast cancer

Results of the study

Maintenance therapy (Tamoxifen)



Cellnumber:
- decrease CETC
- increase CETC
- no change
- decrease-censored
- increase-censored
- no change-censored

log rank p < 0.001

179 Patients were included in this study

K. Pachmann et al, J Cancer Res Clin Oncol 2011, 137: 821-828

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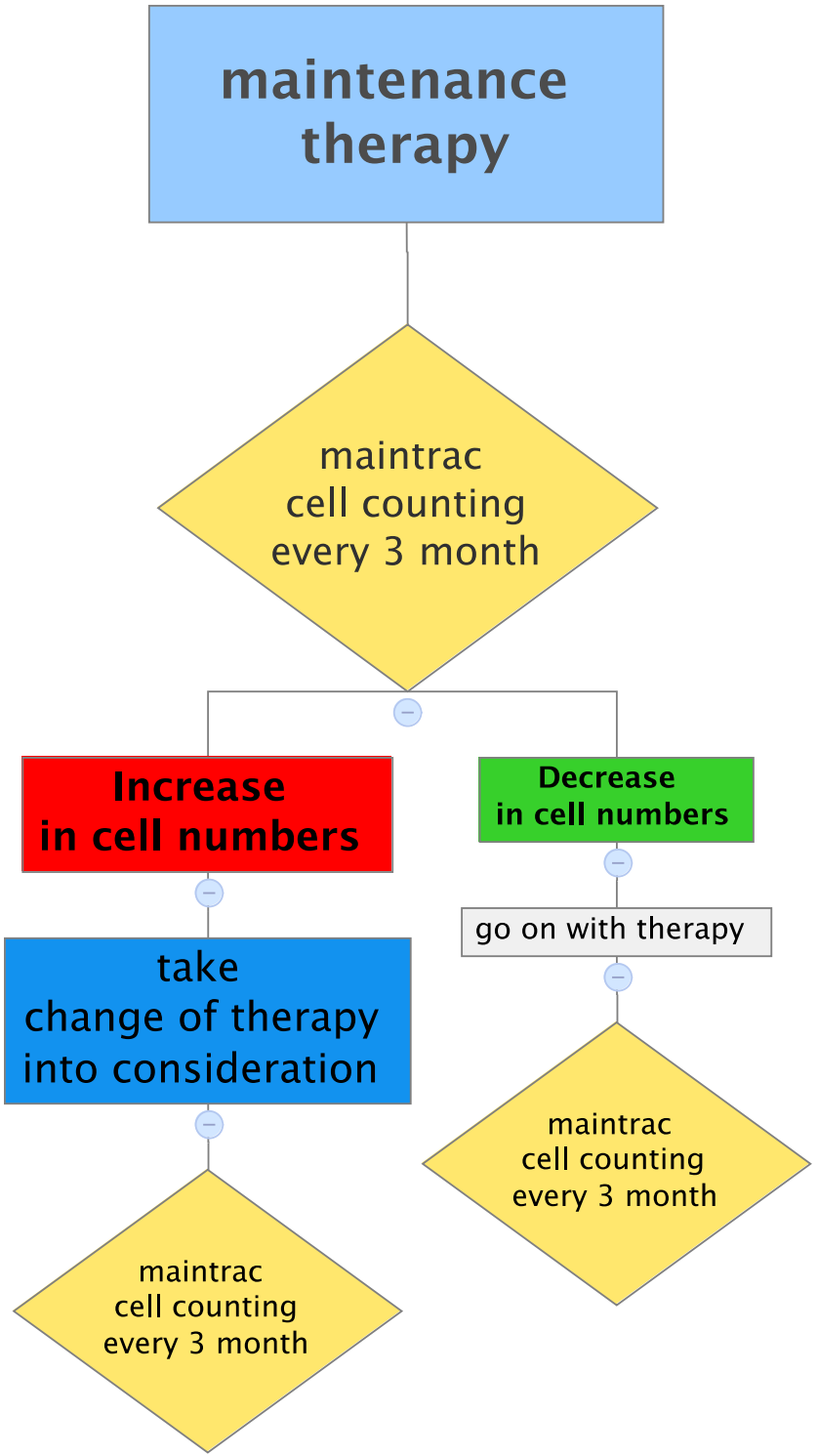
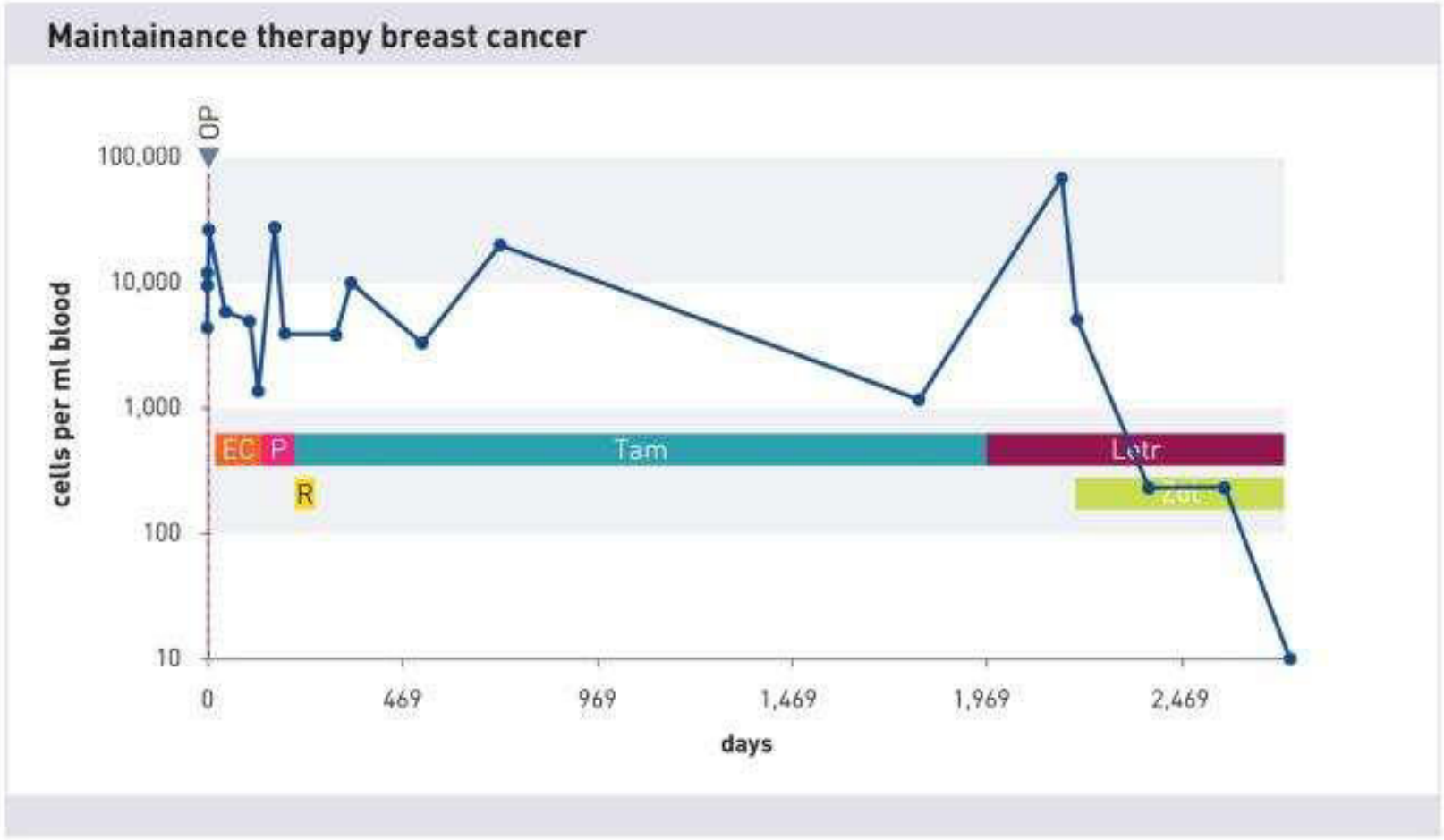
If cell numbers
increase,

