AONM WEBINAR SERIES

The Science and Clinical Utility of CTCs as a Tool to Monitor the Effectiveness of Therapy

Prof. Dr. Katharina Pachmann

Tuesday 19th Feb. 2019, 6.30 pm

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ACADEMY OF NUTRITIONAL MEDICINE

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The science and clinical utility of CTCs as a tool to monitor the effectiveness of therapy.

Katharina Pachmann

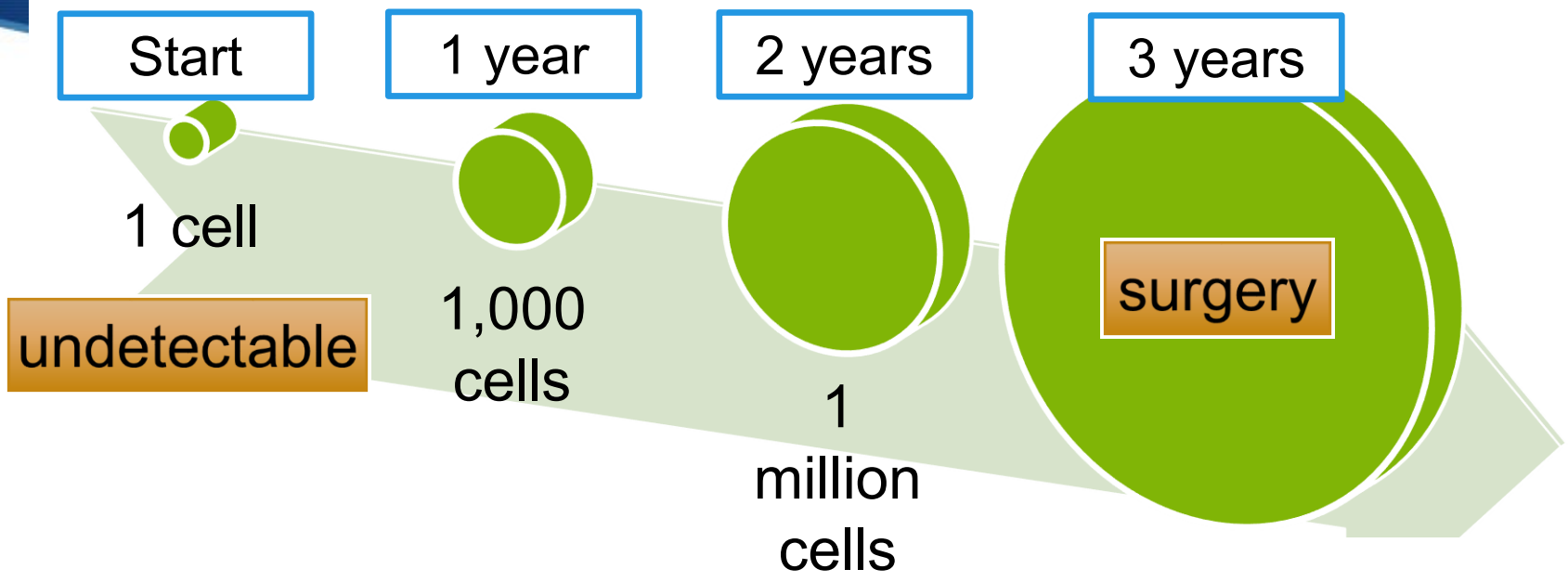
"Cancer-free" – ?

- 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

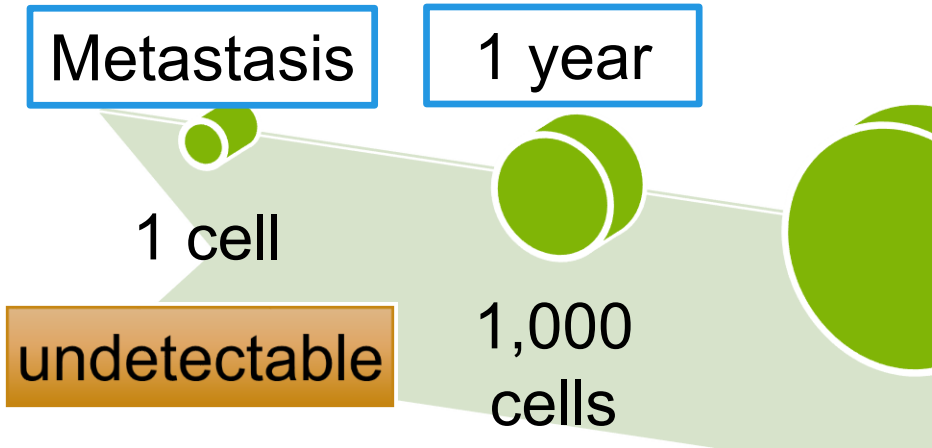
How do metastases develop?

- **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

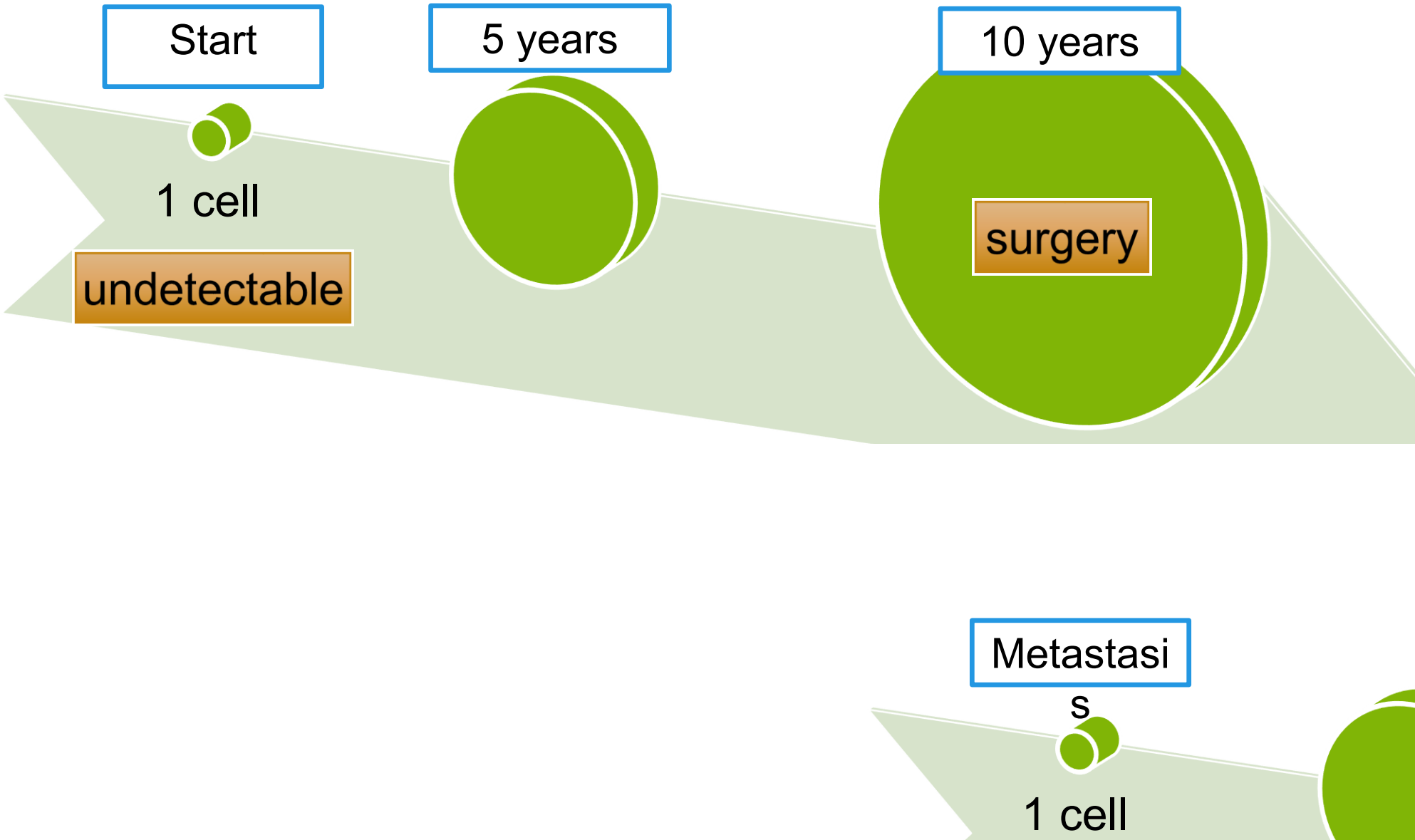
Development of metastases



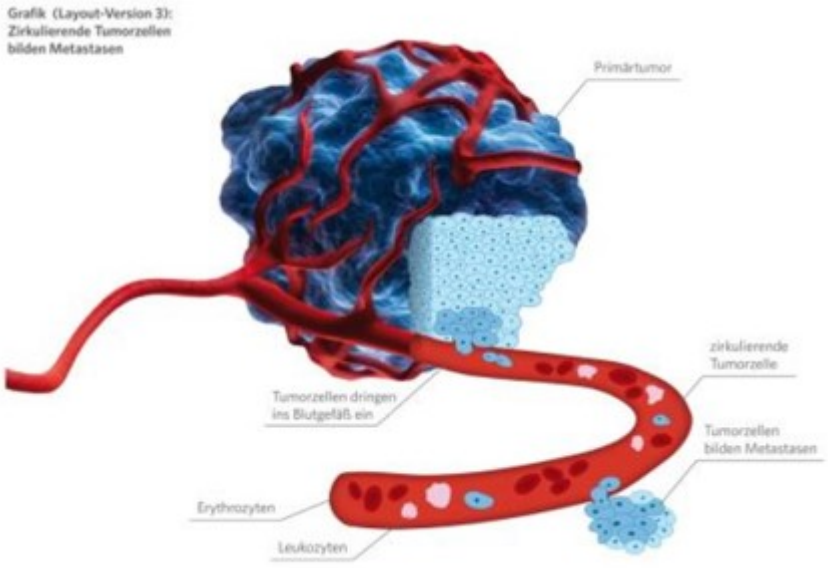
- Fast-growing tumour
  Doubling time 36 days



Development of metastases



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

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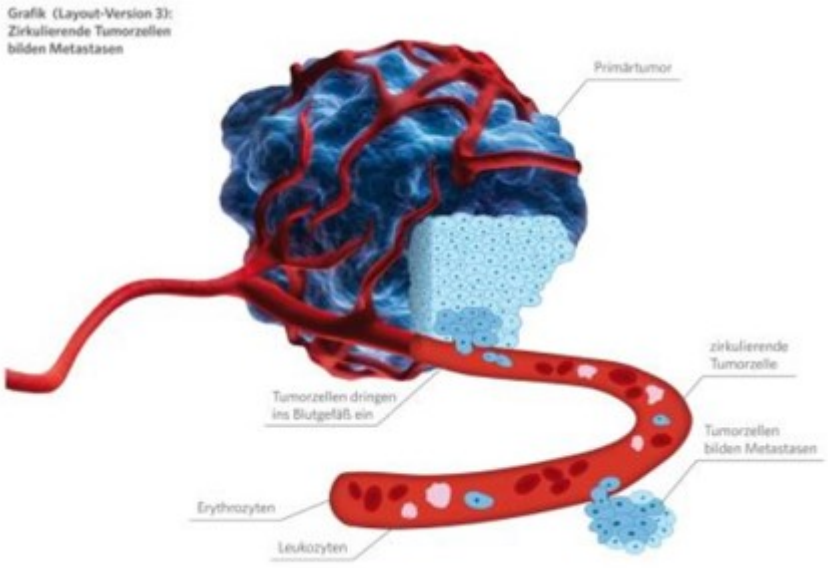
Circulating tumour cells from solid tumours



