maintrac® – What's the future in precision diagnostics? From screening to stem cells and back!

Cancer is a frightening diagnosis: why?

- Malignant tumours are detectable when they have reached a size of about 1cm.
- The first therapeutic action in most cases is complete surgical removal of the tumour.
- But metastases can appear at distant locations even after complete resection of the primary tumour.

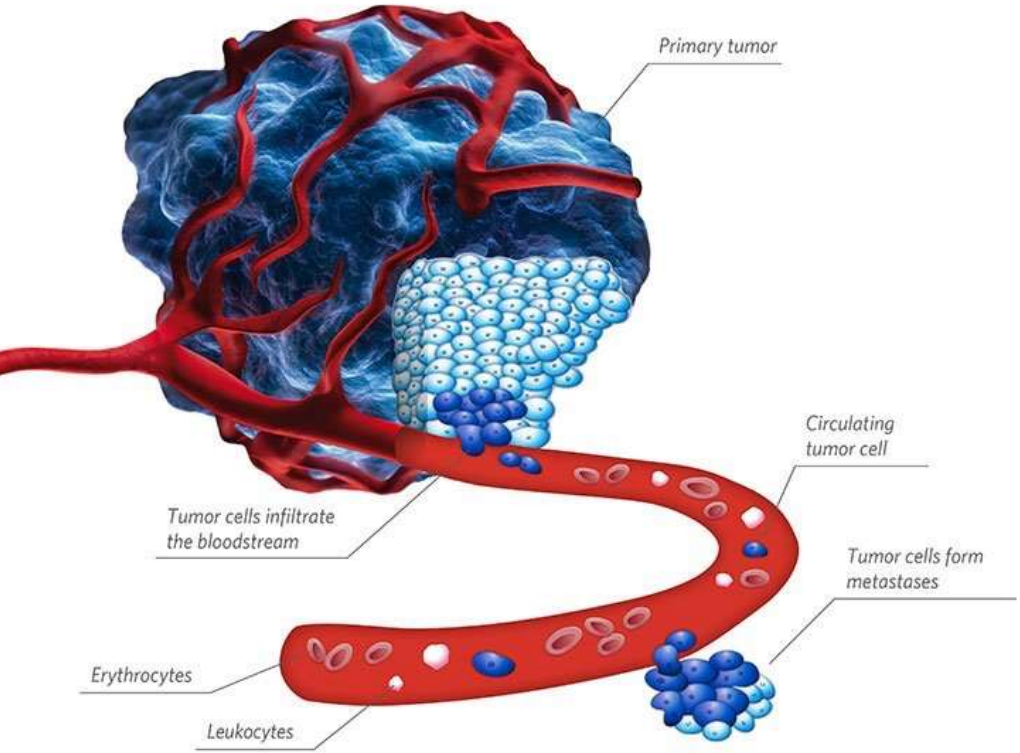
Cancer is a frightening diagnosis: why?

- Such metastases occur in 25-50% of cases after „successful“ surgery.
- They are found most frequently in vital organs like liver, lung and bone marrow
- Metastases are able to destroy these organs leading to fatal outcome.

How do metastases develop?

- **Cells can break away from tumours during tumour growth.**
- After the tumour has been removed, the cells **left behind in the patient‘s body** count.
- They are responsible for the formation of **distant metastases.**

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

TZB

Detection

Arbeitsgemeinschaft TZB

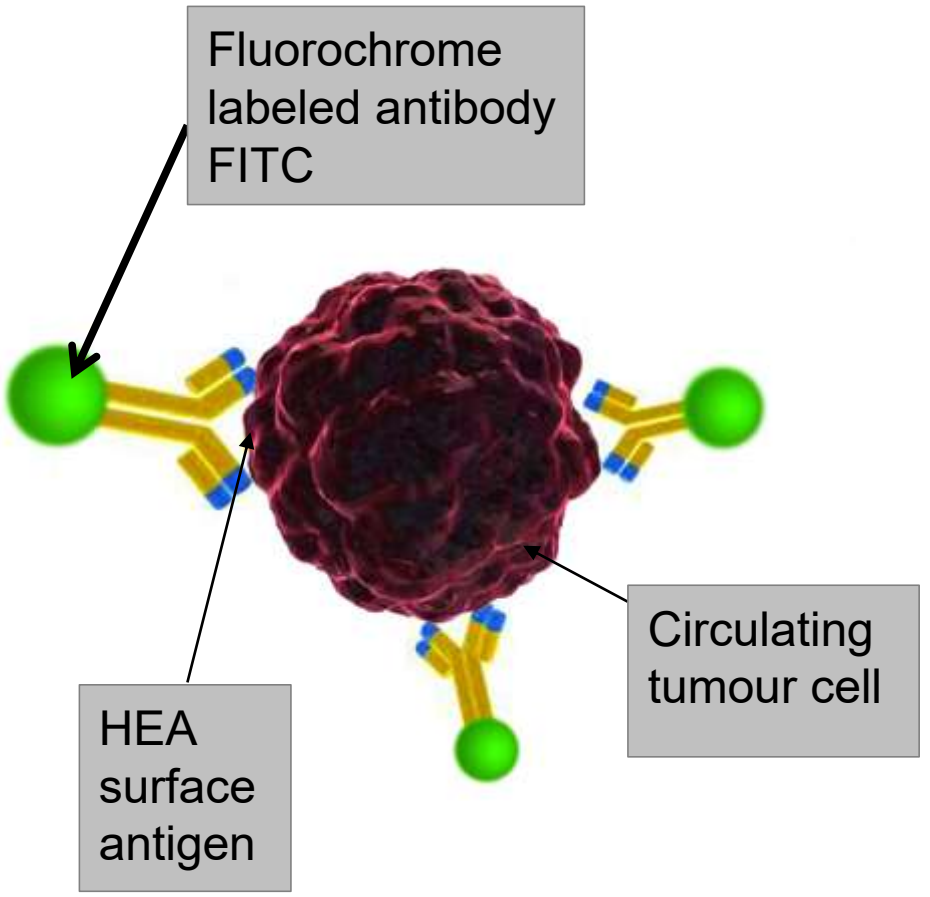
Method

maintrac **liquid biopsy** cell staining allows **quantitative** detection of **vital** circulating tumour cells

NO fixation.

NO isolation.

NO enrichment.



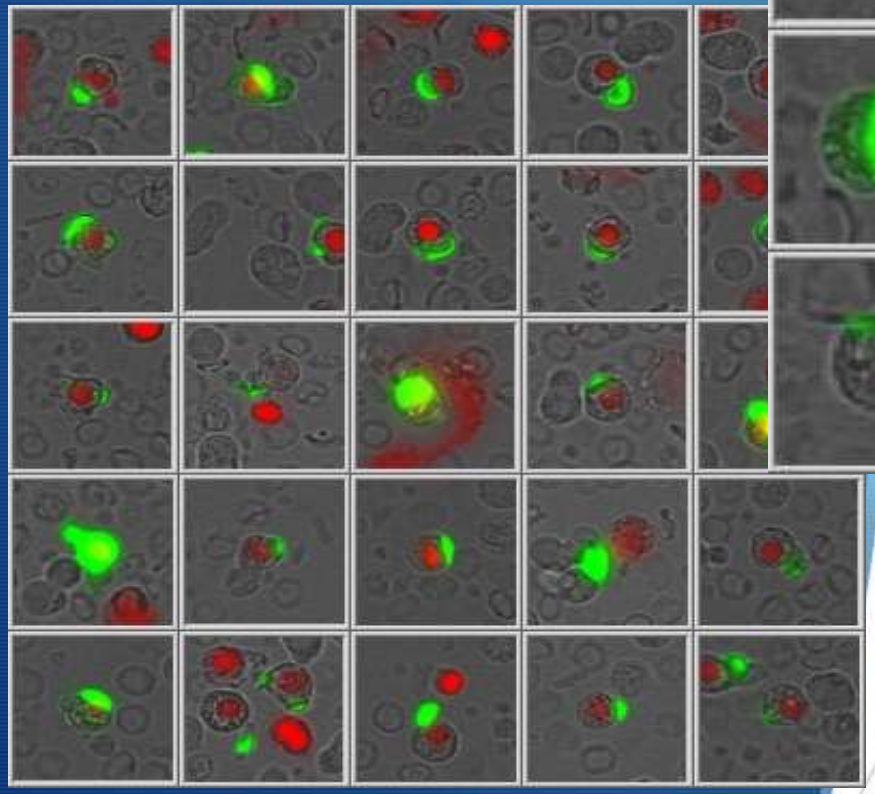
Testing

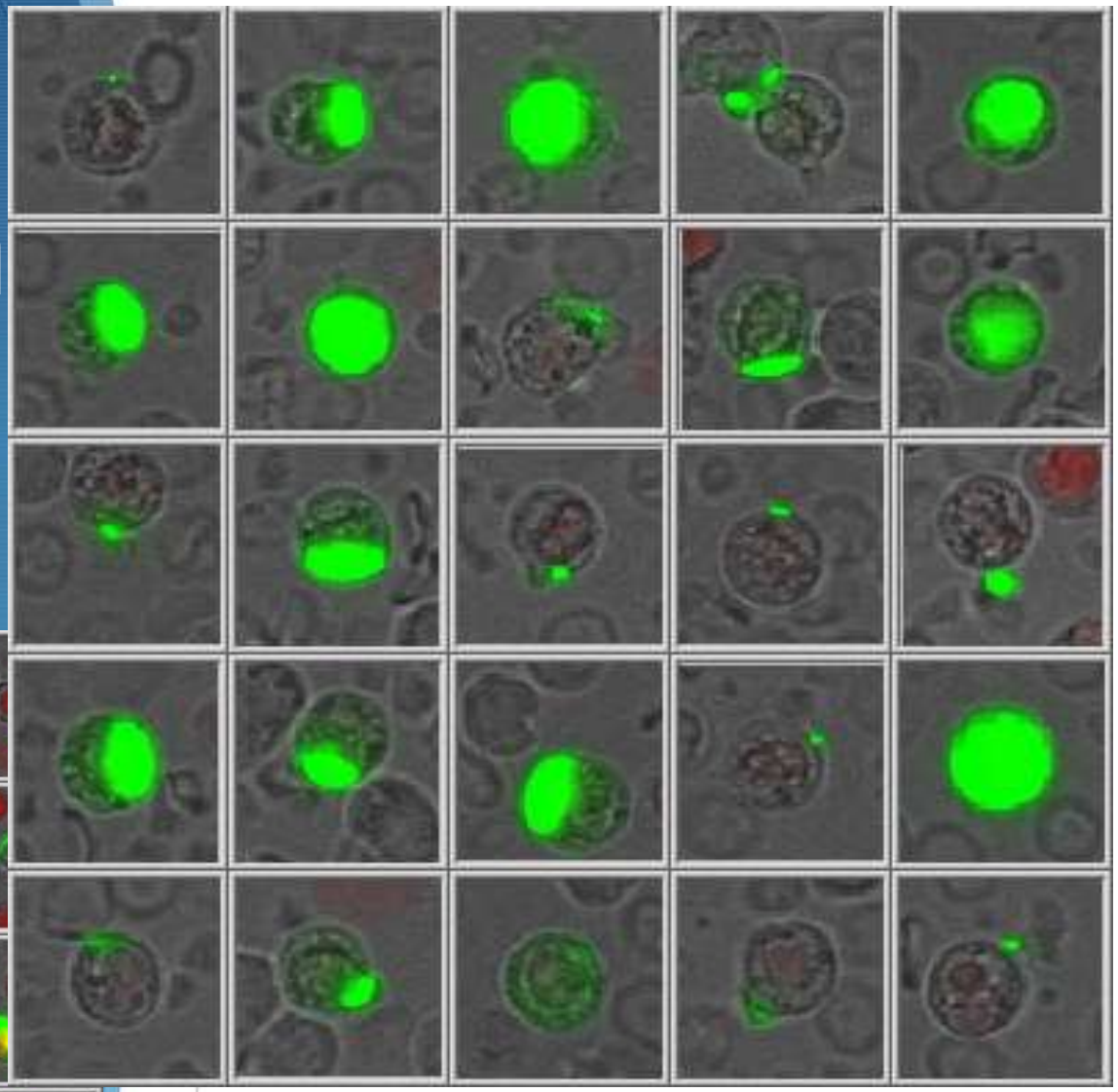
Microscope based semi-automated image evaluation

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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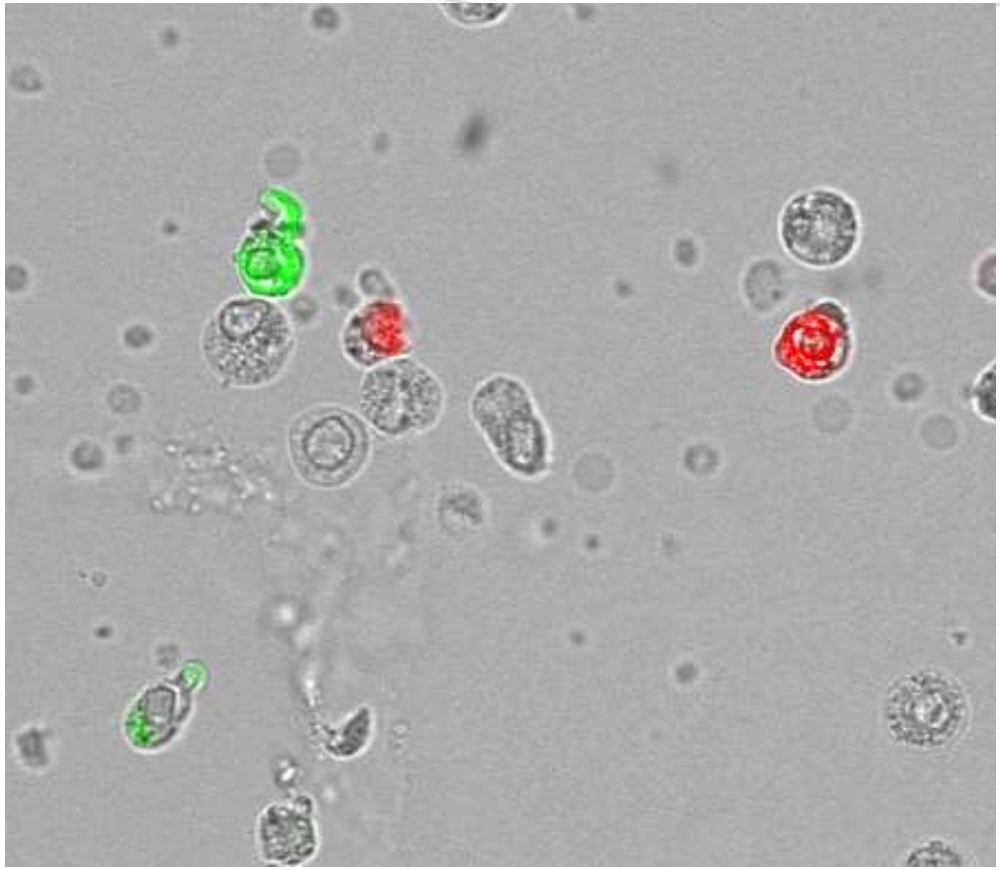
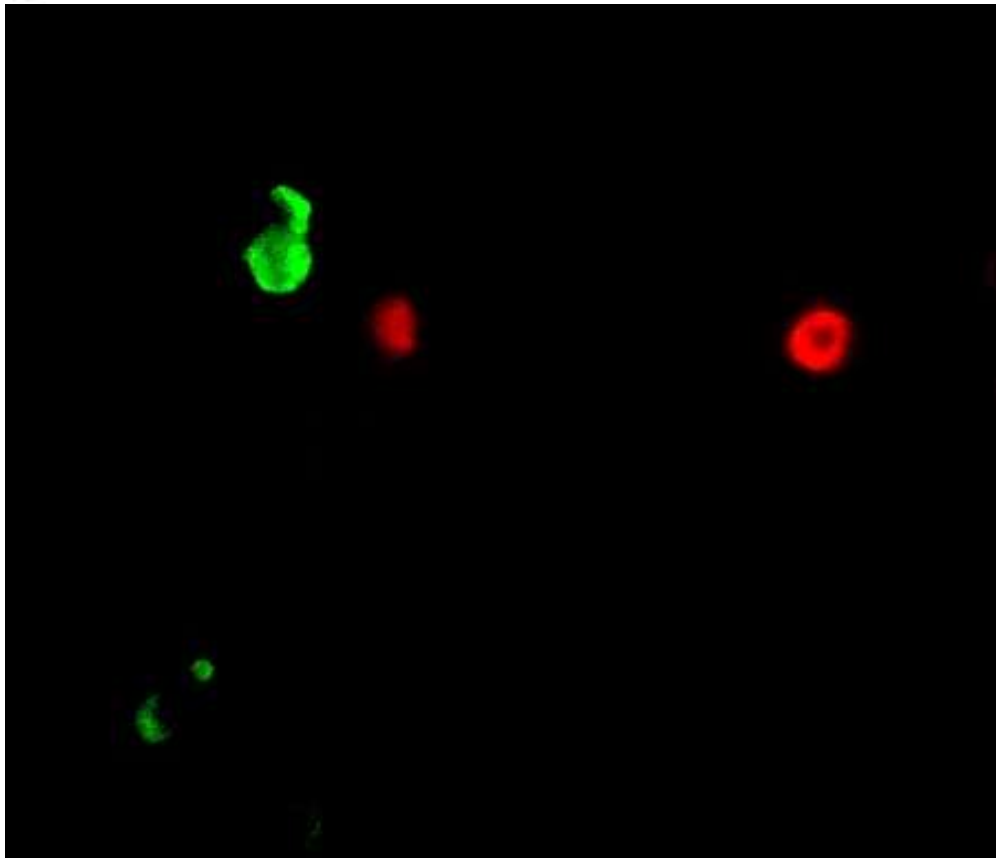
Validation