change of therapy
may be considered

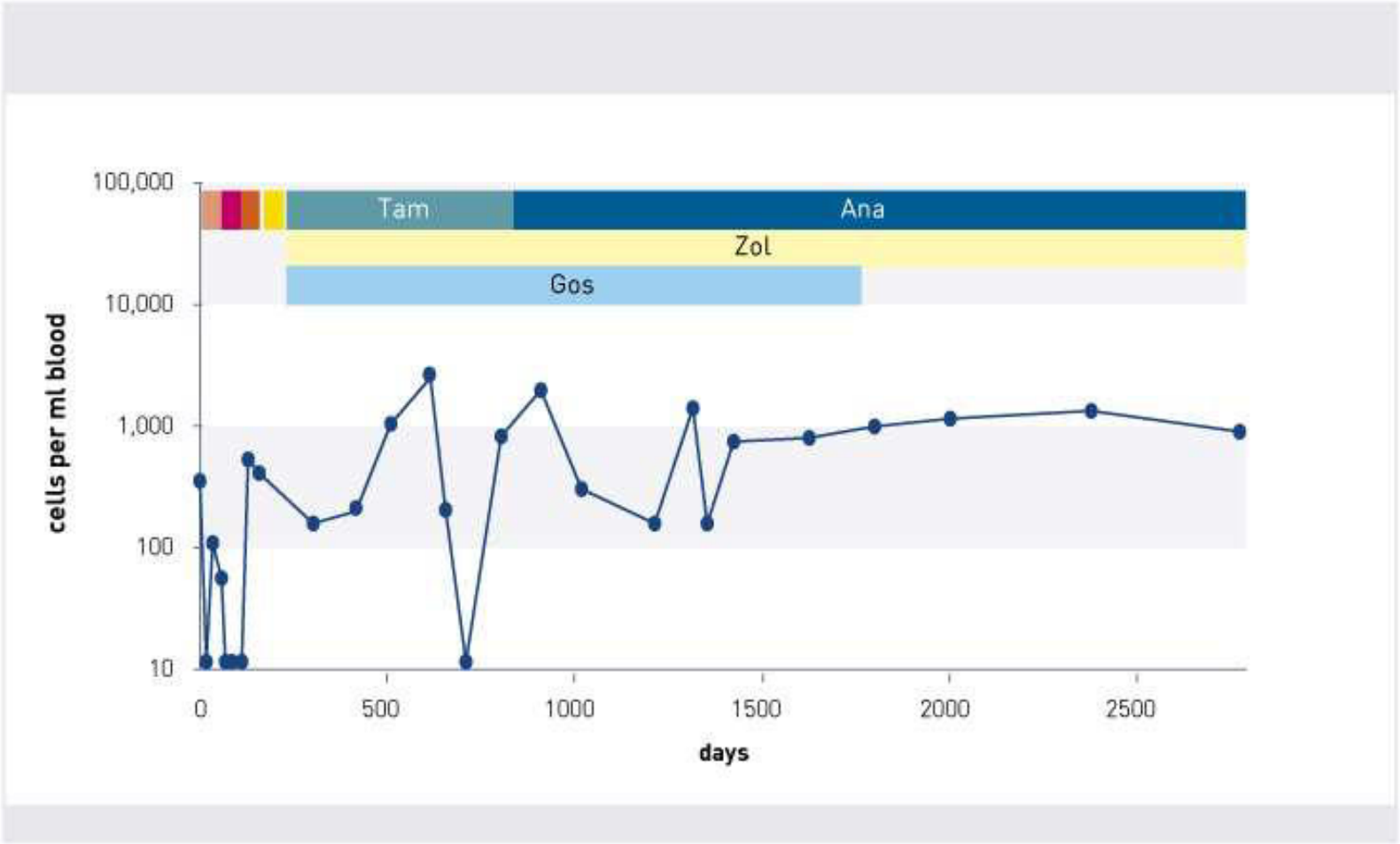
monitor every
3 months



Effect of changes in therapy

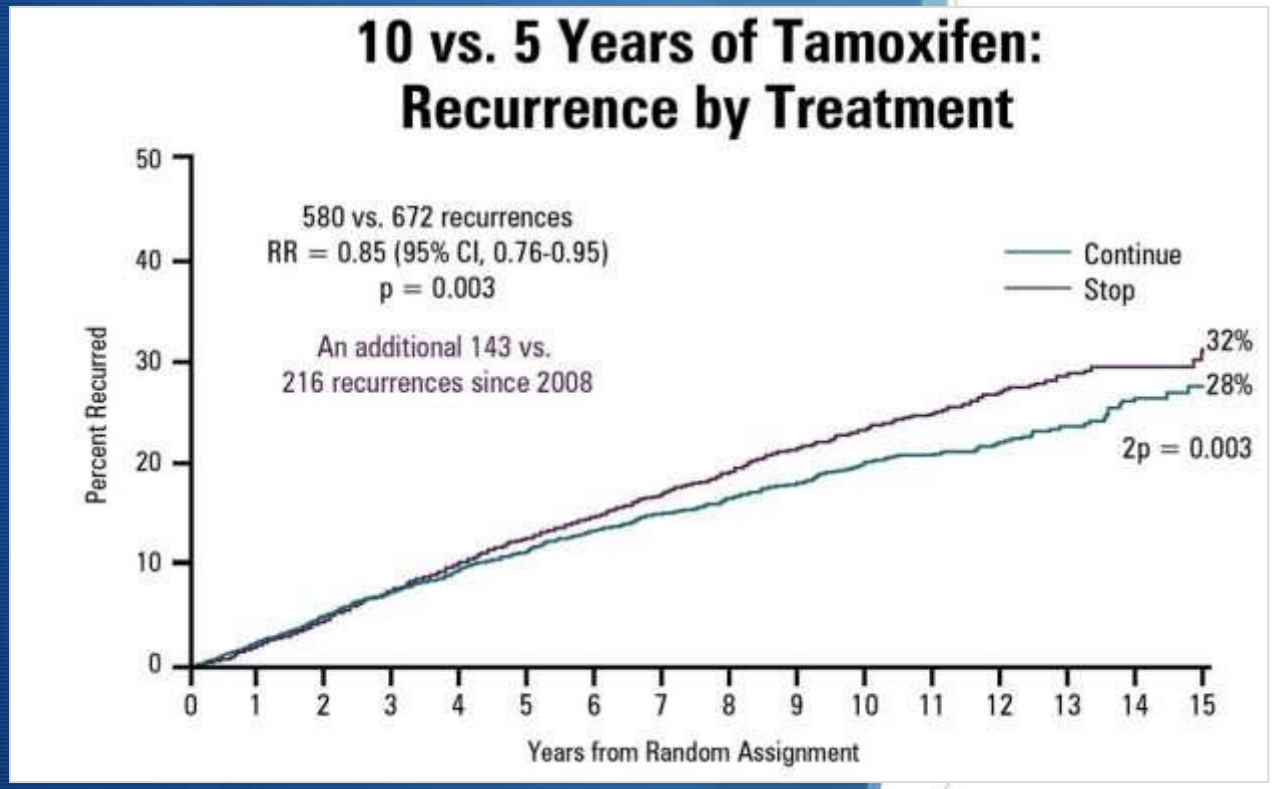


Effect of changes in therapy



Long-term surveillance after maintenance therapy

Endocrine therapy

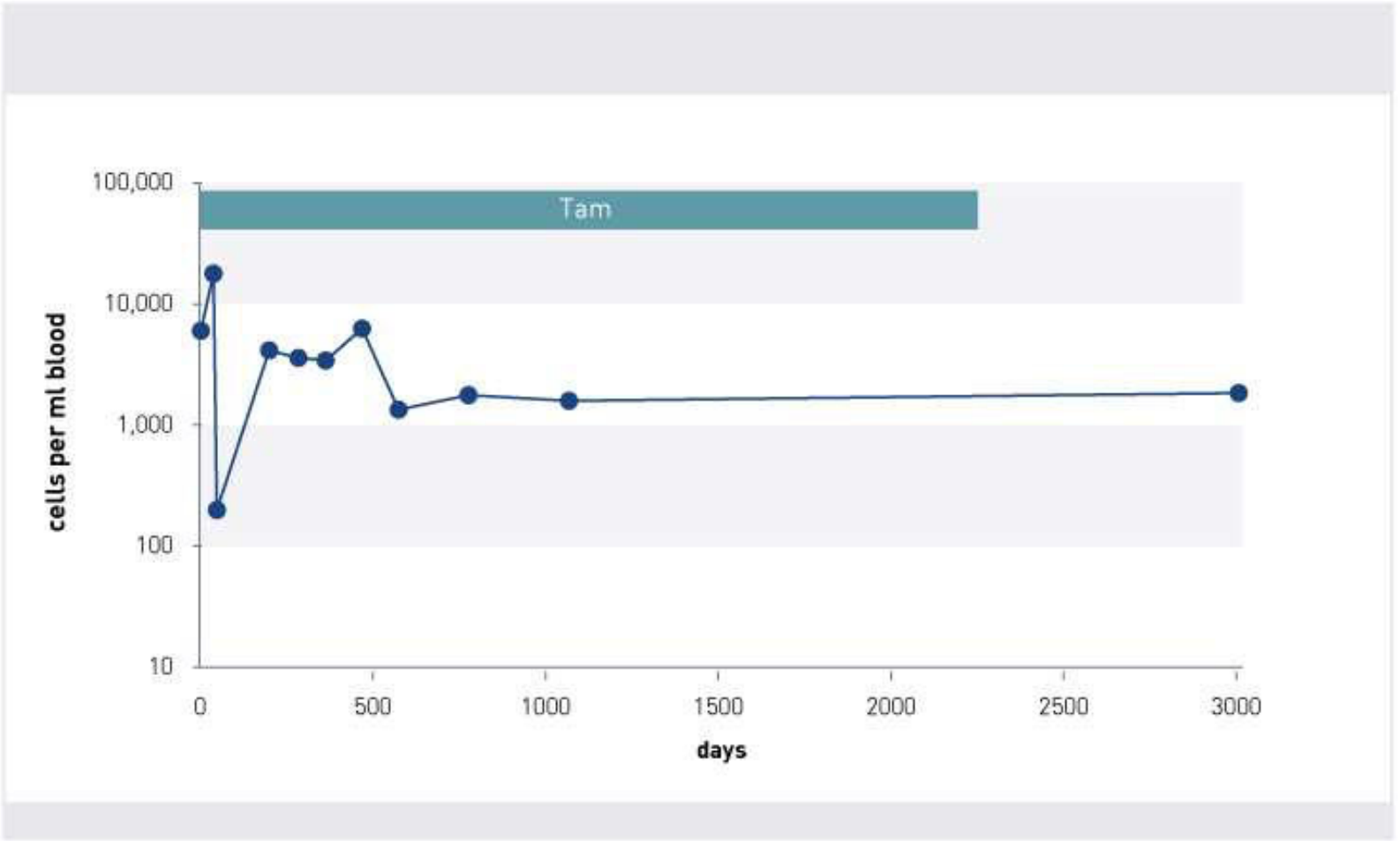


A debate is currently ongoing as to whether it would be better to take Tamoxifen for 10 years instead of stopping after 5 years.

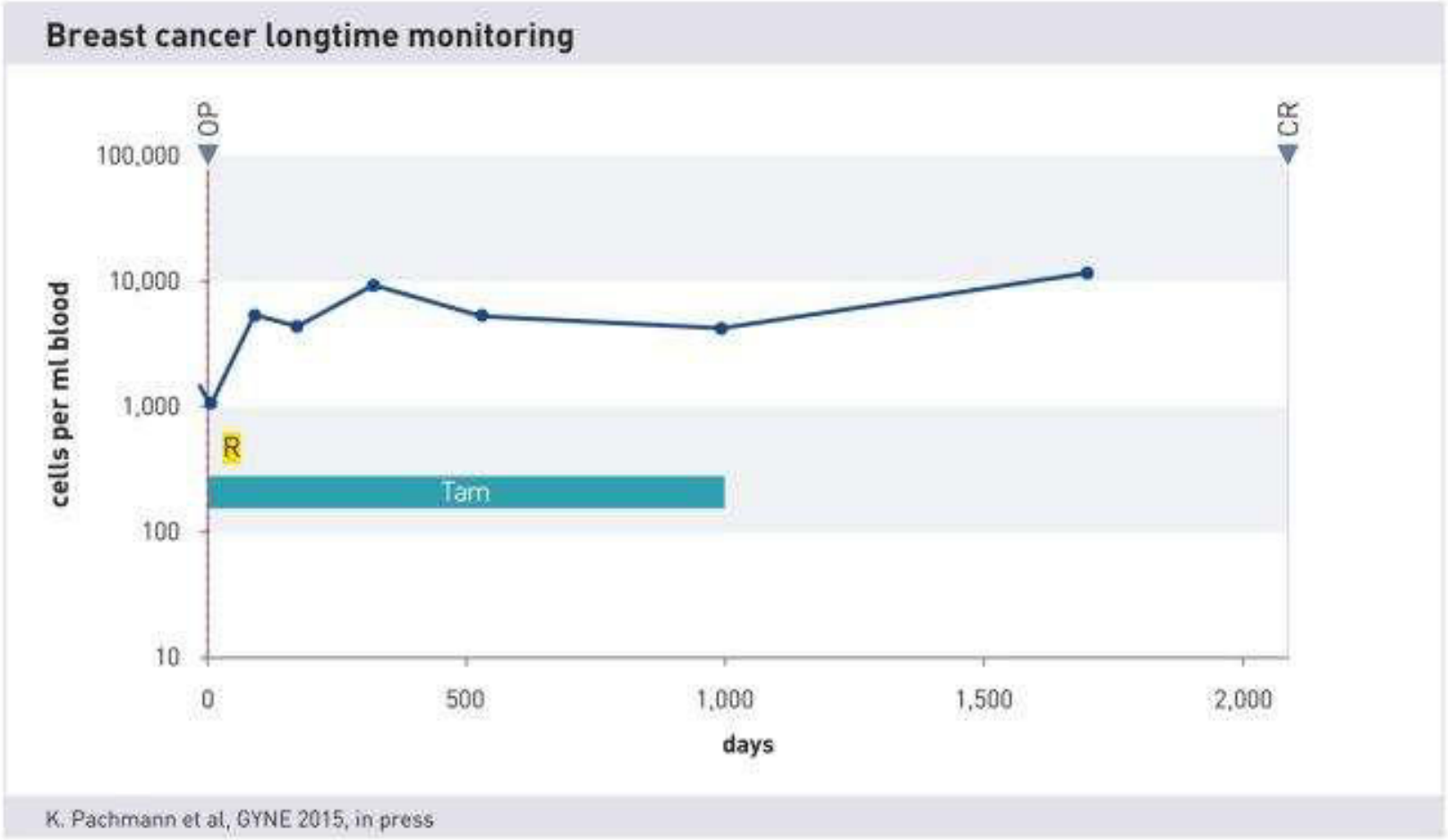
http://am.asco.org/extending-adjuvant-tamoxifen-reduces-breast-cancer-recurrence-mortality