- 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

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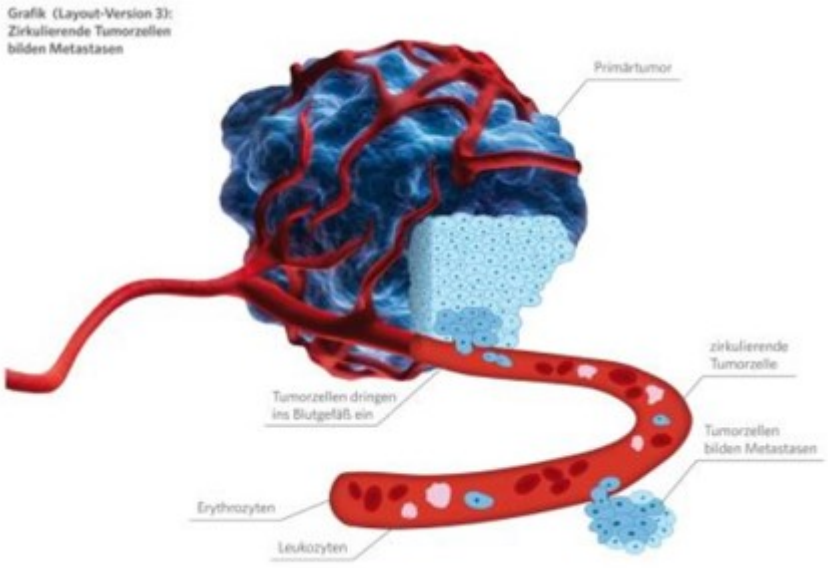
Circulating tumour cells from solid tumours



- 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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Maintrac method for detecting tumour cells

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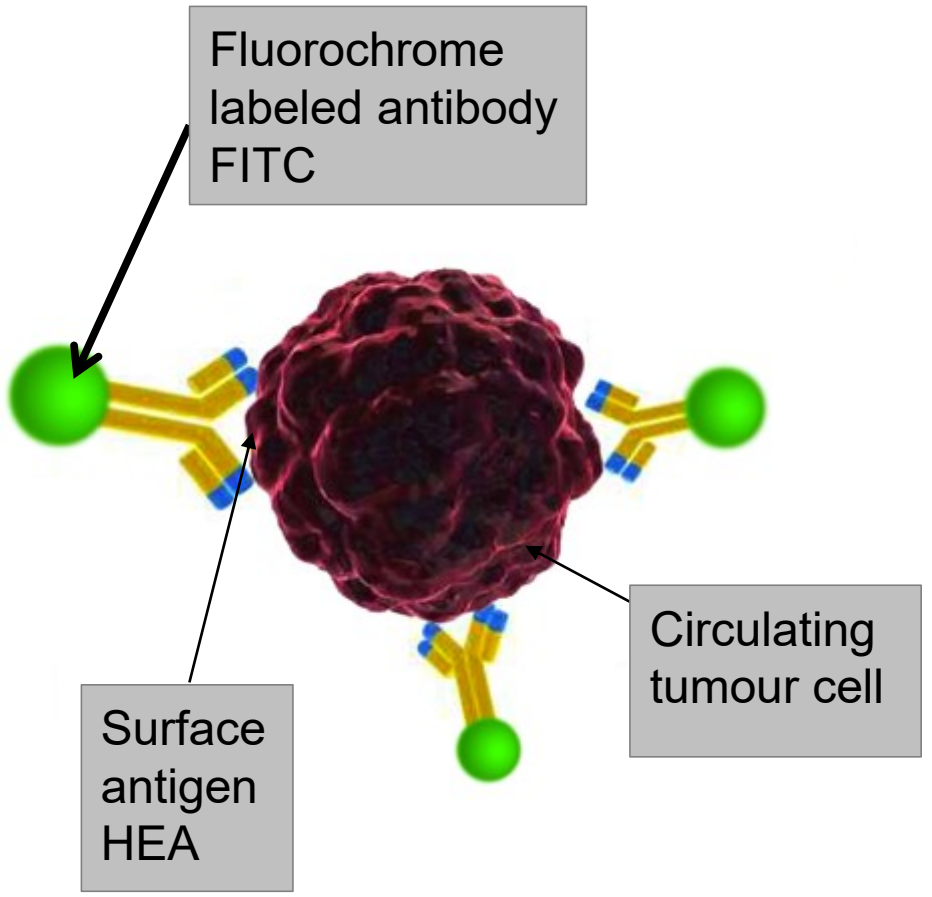
Liquid biopsy technique

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

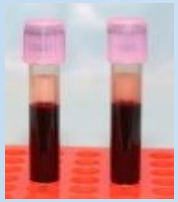
NO fixation.

NO isolation.

NO enrichment.

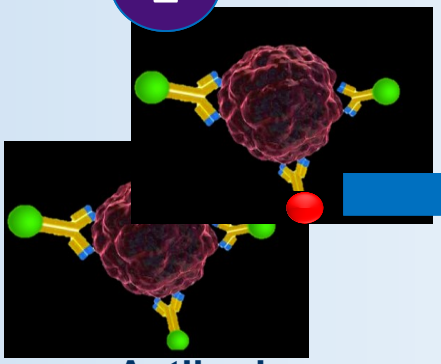


1



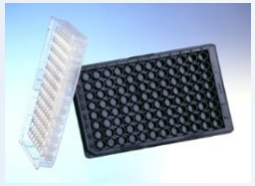
Blood sample

2



Antibody staining

3

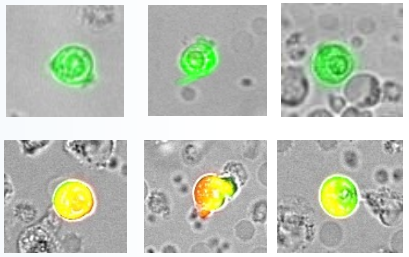


96-well plate

4

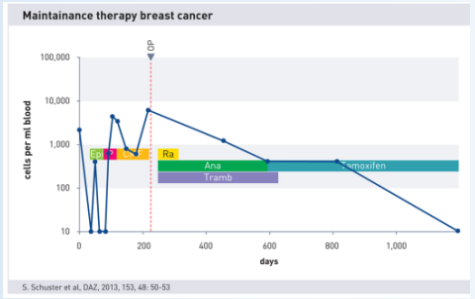
Microscopic identification of living cells