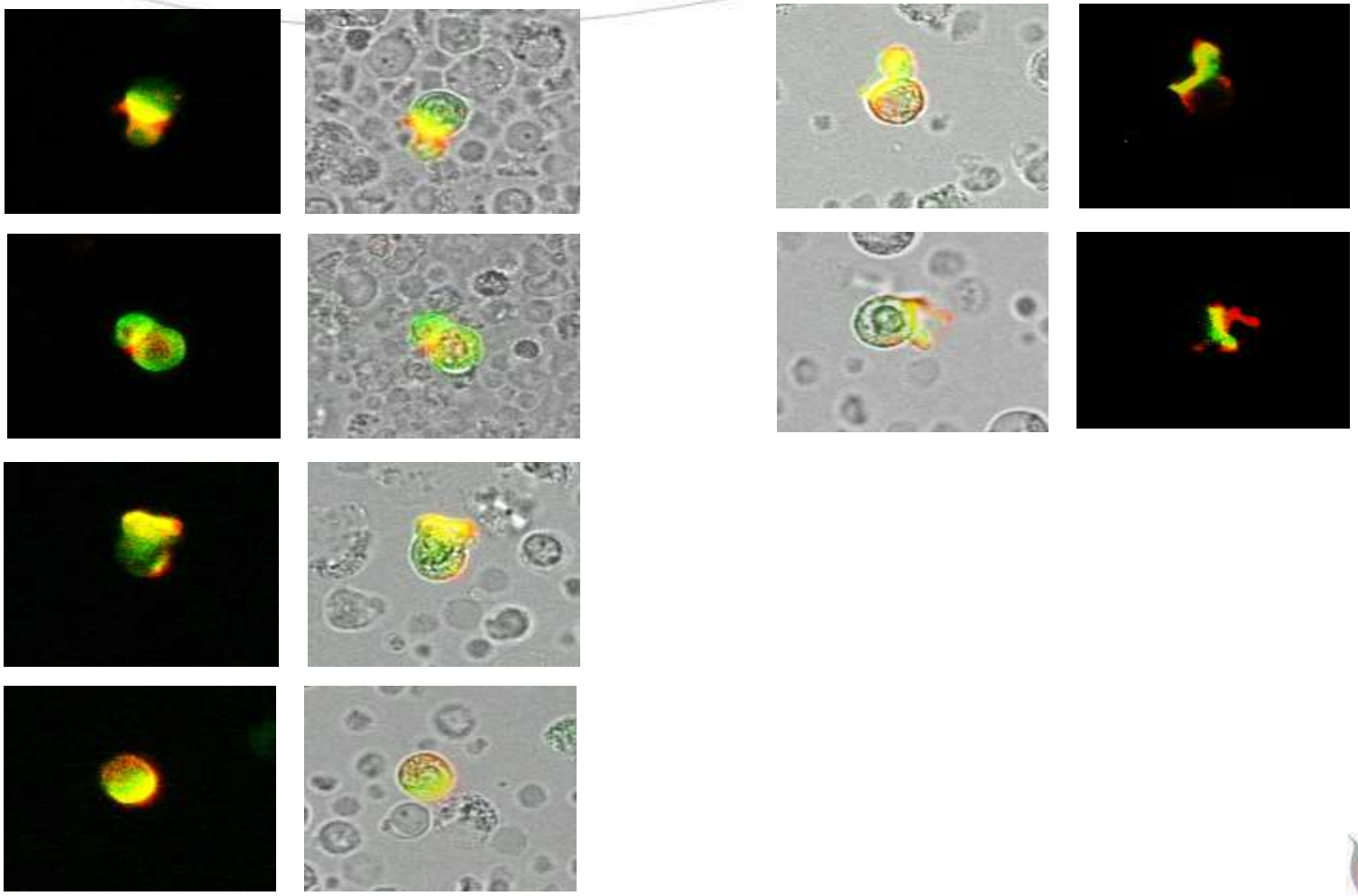
Arbeitsgemeinschaft TZB

Counterstaining CD45/EpCAM



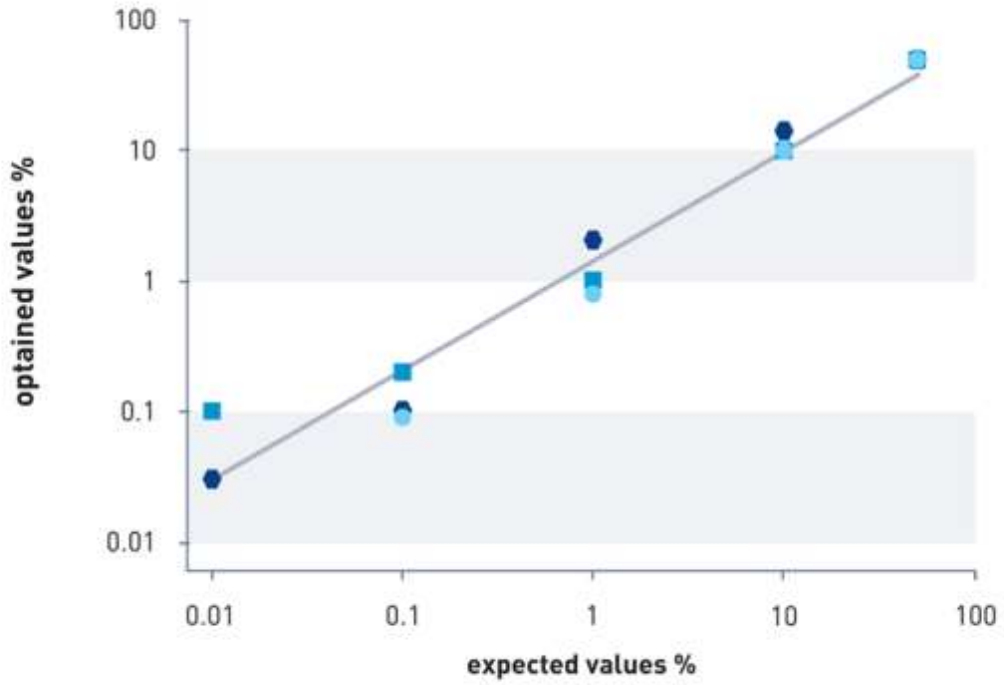


Counterstaining Cytokeratin/EpCAM



Spiking Tests

Spiking tests



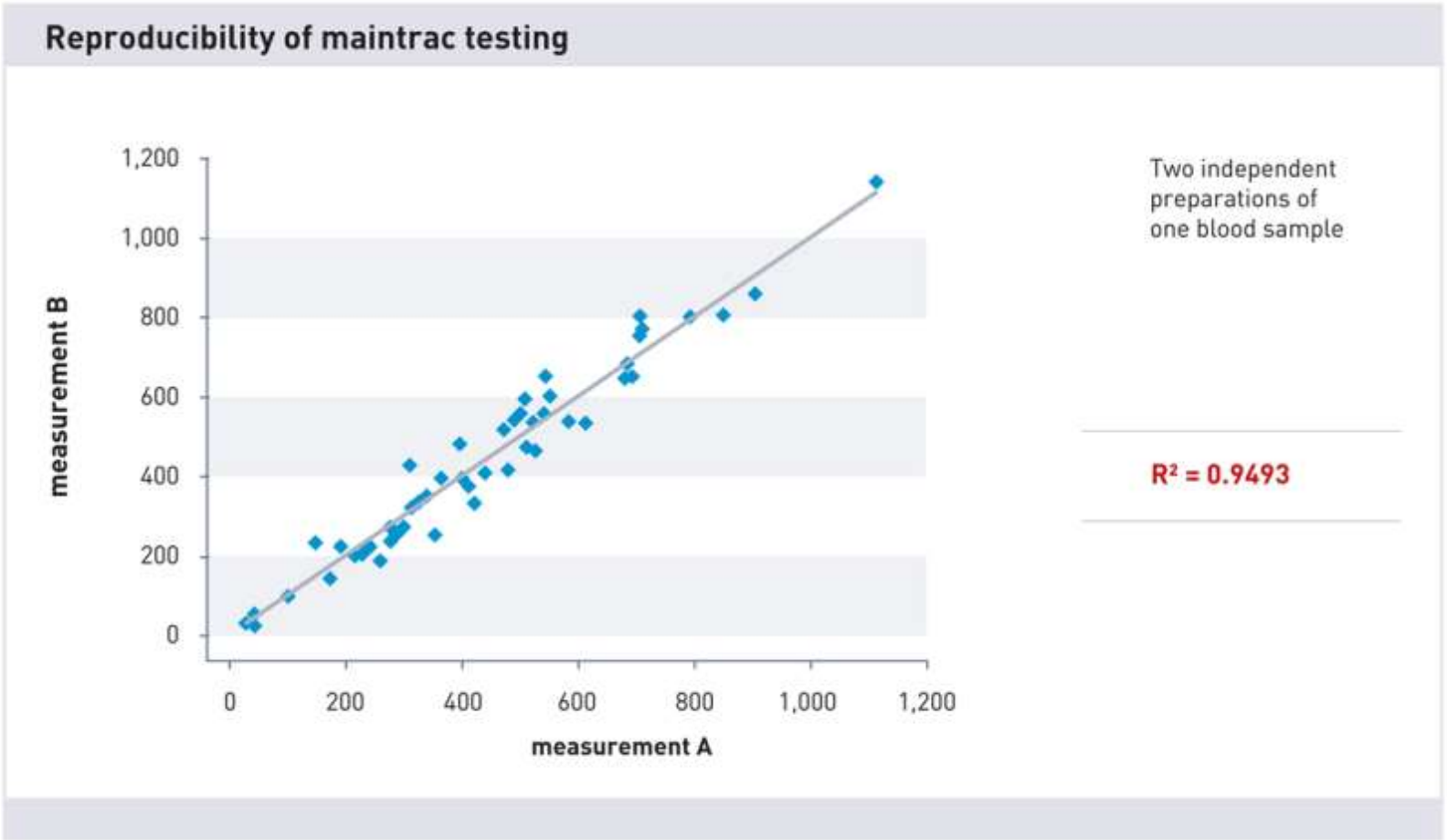
Correlation between expected and observed values of SKBR2 tumor cells in peripheral blood

$R^2 = 0.9859$

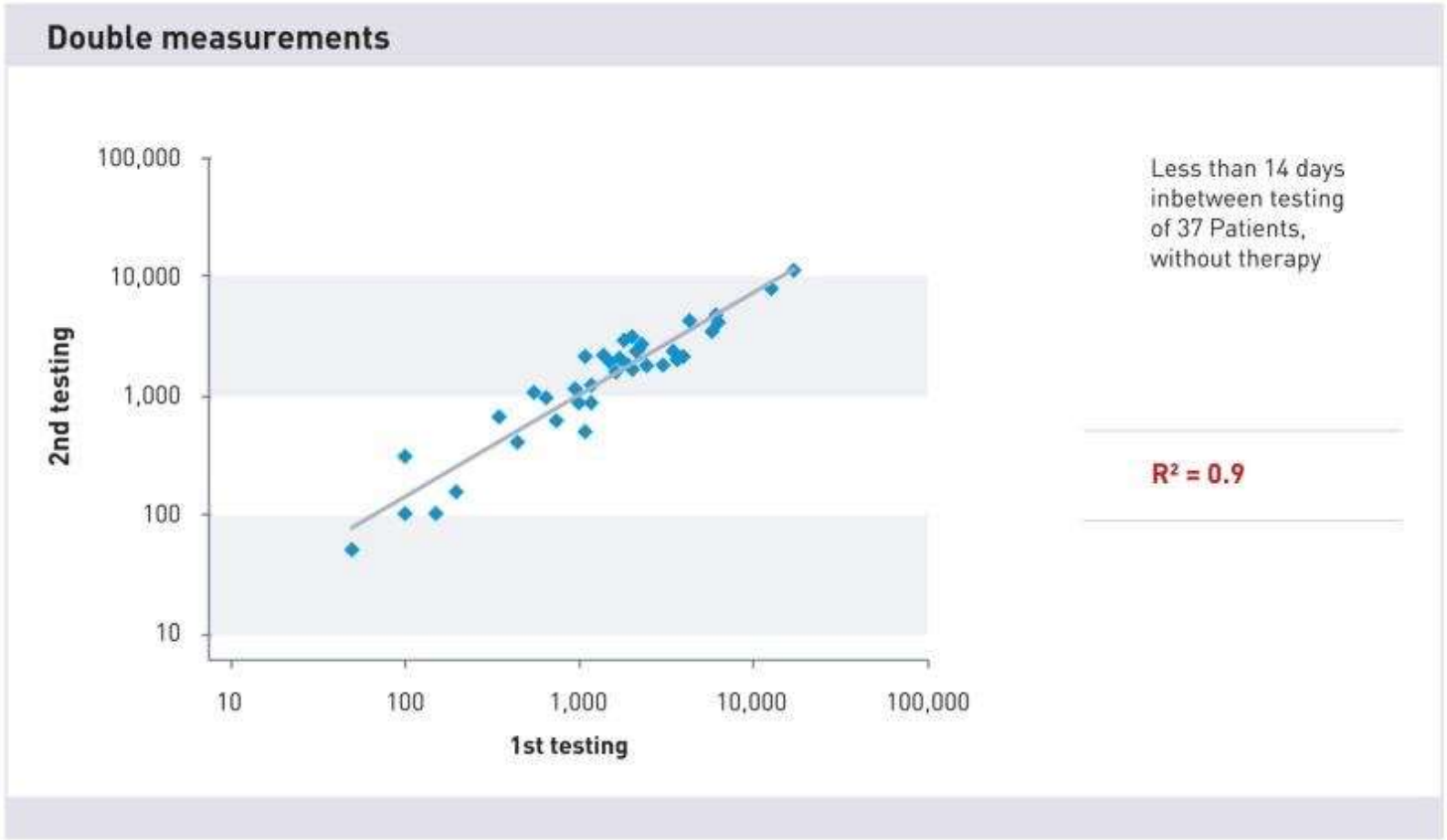
K. Pachmann et al., Clin Chem Lab Med 2001, 39: 811-817

TZB

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 billion
Monocytes	200 – 800 million
Thrombocytes	300 million
Circulating tumour cells	10 – 100.000

TZB

CETC comparison to 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

Screening

Screening Healthy Individuals
maintrac is not sufficiently specific

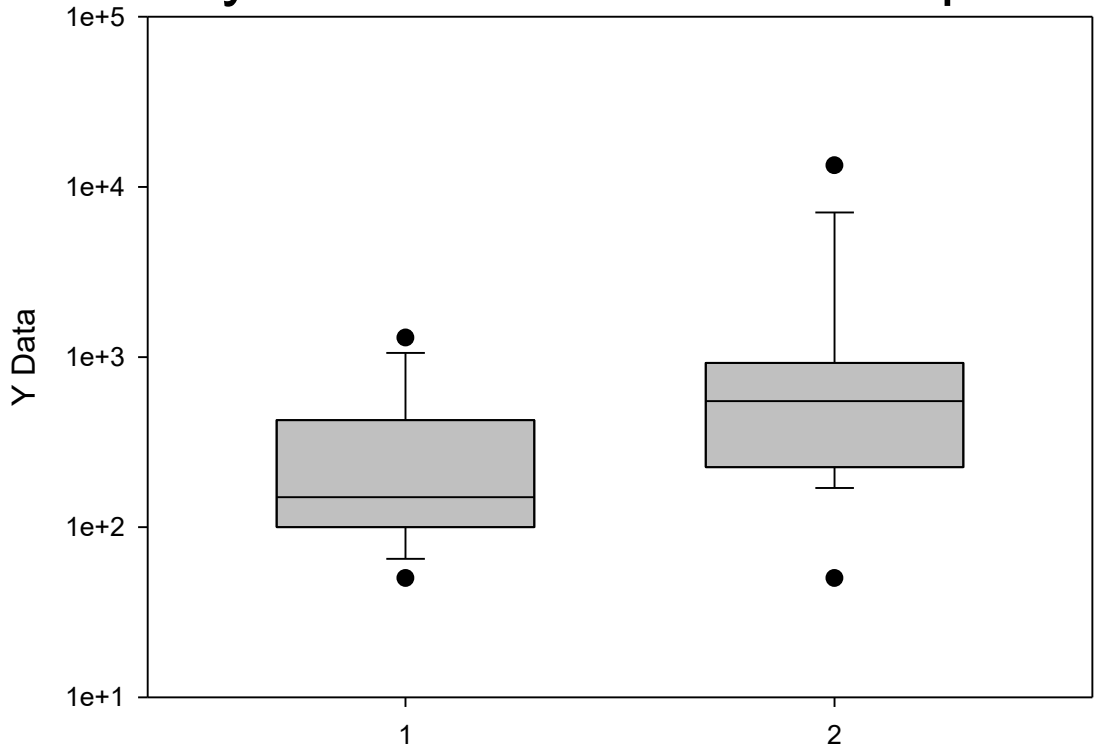
- Detection of suspect cells can unnecessarily frighten individuals
- Such a method should detect cancer cells with than 99.9% certainty
- It may not be confounded by other deseases

Screening individuals at risk

- Patients must be aware of the problematic issues
- Increasing numbers of circulating supect 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



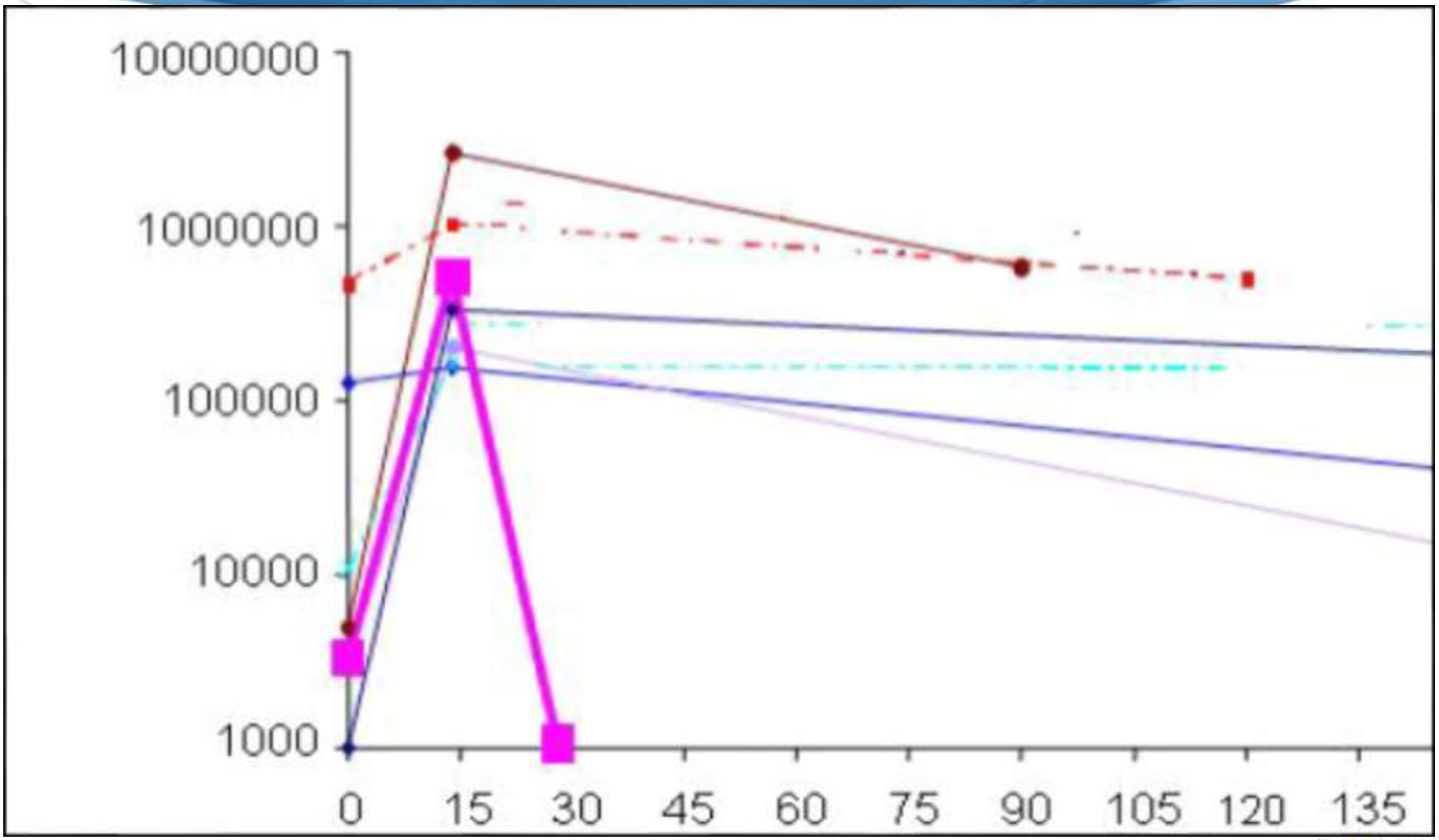
Monitoring therapy using Circulating tumour cells

Surgery

The main application of Circulating tumour cells is monitoring of therapy

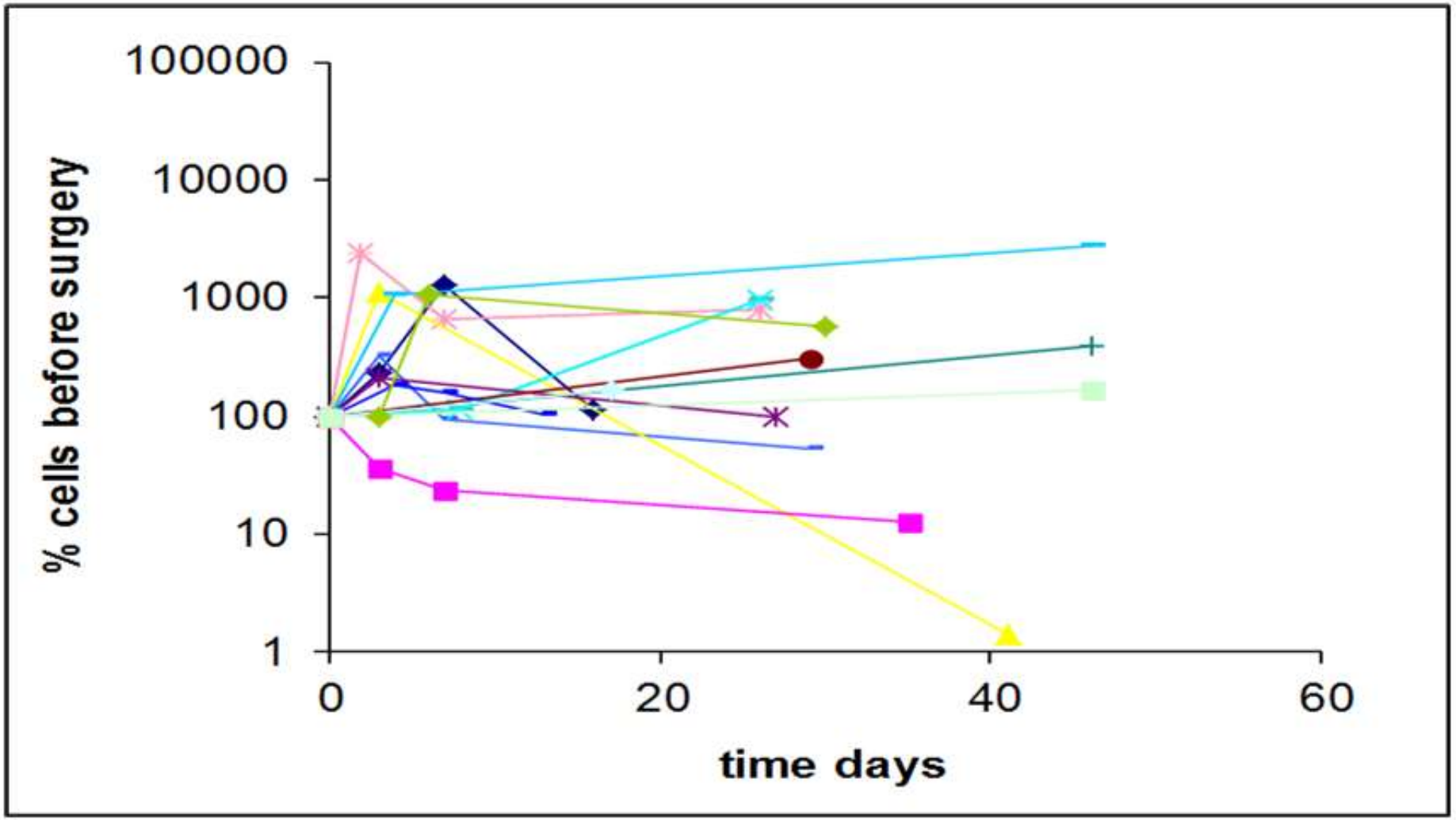
Surgery

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)



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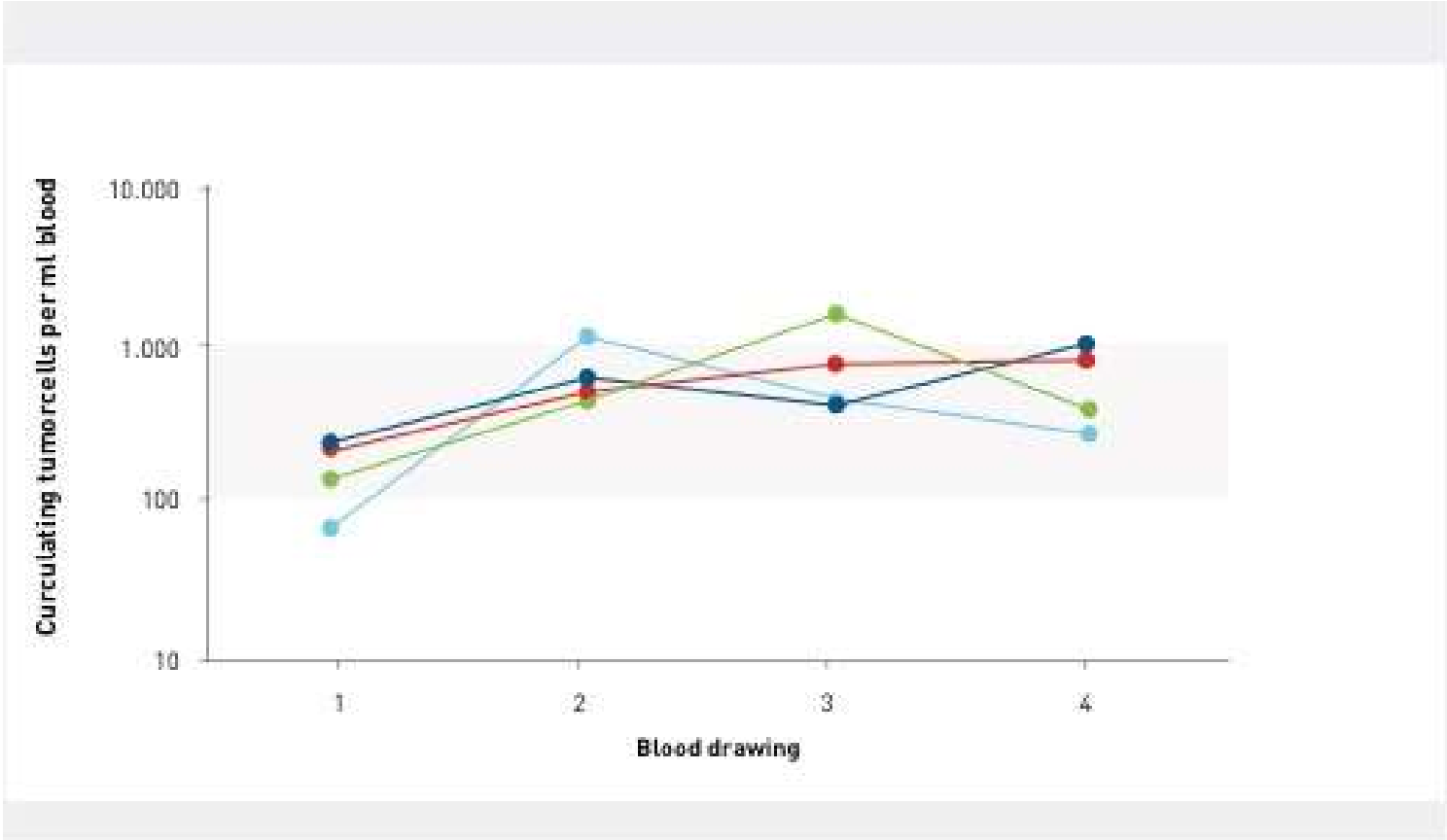
Patterns of CETCs before and after surgery (colon)