TZB

Long-term surveillance

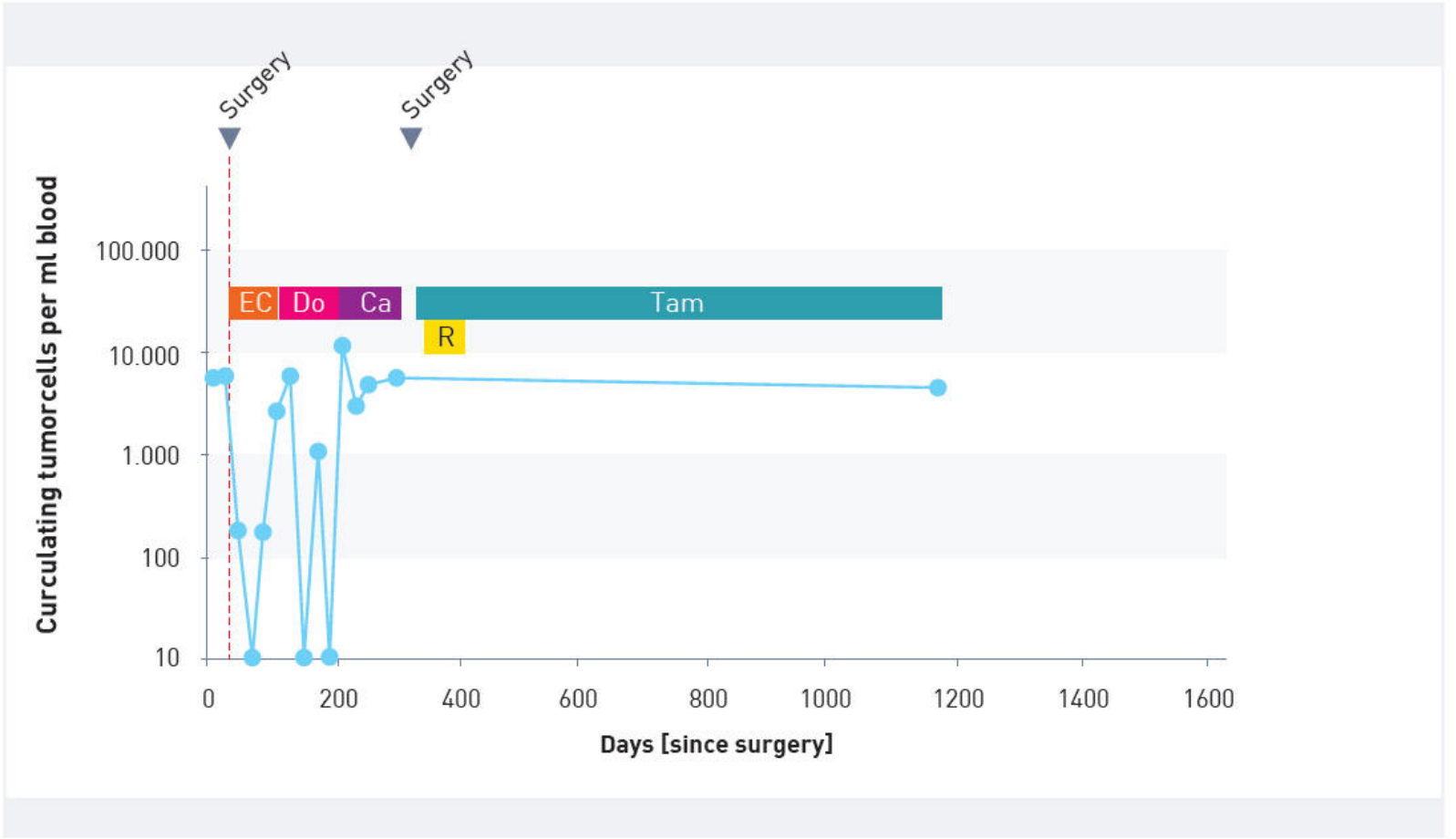


Long-term surveillance



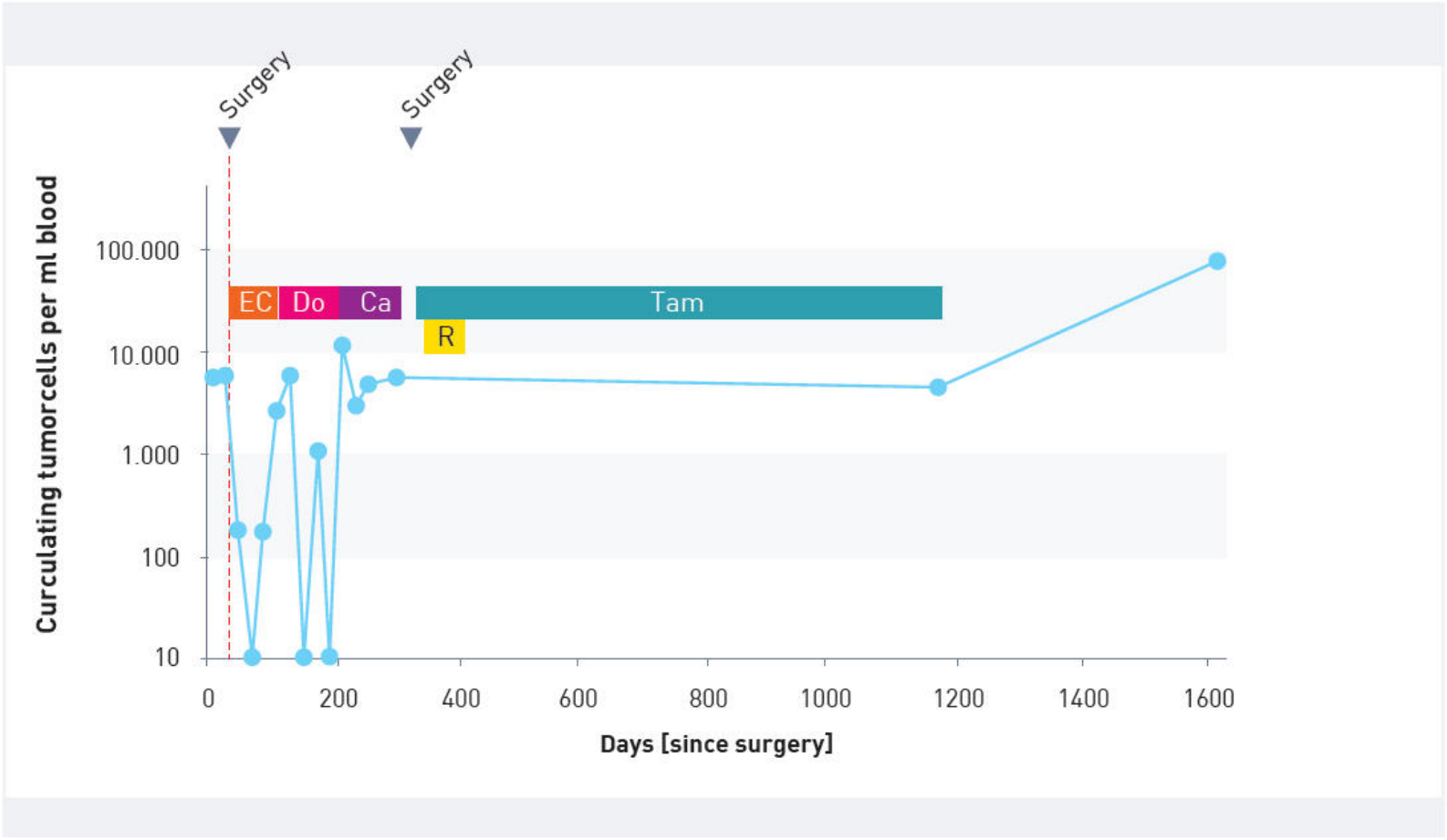
Long-term surveillance

Case report 1



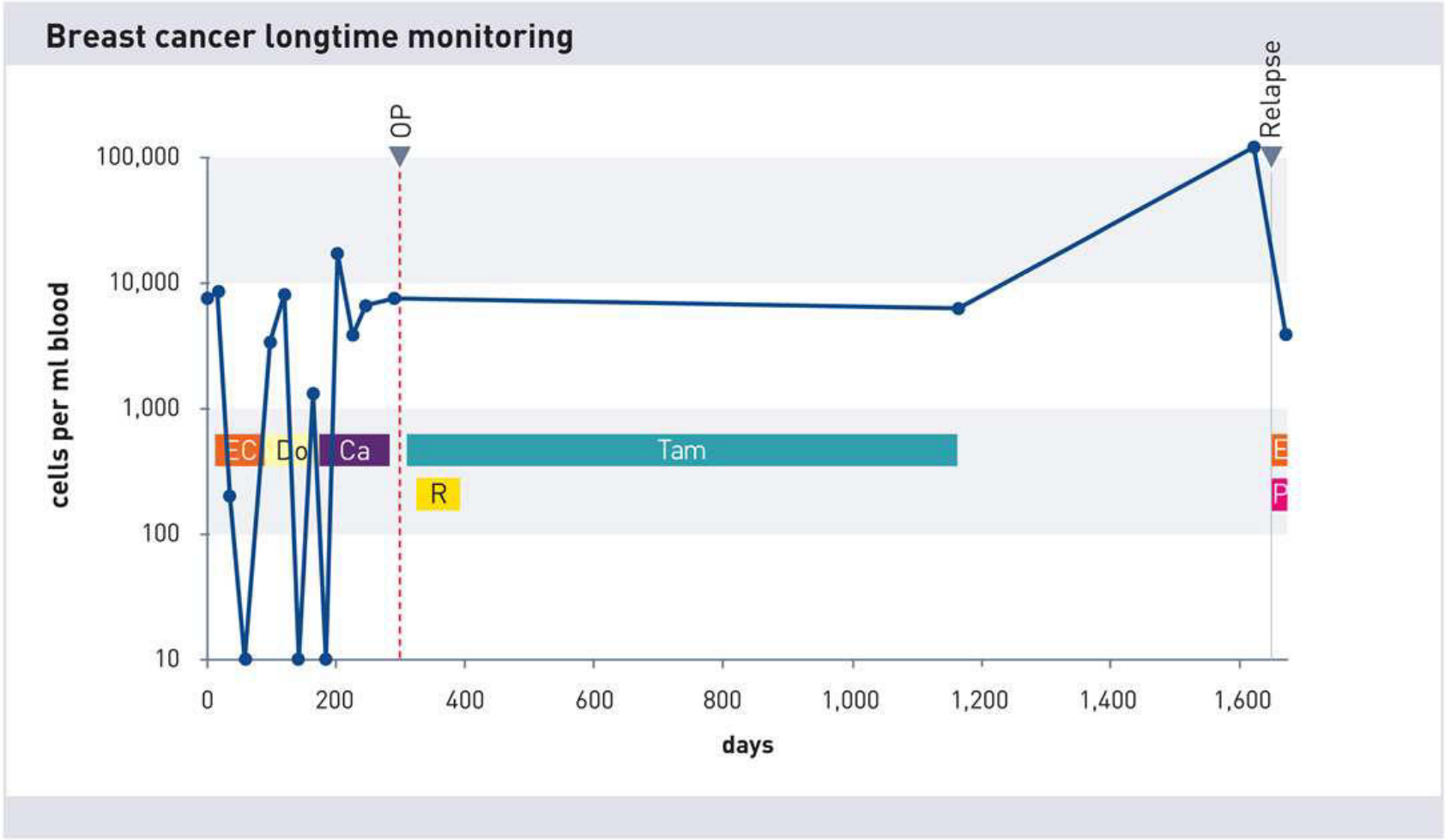
Long-term surveillance

Case report 1

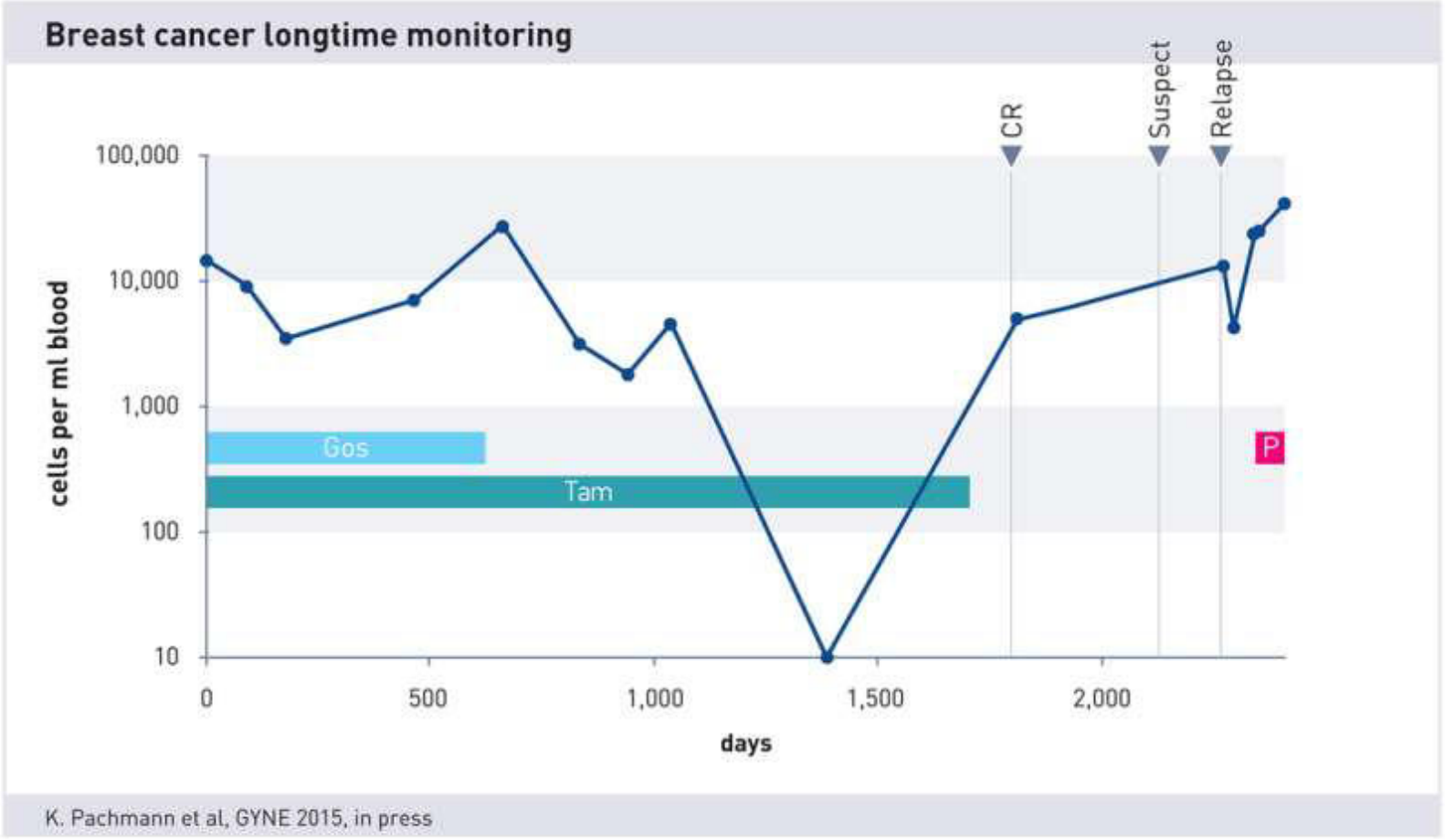


Long-term surveillance

Case report 1

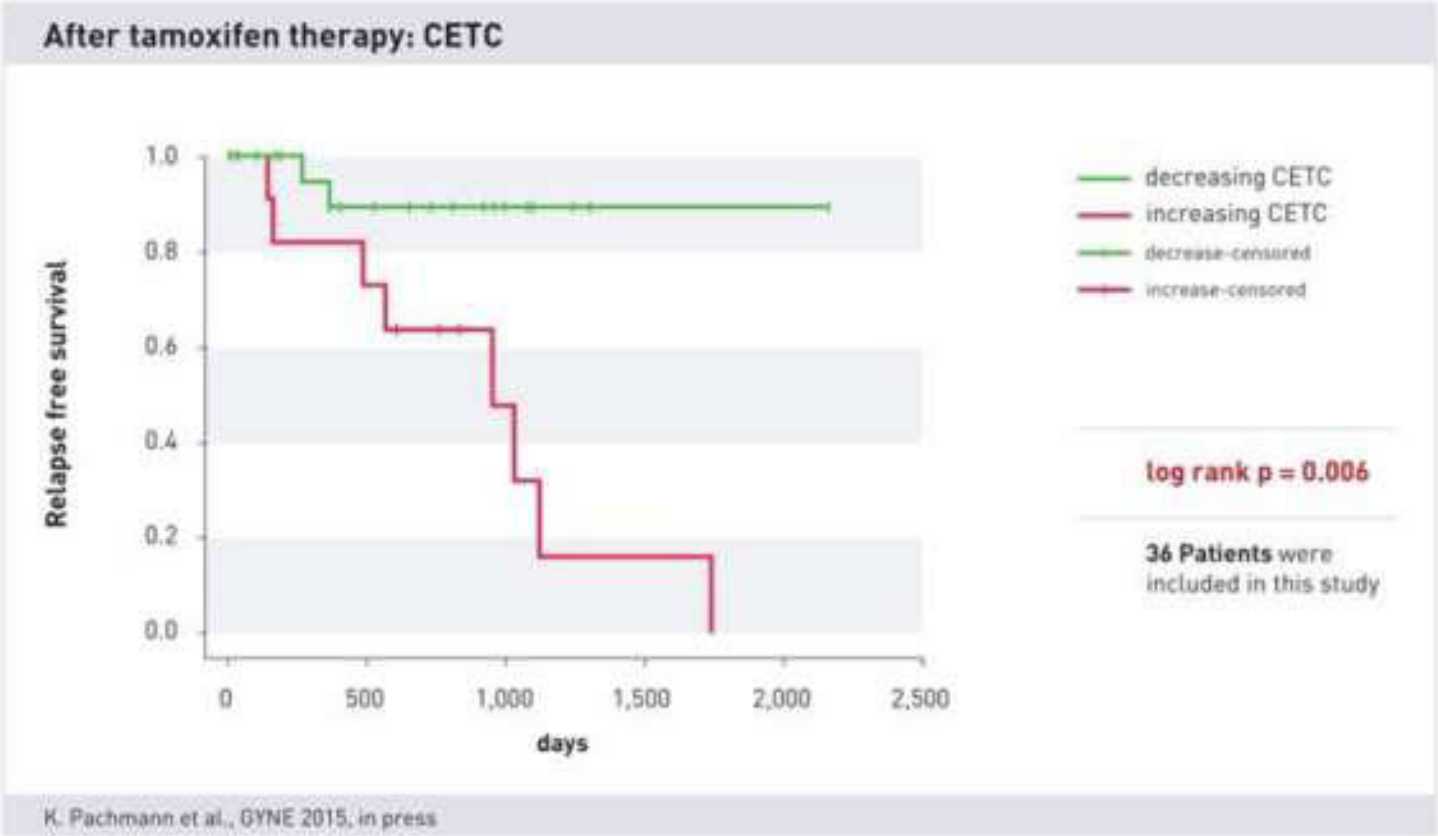


Long-term surveillance



Long-term surveillance

Impact of monitoring CETCs after the end of endocrine therapy



Patients with **increasing** cell numbers

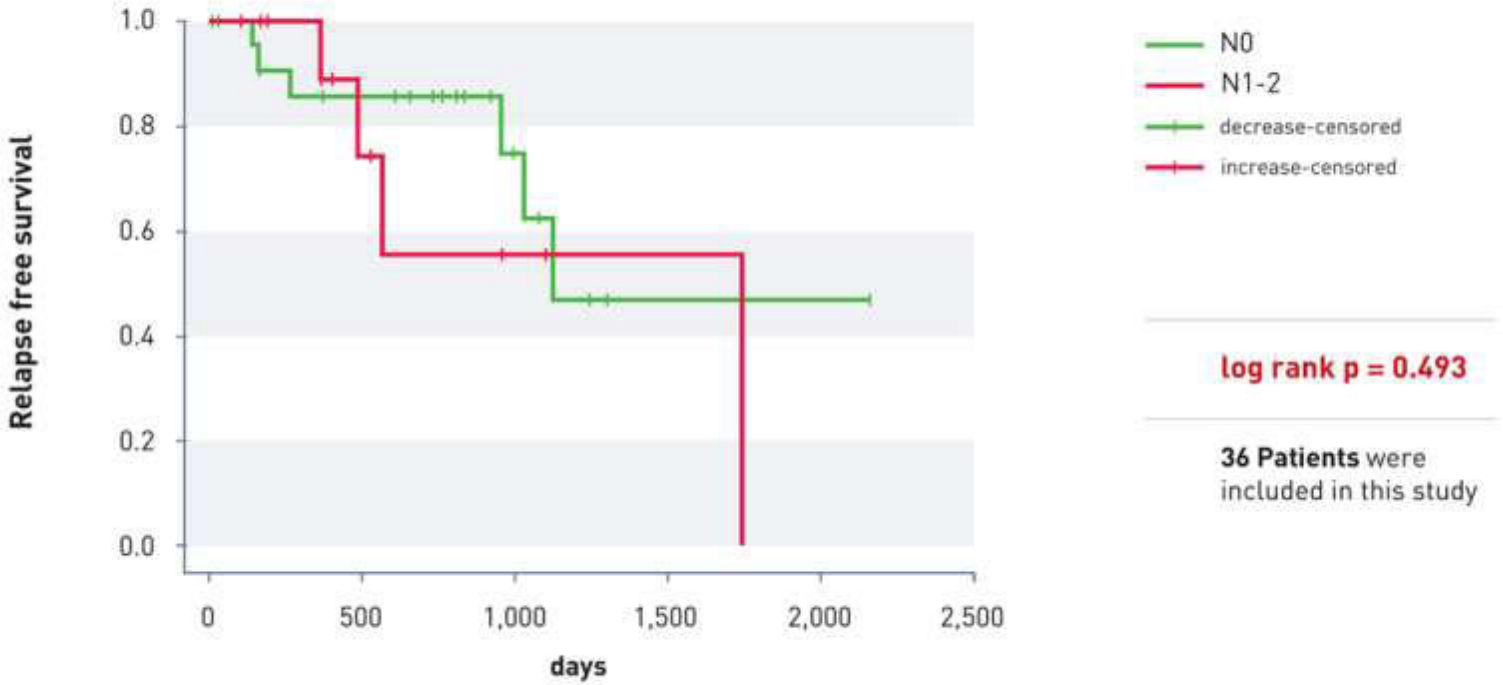
after the end of maintenance therapy

have an **increased risk** of recurrence

Long-term surveillance

After the end of endocrine therapy impact of lymph node status

After Tamoxifen therapy: nodal status

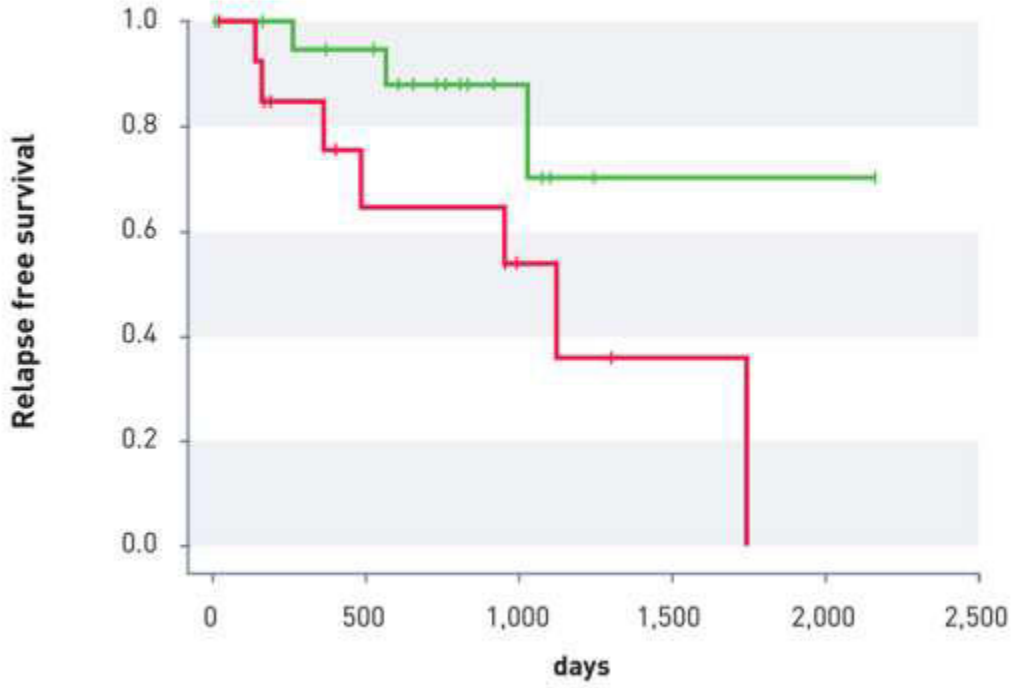


K. Pachmann et al, GYNE 2015, in press

Long-term surveillance

After the end of endocrine therapy impact of tumor size

After Tamoxifen therapy: tumor size



log rank p = 0.058

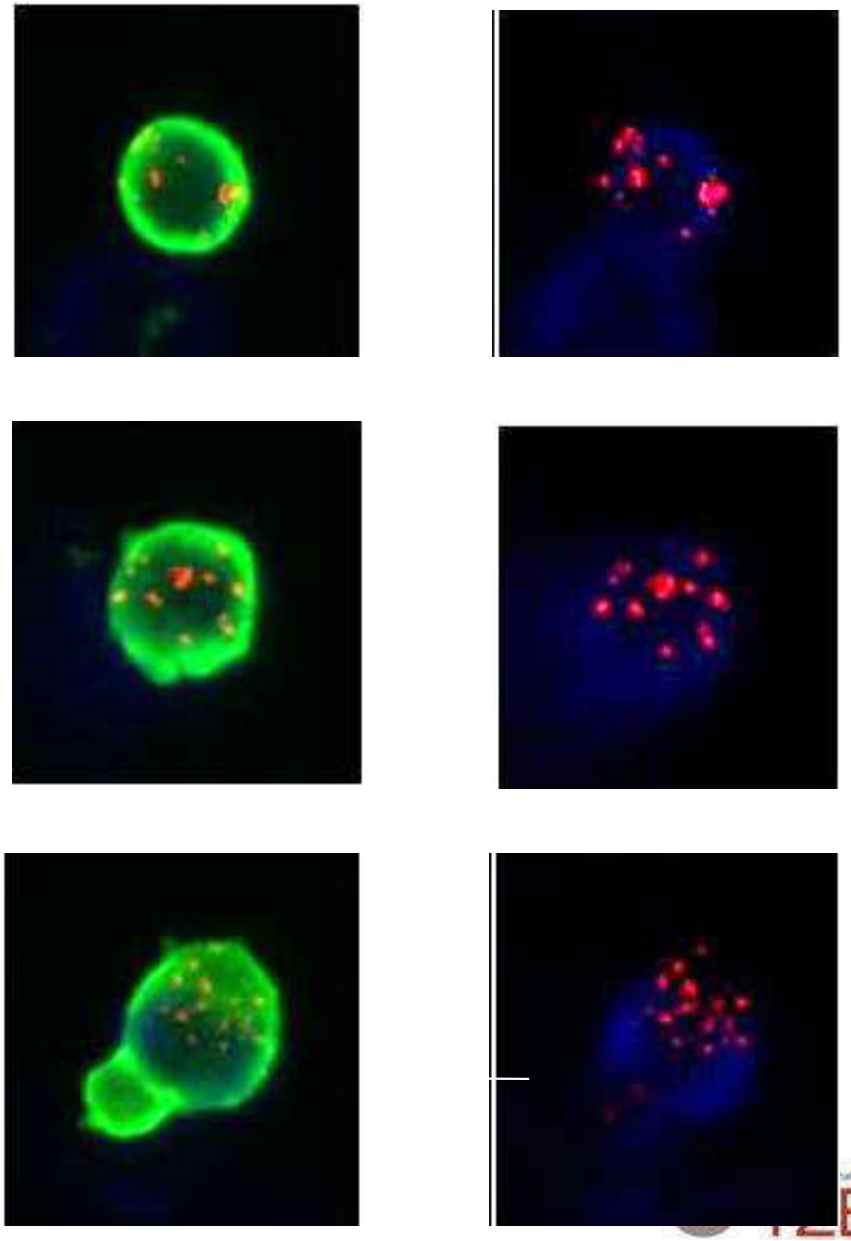
36 Patients were included in this study

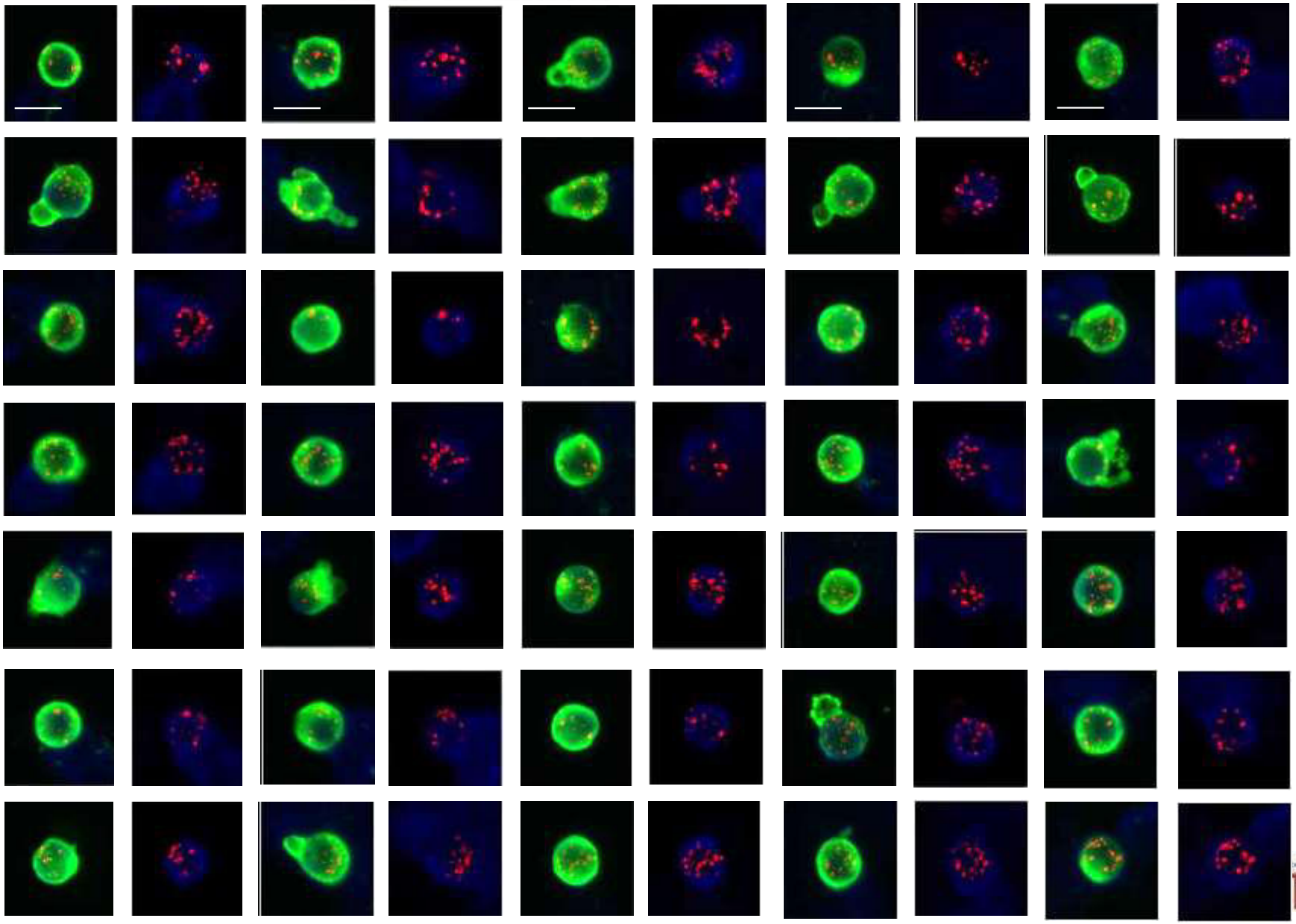
K. Pachmann et al, GYNE 2015, in press

Additional investigations of circulating tumour cells

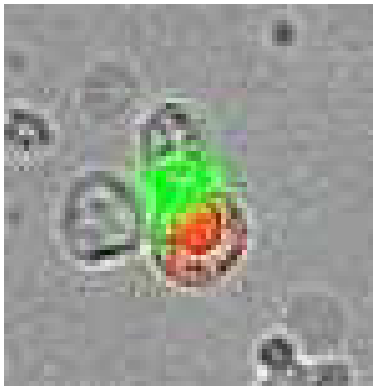
Markers

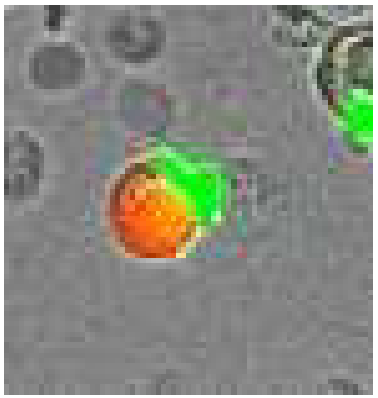
- Estrogen receptor
- Androgen receptor