5



Quantitative enumeration of living cells

6

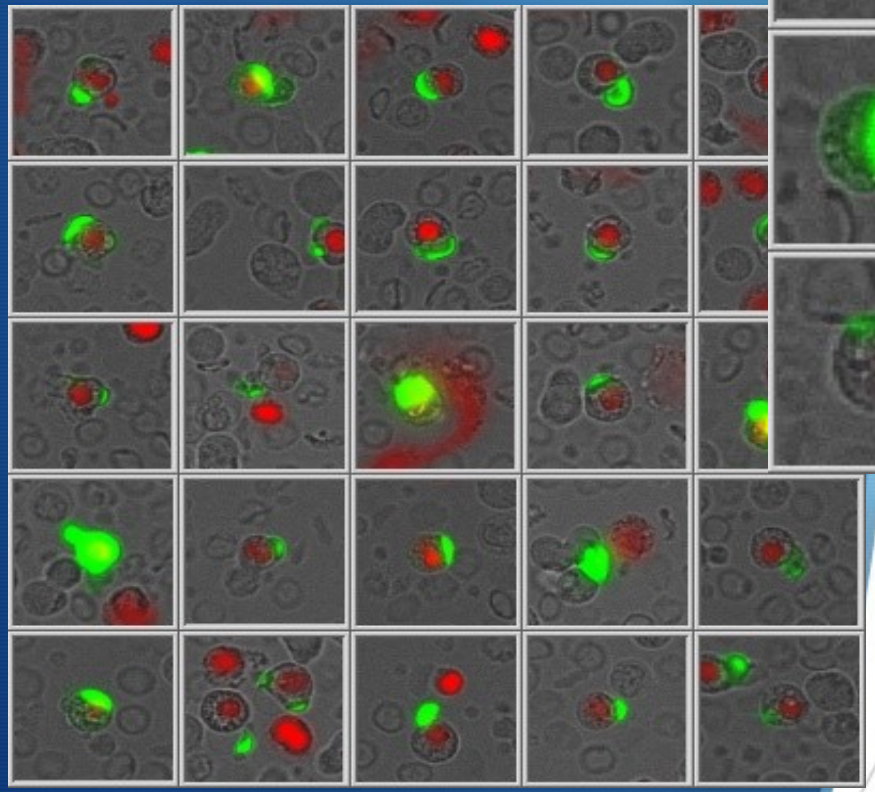


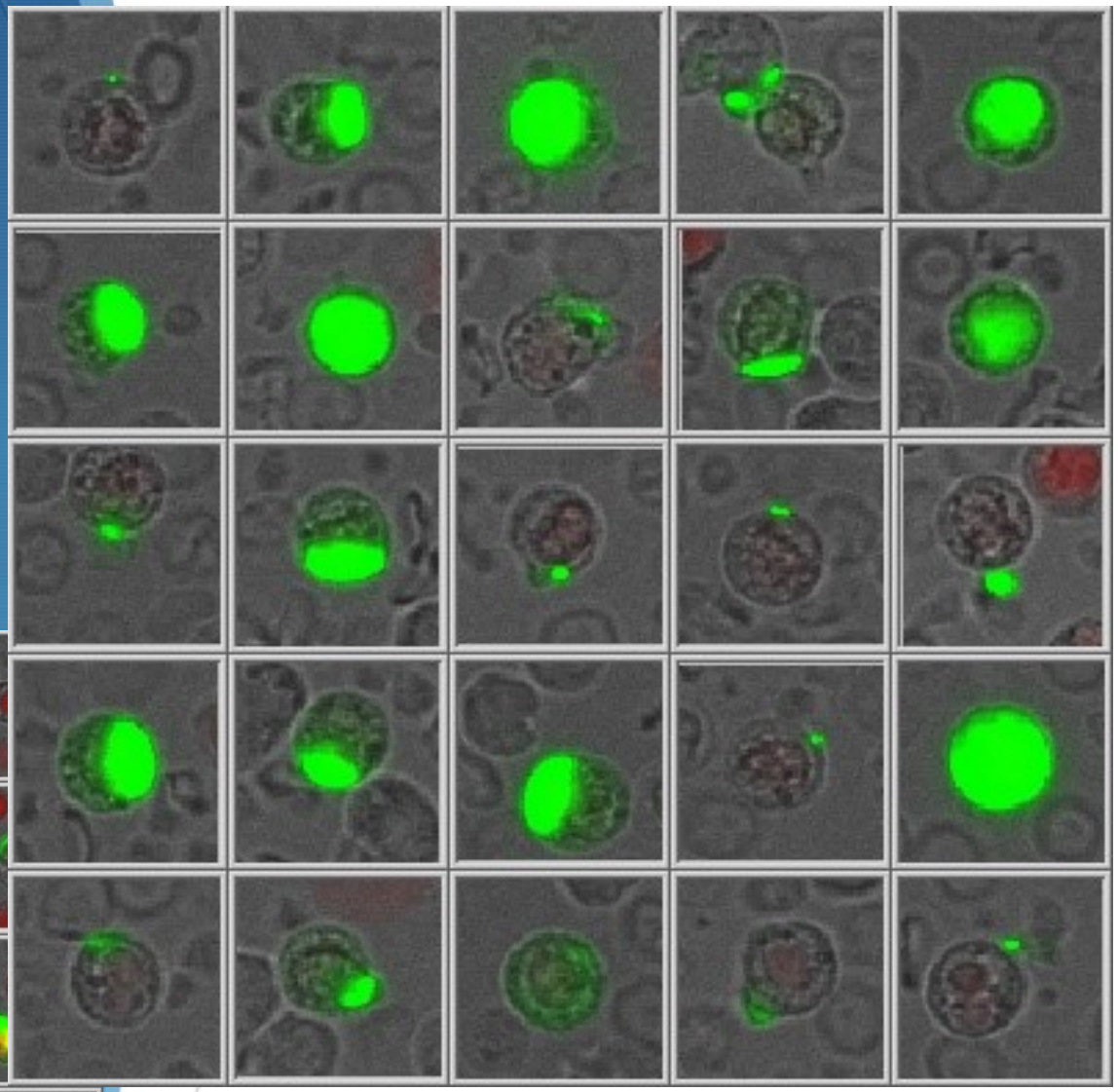
Dynamics of the cell count

Success control:

- Chemotherapy
- Maintenance therapy
- Longtime monitoring

Heterogeneity in cells from one patient





Red-stained nucleus
= dead cell

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How frequent are tumour cells in blood?

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

Comparison with other methods

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?	

CETC comparison with ctDNA

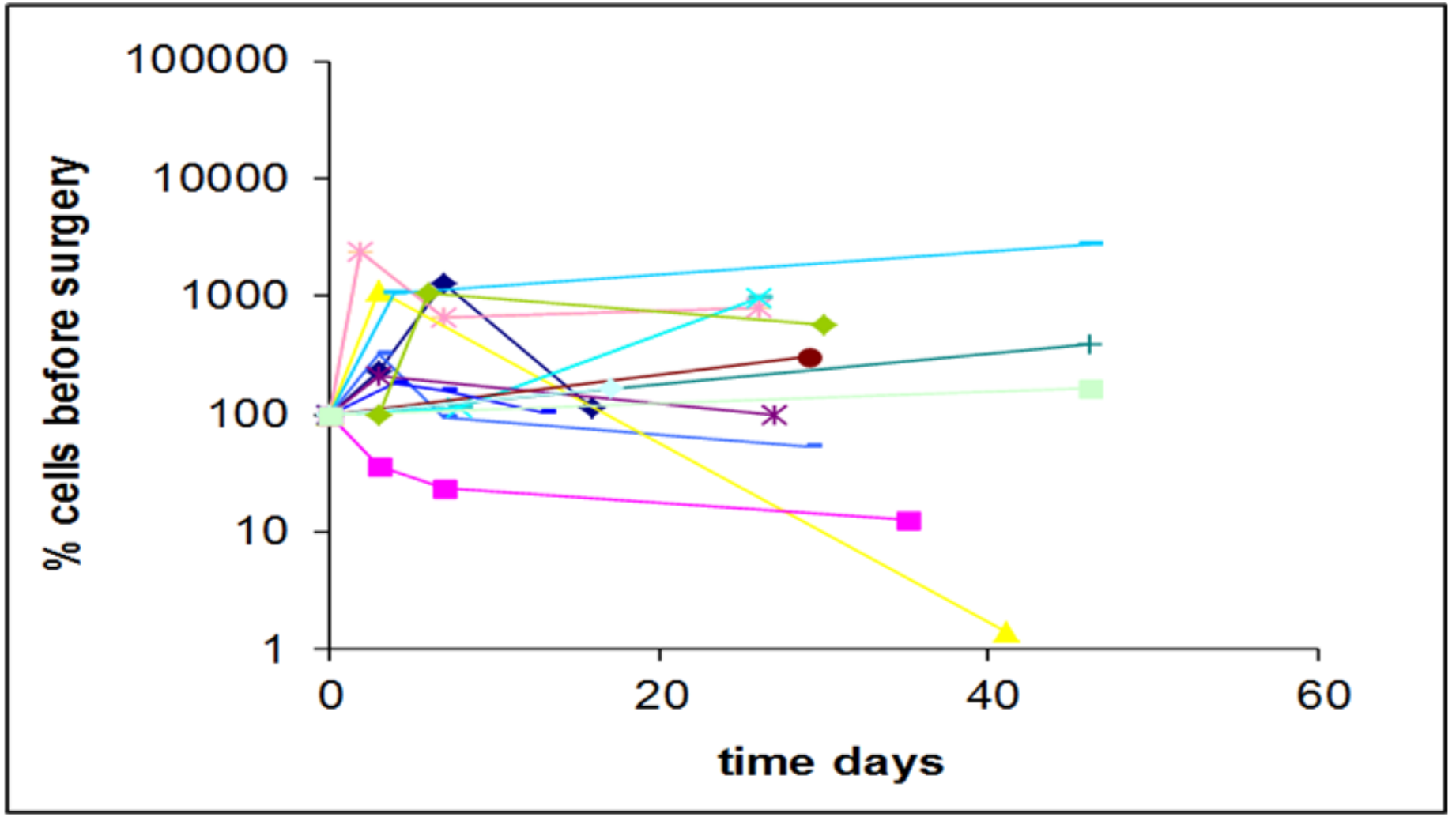
Technique	Problems
Isolation from plasma	DNA derived from destroyed cells.
Derived from dead cells	Stability of tumour DNA
Mutation analysis	Additional mutations due to DNA degradation

Fully accredited laboratory

Monitoring therapy using Circulating Tumor Cells

Surgery

Patterns of CETCs before and after surgery (breast)