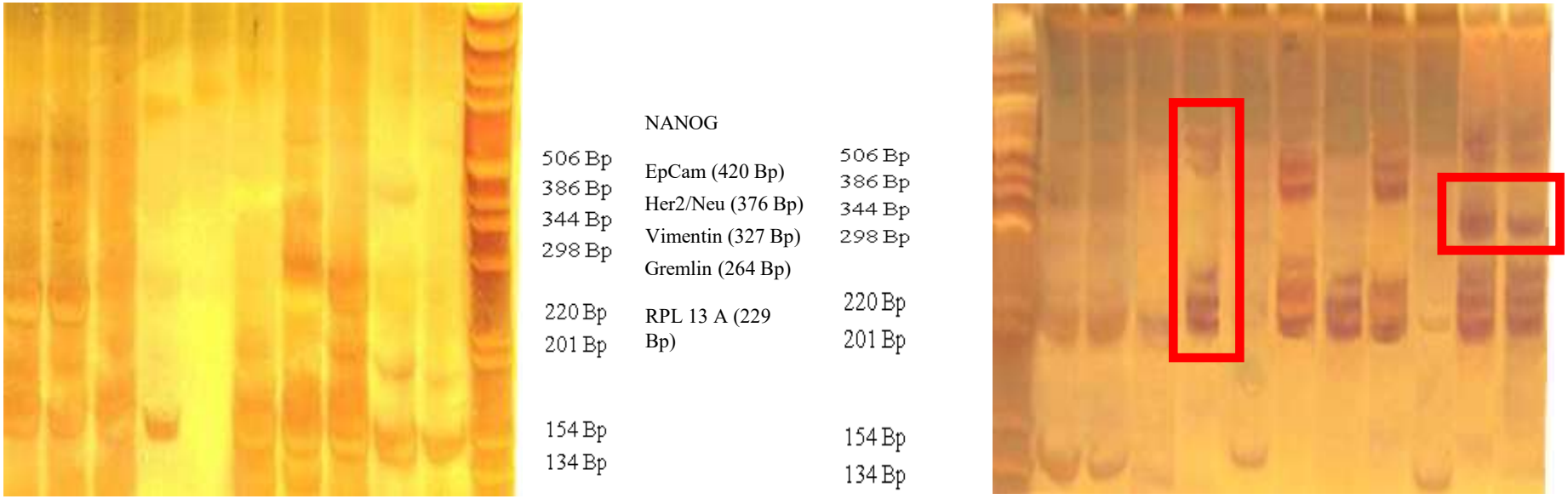


Changes of gene expression in circulating tumour cells after surgery

G, C

Pre OP

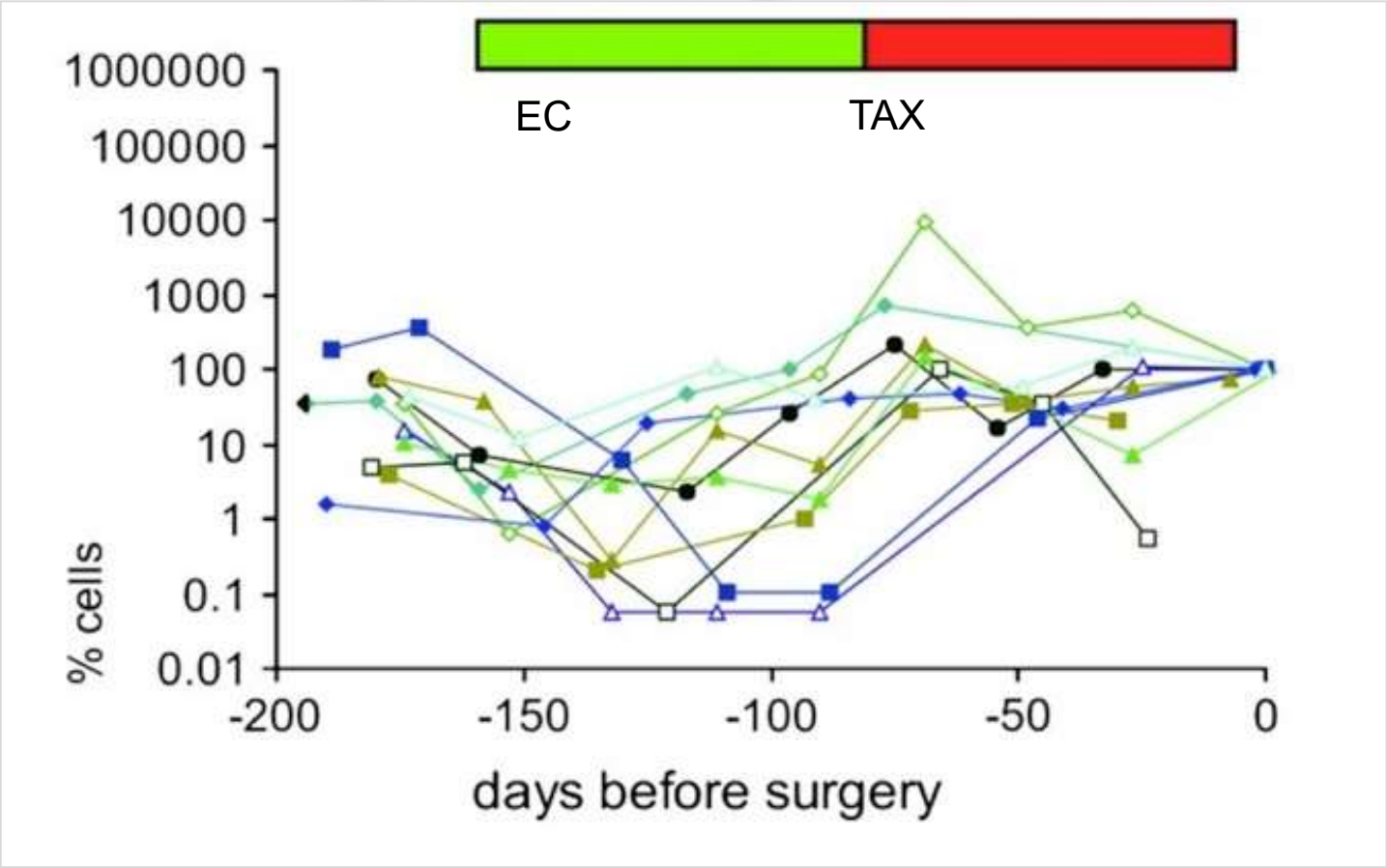
Post OP



Increased expression of stem cell and athesion markers after surgery

Neoadjuvant treatment

Neoadjuvant treatment



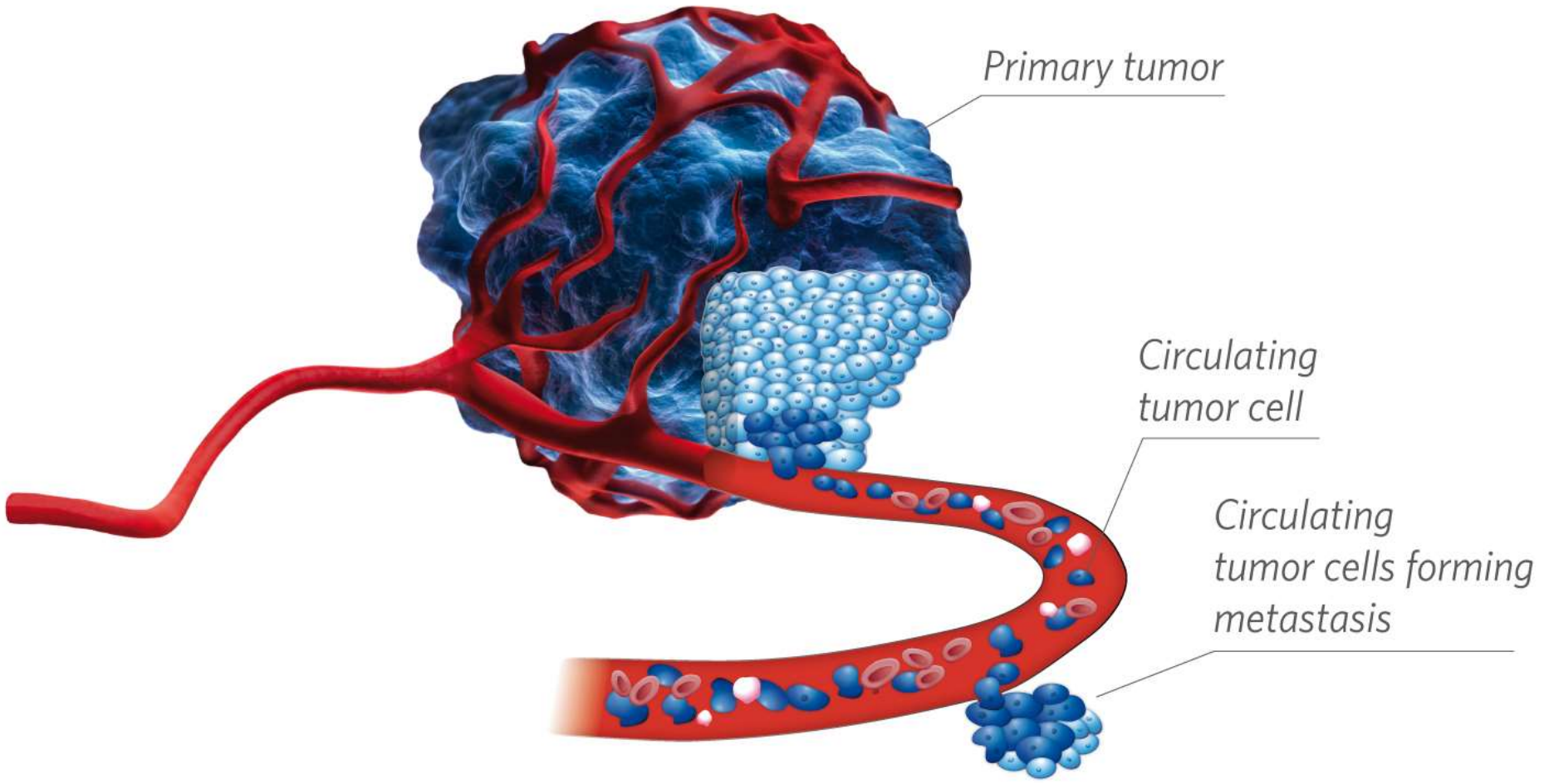
At the end of neoadjuvant therapy almost all patients experience increasing numbers of CECTs !

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

Neoadjuvant treatment

- Neoadjuvant chemotherapy may initially eliminate minimal residual disease (cells circulating in the blood). However, during tumour shrinkage often a re-increase of tumour cells in blood is observed.
- Increasing numbers of CECTs may be due to release of cells in addition to cell death.

Neoadjuvant chemotherapy shrinks the tumour, seeding cells into blood

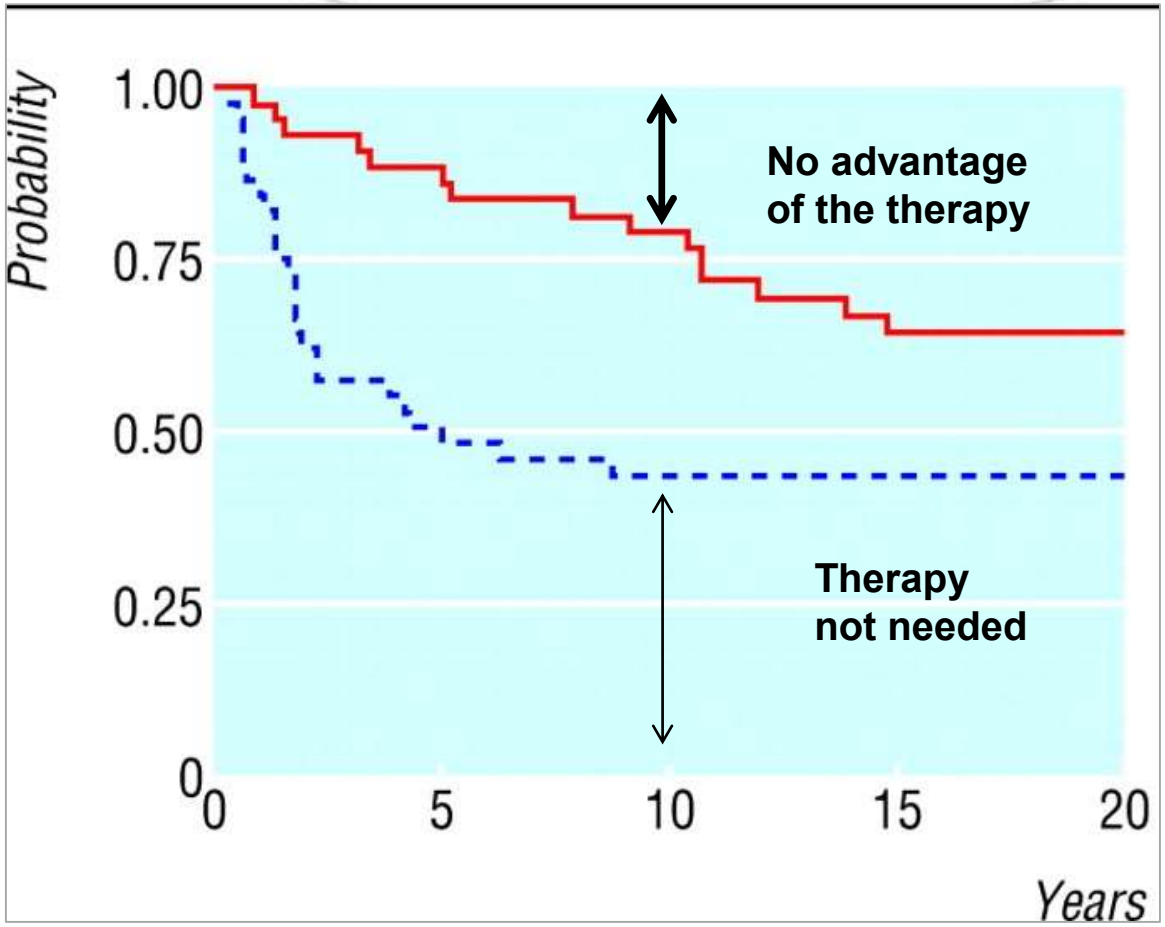


Adjuvant treatment

Adjuvant treatment Background

- From experimental systems it was assumed that minimal residual disease can be left in the body after surgery.
- **Systemic adjuvant therapy was established to eliminate these cells remaining in the body.**

30 years of adjuvant CMF therapy



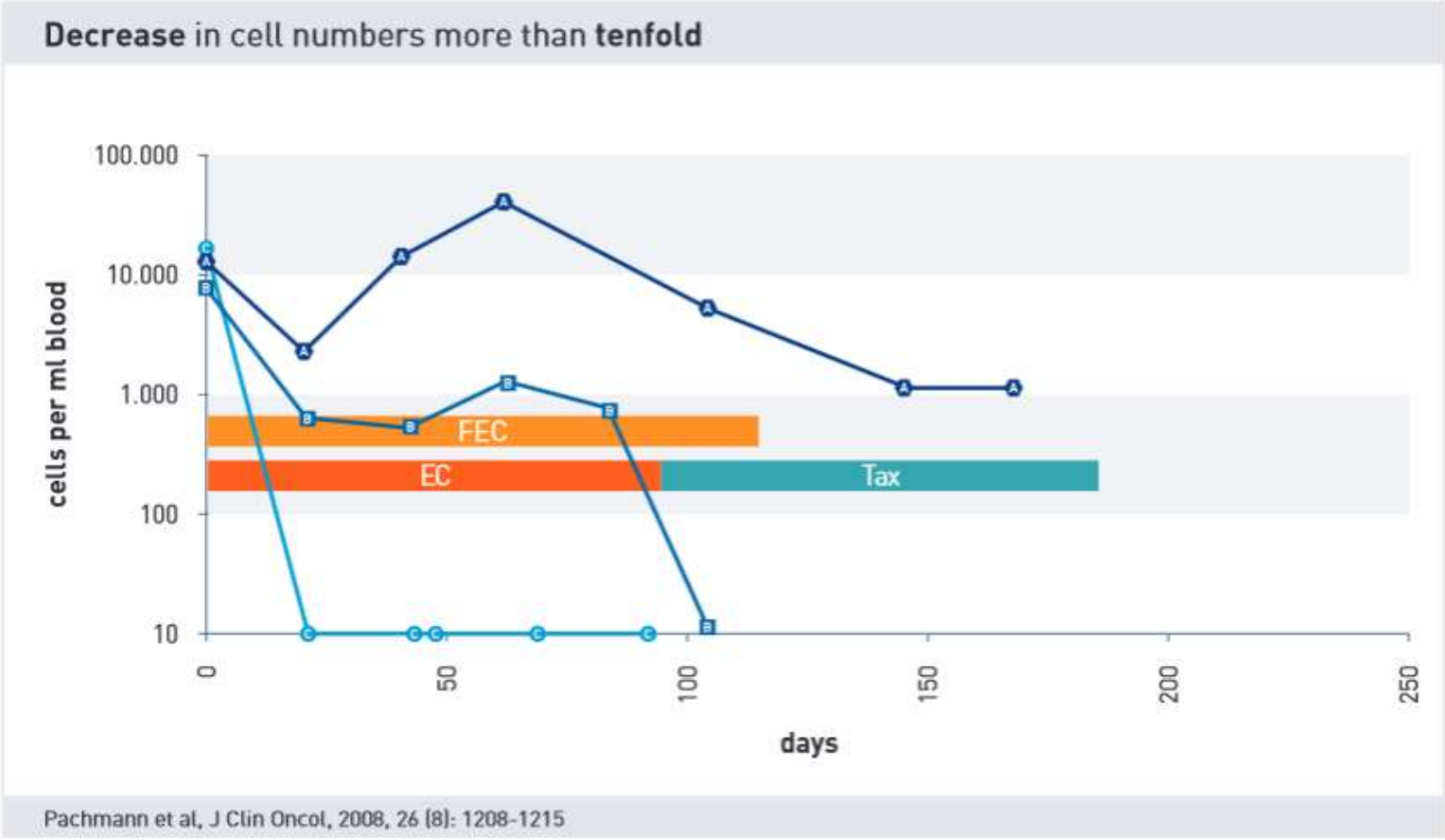
- Relapse-free survival
- Lymph node negative, ER-negative patients

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

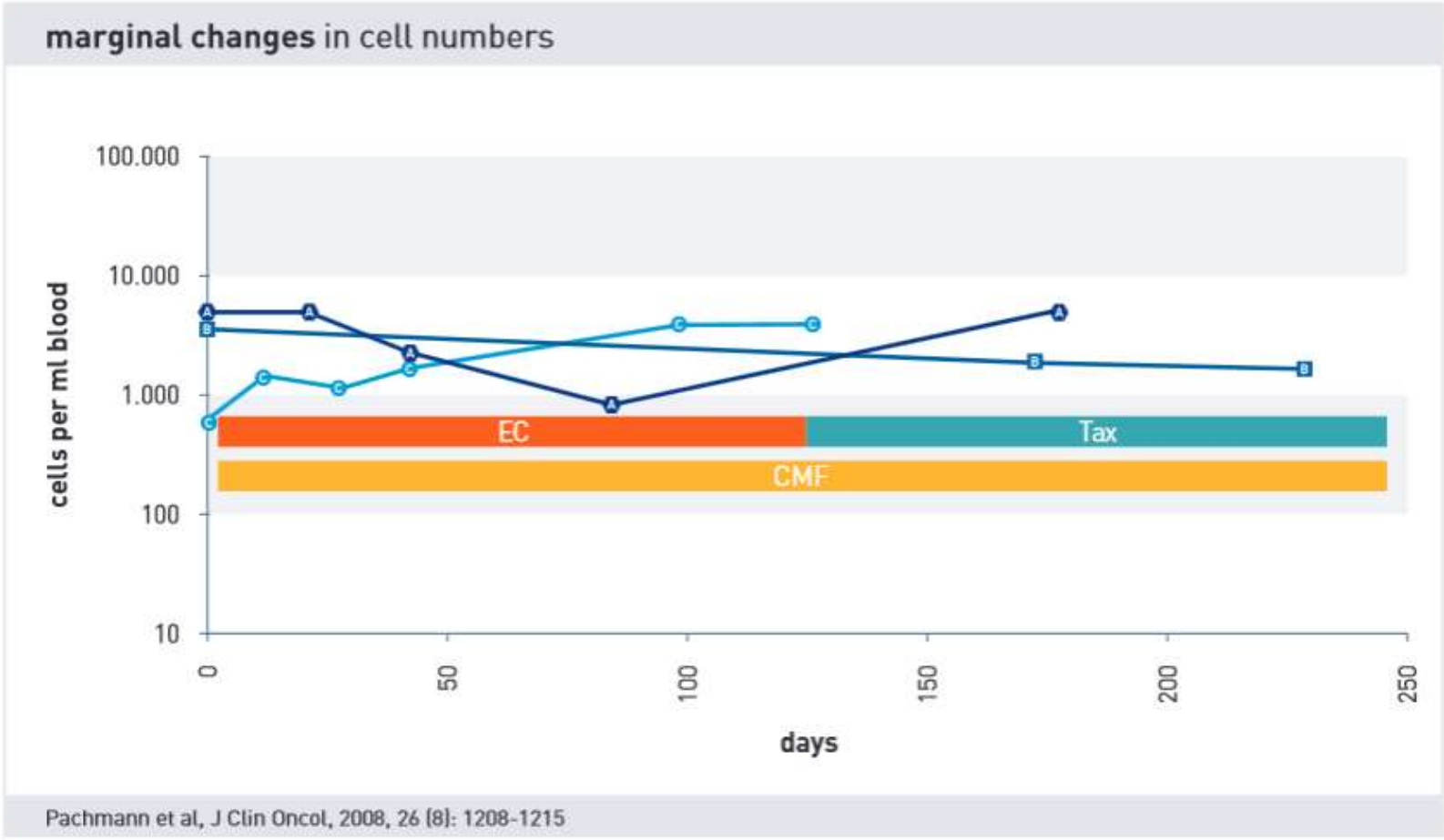
Adjuvant treatment Background

- Regrowth of tumors from these minimal numbers of cells remaining in the body is what accounts for relapse
- We **count the changes** in numbers of these cells in **response to therapy**