- Progesterone receptor
- PSA/PSMA
- FISH EGFR
- Ki67
- PDL1
- HER2/DAPI
- ...

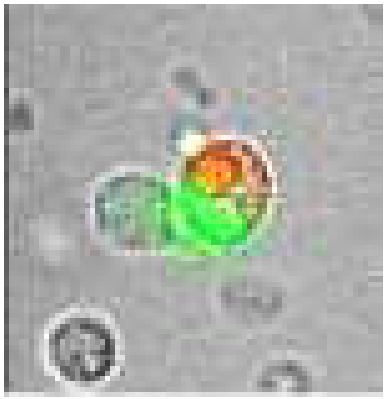



EpCAM/HER2/ DAPI
HER2/DAPI
EpCAM/HER2/ DAPI
HER2/DAPI
EpCAM/HER2/ DAPI
HER2/DAPI
EpCAM/HER2/ DAPI
HER2/DAPI
EpCAM/HER2/ DAPI
HER2/DAPI

Typical estrogen-receptor-positive cells







A PX who was ER/PR negative ....

Labor Dr. med. Ulrich Pachmann · Kurpromenade 2 · 95448 Bayreuth

Bayreuth, 18.07.2017

Your patient: **N, L**
Born: **1946**

Blood collection date: 11.07.2017
Our Lab number: T733890
Initial findings: T732269, T733168

Phone
Fax
Mail:

Report on diagnostic findings on Circulating Tumor Cells (MAINTRAC)

Dear Dr.

Many thanks for sending your examination request regarding the detection of circulating tumor cells. Follow up.

Diagnosis:
invasive Breast Cancer and DCIS
Histology: ER/PR: neg., Her2/neu: +++ (pos.)
- 02.05.2017: partial mastectomy

The automated microfluorimetric image analysis of the **epithelial cell adhesion molecule (EpCAM)**-positive cells with visual control (MAINTRAC) from **1 ml EDTA blood** resulted in following findings (detection limit is at 10 cells/ml):

	Number of potential tumor cells			
Examination parameter	In the sample (1ml)	In circulation (5l) (in millions)	In addit. examination: % of EpCAM-pos. cells	Cell fragments
EpCAM	**1 050**	**5,25**		numerous

