



Circulating epithelial tumor cells in the metastatic pathway

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Description of the method

Aims:

Detection of very rare cells

Fast scanning of multiple samples

Relocation for visual examination

Discrimination of live and dead cells

Antibodies used for detection of tumor cells in blood

- Anti-Human Epithelial Antigen (HEA)
- Anti-Cytokeratin Antibody (CK17,18,19,20)
- Anti-BMP7 for lung cancer
- Anti-Estrogen receptor
- anti-Her2/neu
- Anti-Melan A

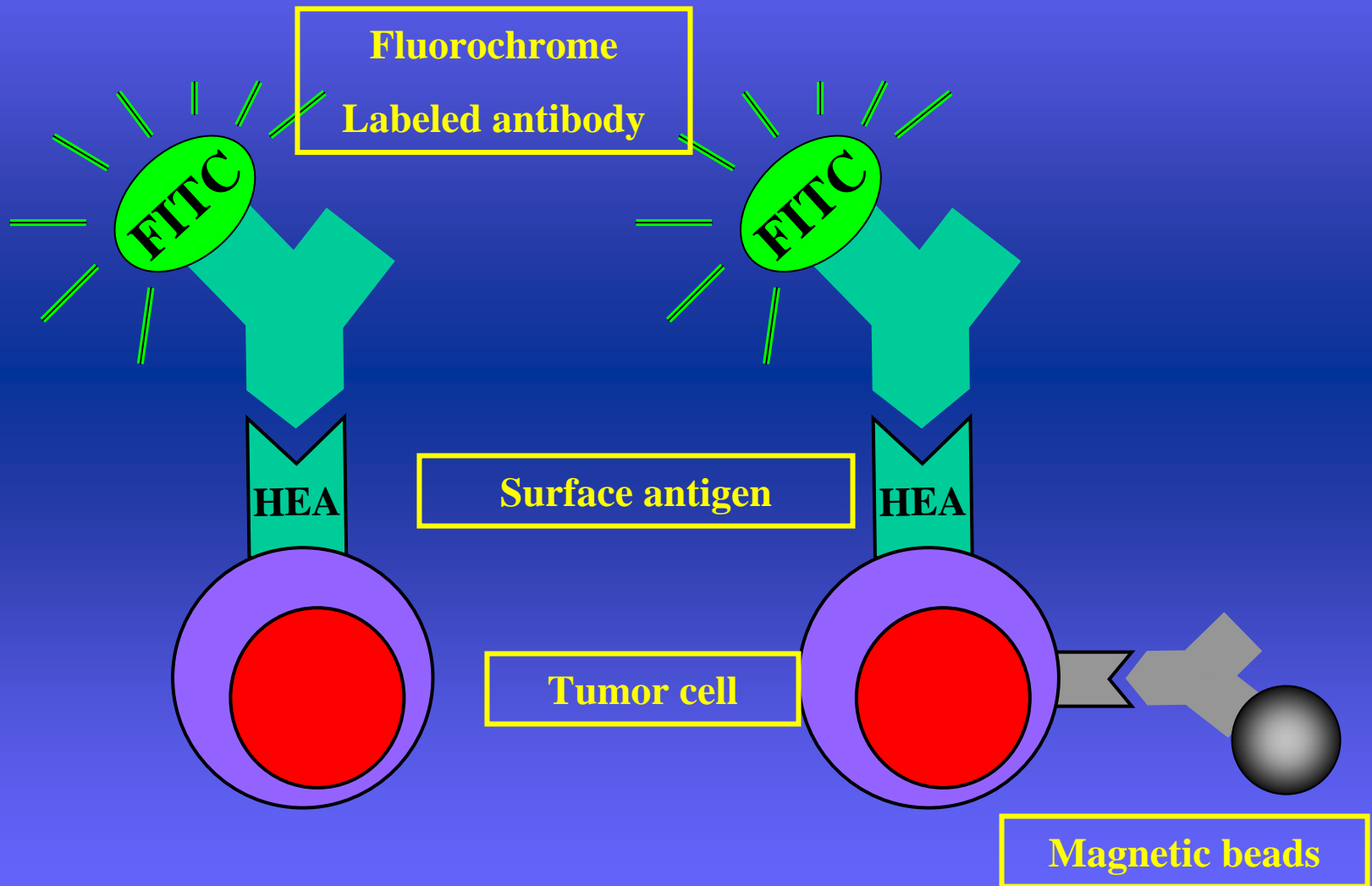