Adjuvant treatment
decreasing cell numbers

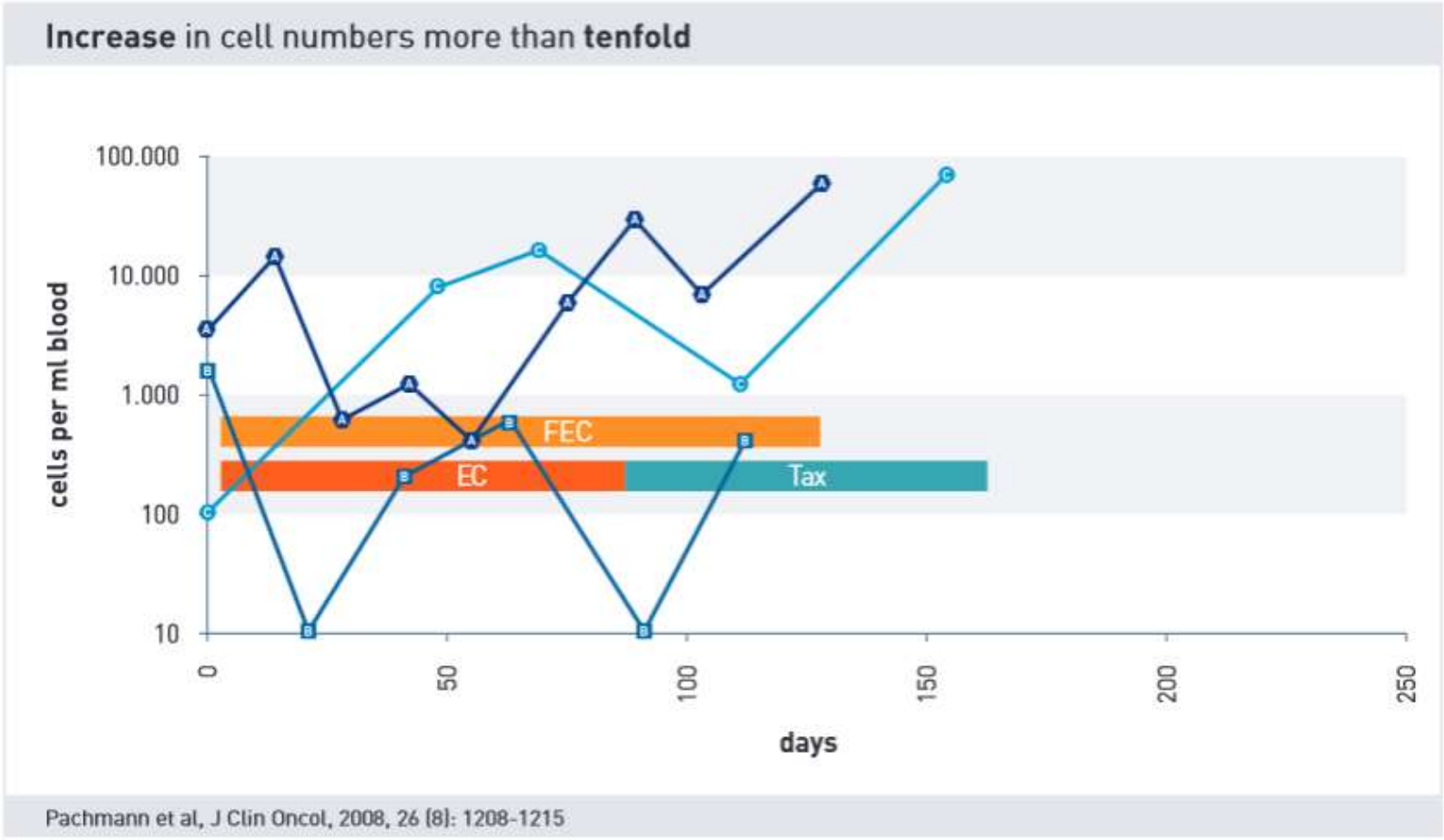


Adjuvant treatment
marginal change in cell numbers

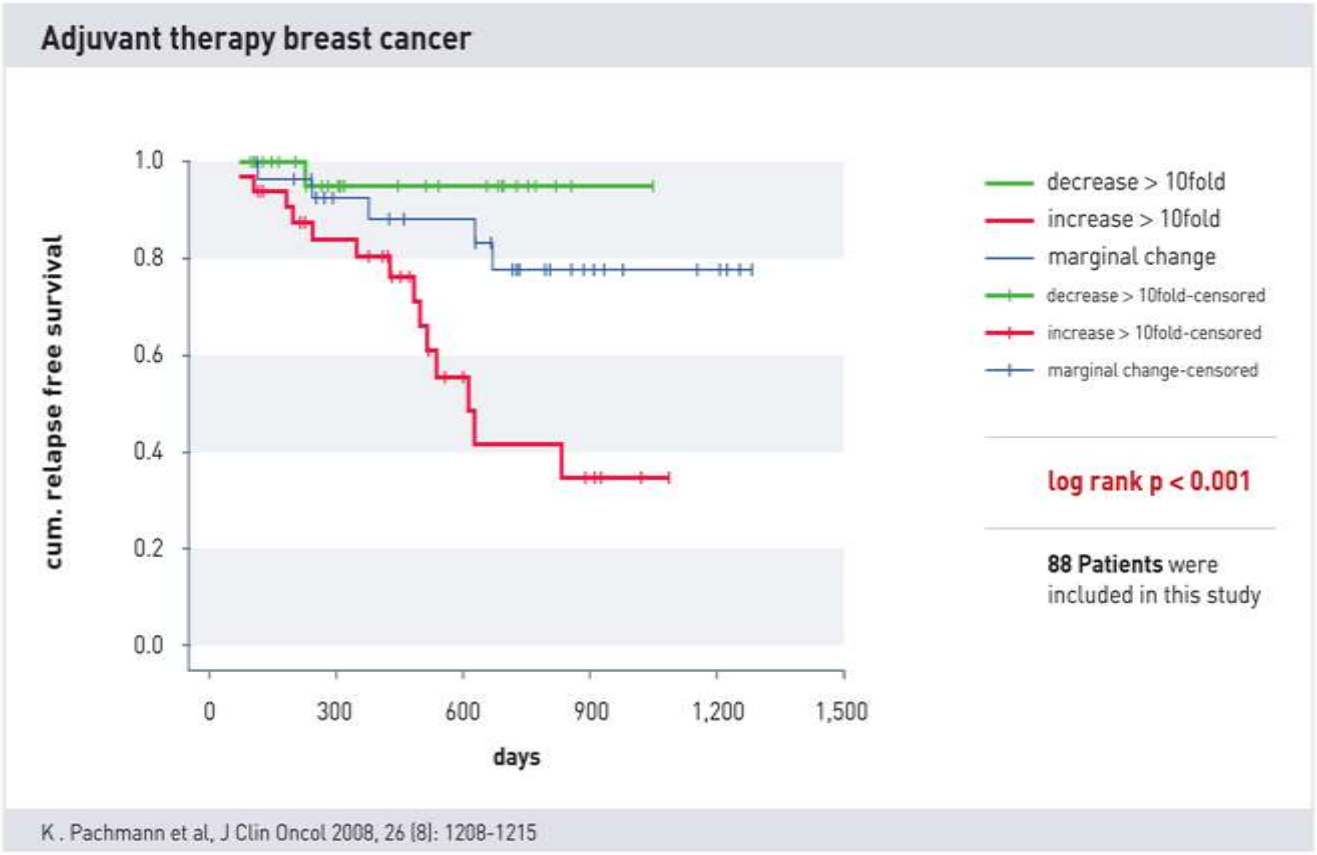


Pachmann et al, J Clin Oncol, 2008, 26 (8): 1208-1215

Adjuvant treatment
increasing cell numbers



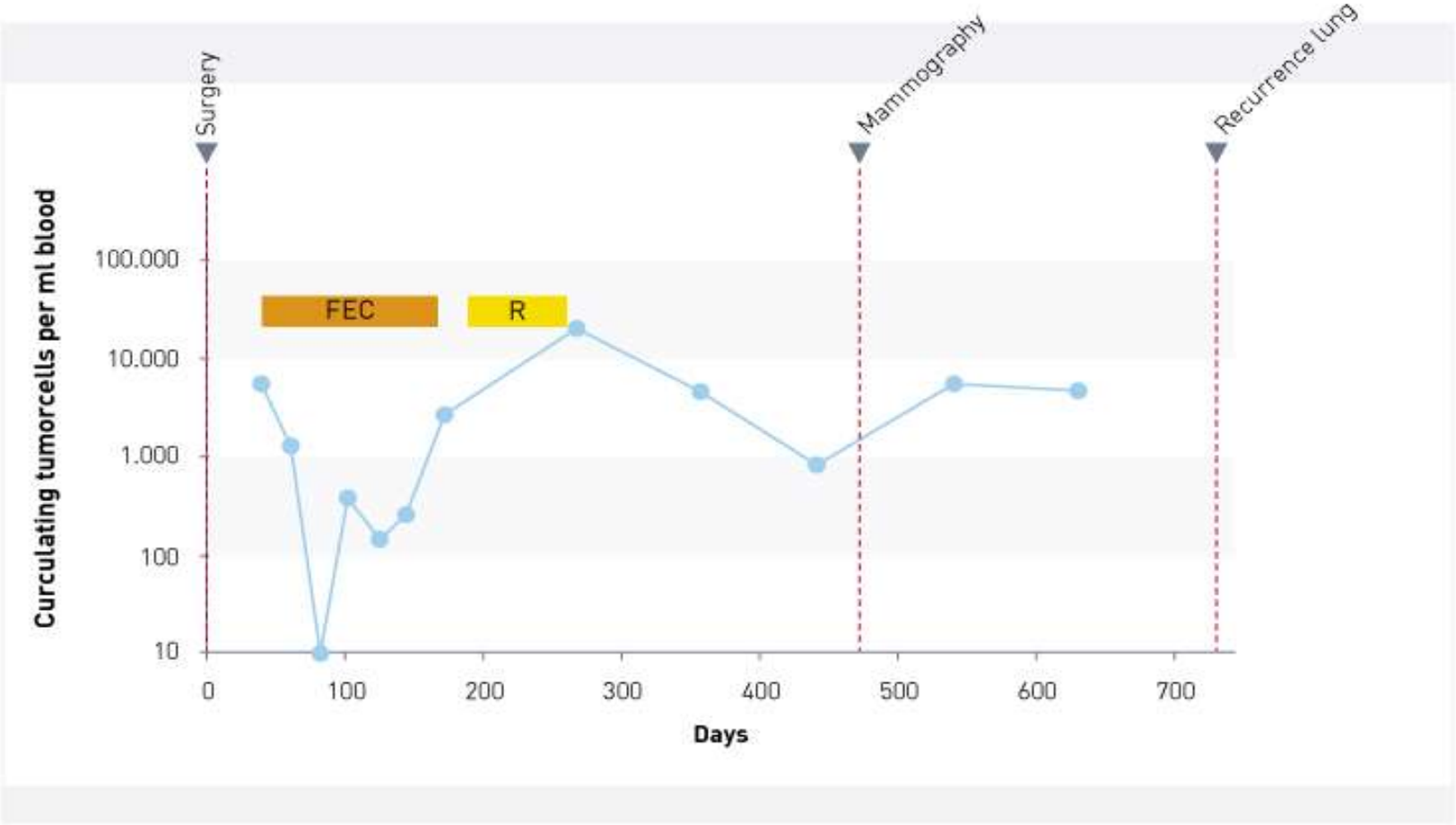
Adjuvant treatment



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

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

Case report
increasing cell numbers



Increasing cell numbers

What can we do?

Chemo-sensitivity

J Cancer Therapy 2013, 4:597-605

Chemosensitivity Testing of Circulating Epithelial Tumor Cells (CETC) in Vitro: Correlation to 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

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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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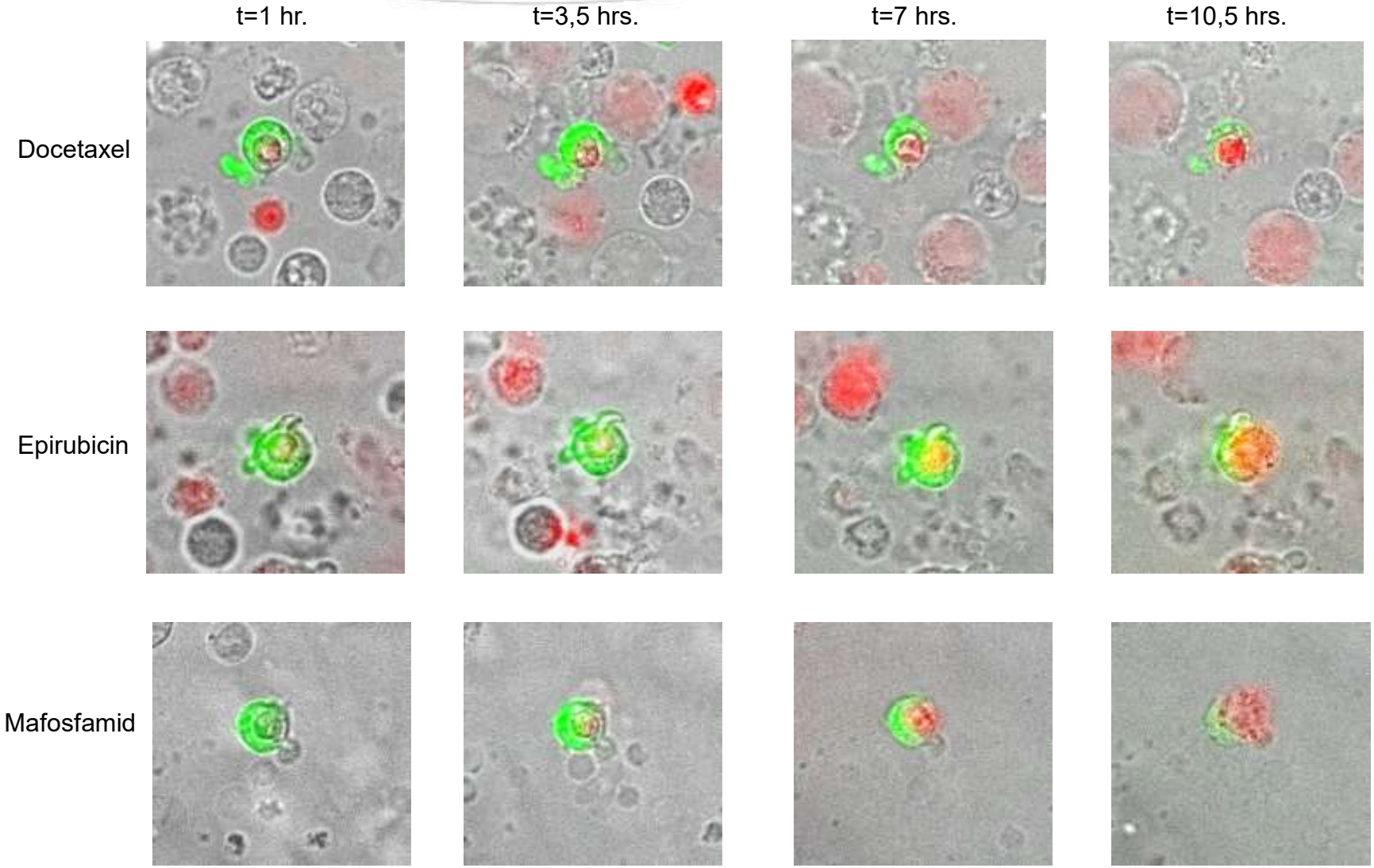
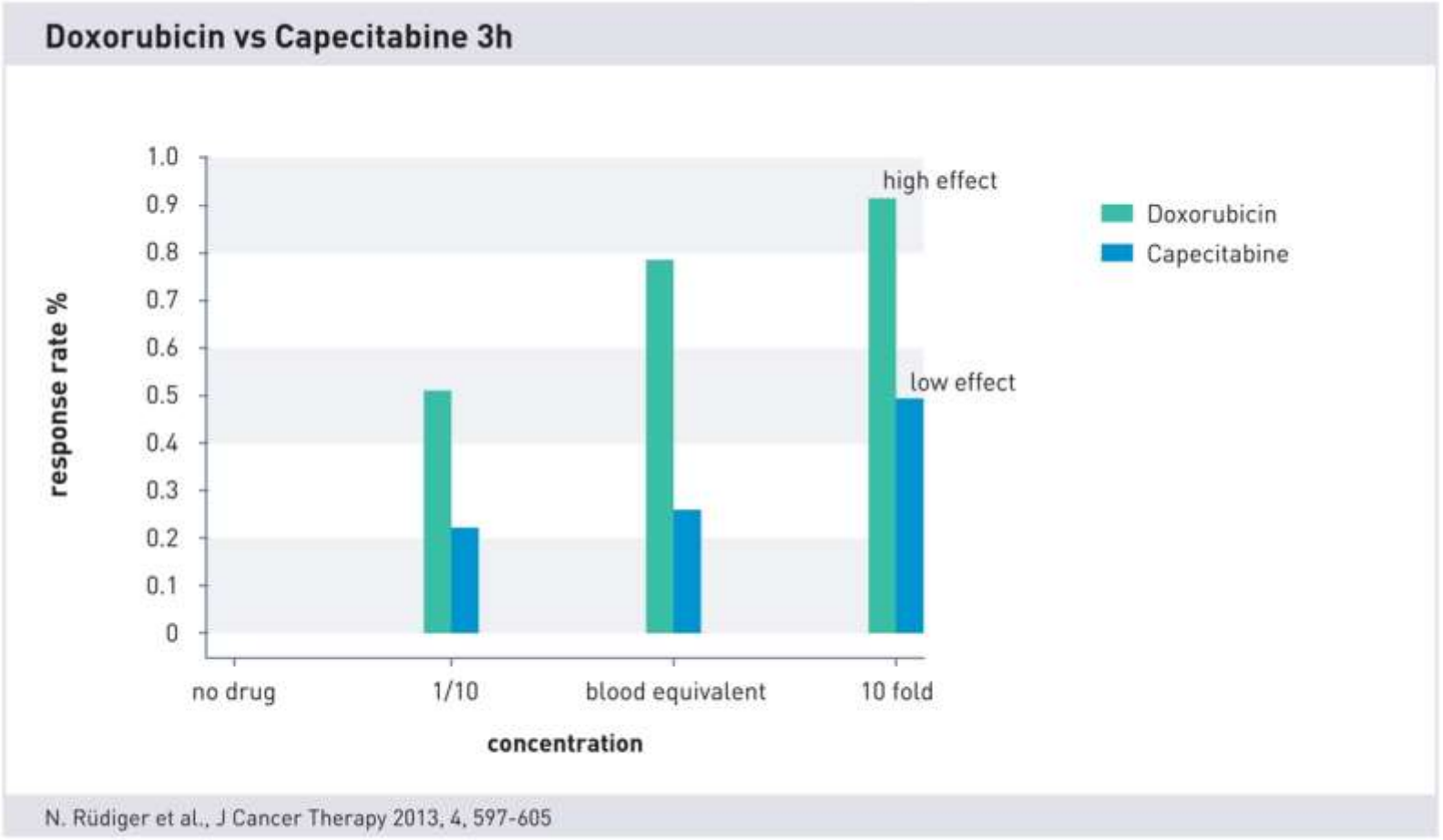
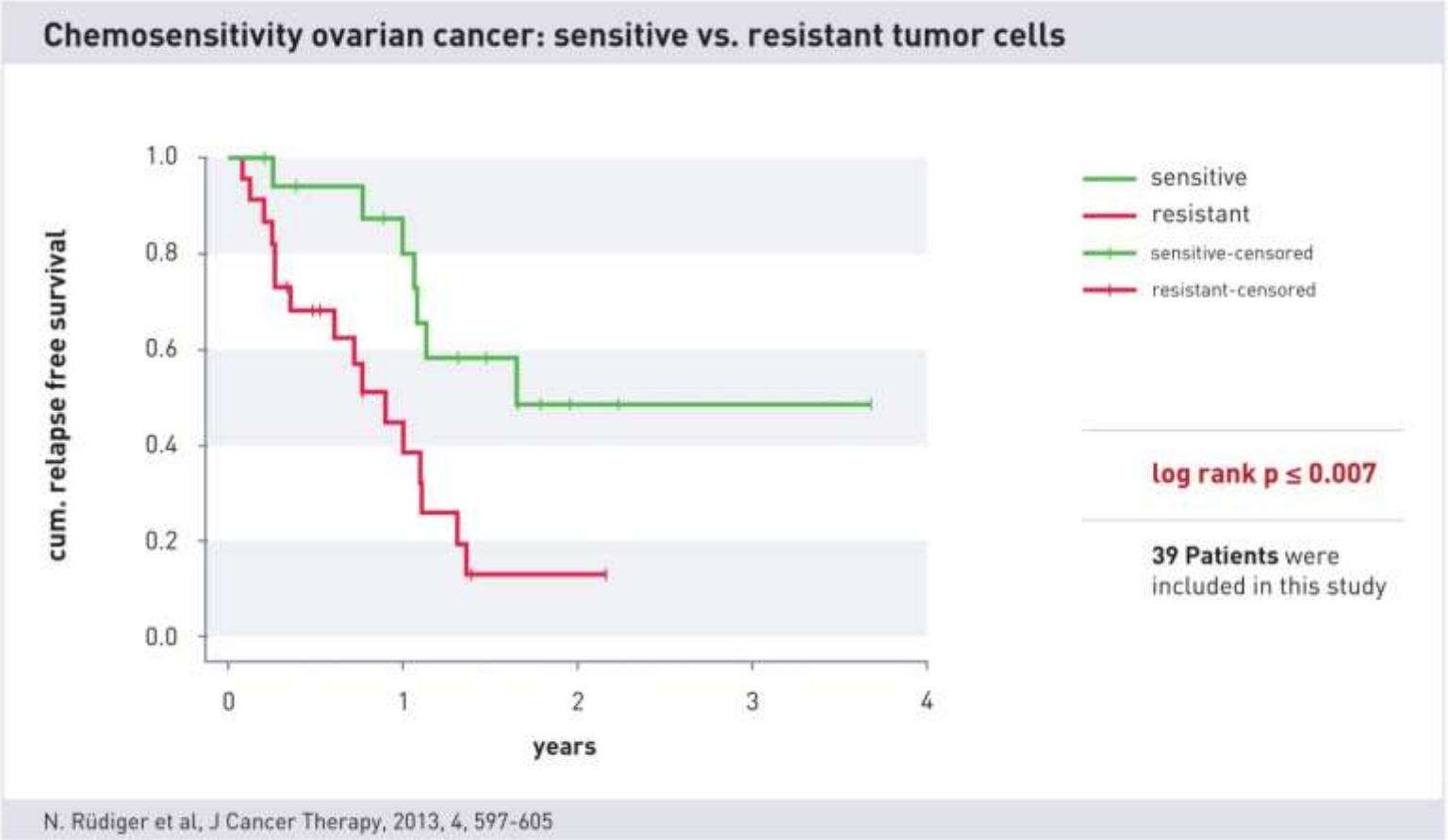
Cell decay