The material for examination could be thoroughly evaluated.
We again found **a moderately increased number of live, potentially malignant tumor cells circulating in the blood. In comparison to the previous findings from May 2017 the number of potential tumor cells has increased slightly.**
In addition, there were numerous specific cell fragments detected. Specific cell fragments occur, for example, as part of an immune response and indicate damaged cells.

Pre-surgery we could detect moderate cell numbers. Post surgery and now, over a period of 2 months, cells remain relatively stable.

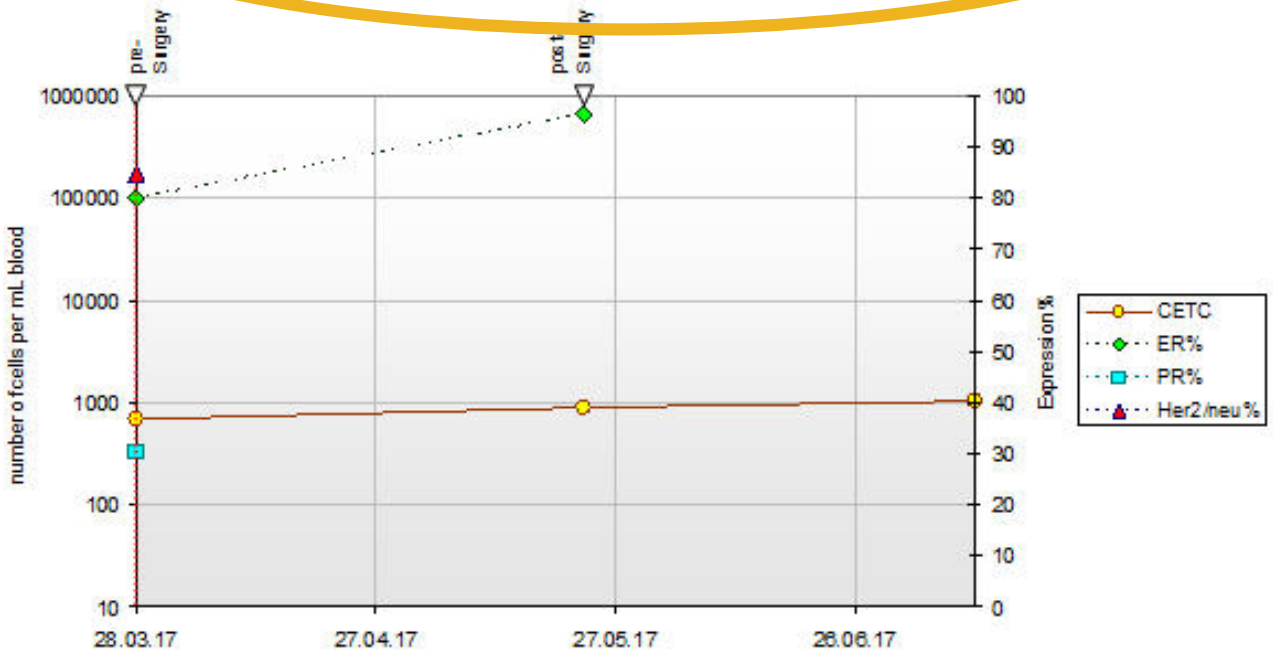
Does the patient receive any therapy after surgery such as chemotherapy or Herceptin?
We would very much appreciate if you could provide us with details of treatments your patient receives so we can individualize our comments regarding the results.

Arbeitsgemeinschaft TZB

Her cells are now intensively expressing estrogen receptors: hormone therapy possible

18.07.2017

We would like to emphasize that in contrast to the histological report the cells in the circulation express to a high extent the estrogen receptor. Endocrine therapy might therefore be taken into consideration.

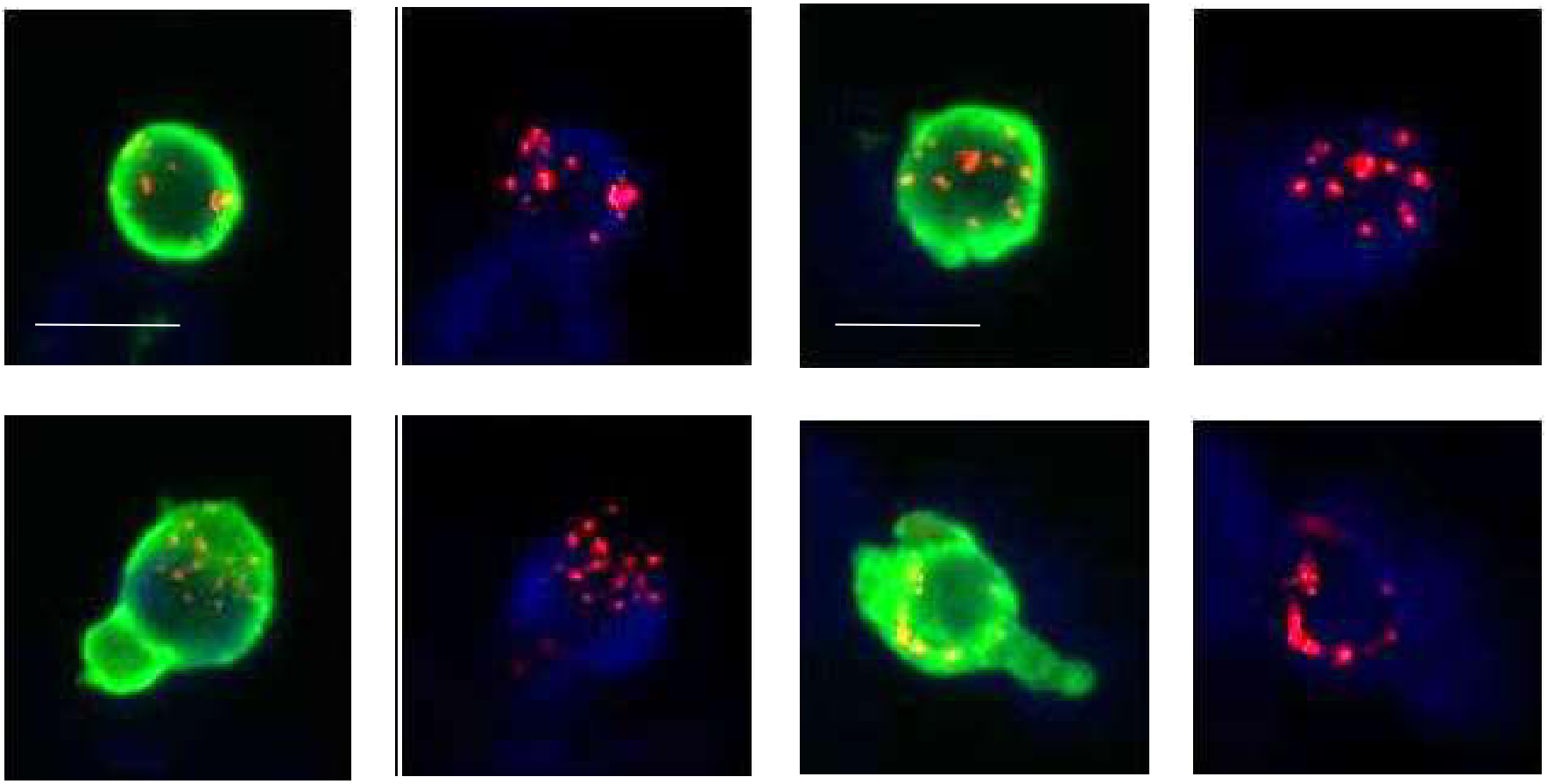


With best regards,
Dr. med. Ulrich Pachmann Prof. Dr. med. Katharina Pachmann Dr. med. Matthias Mäurer