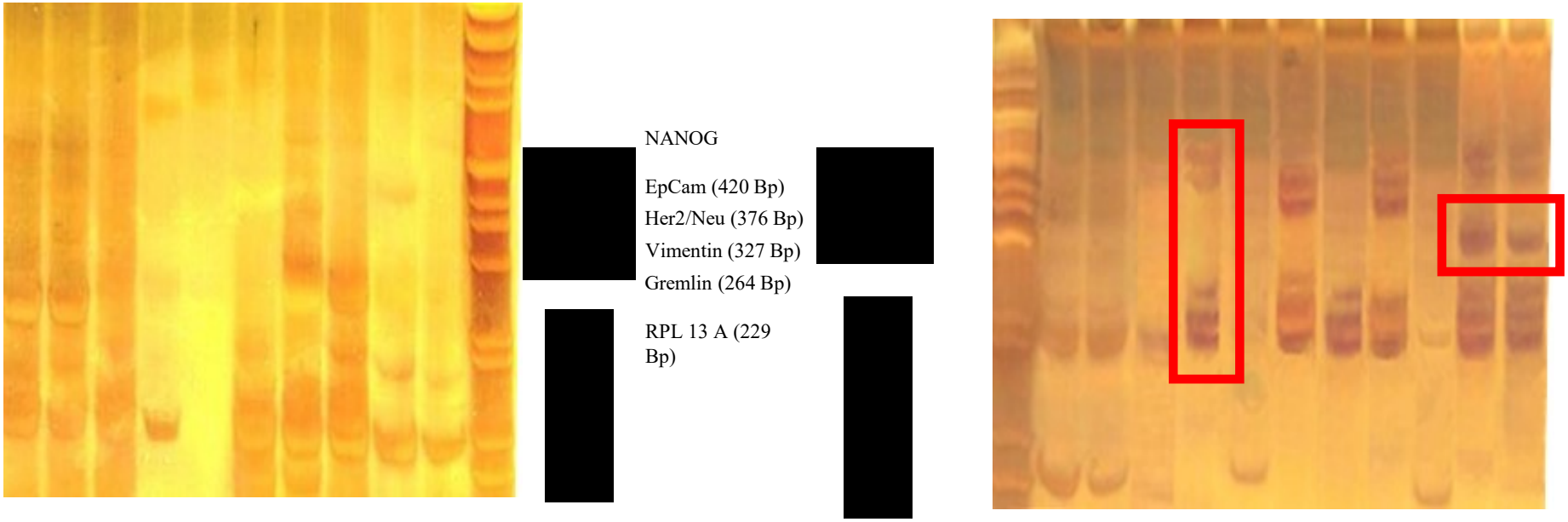


Changes of gene expression in circulating tumor cells after surgery

G, C

Pre OP

Post OP

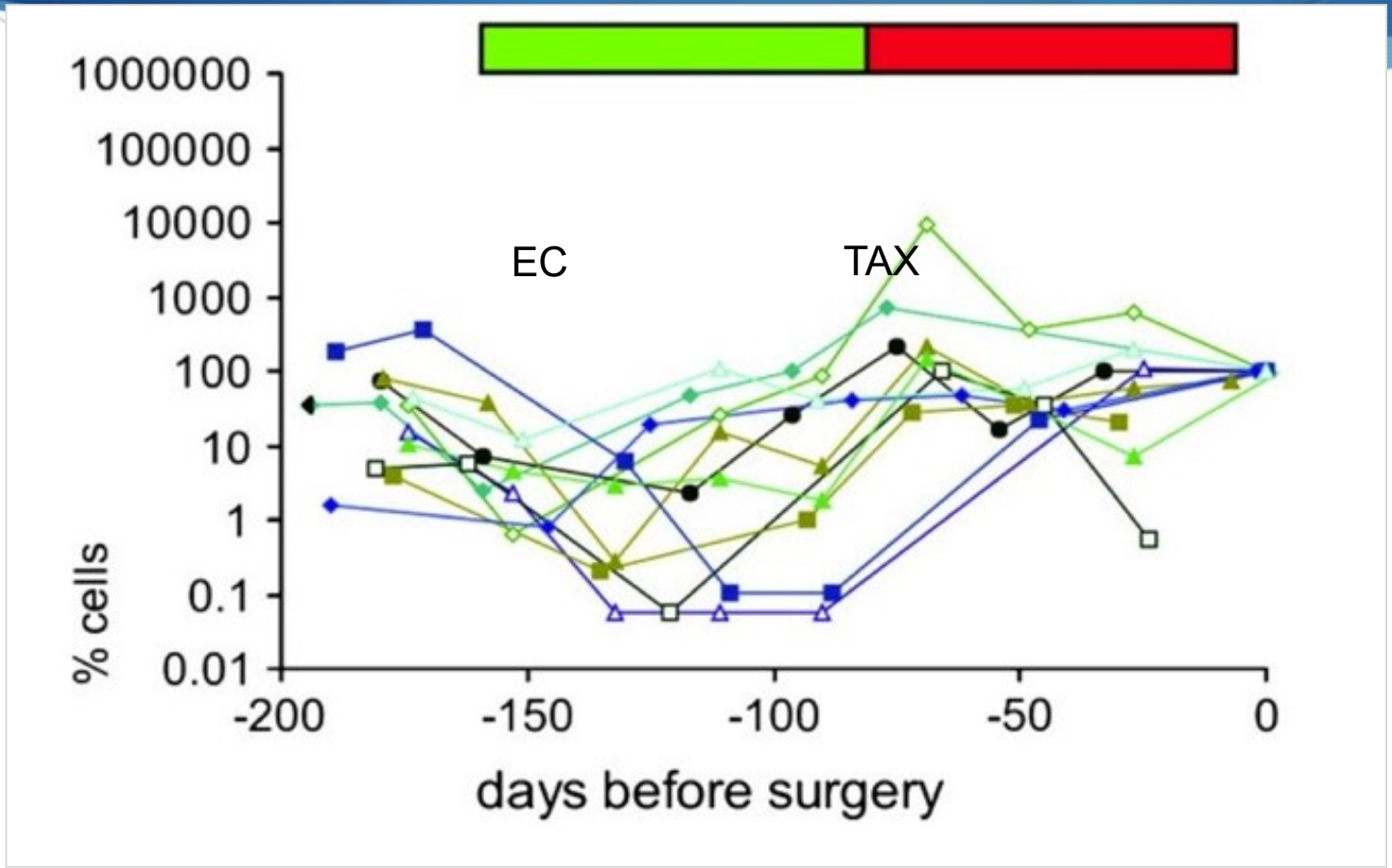


Increased expression of stem cell and athesion markers after surgery

Neoadjuvant treatment

The administration of therapeutic agents before treatment such as surgery in an attempt to shrink the tumour

Neoadjuvant treatment



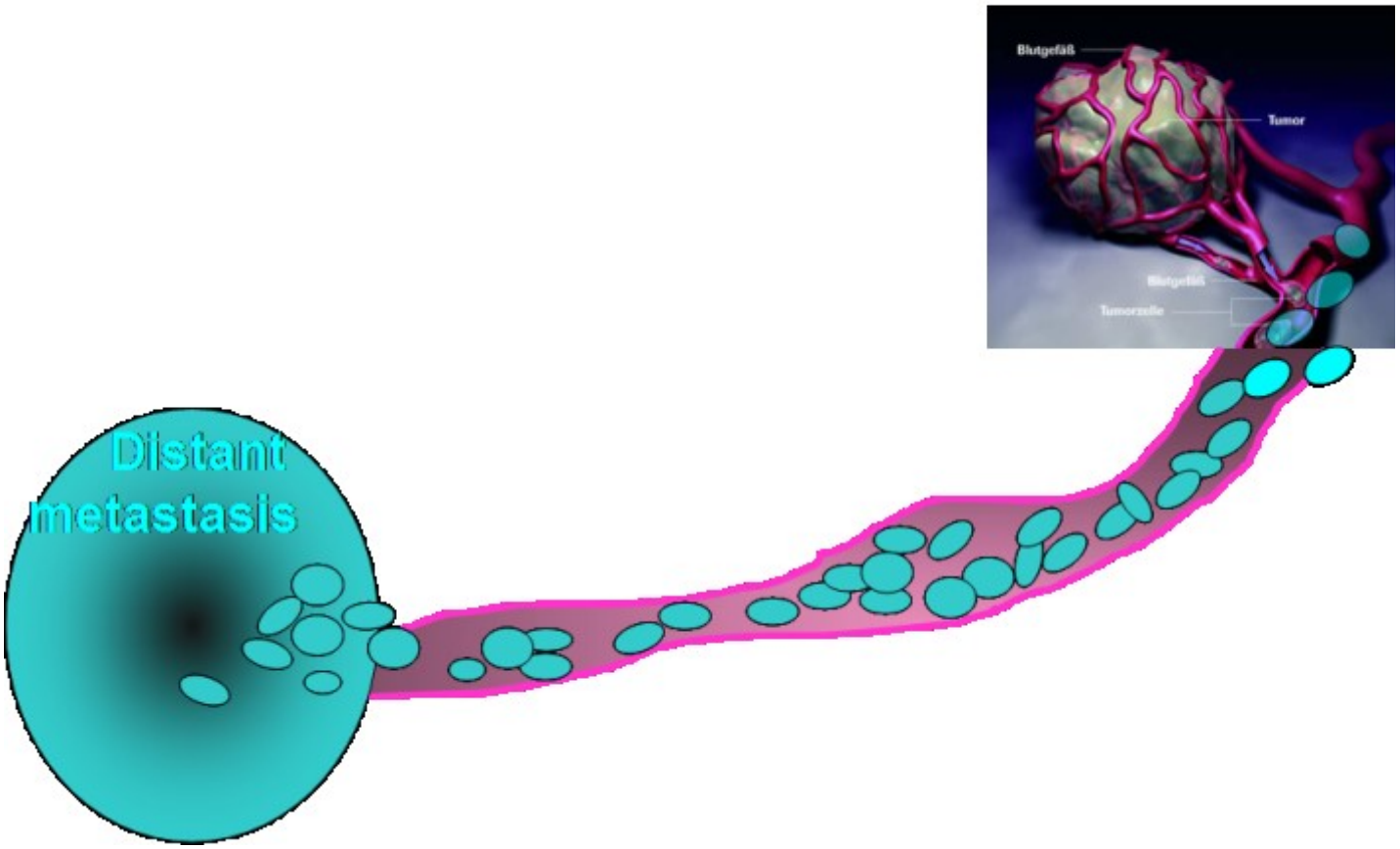
At the end of neoadjuvant therapy almost all patients experience increasing numbers of CECTs !

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

Neoadjuvant treatment

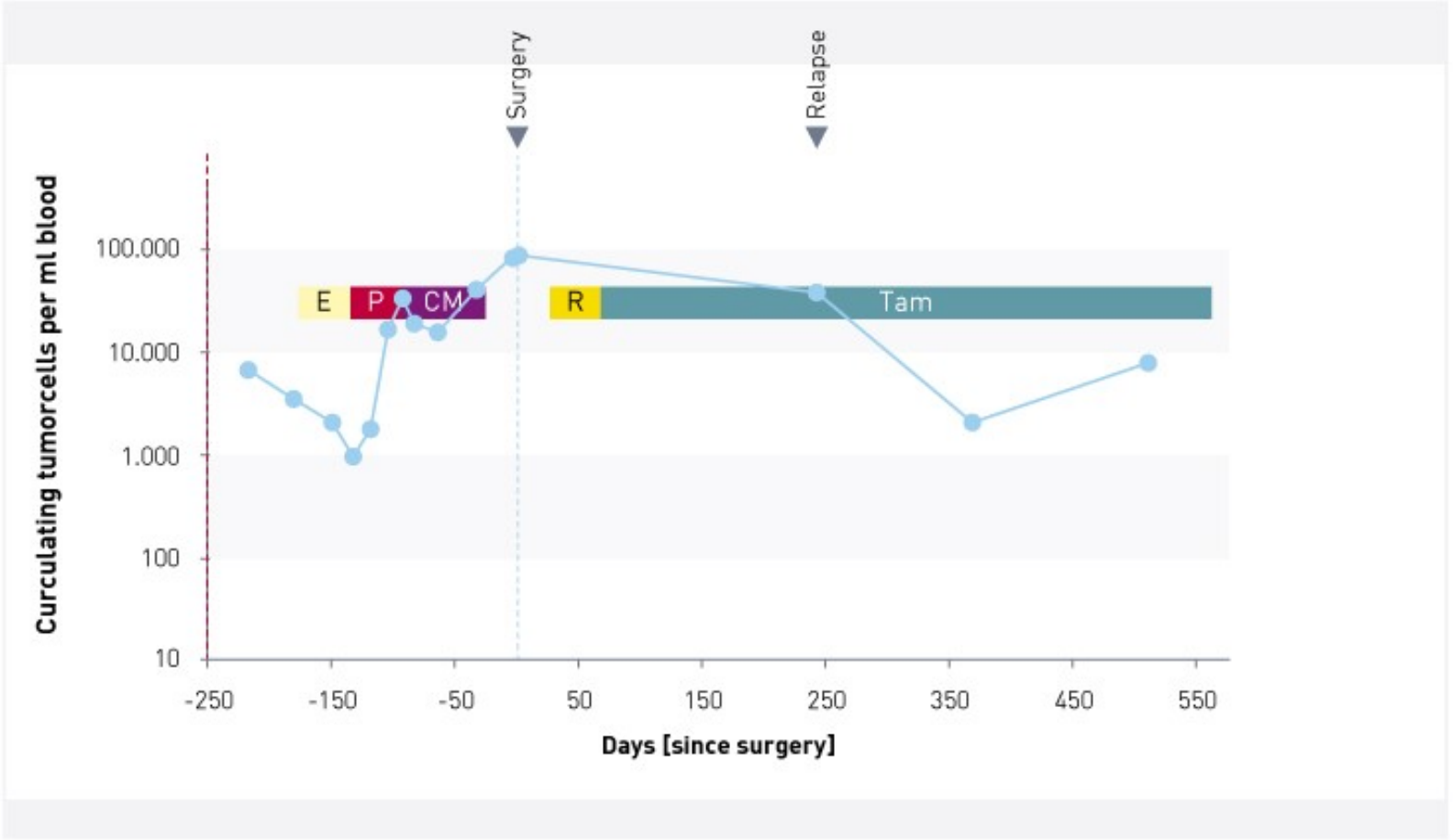
- Neoadjuvant chemotherapy may initially eliminate minimal residual disease (cells circulating in blood). However, during tumor shrinkage often tumor cells in the blood go up again.
- Increasing numbers of CECTs may be due to release of cells in addition to cell death