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

Sensitivity to different drug concentrations

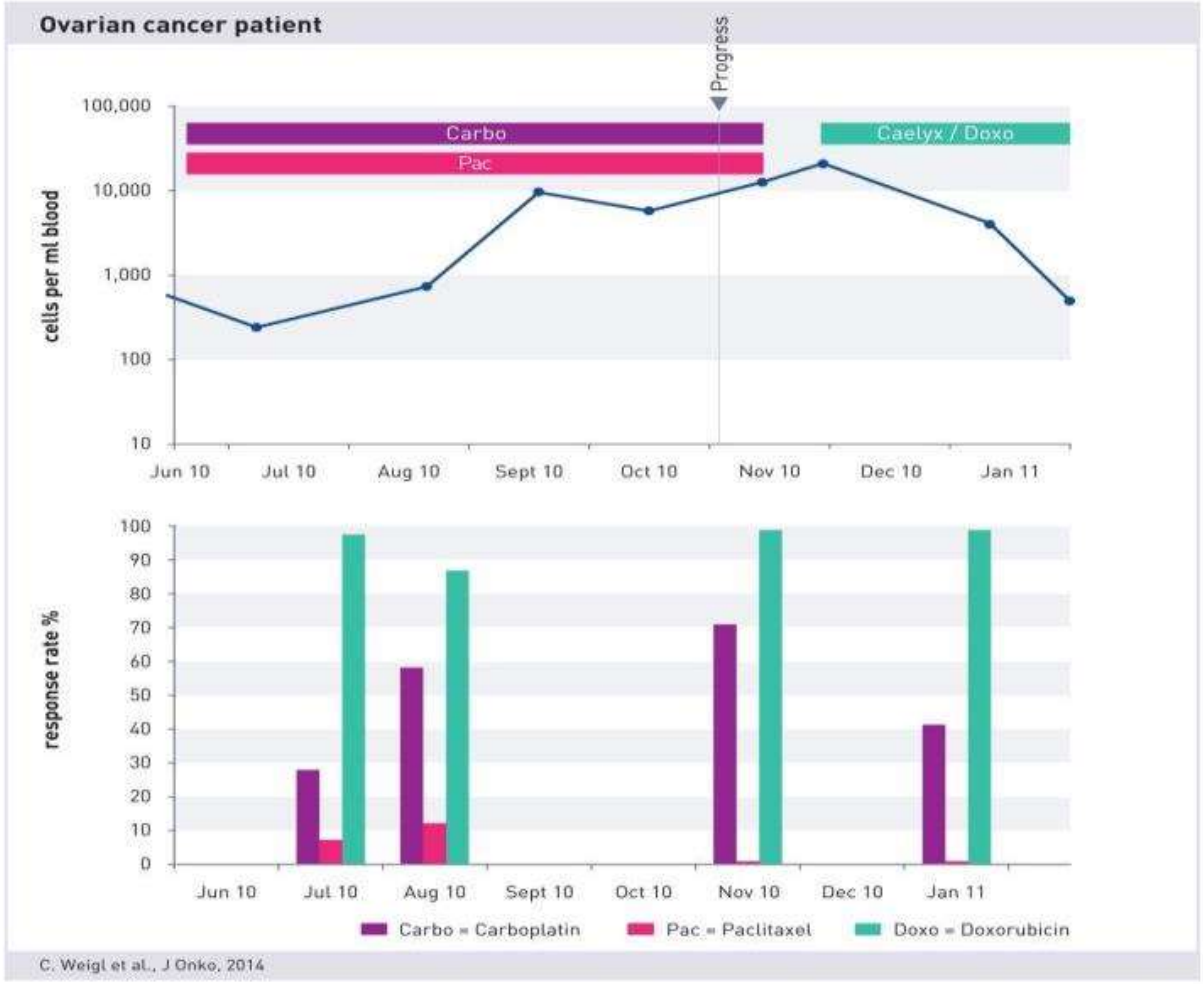


Pilot Study: Relapse free survival of patients with ovarian carcinoma patients with sensitive vs. resistant CETCs



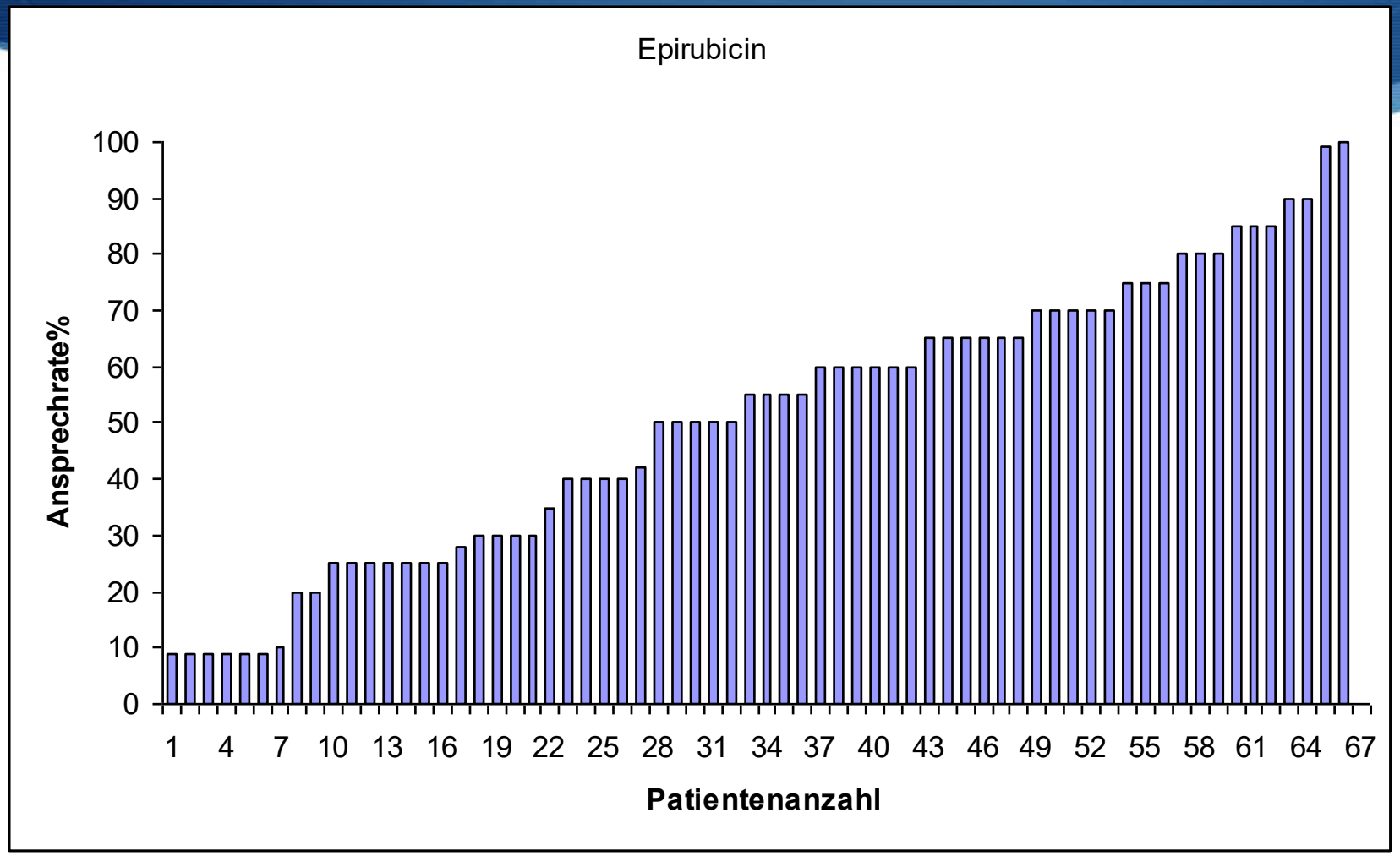
Case report: Ovarian carcinoma

Resistance to guide line drugs with progress, sensitivity to second line drug

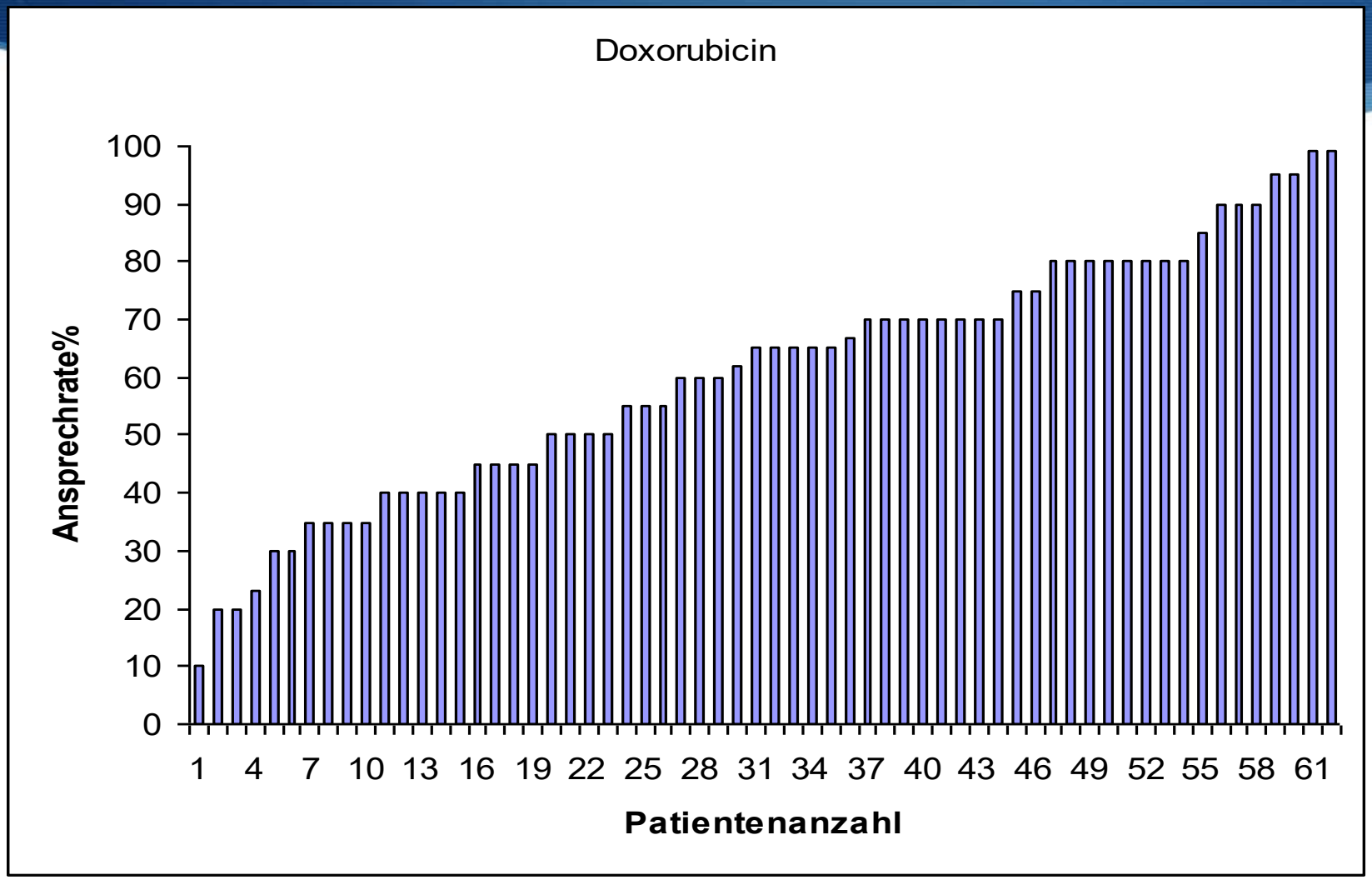


Case report breast cancer

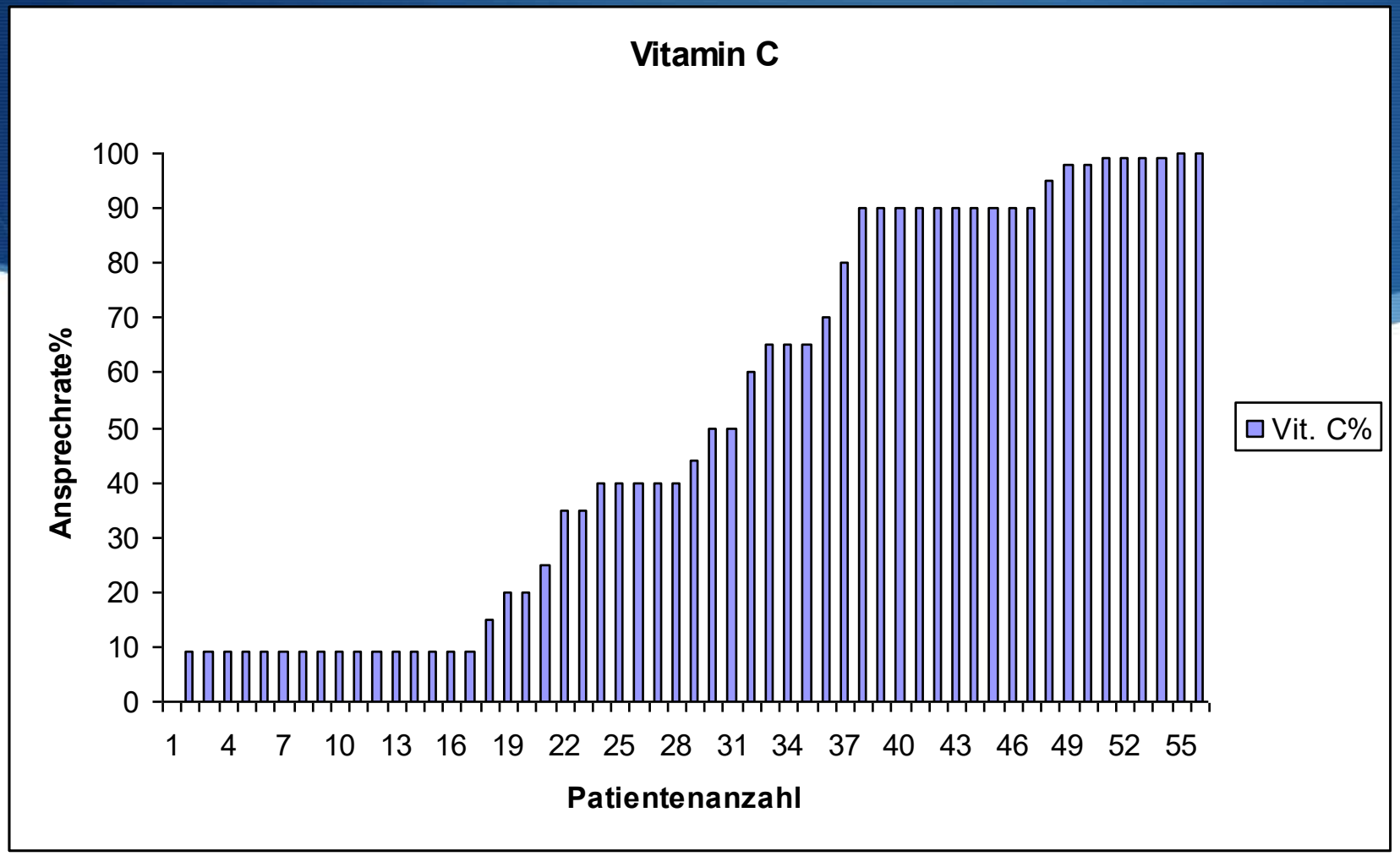
increasing resistance to drugs



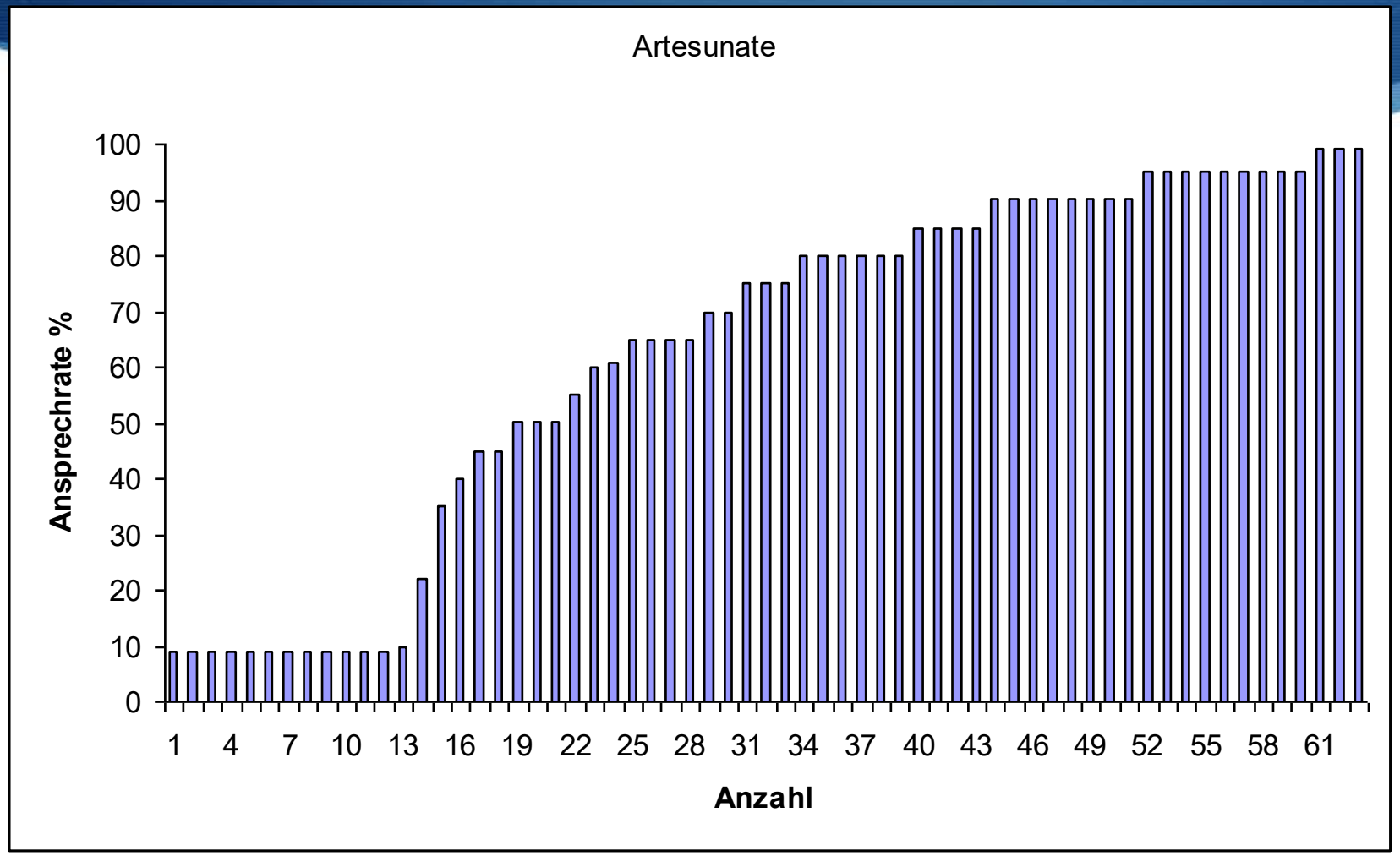
Patients total: 66		
Sensitivity > 50%	34 Patients	52%
Sensitivity < 50%	32 Patients	48%



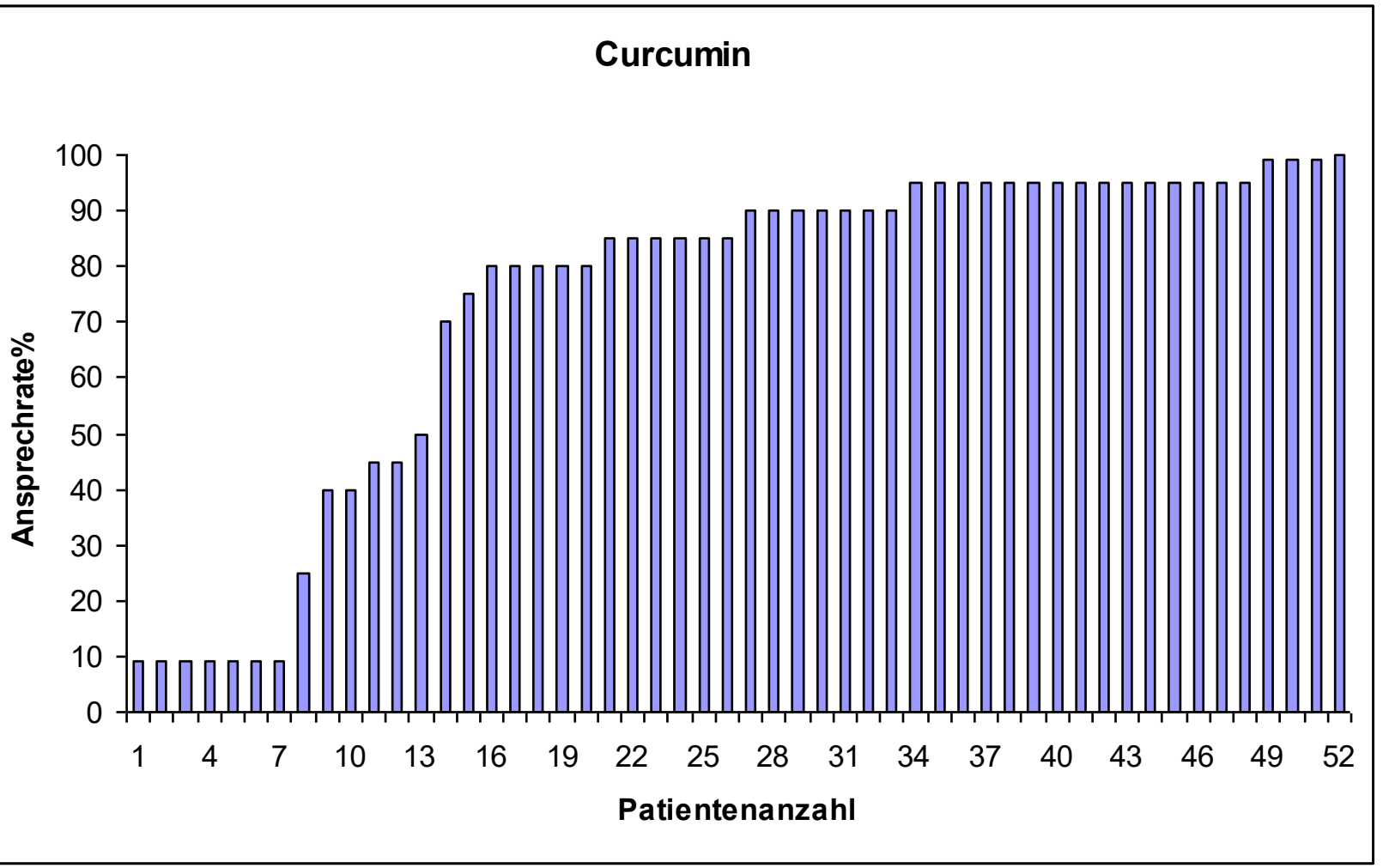
Patients total: 62		
Sensitivity > 50%	39 Patients	63%
Sensitivity < 50%	23 Patients	37%