Her2/neu amplification

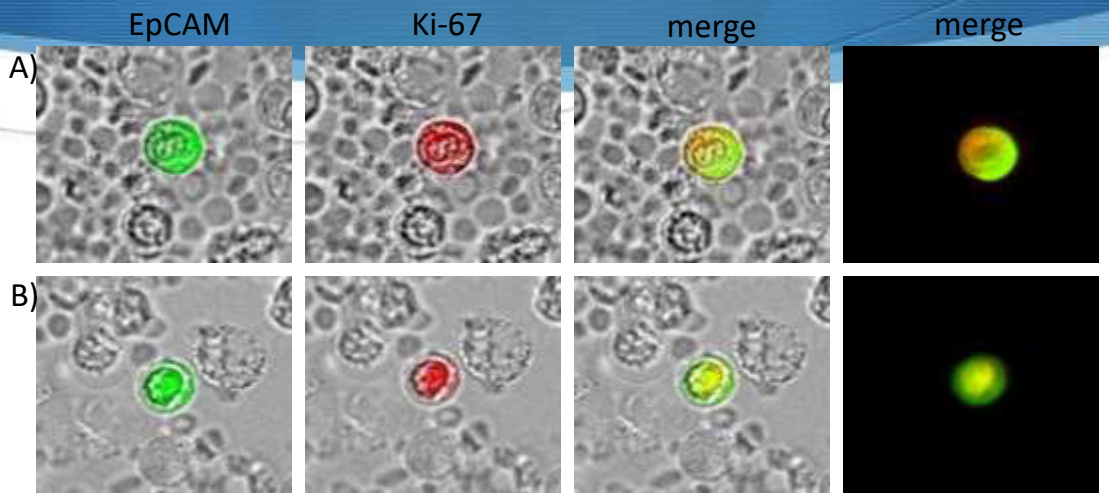
EpCAM /HER2/ HER2/DAPI DAPI

EpCAM /HER2/ HER2/DAPI DAPI

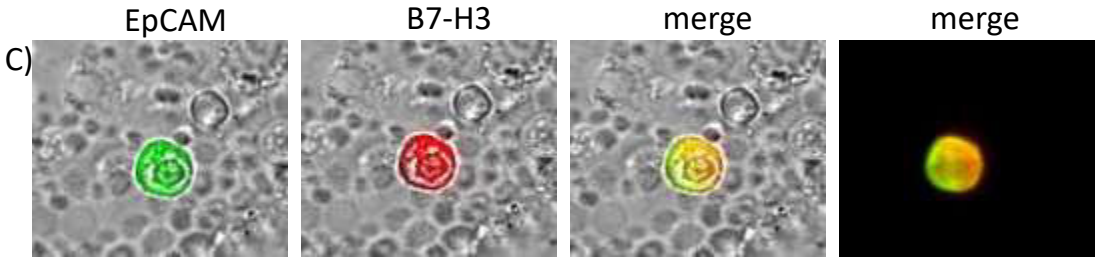


Activation markers

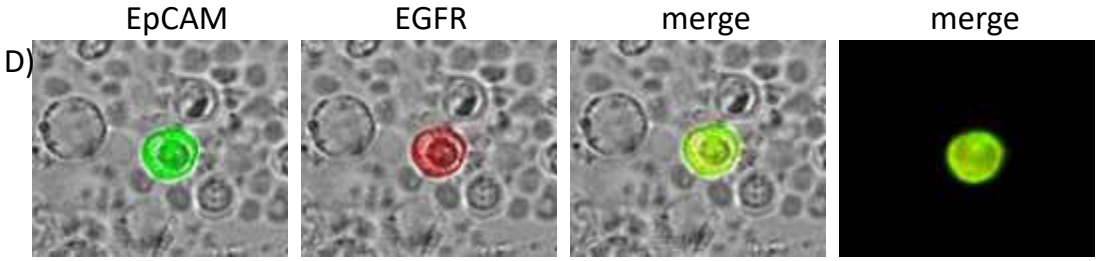
Ki-67



B7-H3



EGFR



Single cell picking

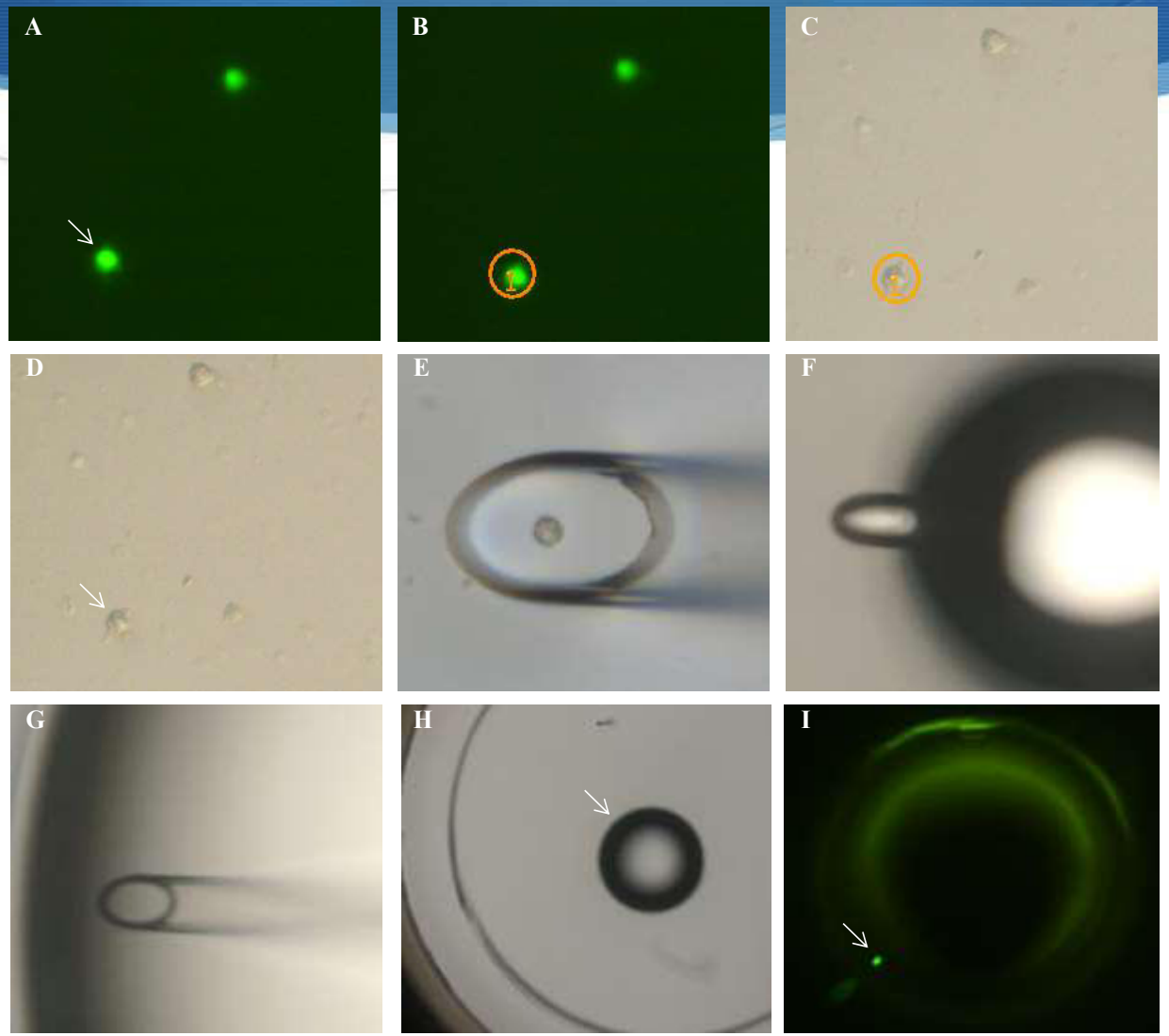
Live circulating epithelial tumour cells

for further (e.g. genetic) investigation or NGS (Next Generation Sequencing).

Already available at maintrac

TZB

Picking steps



Mutation Analysis

Sample ID	Test Result	Mutation Result
15390 Cell 1	Mutation not detected	N/A
15390 Cell 2	Mutation not detected	N/A
15390 Cell 3	Mutation detected	Codon 61
15390 Cell 4	Mutation not detected	N/A
15390 Cell 5	Mutation detected	Codon 61
15390 Cell 6	Mutation not detected	N/A
15390 Cell 7	Mutation not detected	N/A

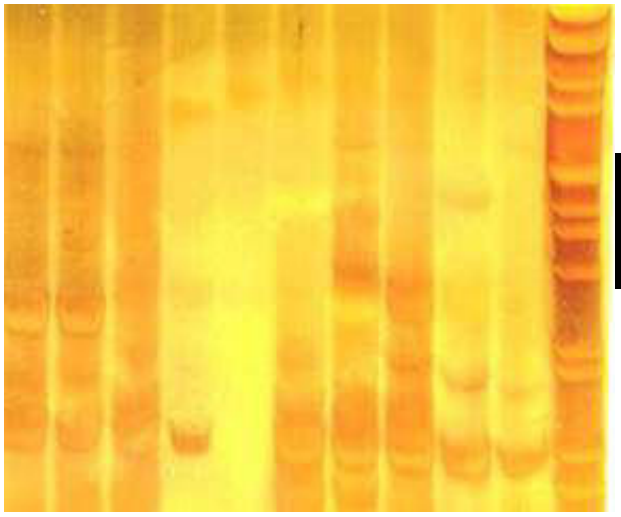
Detection of mutations

	Number of isolated CETCs with wild type (%)	Number of isolated CETCs with detected mutation (%)	Invalid samples (%)
Colorectal Cancer (KRAS)	5/7 (71.4)	2/7 (28.6) (Codon 61)	--
Malignant melanoma (BRAF)	3/8 (37.5)	3/8 (37.5) (V600)	2/8 (25)
Non-small cell lung cancer (EGFR)	5/8 (62.5)	1/8 (12.5) (Exon 20)	2/8 (25)

Changes of gene expression in circulating tumour cells

G, C

Pre OP

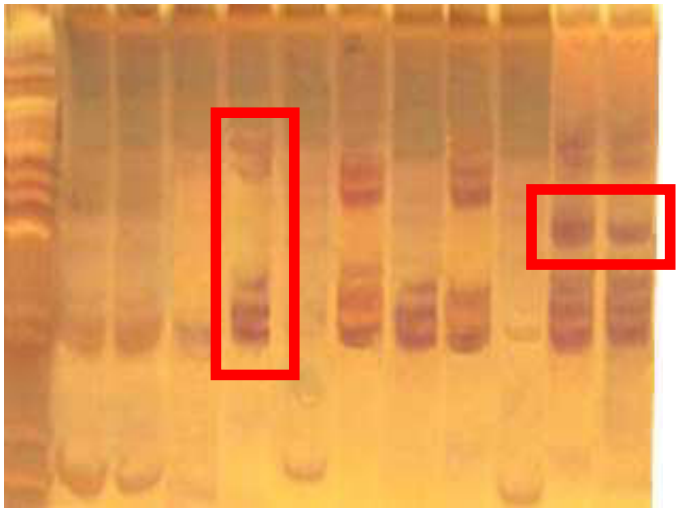


NANOG

EpCam (420 Bp)
Her2/Neu (376 Bp)
Vimentin (327 Bp)
Gremlin (264 Bp)

RPL 13 A (229 Bp)

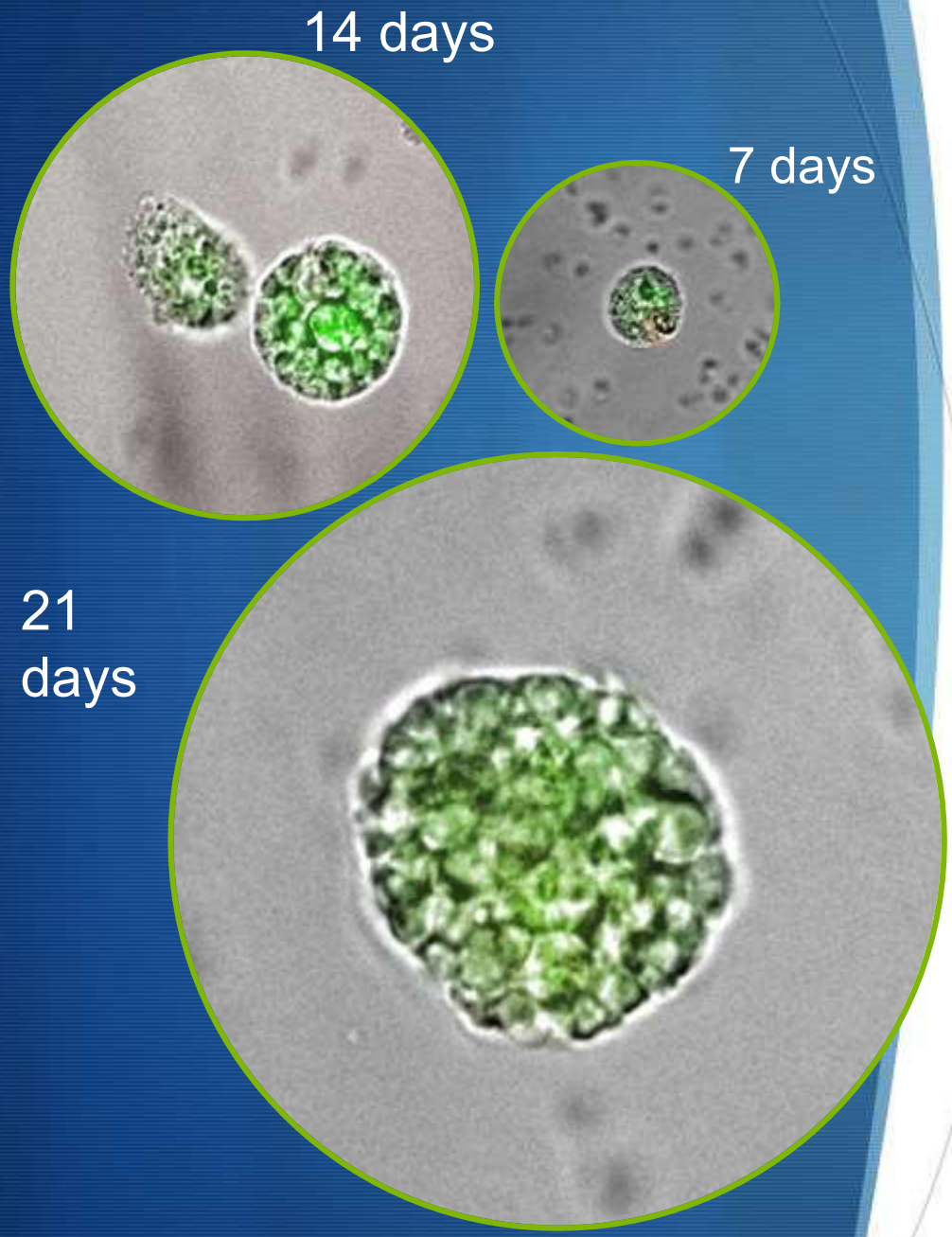
Post OP



Increased expression of stem cell and adhesion markers after surgery

TZB

Circulating Epithelial Tumour Cells – Next Generation



Tumour spheres from CETCs

Spheres were detected in 86 out of 109 patients **(78.9%)**;

Number of spheres varied between 50 and 1700/ml (median 200)

All spheres detected **are positive for EpCAM**.