Neoadjuvant chemotherapy shrinks the tumour, seeding cells into blood



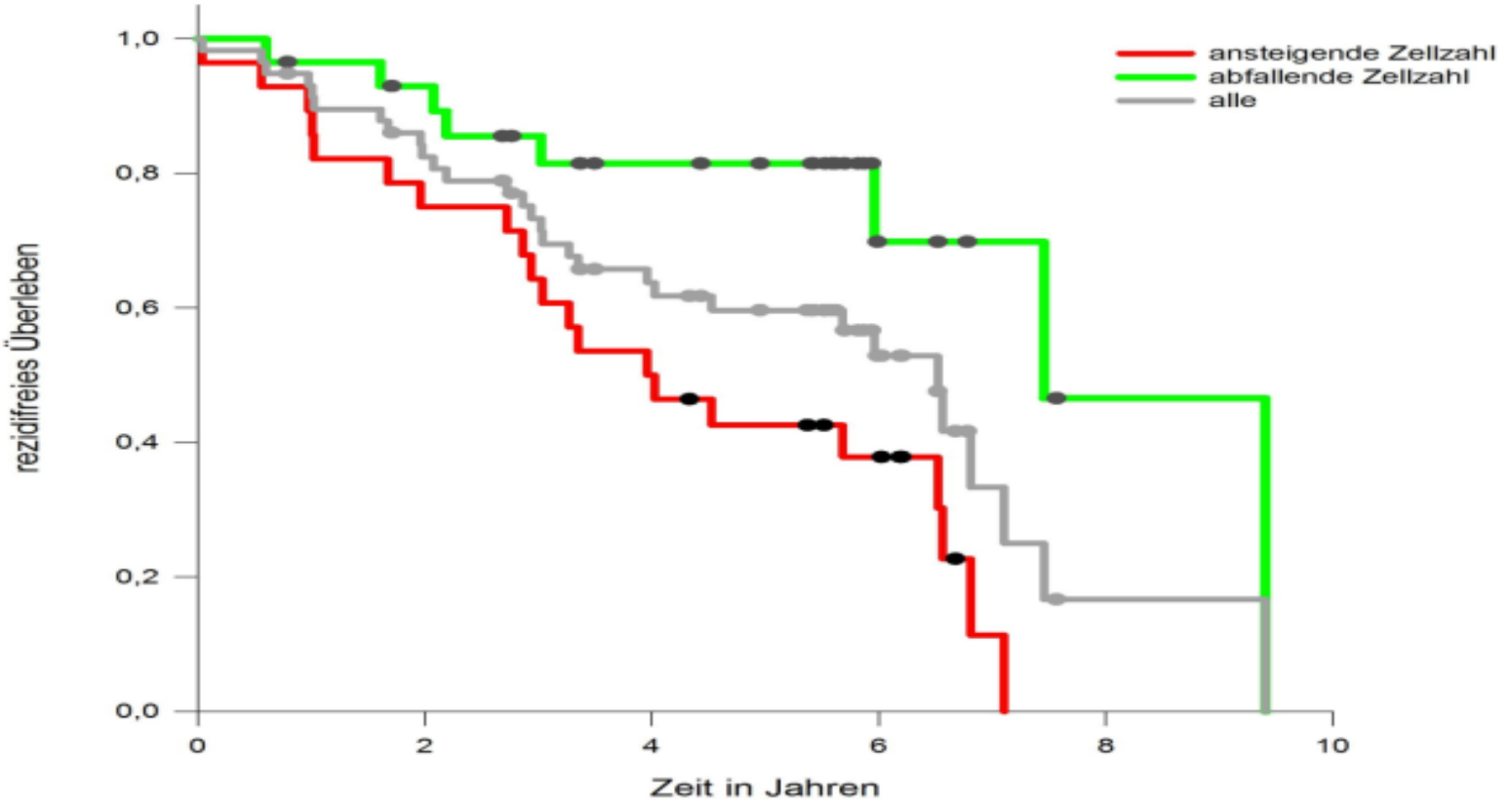
Neoadjuvant treatment

typical course of disease



Kaplan-Meyer survival results

relevance of circulating tumor cells during neoadjuvant therapy



Gehan-Breslow Test:

Statistic	DF	P Value
7,647	1	0,006

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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

Chemotherapeutic agents suggested by maintrac

maintrac drug testing (does not include cell counting)

	daily dose
○ Docetaxel	
○ Paclitaxe	
○ Cyclophosphamide	
○ Epirubicin	
○ 5-Fluoruracil	
○ Doxorubicin	
○ Gemcitabine	
○ Vinorelbine	
○ Cisplatin	
○ Carboplatin	
○ Oxaliplatin	

○ *Further substances:*

○ *Combination testing:*

Results of chemosensitivity tests, example

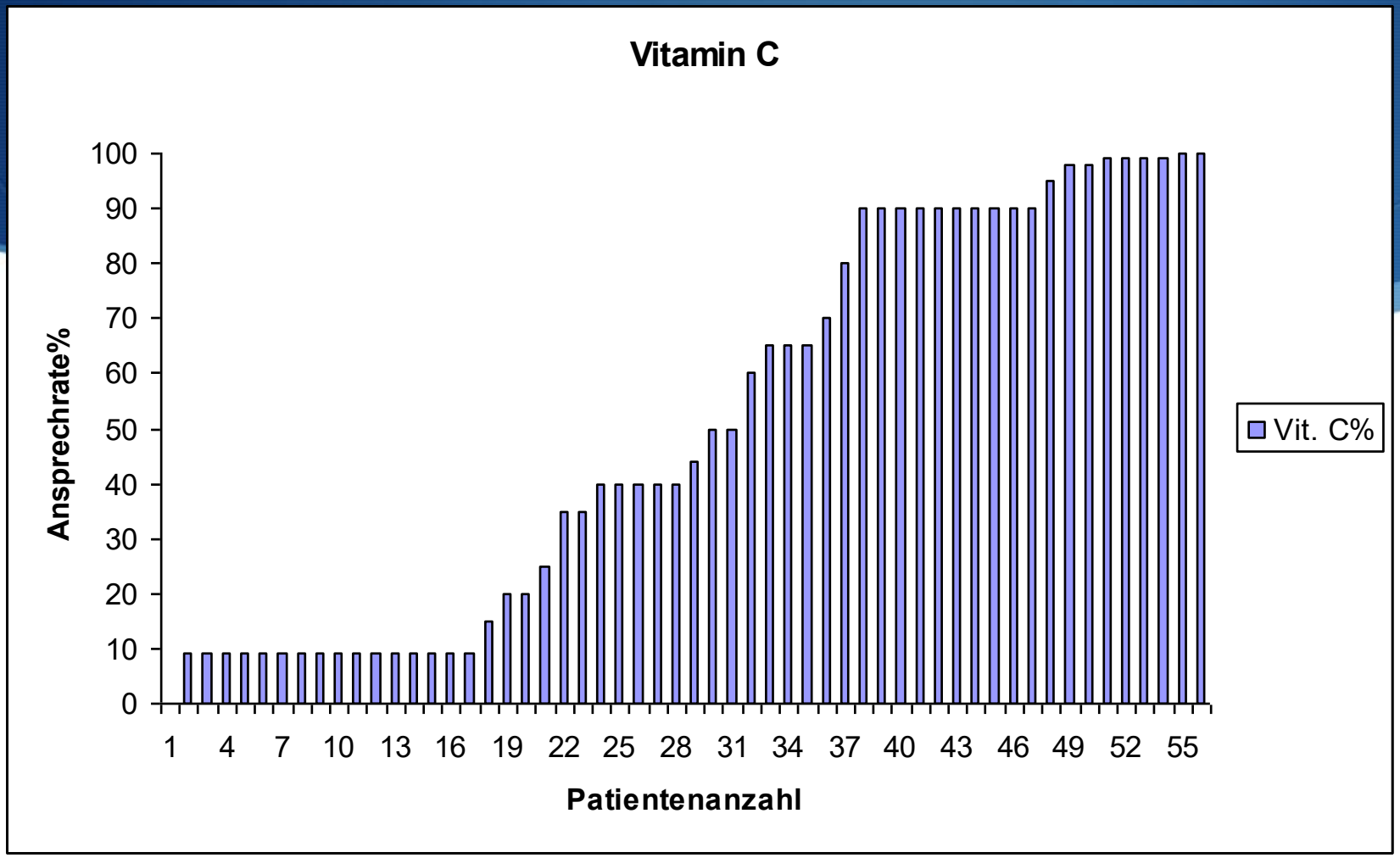
	Number of potential tumor cells			
Examination parameter	In the sample (1ml)	In circulation (5l) (in millions)	In addit. examination: % of EpCAM-pos. cells	Cell fragments
EpCAM	**200**	**1**		some

in-vitro-vitality reduction in relation to concentration and time (in%) with eutherapeutic concentrations of				
Vitamin C	**<10**	Capecitabine	**80**	The ideal is a reduction by 100% in short-term cell culture
Artesunat	**50**			

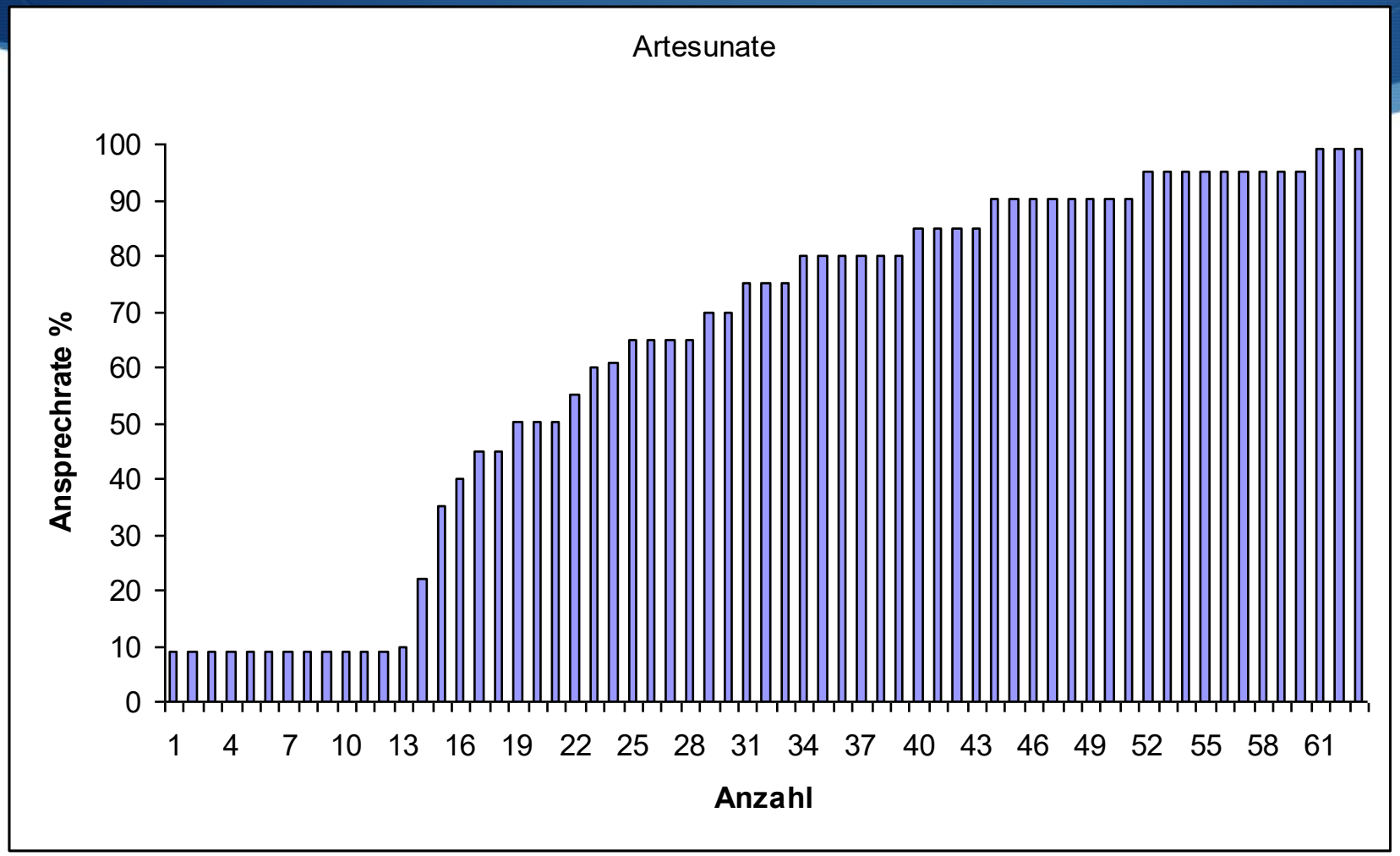
The material for examination could be thoroughly evaluated.
Under current therapy we found only **a slightly increased number of live tumor suspected cells circulating in the blood.**
In comparison to the previous findings from March 2017 the number of potential tumor cells has decreased somewhat.