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



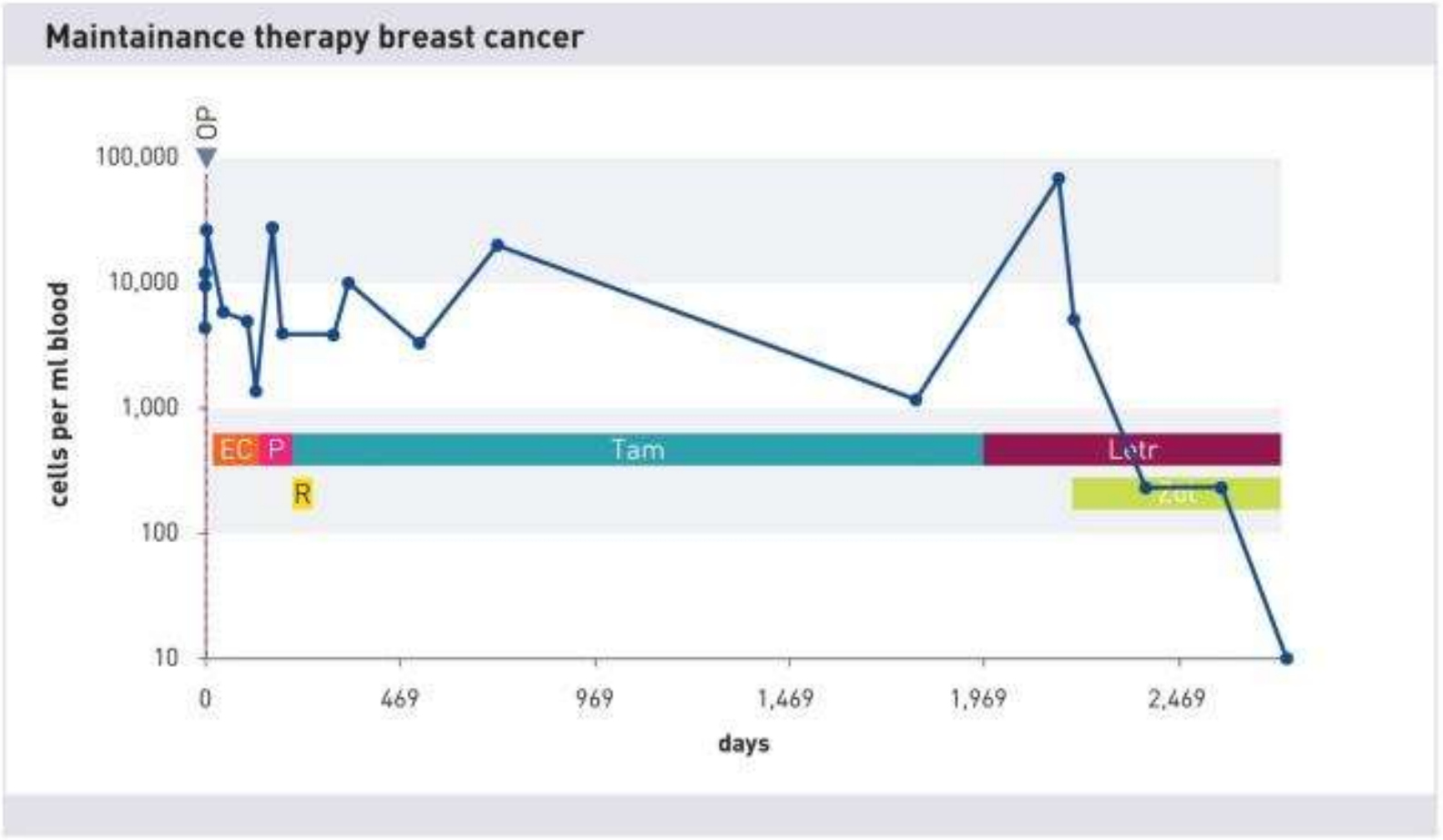
Patients total: 63		
Sensitivity > 50%	42 Patients	67%
Sensitivity < 50%	21 Patients	33%



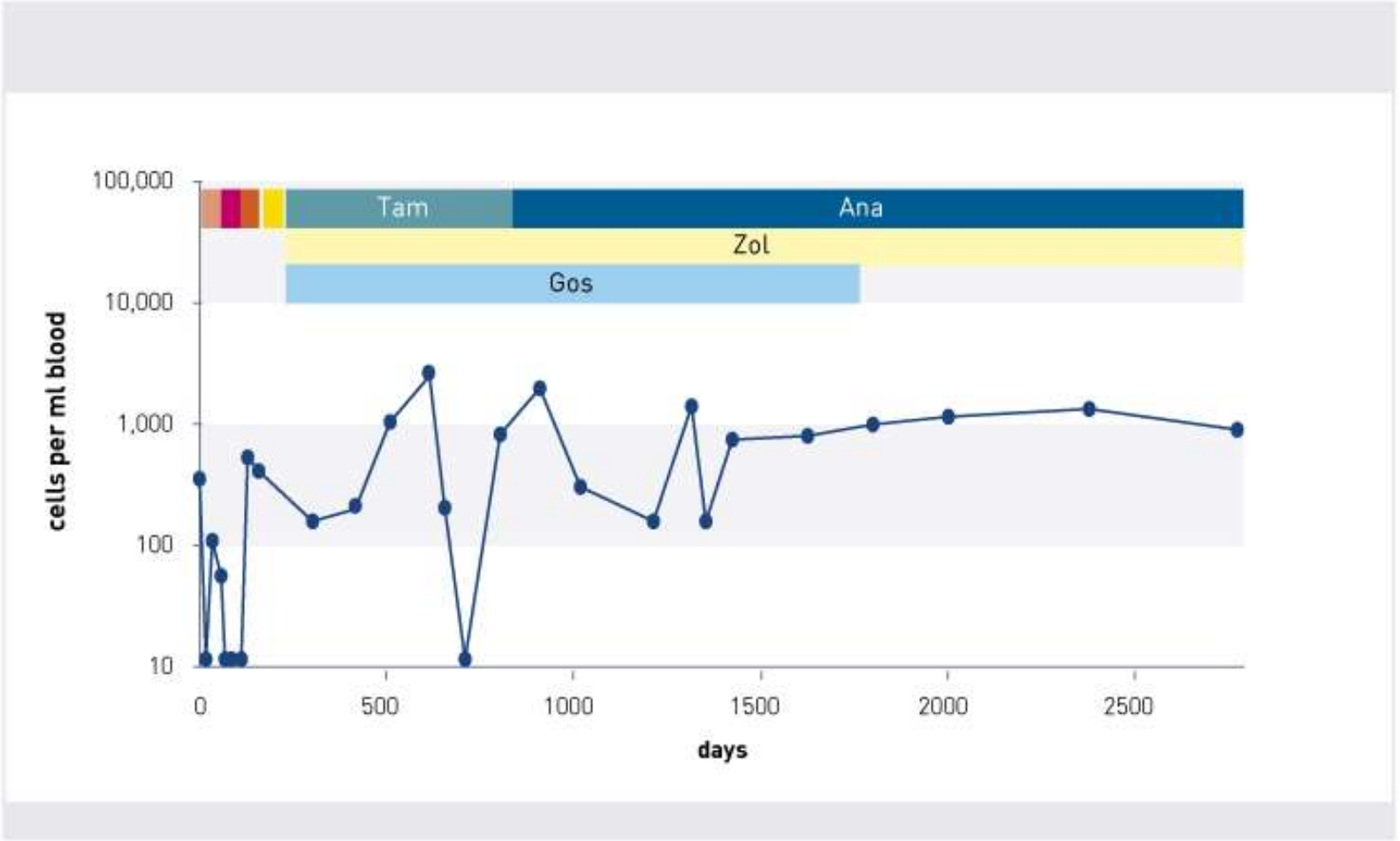
Patients total: 52		
Sensitivity > 50%	39 Patients	75%
Sensitivity < 50%	13 Patients	25%