Clonal expansion of circulating tumour cells

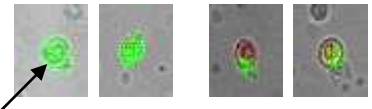
Methodology

1 ml EDTA Blood

Lysis of red blood cells

One centri-fugation step

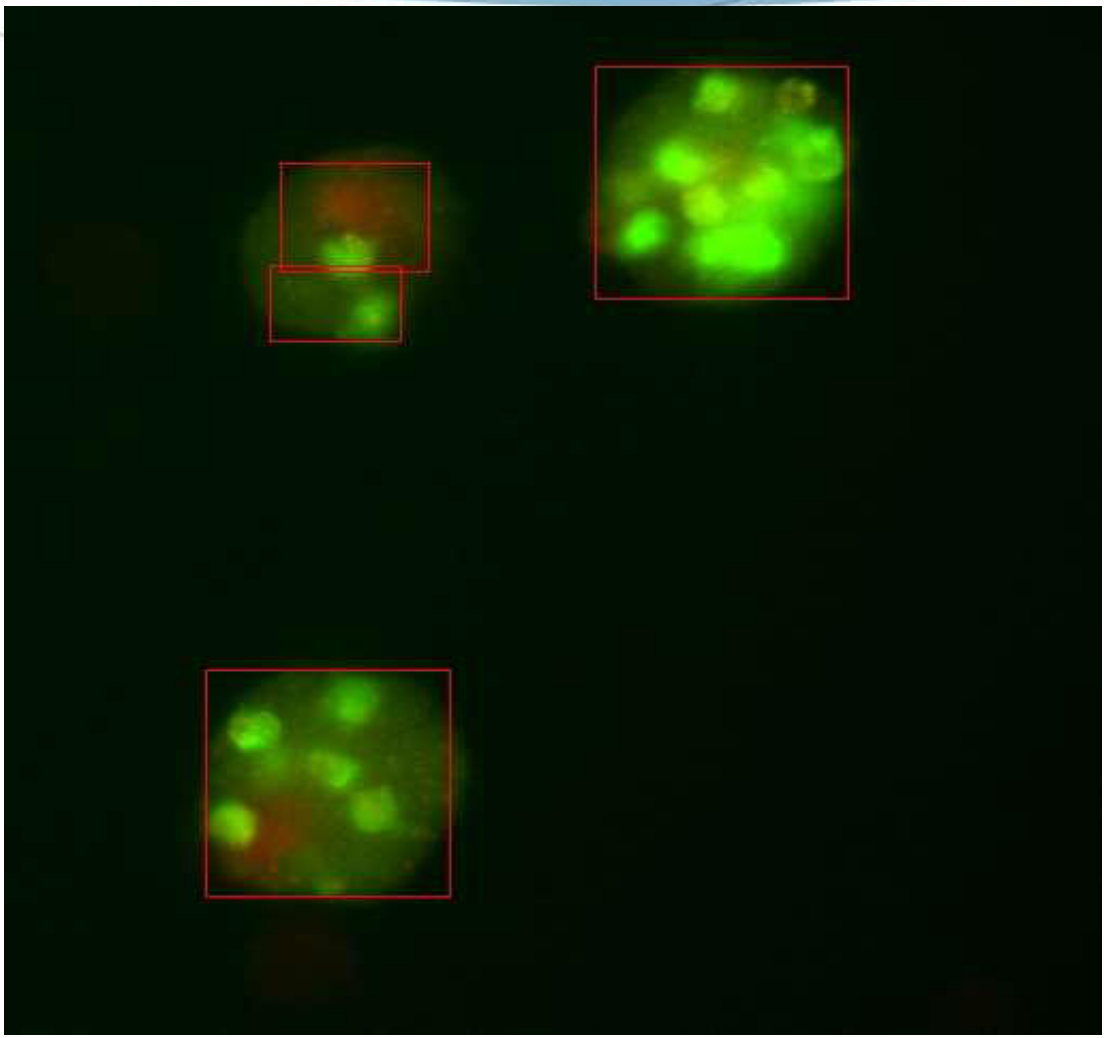
+ Anti-EpCAM



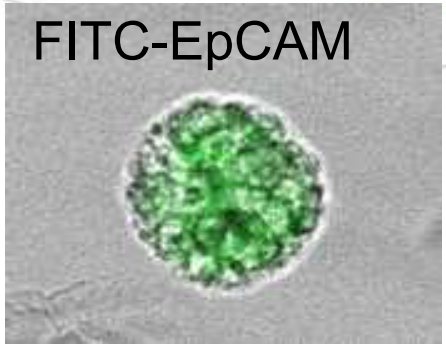
Determination of vital and dead EpCAM positive cells among the white blood cells

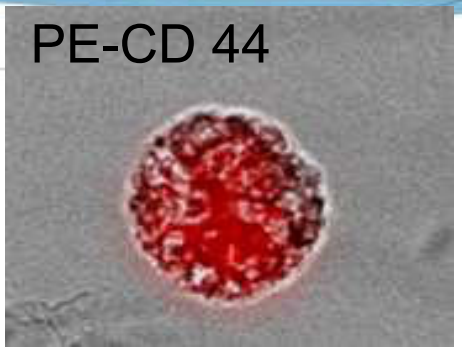
Culture of all white blood cells under conditions favouring growth of epithelial cells and determination of sphere formation at different times of culture

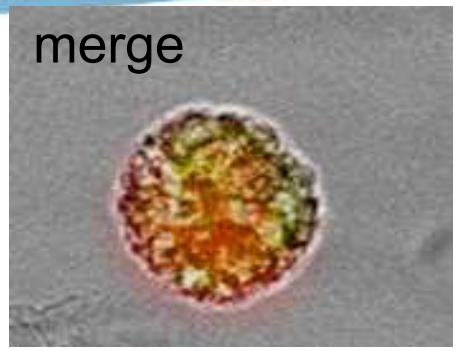
EpCAM expression in tumour spheres

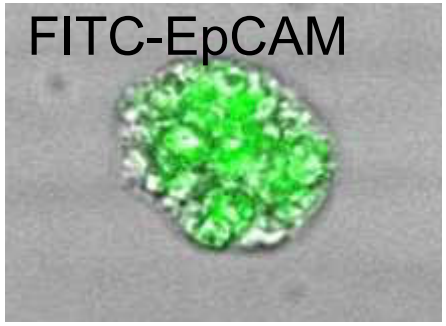


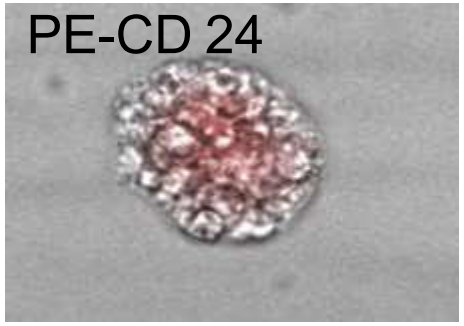
Stem cell marker expression in tumour spheres