Natural agents suggested by maintrac

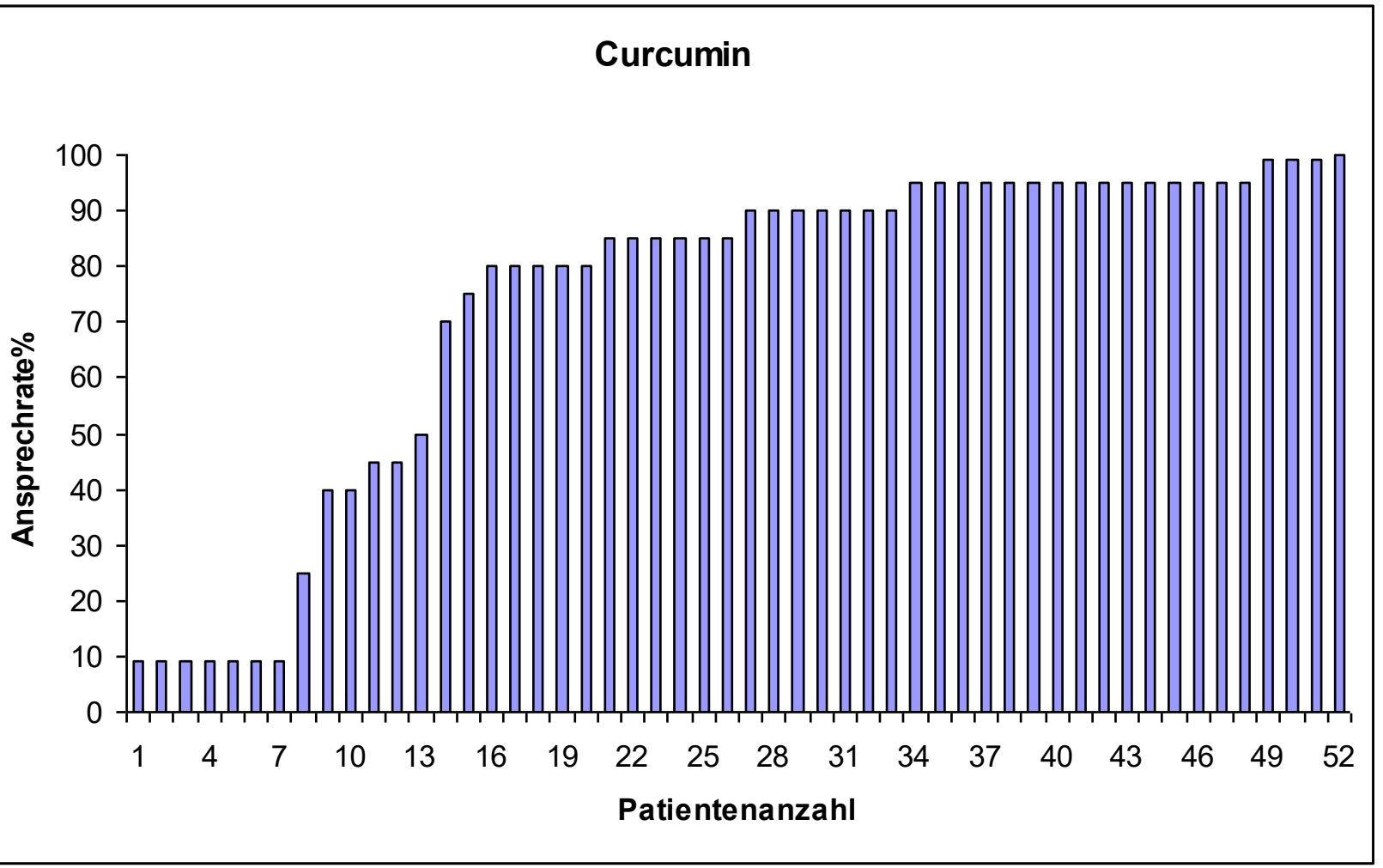
○ Helixor A ; M ; P	○ Further substances:
Please name manufacturer:	
daily dose	
○ Vitamin C	
○ Graviola	
○ Iscador M; Q; U; P	
○ DCA (Dichloracetat)	
○ Amygdalin	○ Combination testing:
○ Sulforaphan	
○ Hypericin	
○ Curcumin	
○ Artesunat	



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%

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

Labor Dr. med. Ulrich Pachmann · Kutzenreuthe 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

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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
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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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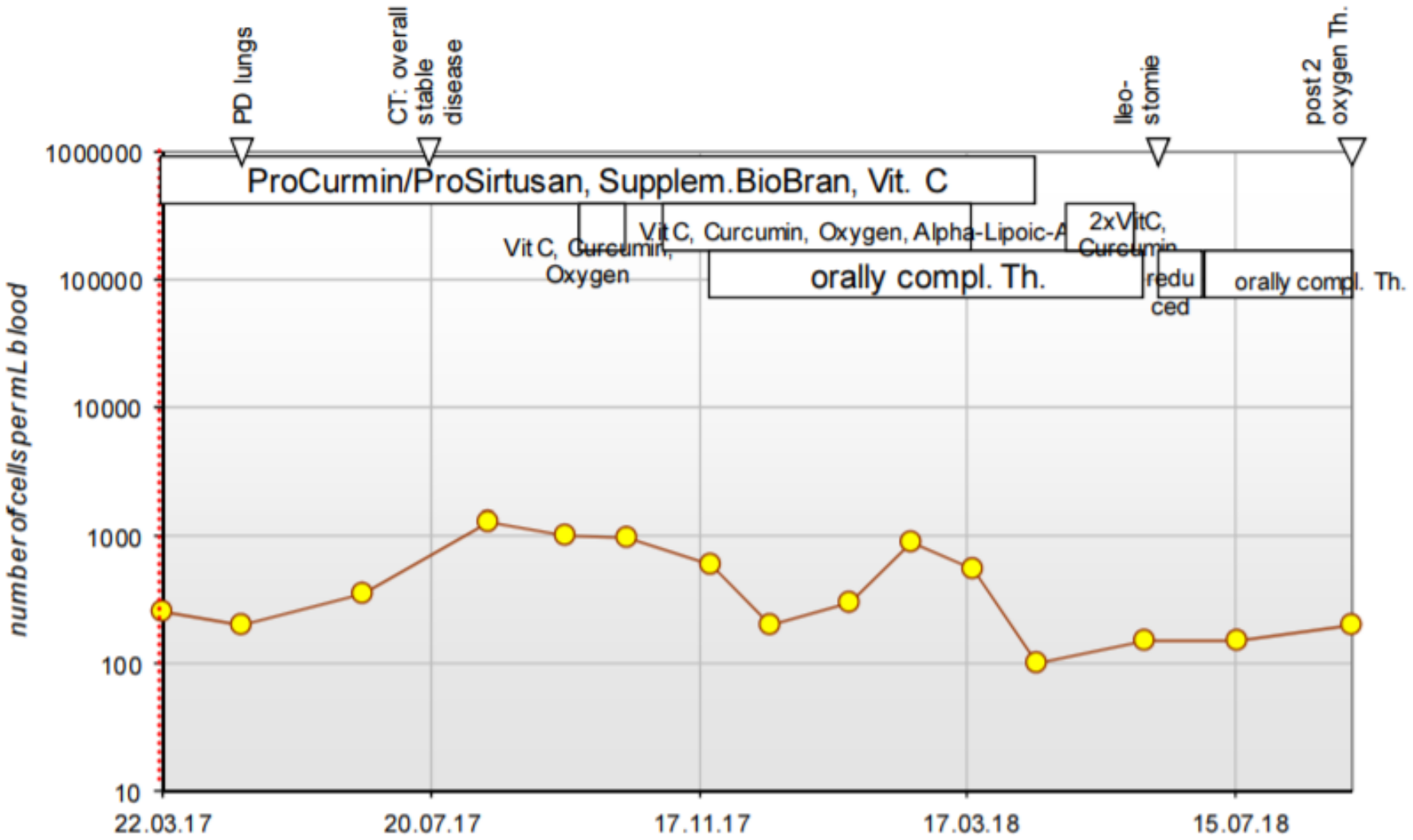
Results now available in three levels of concentration

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.**

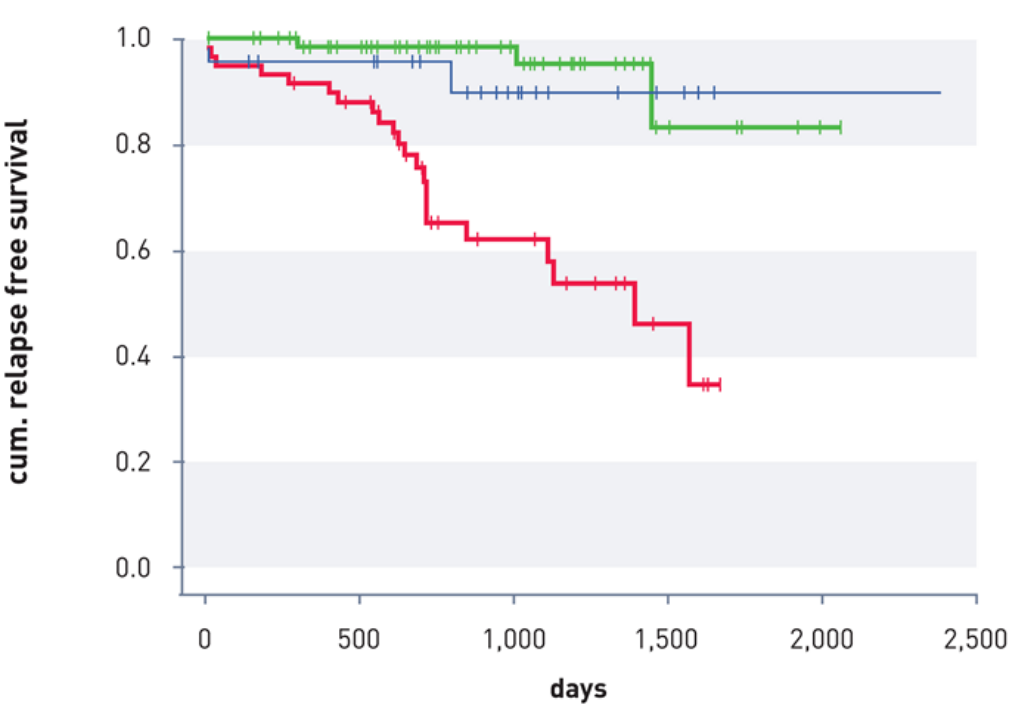
The results build to form a trajectory



Maintenance therapy

Endocrine Therapy Breast cancer (Tamoxifen)