Maintenance therapy

Effect of changes in therapy

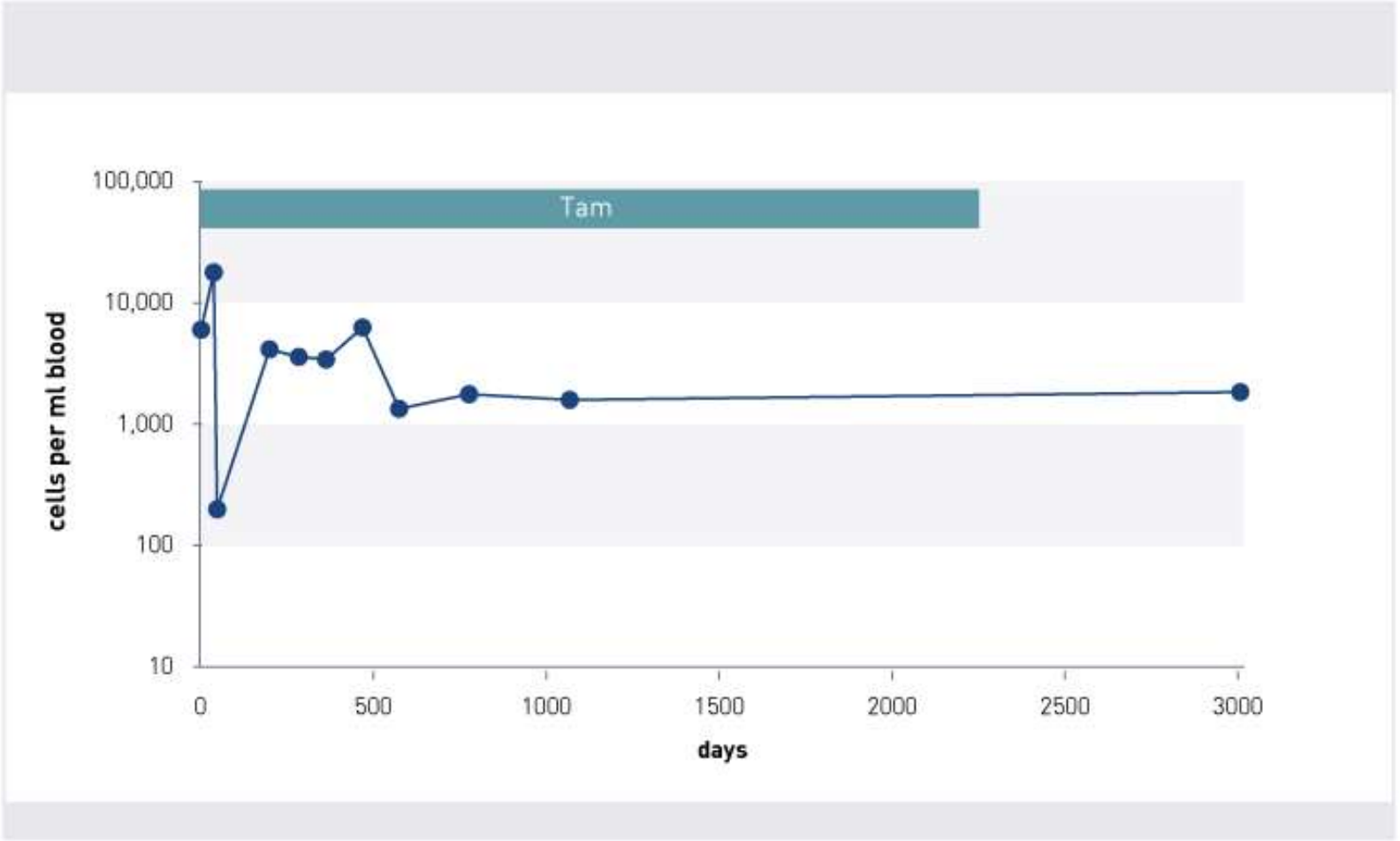


Effect of changes in therapy

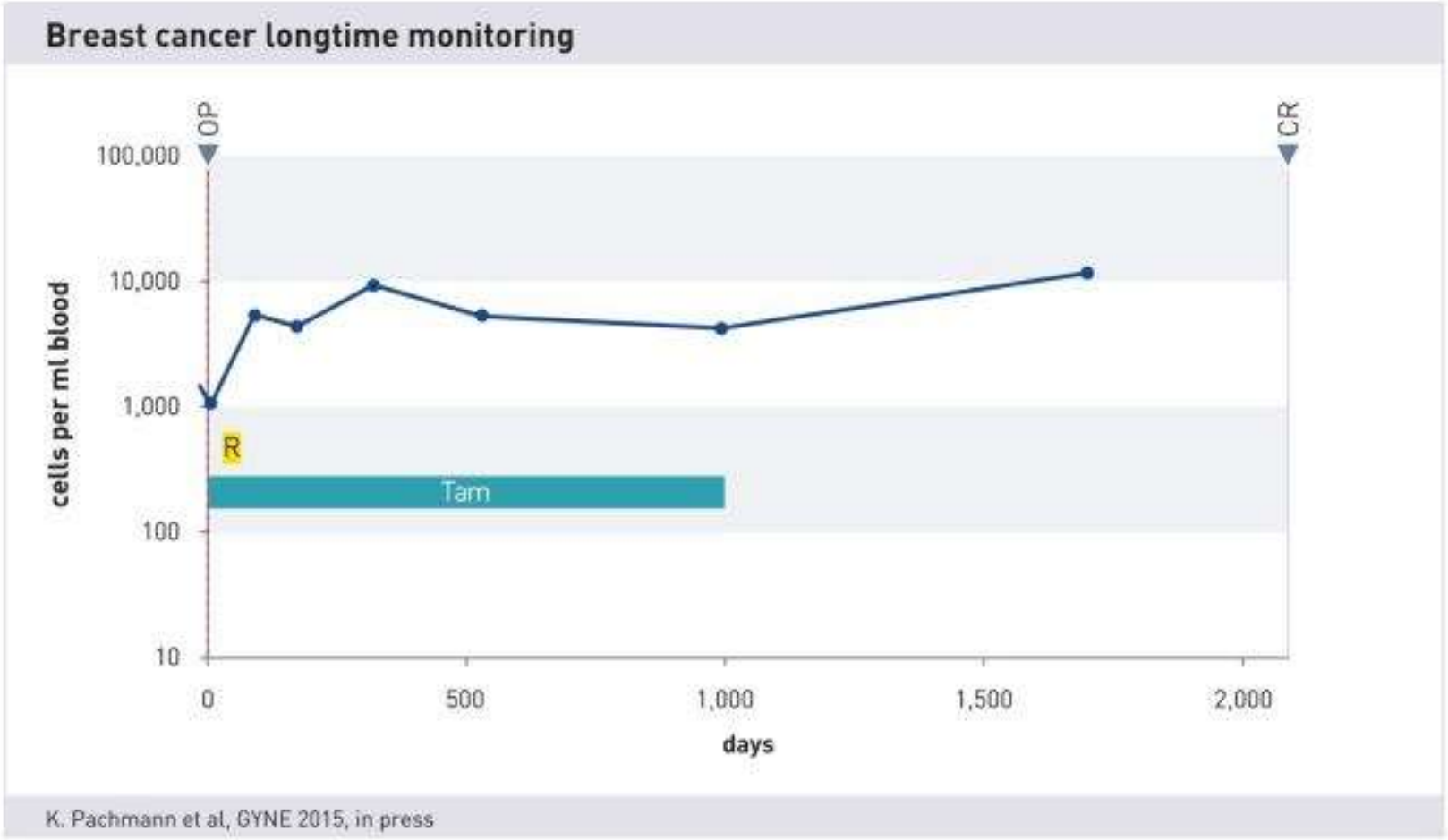


Long term surveillance after maintenance therapy

Long term surveillance

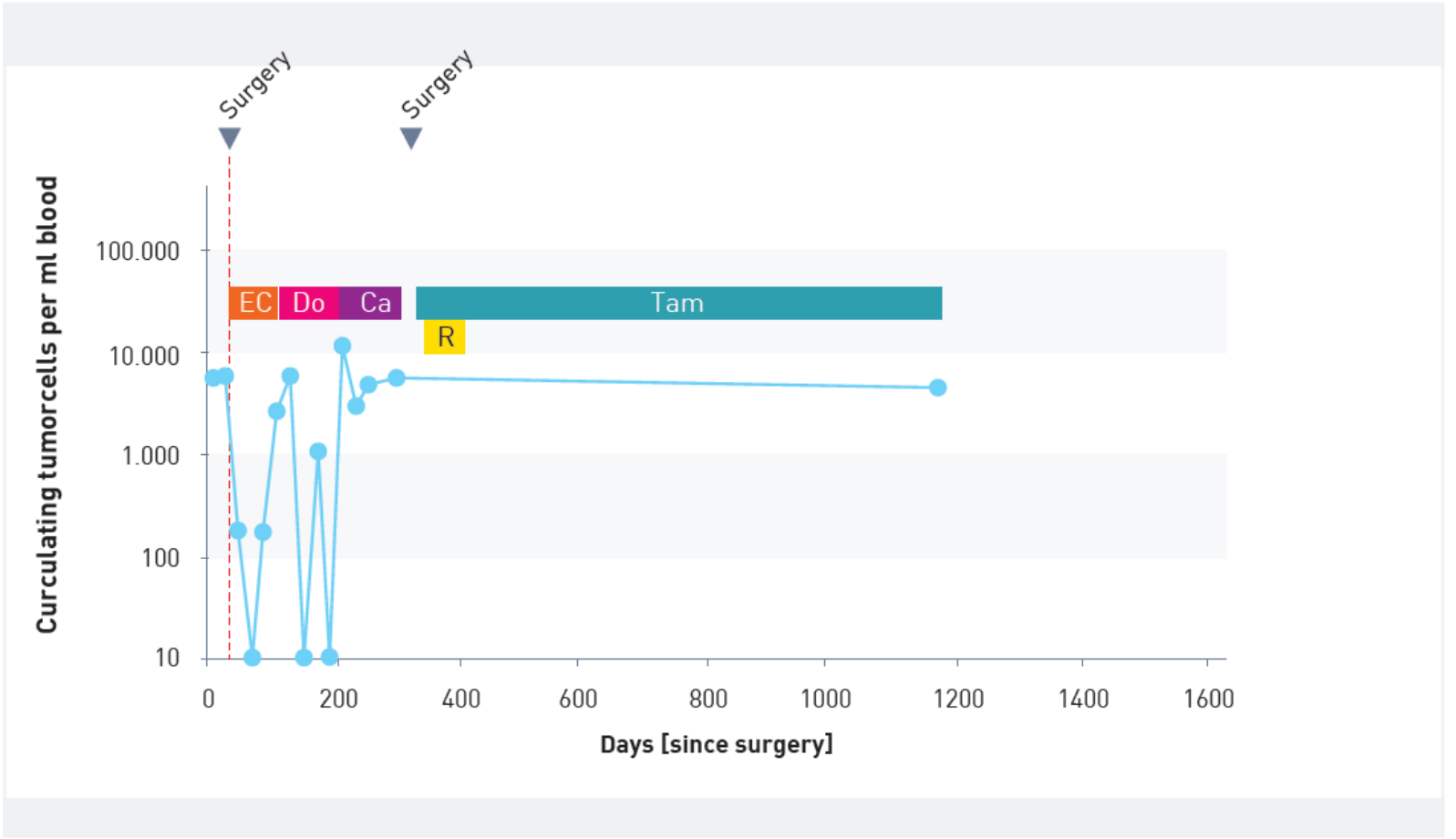


Long term surveillance



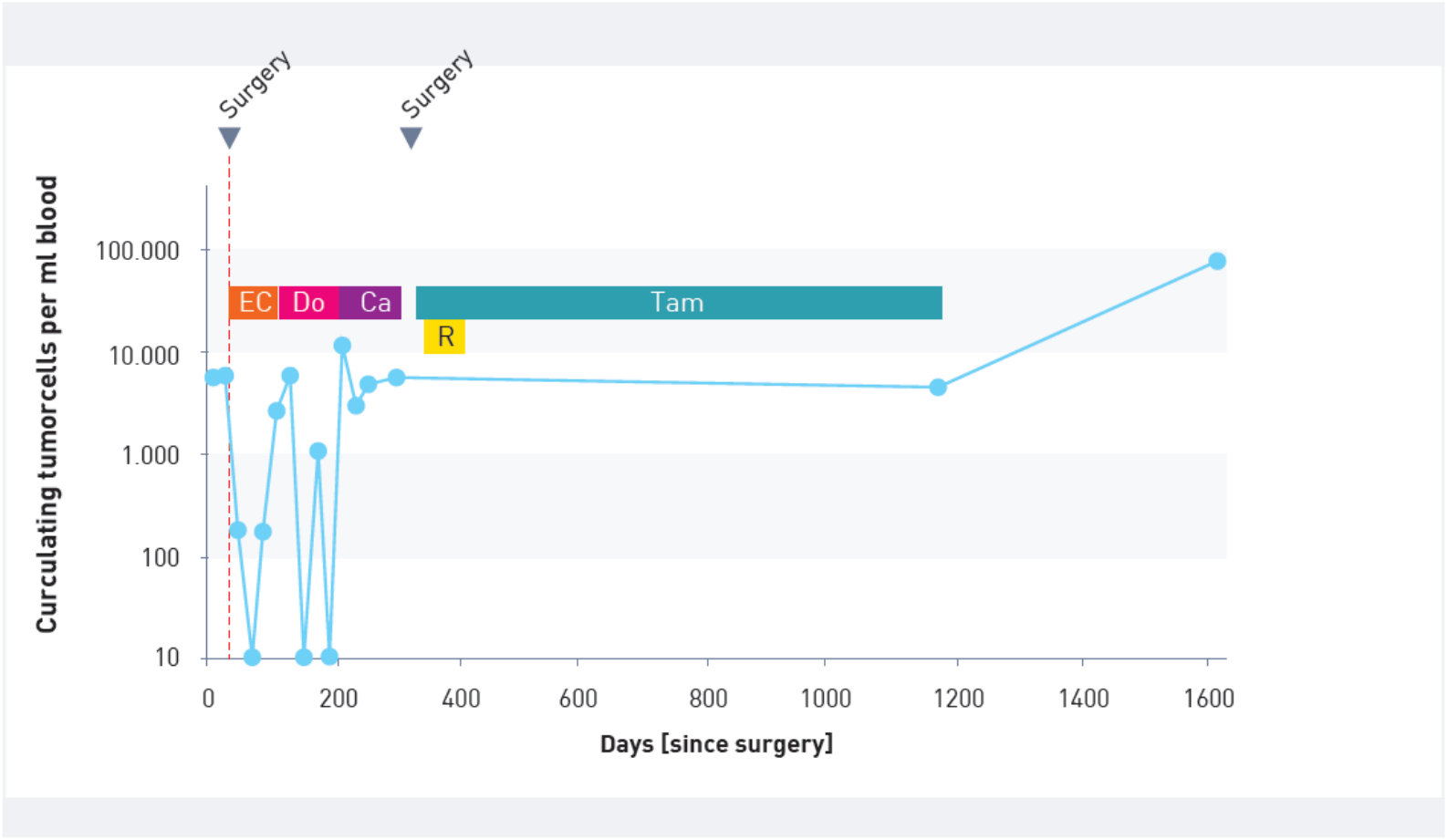
Long term surveillance

Case report 1



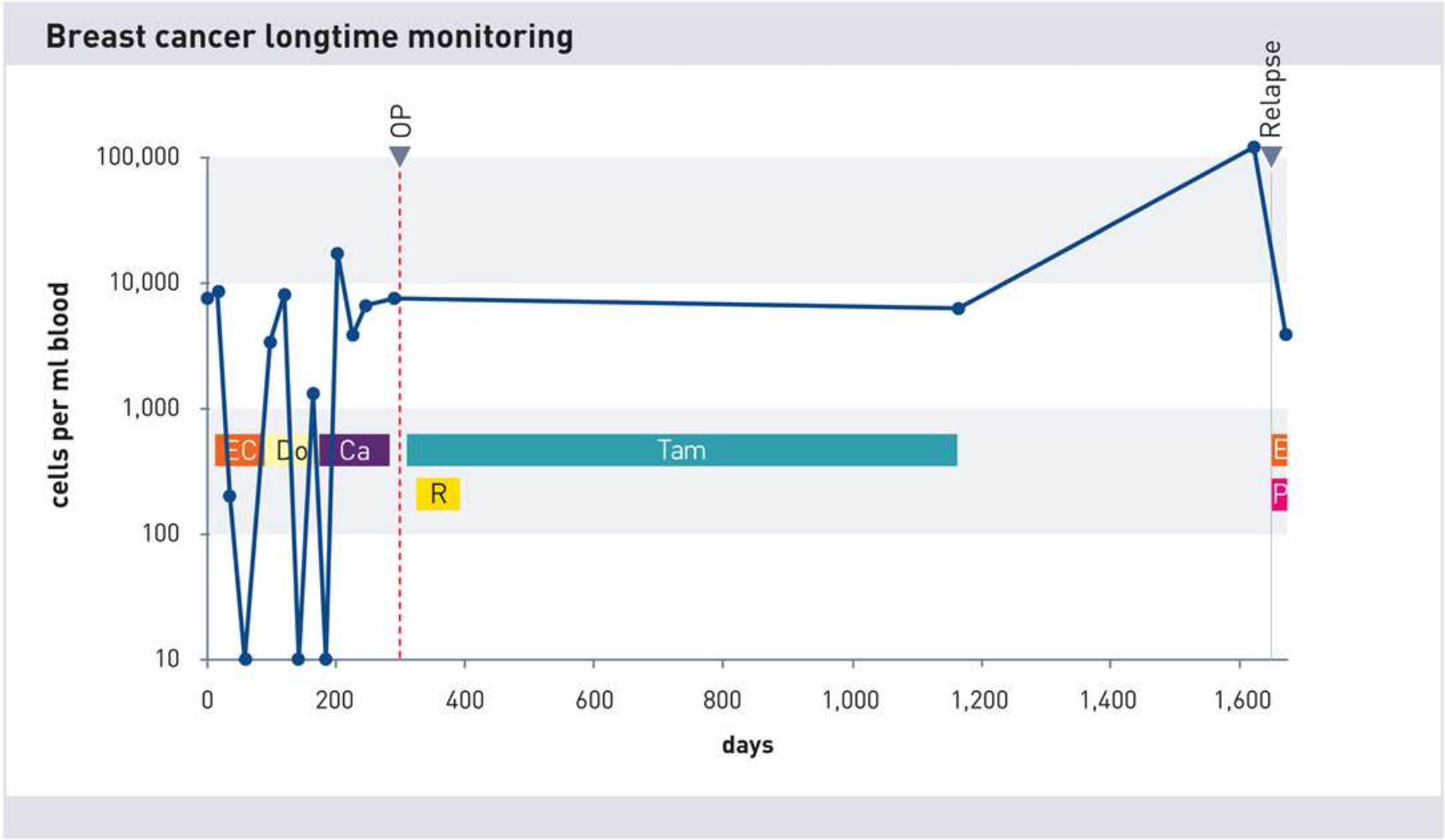
Long term surveillance

Case report 1



Long term surveillance

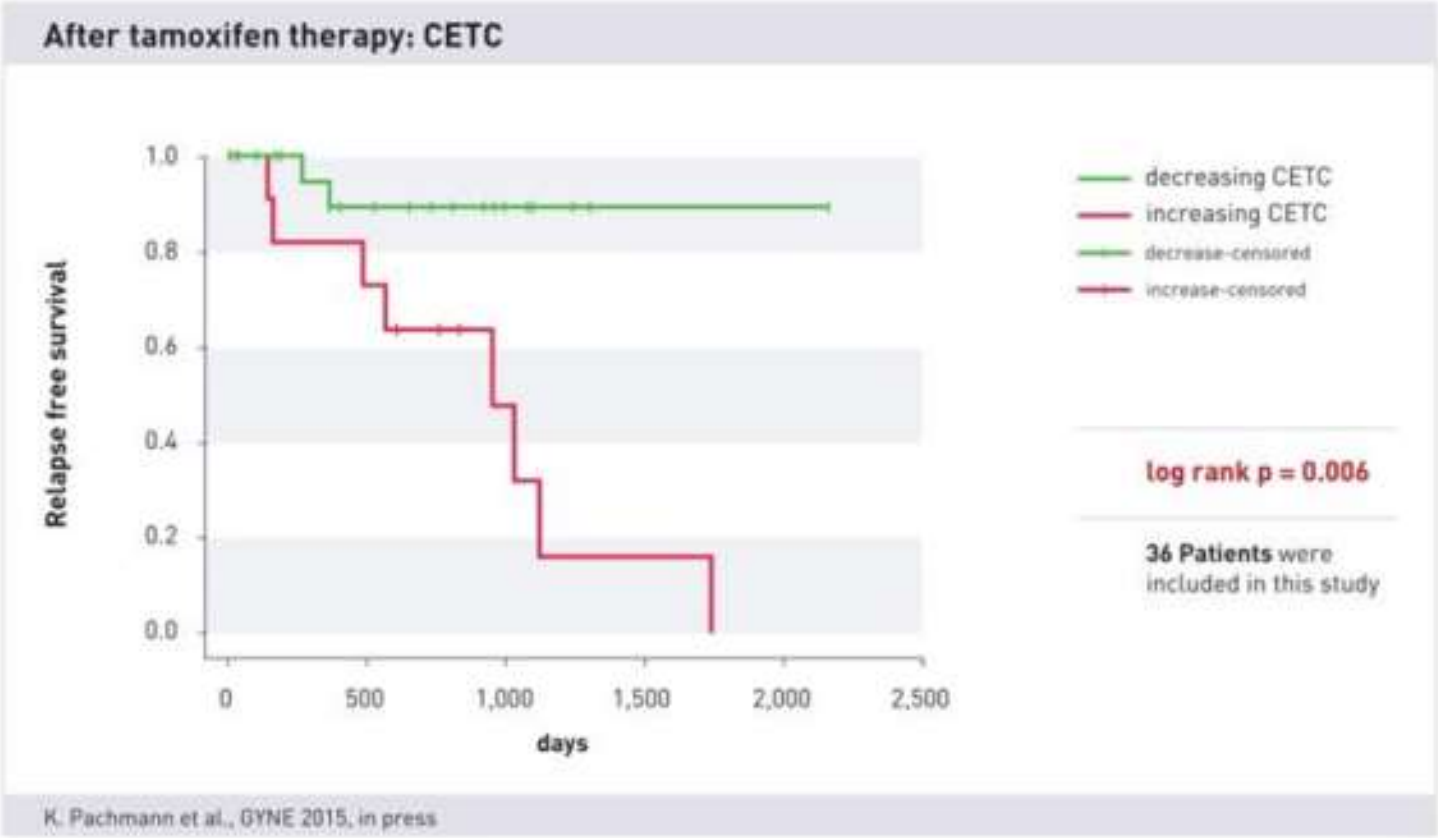
Case report 1



Long term surveillance

Long term surveillance

After the end of endocrine therapy impact of CETC



Patients with **increasing** cell numbers

after the end of maintenance therapy

have an **increased risk** of recurrence

Metastatic disease

Background Metastatic disease

- **In metastatic disease systemic therapy is used to reduce the size of the solid masses**
- In this situation **the interaction between tumor and blood** needs to be taken into consideration
- **Changes** in numbers of cells can be due to elimination as well as reseeding due to tumor tissue disintegration in **response to therapy**

Metastatic disease

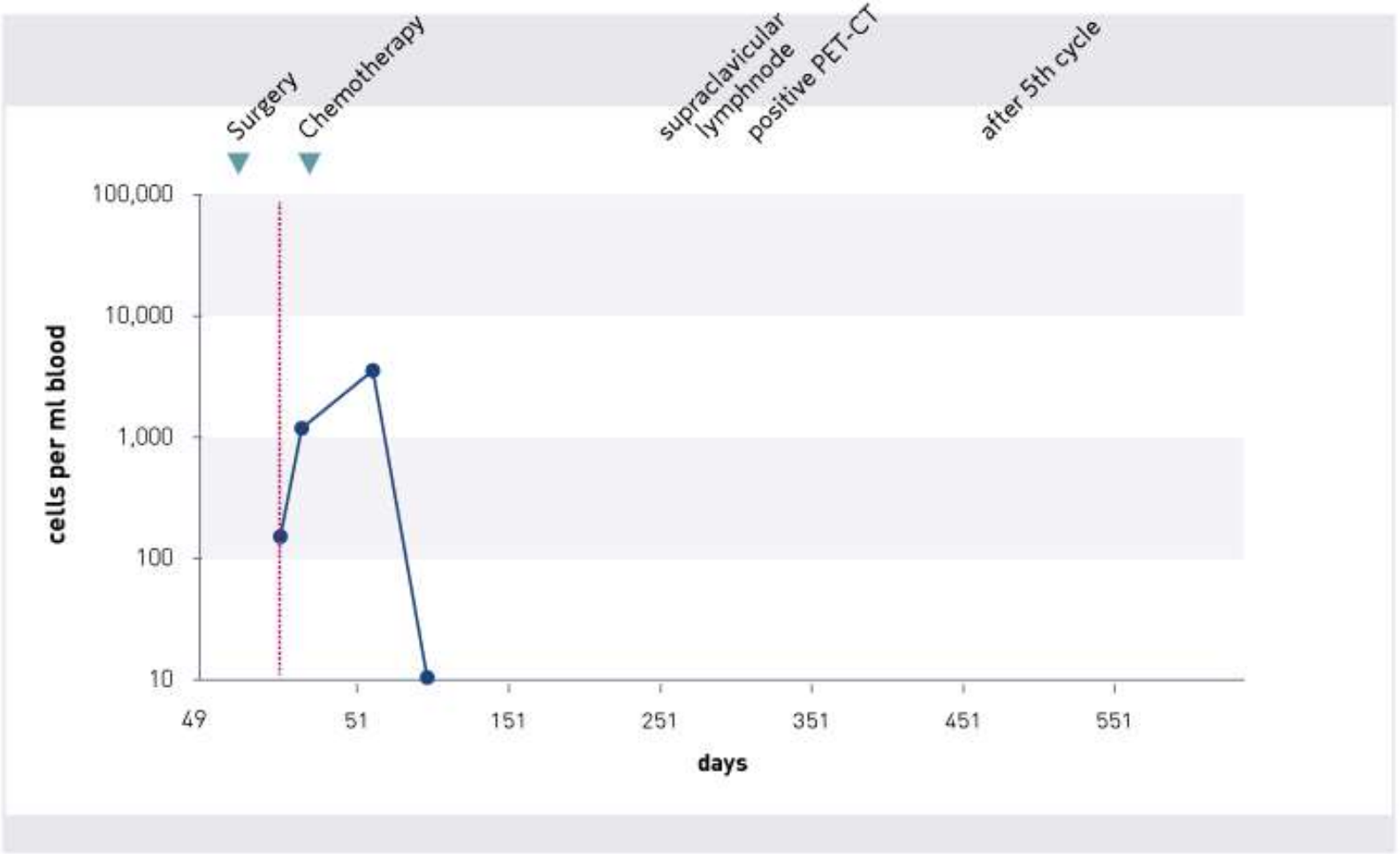
- Cells in blood respond but the metastases grow. Sufficient concentrations of the drug are not reached inside the **solid masses** due to **high intrartumoral pressure**.
- **This is the most frequent cause of treatment failure** in metastatic disease.

Metastatic disease

An increase in circulating cell numbers may be due to release of cells in addition to cell death
during tumor shrinkage

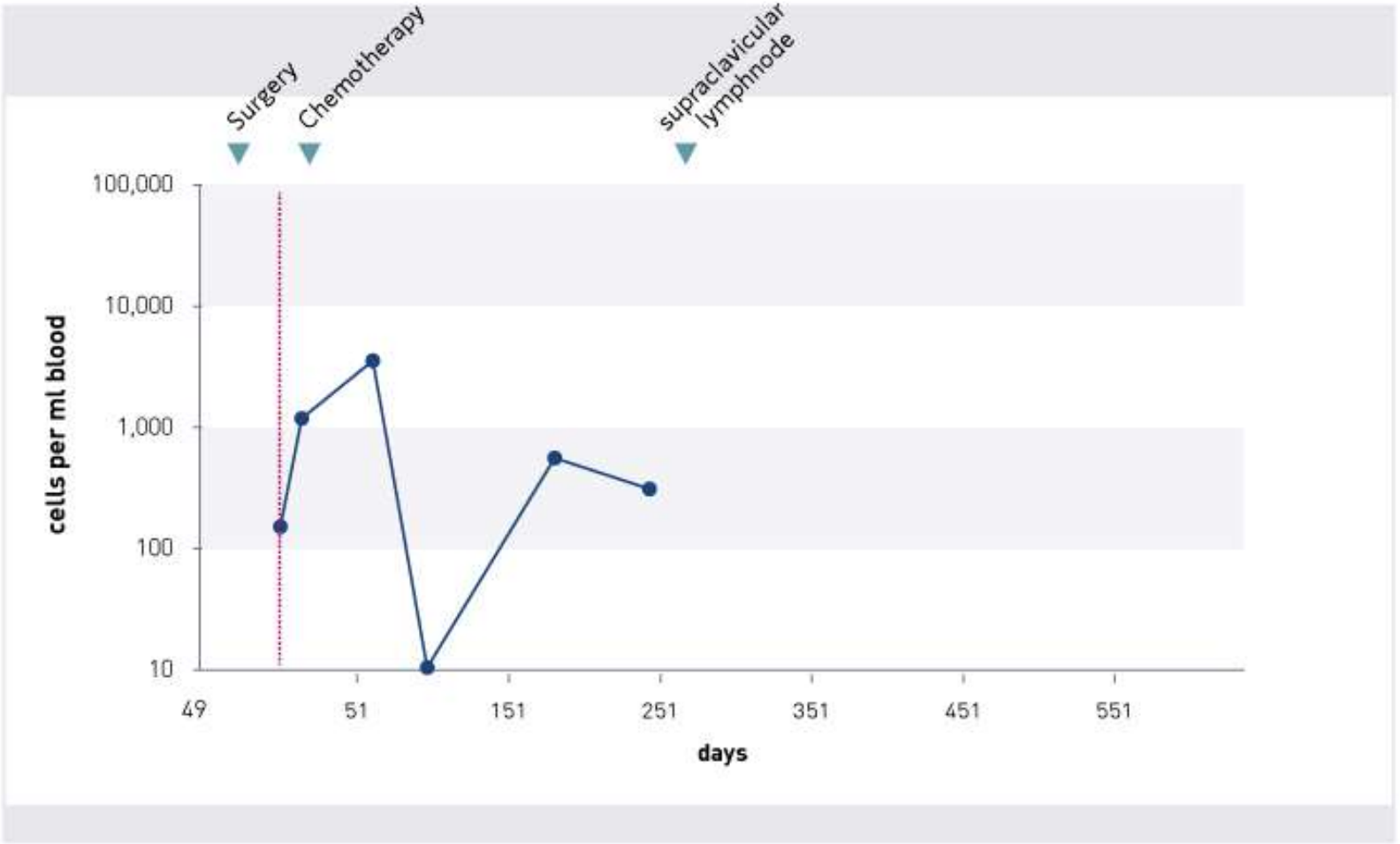
Metastatic disease

Case report 1 (ovarian cancer)



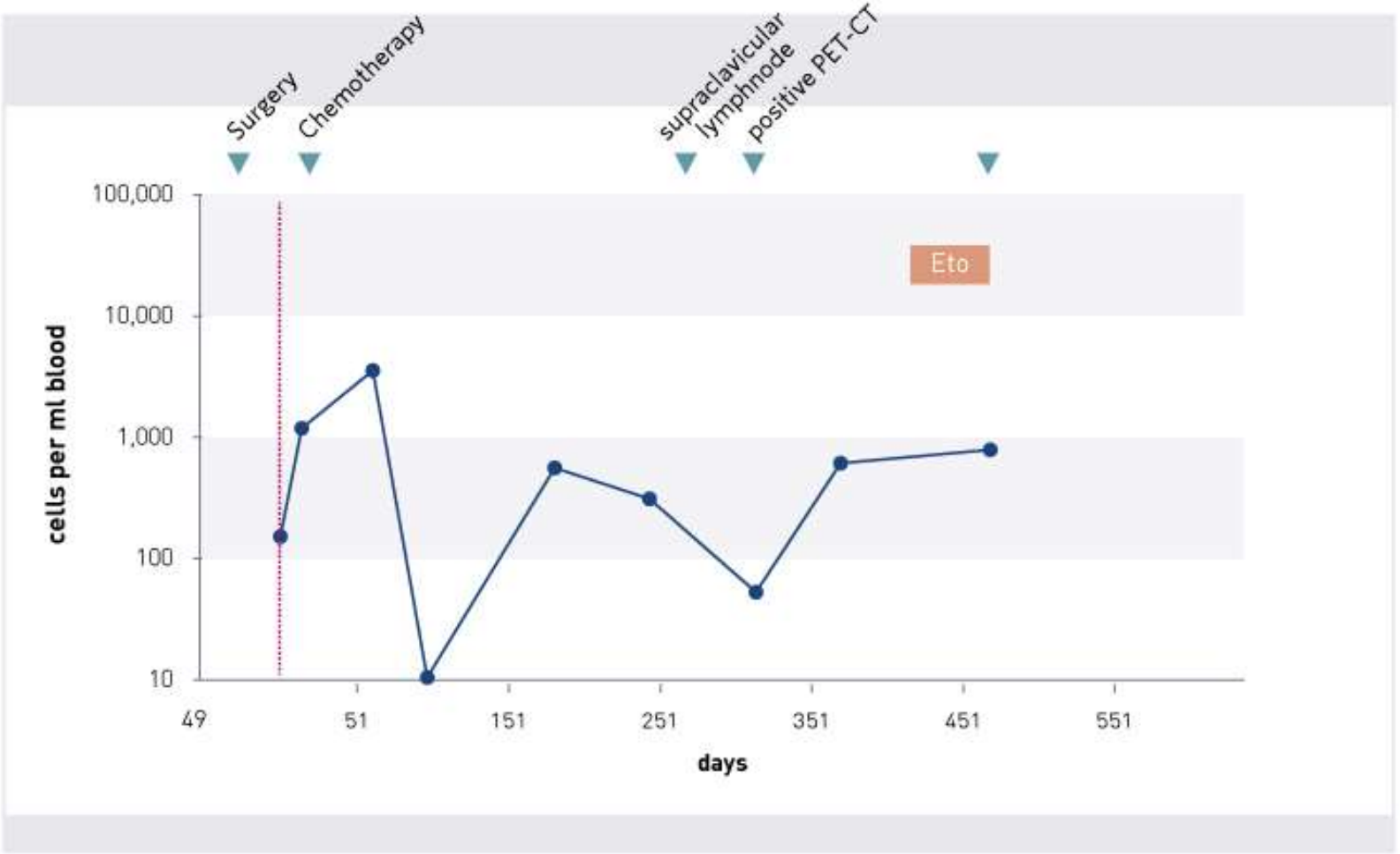
Metastatic disease

Case report 1 (ovarian cancer)



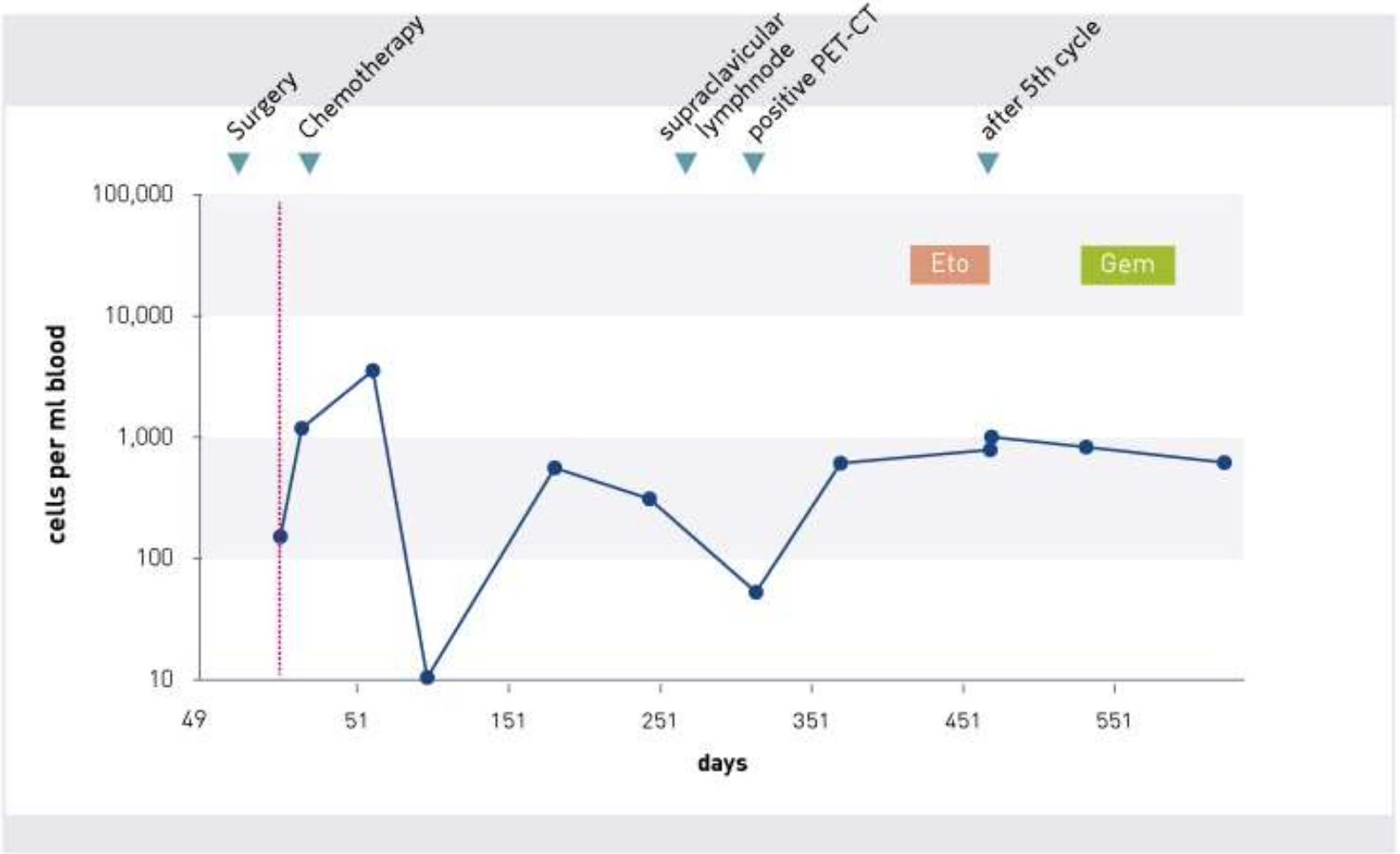
Metastatic disease

Case report 1 (ovarian cancer)