Description of the method

anticoagulated blood



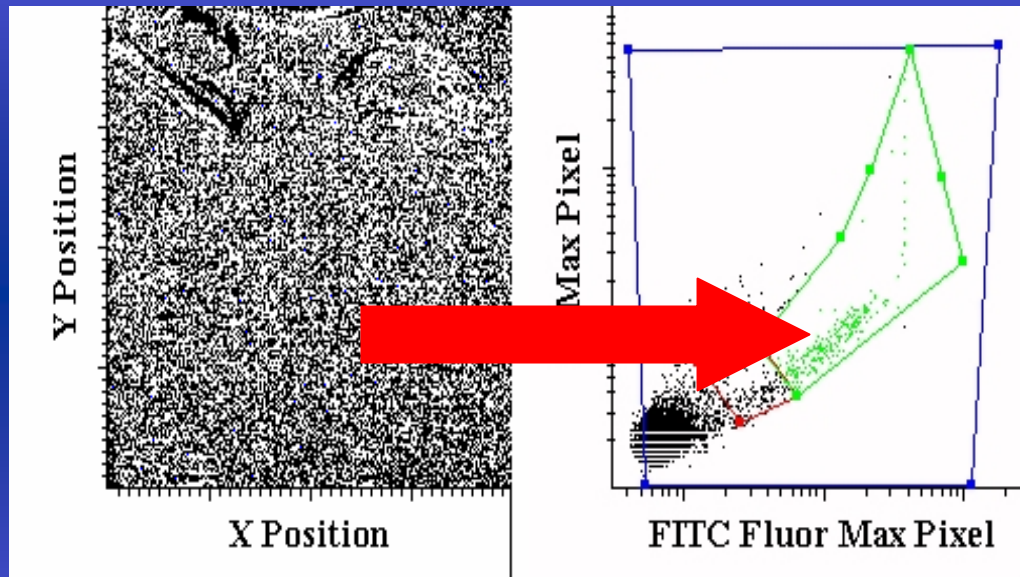
**Red blood cell
lysis**

Description of the method

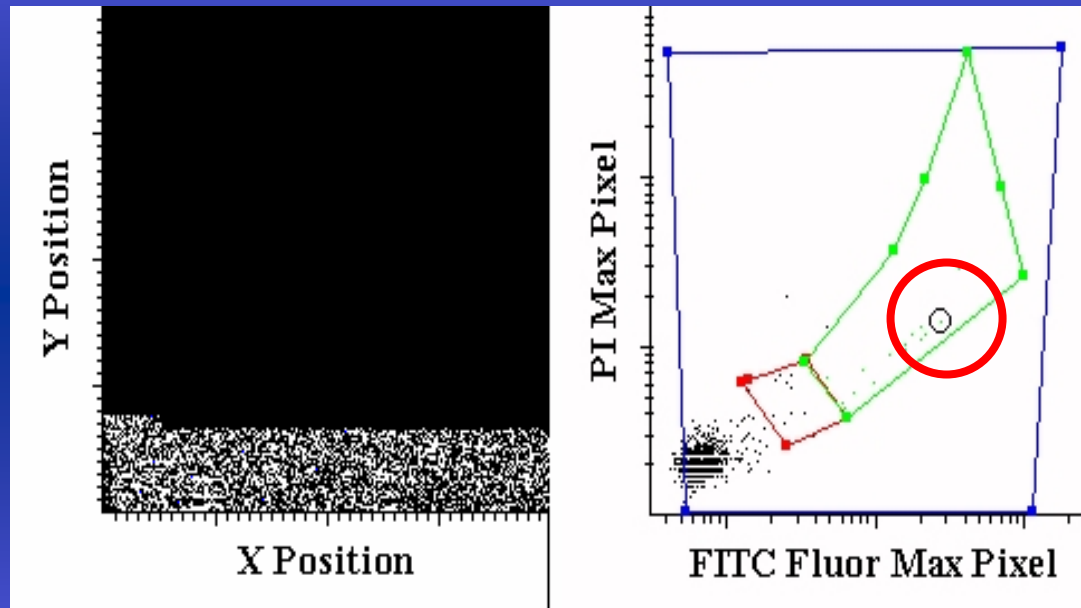


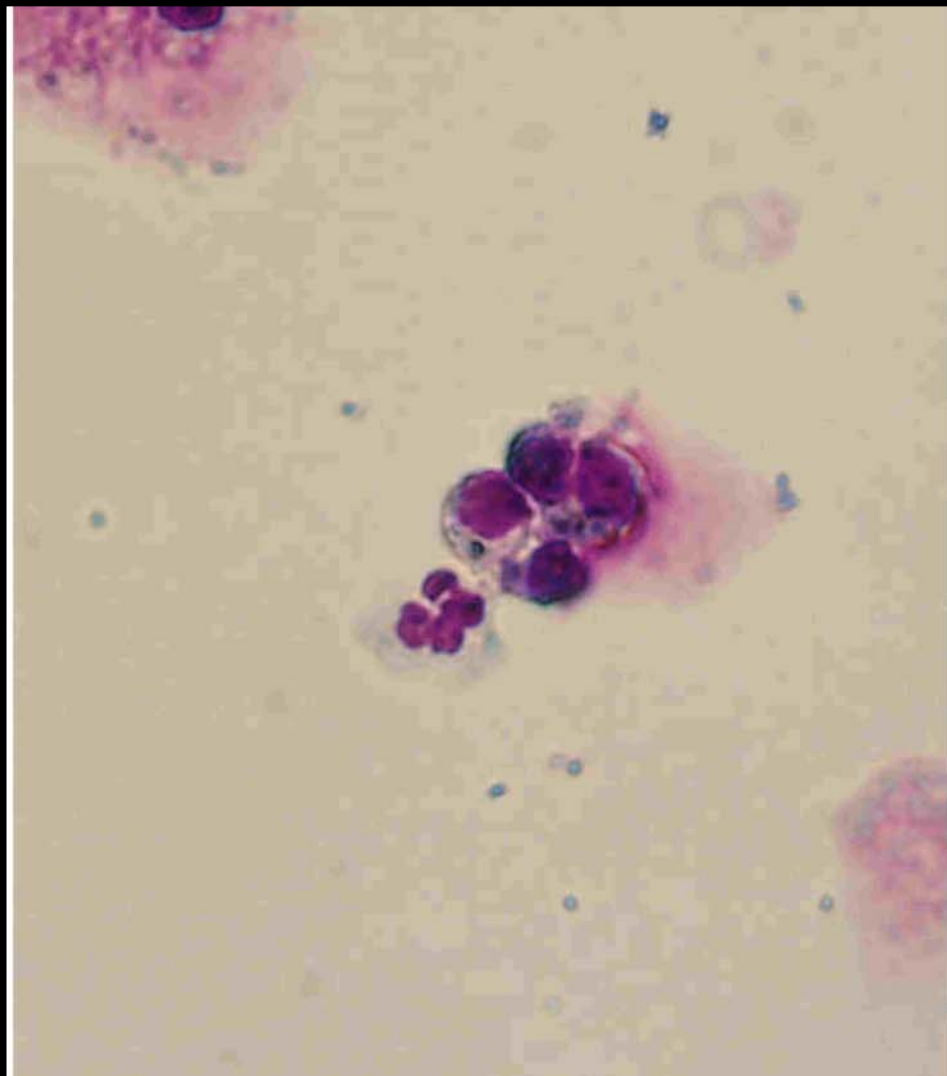
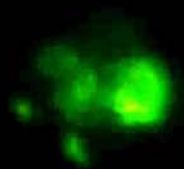