Maintenance therapy (Tamoxifen)



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

log rank p < 0.001

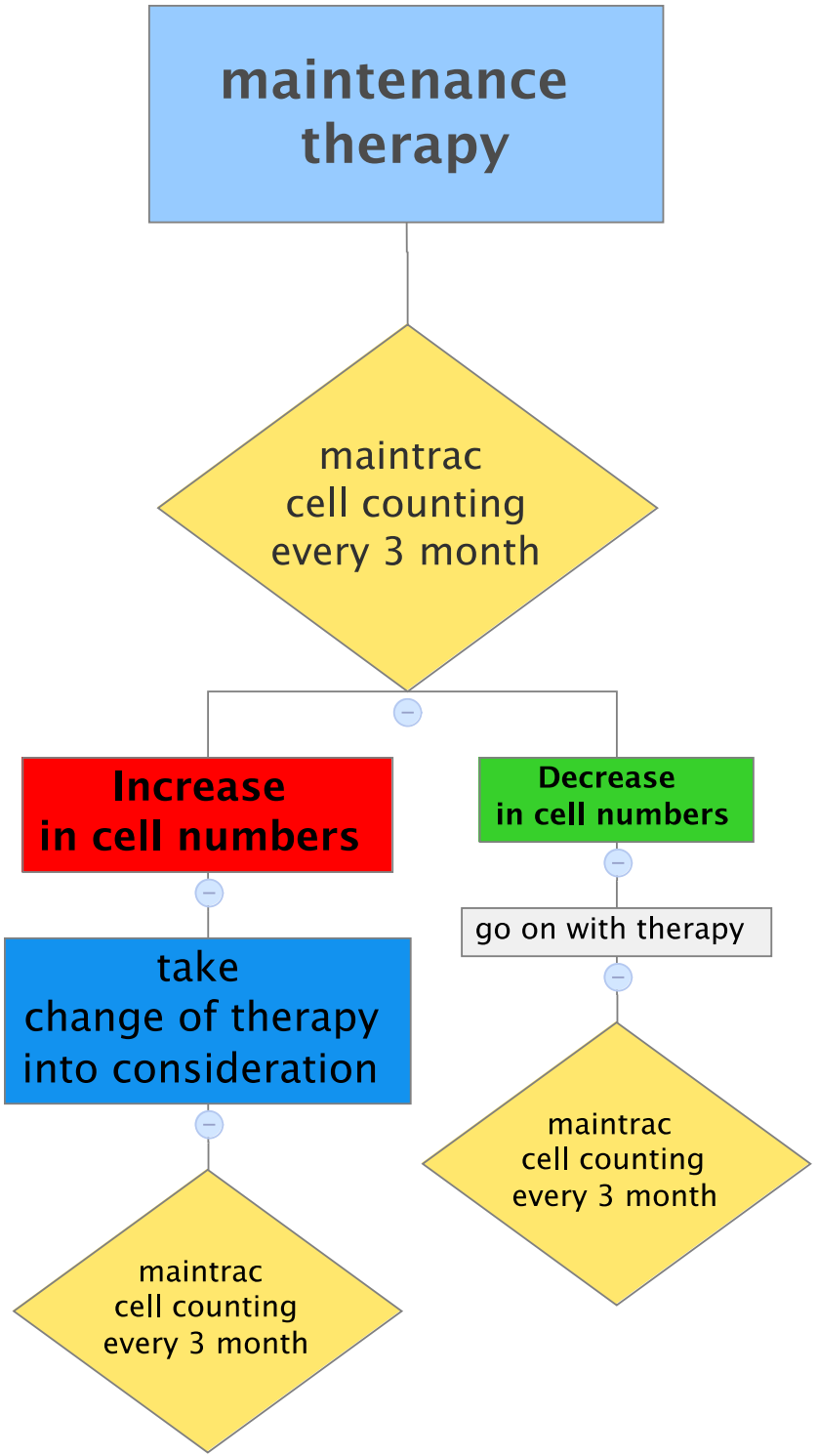
179 Patients were included in this study

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

If cell numbers **increase**,

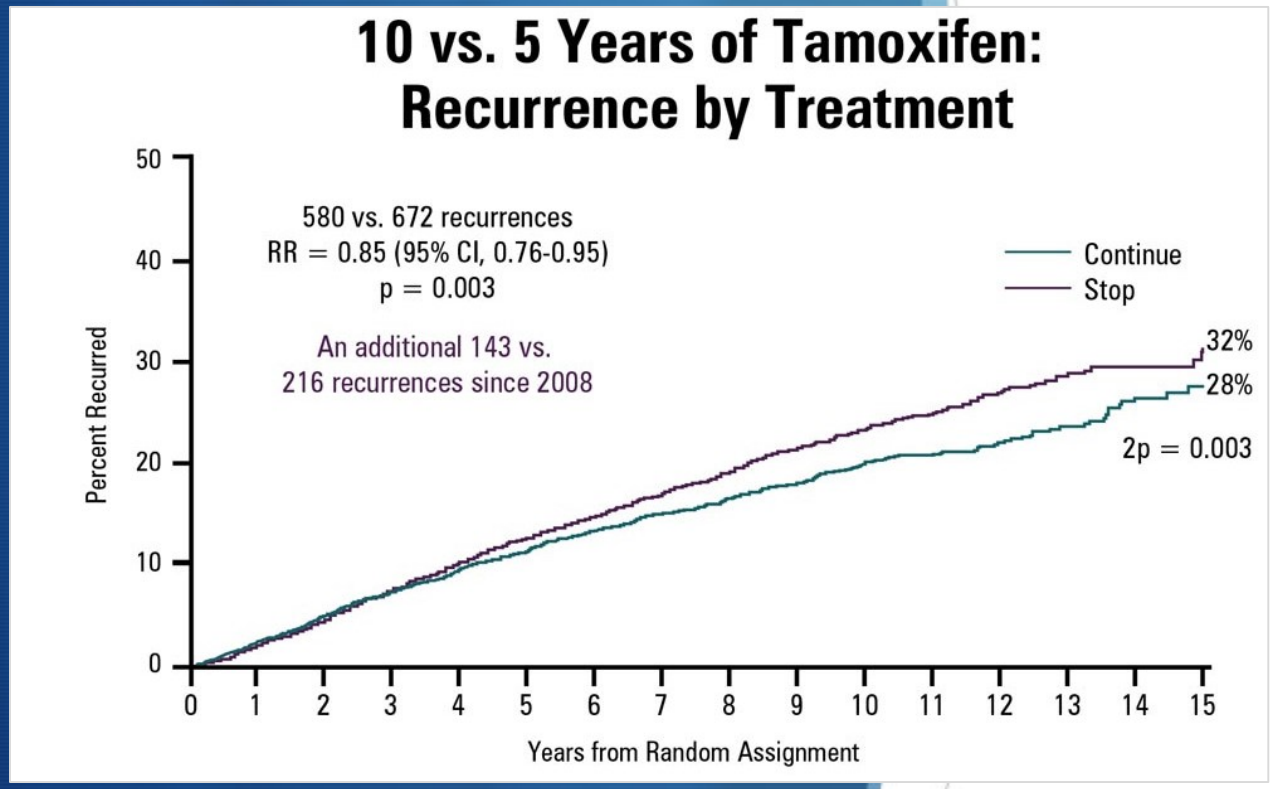
change of therapy may be considered

monitor every 3 months



Long-term surveillance after maintenance therapy

Endocrine Therapy



Is it worth taking Tamoxifen for 10 years, or only 5? ....