Metastatic disease

Case report 1 (ovarian cancer)



Metastatic disease

Case report 2 (triple negative breast cancer)

Metastatic disease

Case report 2 (triple negative breast cancer)

Metastatic disease

Case report 2 (triple negative breast cancer)

Metastatic disease

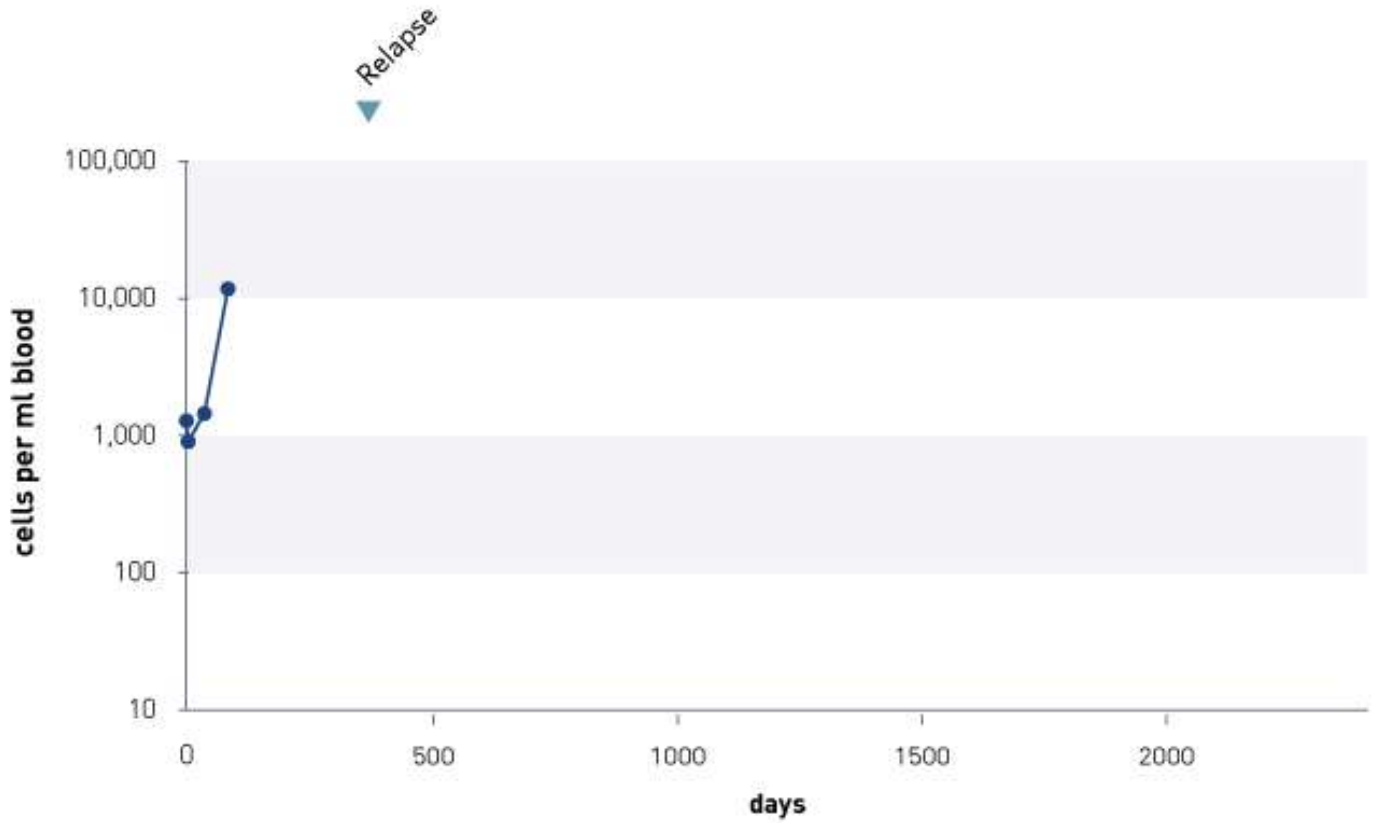
Case report 2 (triple negative breast cancer)

Metastatic disease

Case report 2 (triple negative breast cancer)

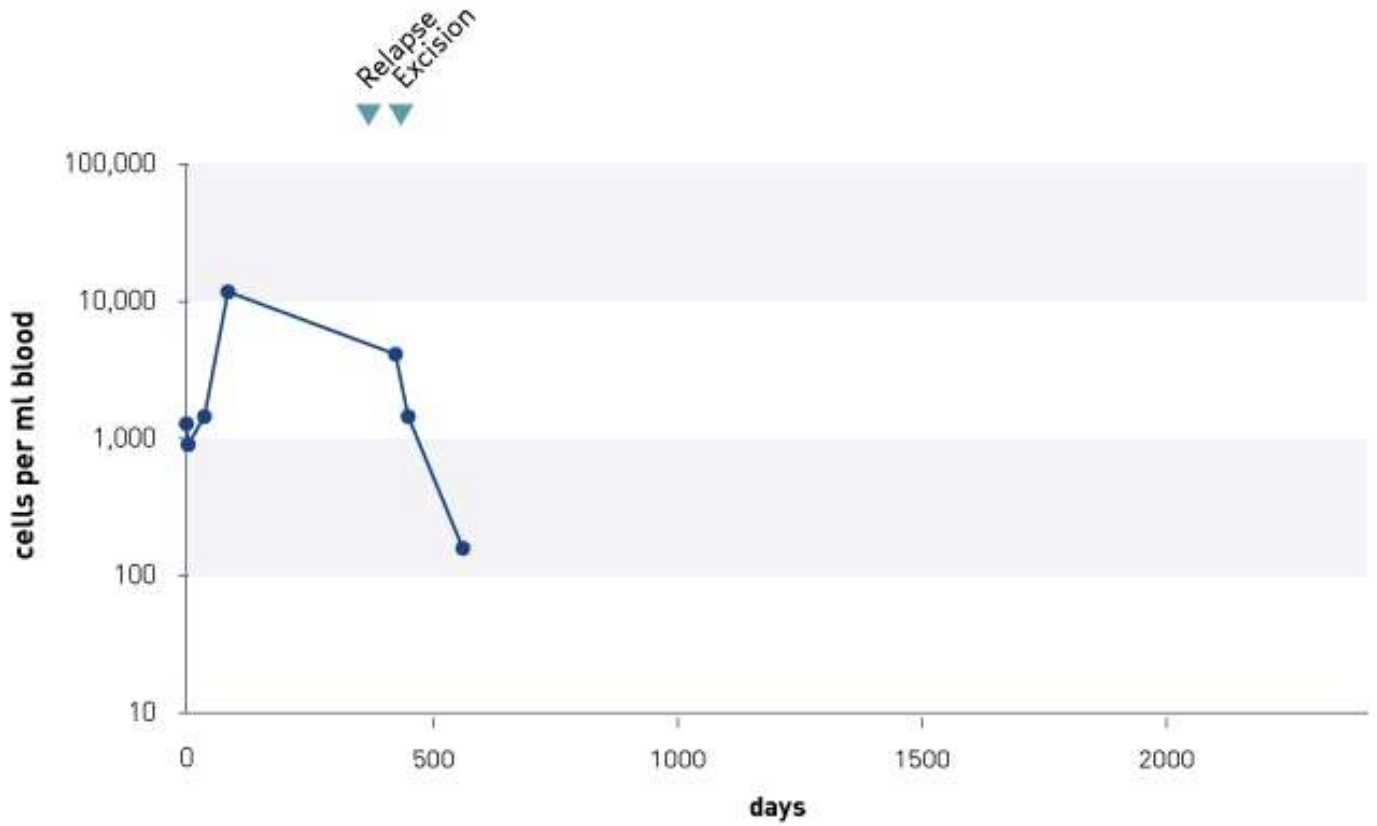
Metastatic disease

Case report 3 (triple negative breast cancer)



Metastatic disease

Case report 3 (triple negative breast cancer)



Metastatic disease

Case report 3 (triple negative breast cancer)

Metastatic disease

Case report 3 (triple negative breast cancer)

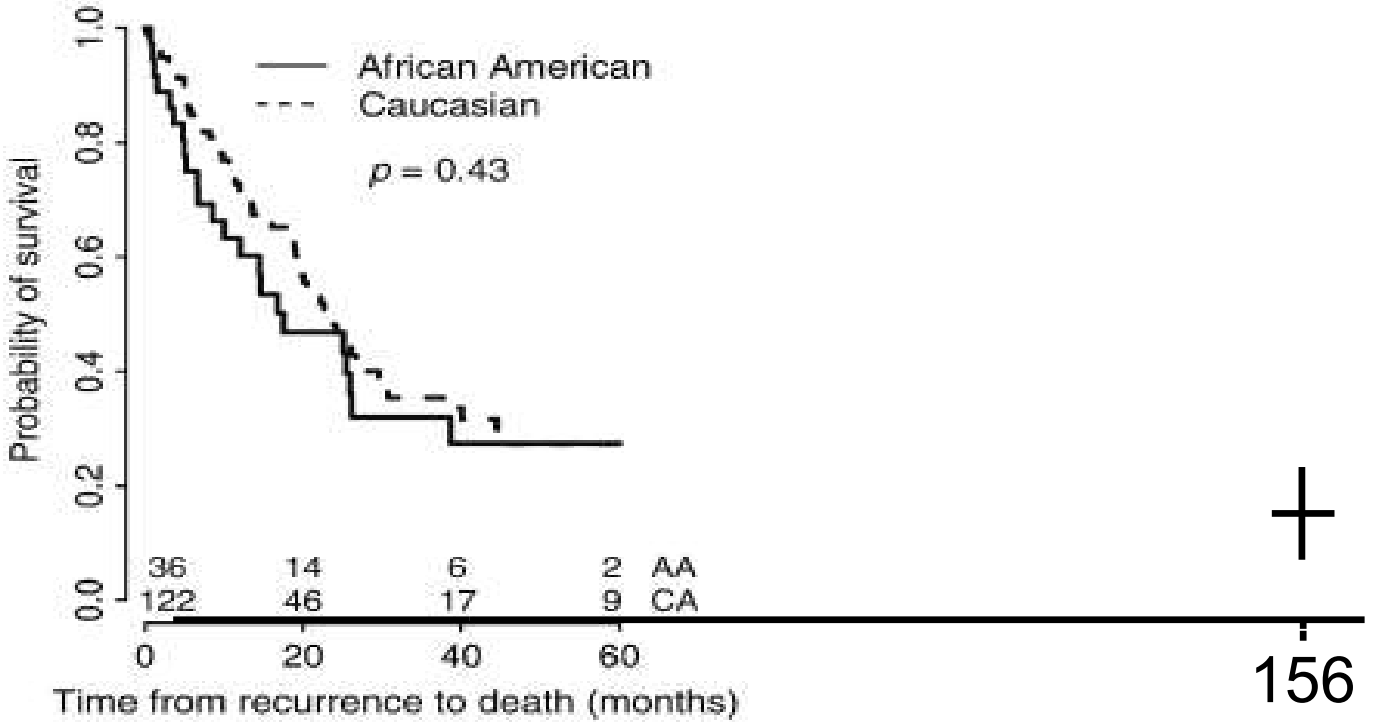
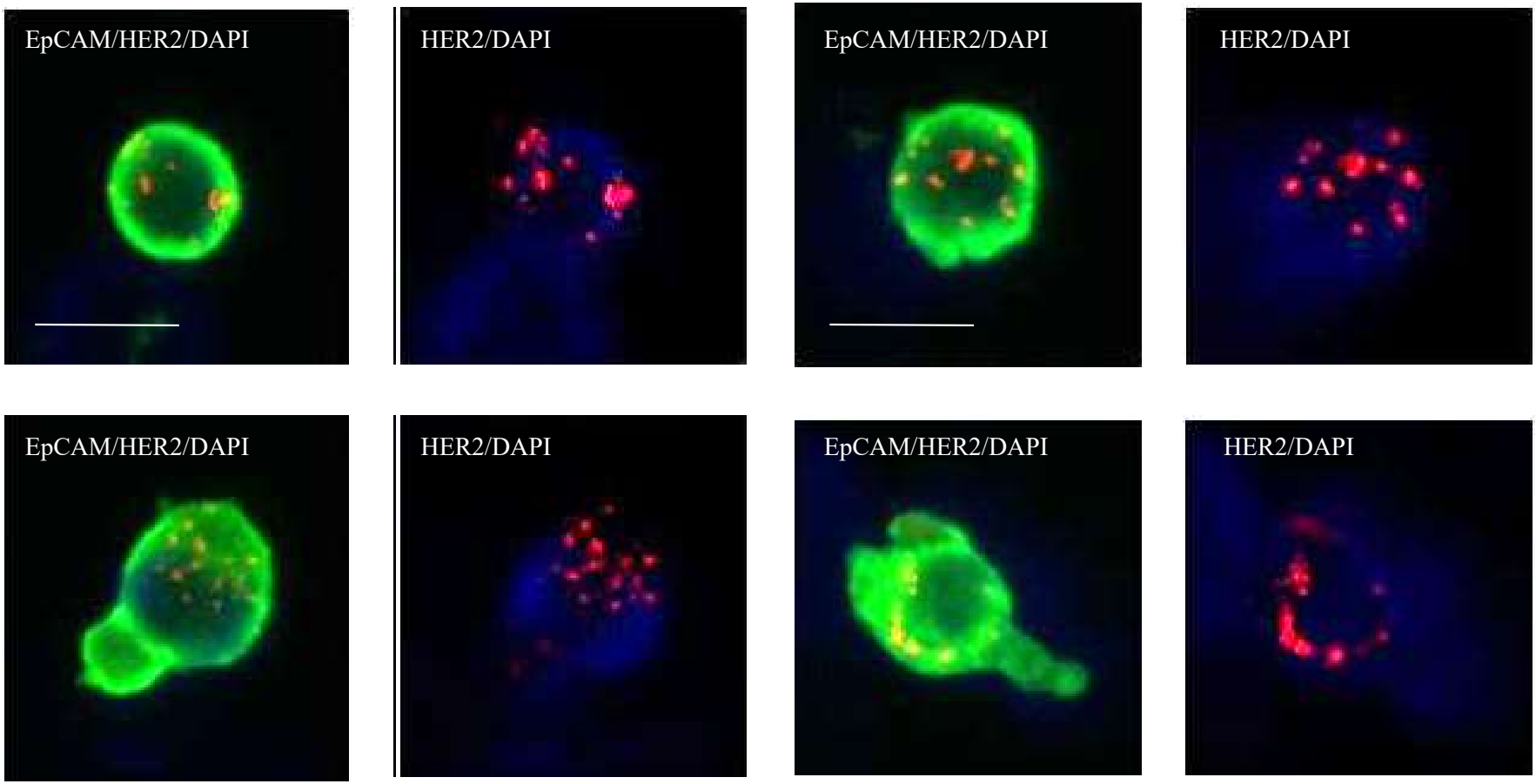


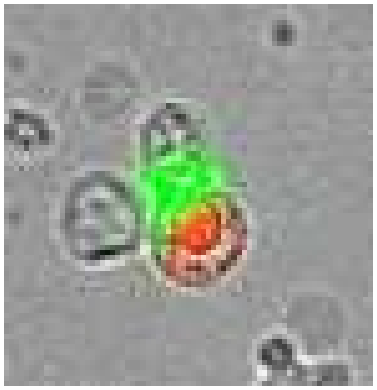
Fig. 1.
Comparison of survival outcomes between African American and Caucasian Women. K-M survival curves of OS for all patients (**a**), DFS for patients with early stage breast cancer (**b**), OS for patients with recurrent or stage IV at presentation (**c**), by race are shown

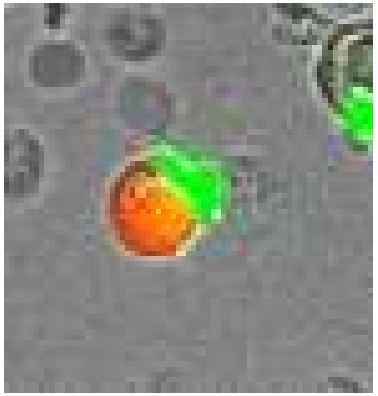
Additional analyses on CETCs

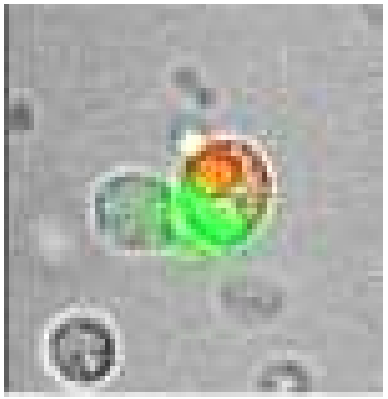
Her2/neu amplification



Estrogen receptor-positive cells







Single cell picking

Vital circulating epithelial tumor cells

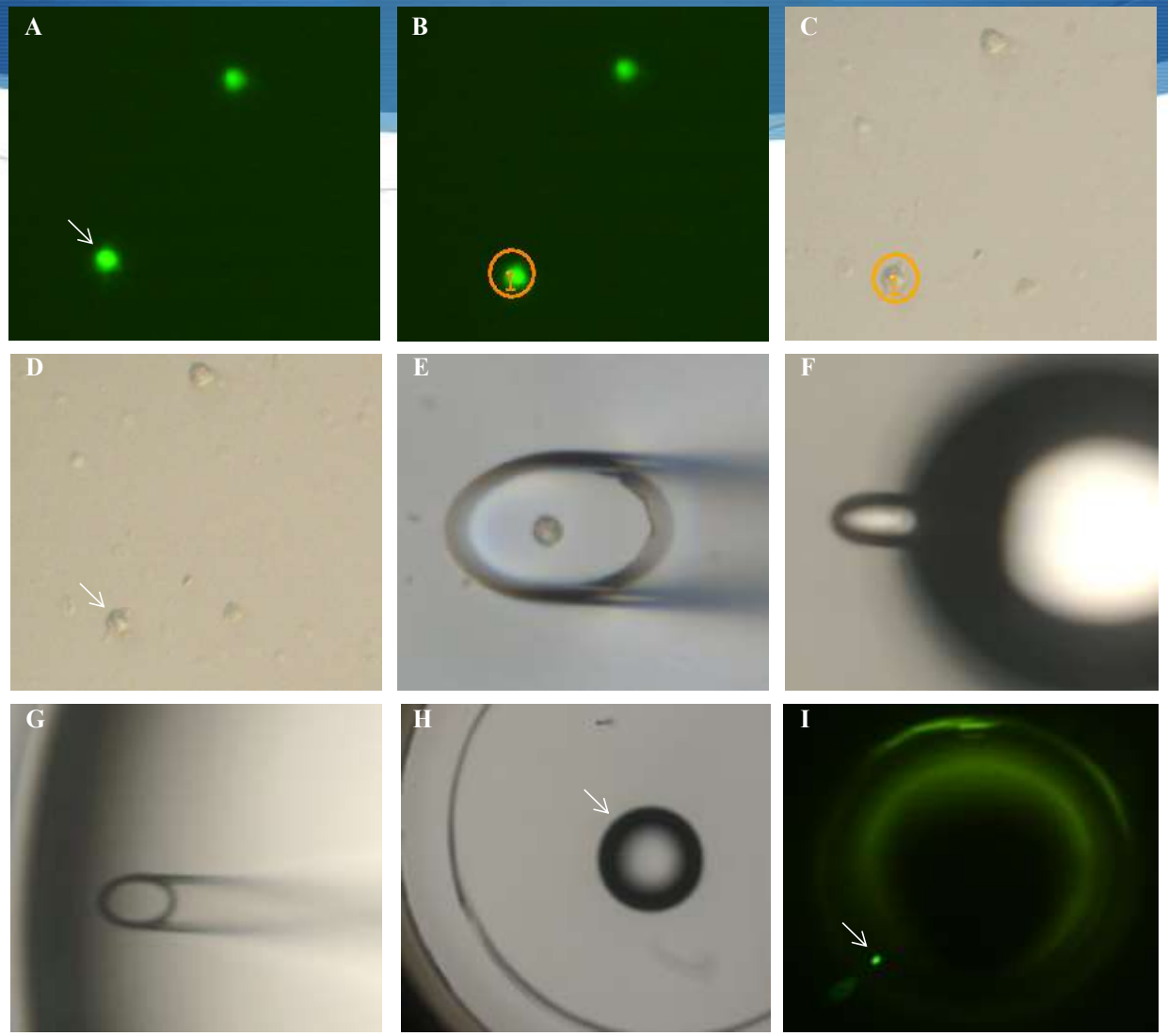
for further (e.g. genetic) investigation or NGS.

Already availible at maintrac

TZB

Single cell picking

Picking steps



Single cell picking

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

TZB

Single cell picking

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)

Conclusion

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

- CETCs can be **identified** and **characterized** already in primary diagnosed cancer patients
- Maintrac is **quantitative**
- **Efficacy of medication** can be measured

→ Treatment decisions can be made with maintrac

Shipping and results

Within 48 to max. 72 h
at room temperature

to our lab in Bayreuth,
Germany

Results will be sent usually
5 days after receiving the
sample.

Thank you
for your attention

Association Transfusion Medicine Center in Bayreuth - TZB

SIMFO Specialized Immunology Science + Development GmbH
& Laboratory Dr. Ulrich Pachmann

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