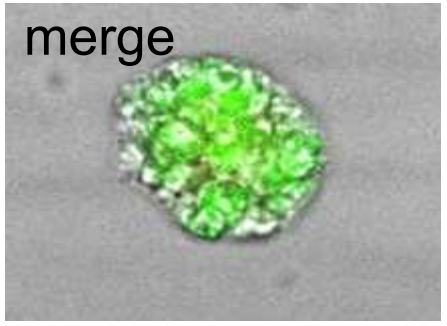


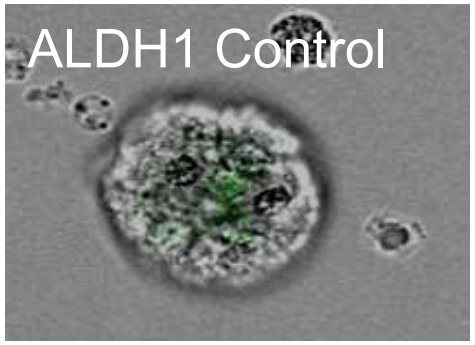


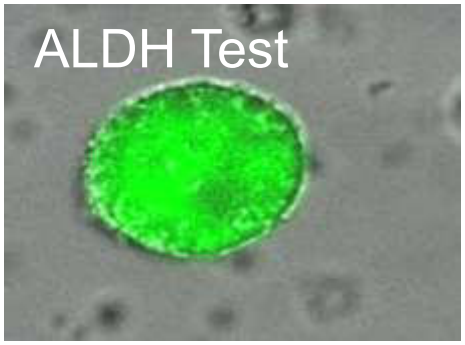






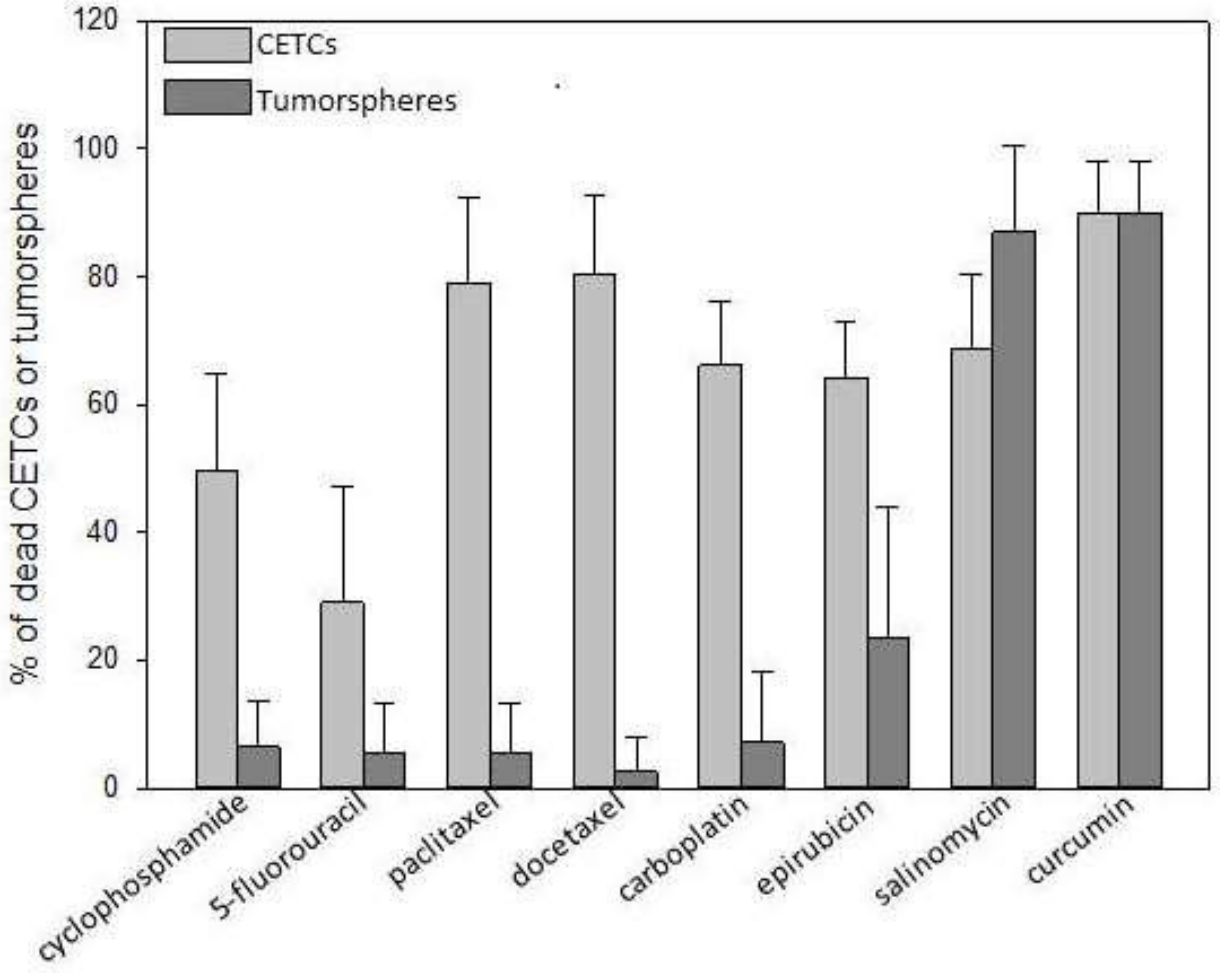




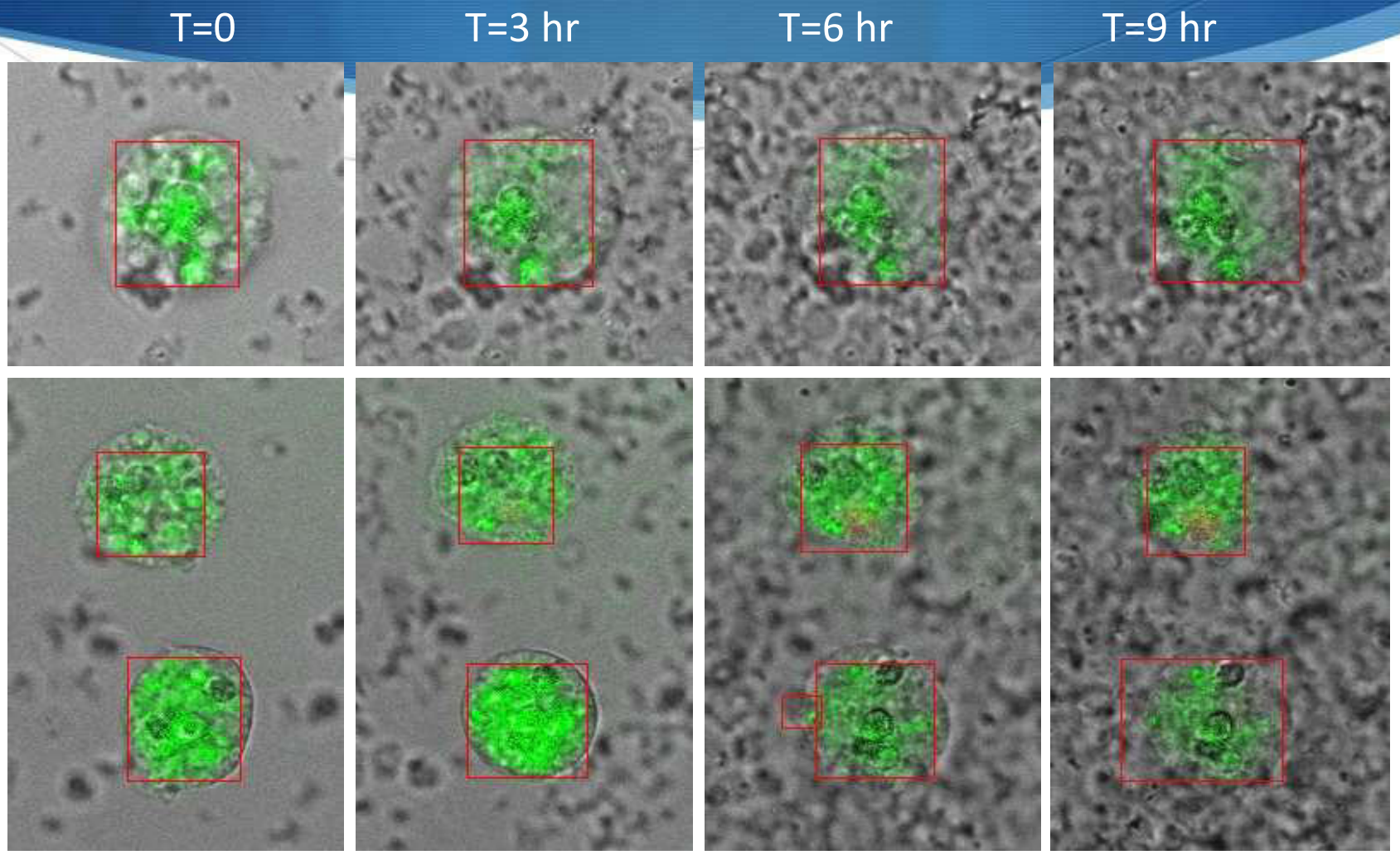


Chemo-sensitivity of tumour spheroids

Chemosensitivity of tumour spheroids vs. CETCs



Cancer stem cells are particularly sensitive to curcumin



Tumour spheres

Cancer Res 2013;73(24 Suppl): Abstract nr PD6-1

Tumour spheres growing from peripherally circulating tumour cells exhibit stem cell features

Abstract

Background: Among the cells that are disseminated from a malignant tumour only very few are capable to resettle in distant organs and grow into life-threatening metastases. Therefore, the question arises how and whether such cells which have the potential to grow into metastases can be detected. It has been shown that a subpopulation of cells from breast cancer tissue can form so-called mammospheres with stem cell features. Here we show that such tumour spheres can also be grown from peripherally circulating tumour cells from breast cancer patients in different stages of disease

Materials and Methods: Using a nondissipative approach with only one enrichment step of red blood cell lysis, the cells from the pellet, containing the white blood cells together with the putative tumour cells were cultured under conditions favoring the growth of epithelial cells. At 7, 14 and 21days the cell cultures were inspected for the appearance of spheroids staining with anti-EpCAM, anti-CD24 and anti-CD44 antibody and.expressing ALDH1.

Results: Peripherally circulating cells from patients with malignant tumours in different stages of disease were analyzed for the presence of circulating epithelial tumour suspect cells and the frequencies of tumourspheres. tumourspheres could so far be grown from 79% of 36 patients in whom more than 1700/ml epithelial tumour suspect cells were detected. Numbers of tumourspheres varied from 1 to 29 /ml and correlated with the aggressiveness of the tumour. Surprsingly the numbers were highest in patients after surgery who had not yet received any systemic therapy. The size of the spheres increased from day 7 to day 21. The spheres were negative for CD24 and positive for CD44. They highly express ALDH1 and thus exhibite typical features of stem cells.

Conclusion: Here, we demonstrate that the circulating tumour cells, detected in our approach contain a subpopulation with stem cell-like properties capable of growing into tumourspheres. The frequency and growth potential of cells capable of forming spheres seems to be dependent from the properties of the primary tumour. The possibility to grow tumourspheres from peripherally circulating tumour cells may open up a new field, where the relevant cells with stem cell properties from individual patients can now be specifically analysed further for genetic endowment, transcriptional activity, heterogeneity and stem cell markers.

http://cancerres.aacrjournals.org/content/73/24_Supplement/PD6-1.short

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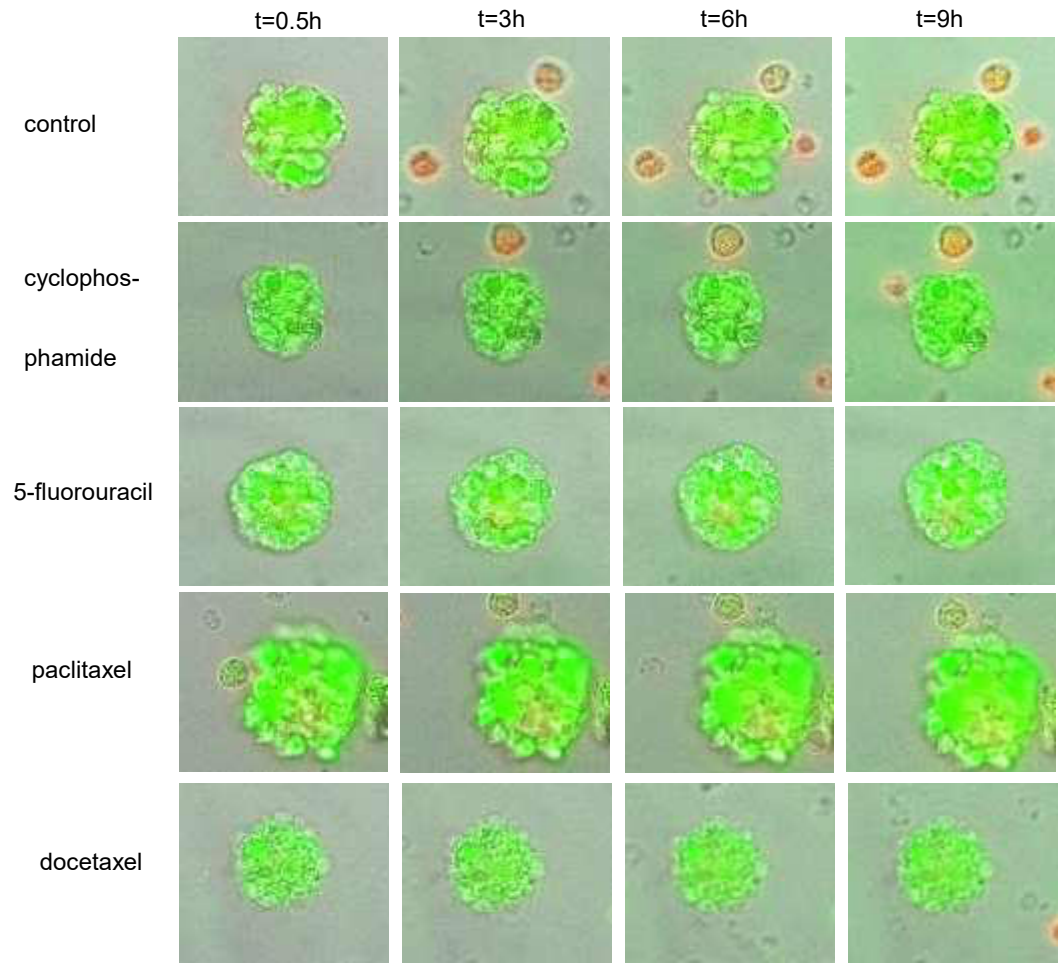
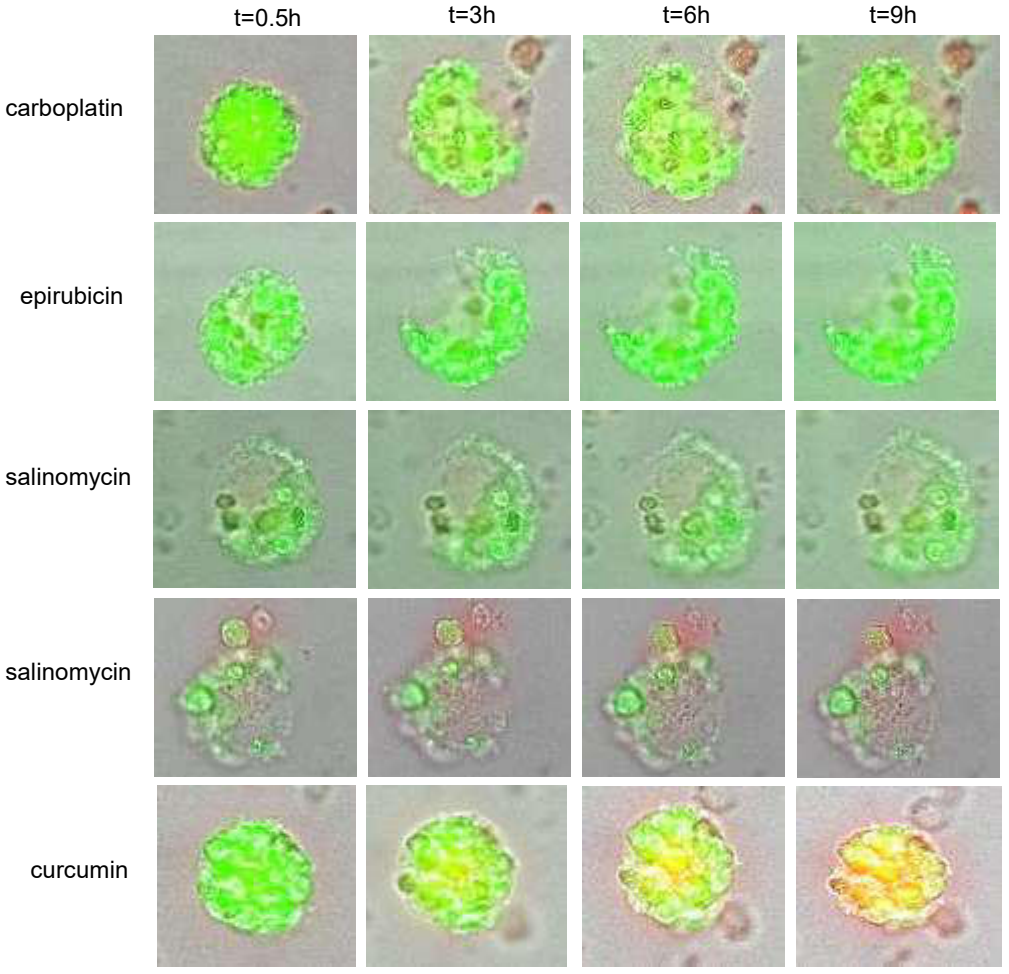
Fascinating to see the effectiveness of salinomycin and curcumin



Examples of tumourspheres with chemoresistance to cyclophosphamide, 5-fluorouracil, paclitaxel and docetaxel. tumourspheres remain alive during short time culture (0-9h).



tumourspheres sensitive to carobplatin, epirubicin, salinomycin and curcumin. Carboplatin and epirubicin lead to disintegration of tumourspheres with destruction of part of the cells in the spheroids. The strong cytotoxic effect of salinomycin is already observed at the first point of measurement with almost total destruction of all cells. Curcumin works by inducing cell death in all cells of the tumourspheres leading to nuclear staining with propidium iodide.

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New maintrac test: stemtrac

eichend für die
dikamenten.

○ Vinorelbin	○ Sulforaphan	
○ Cisplatin	○ Hypericin	
○ Carboplatin	○ Curcuma	
○ Oxaliplatin	○ Artesunat	

Personalisierte Medikamentenpakete *(bitte oben auswählen)*

○ 3 Medikamente	○ 5 Medikamente	○ 7 Medikamente

15 ml EDTA Blut
werden benötigt.

Zusatzuntersuchungen

○ *Immunstatus*	*Lymphozyten-Subpopulationen, NK-Zellen und Monozyten bei Sonderindikation 32011*	
○ ***stemtrac®***	*Zirkulierende Krebsstammzellen (Tumorsphären) Kultivierung über einen Zeitraum von bis zu 21 Tagen*.*	NEU
○ *thrombotrac* *Rücksprache erforderlich!*	*Thromboserisiko-Analyse (Gutachten und Laboruntersuchung) Bei Tumoren besteht erhöhtes Thromboserisiko. bei Sonderindikation 32011*	*0*

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Conclusion

Dynamics of CETCs as a **parameter**
for personalised therapy decisions

- CETCs can be **identified** and **characterised** in patients who have received a diagnosis of primary cancer
- maintrac is **quantitative**
- **Efficacy of medication** can be measured

→ **maintrac® can support therapeutic decisions**

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Within 48 to max. 72 h
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Results will usually be sent
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sample.

Thank you
for your attention

Arbeitsgemeinschaft TZB

Publications 2015

Treatment of advanced solid tumours with NSAID. Correlation of quantitative monitoring of CTCs to PET imaging. Hochmuth-Willecke R

Prognostic Role of Circulating tumour Cells during Induction Chemotherapy Followed by Curative Surgery Combined with Postoperative Radiotherapy in Patients with Locally Advanced Oral and Oropharyngeal Squamous Cell Cancer. Inhestern J, Oertel K, Stemmann V, Schmalenberg H, Dietz A, Rotter N, Veit J, Görner M, Sudhoff H, Junghanß C, Wittekindt C, Pachmann K, Guntinas-Lichius O. PLoS One. 2015 Jul 17;10(7):e0132901. doi: 10.1371/journal.pone.0132901. eCollection 2015.

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