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

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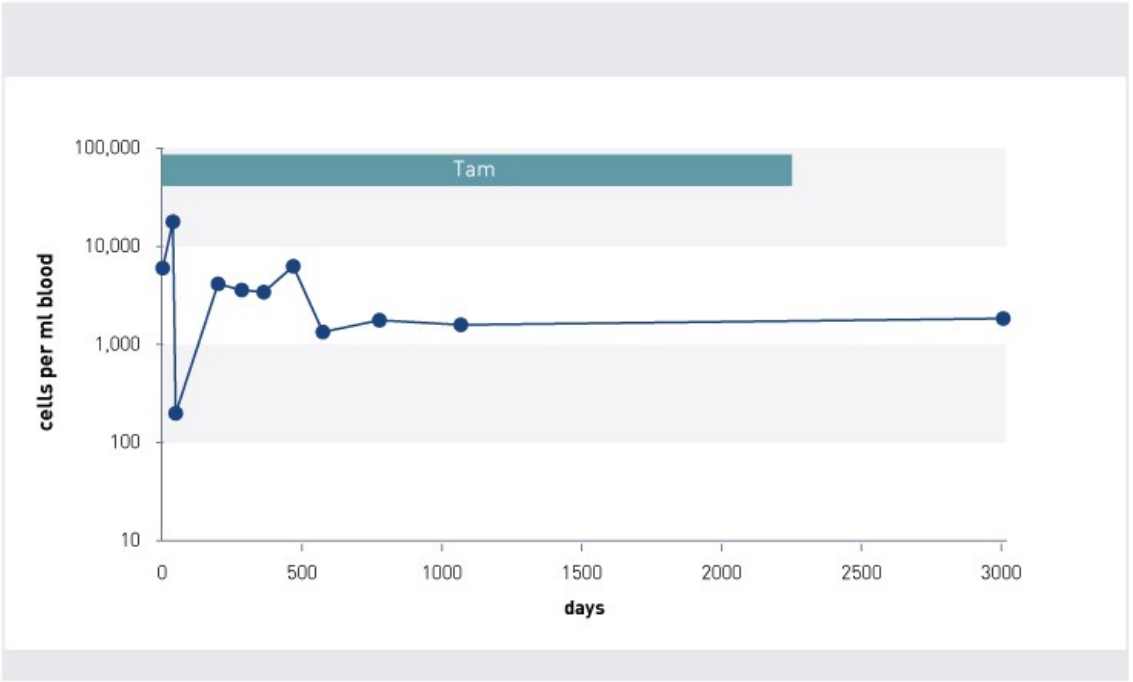
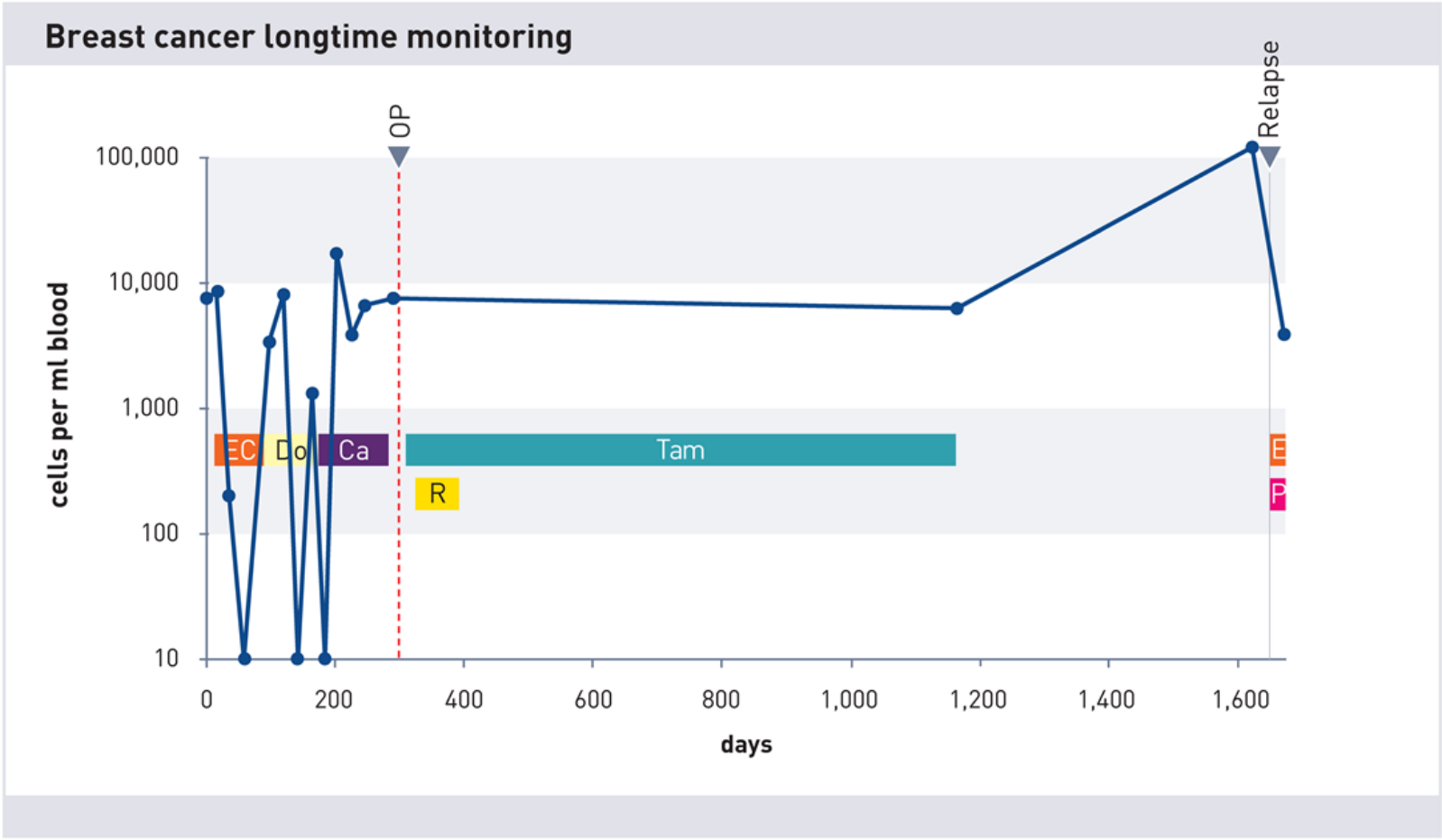
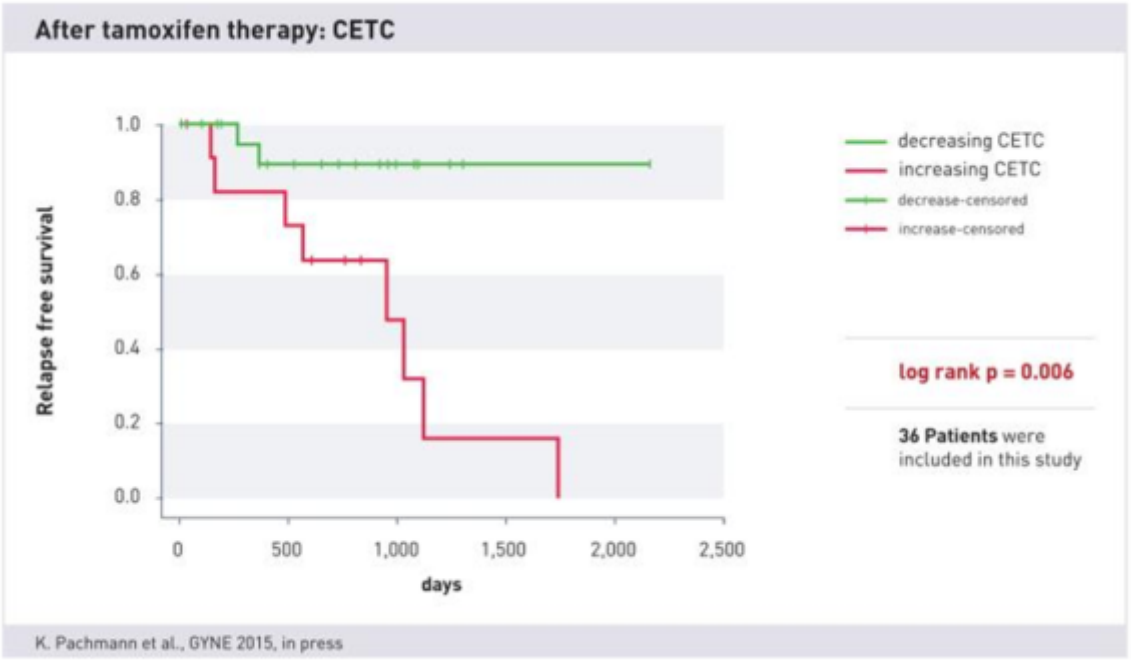
Long-term surveillance after stopping Tamoxifen therapy



Long-term surveillance after stopping Tamoxifen therapy



Long-term surveillance after stopping Tamoxifen therapy



Patients with **increasing** cell numbers after the end of maintenance therapy have an **increased risk** of recurrence

Metastatic disease

Background

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

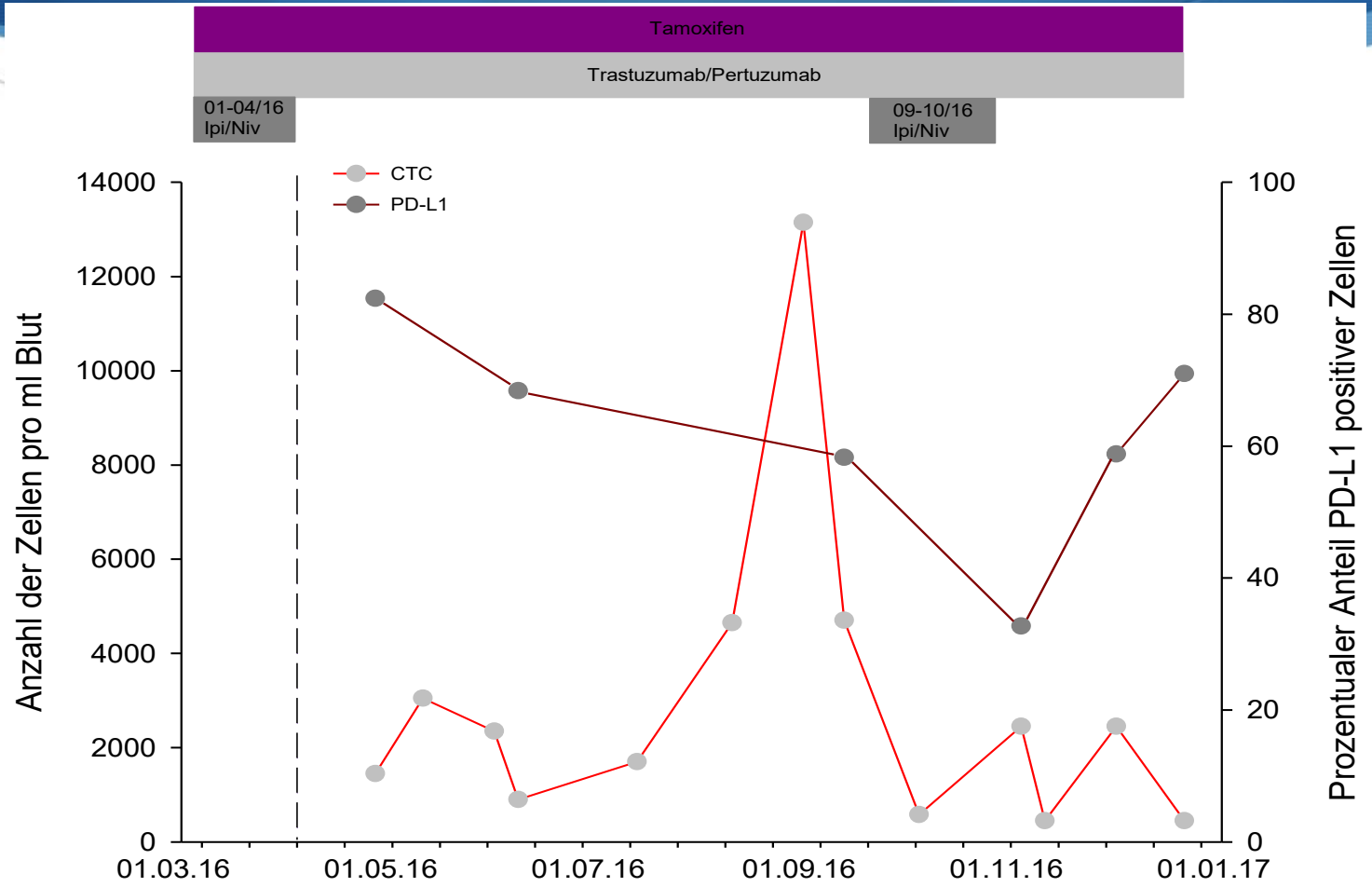
Metastatic disease

- 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

Case report: metastatic breast cancer



Sharp increase in cell numbers before and decrease after immunotherapy

free of progression

Transfusion Medicine Center in Bayreuth - TZB

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

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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.

For more information about maintrac and CTC testing please contact

info@aonm.org

+44(0)3331 210 305

www.aonm.org/maintrac/