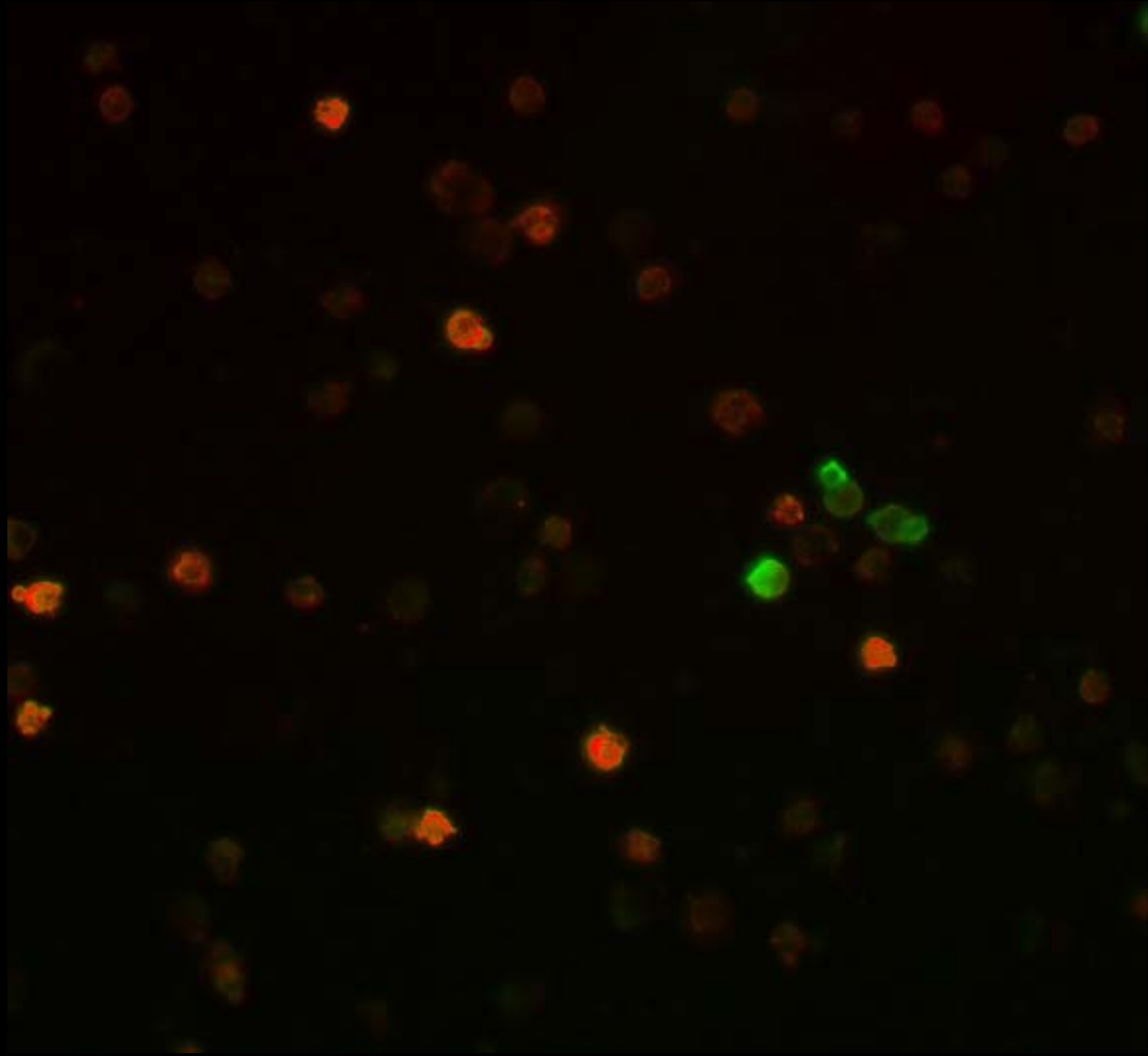
Relocalisation of positive cells

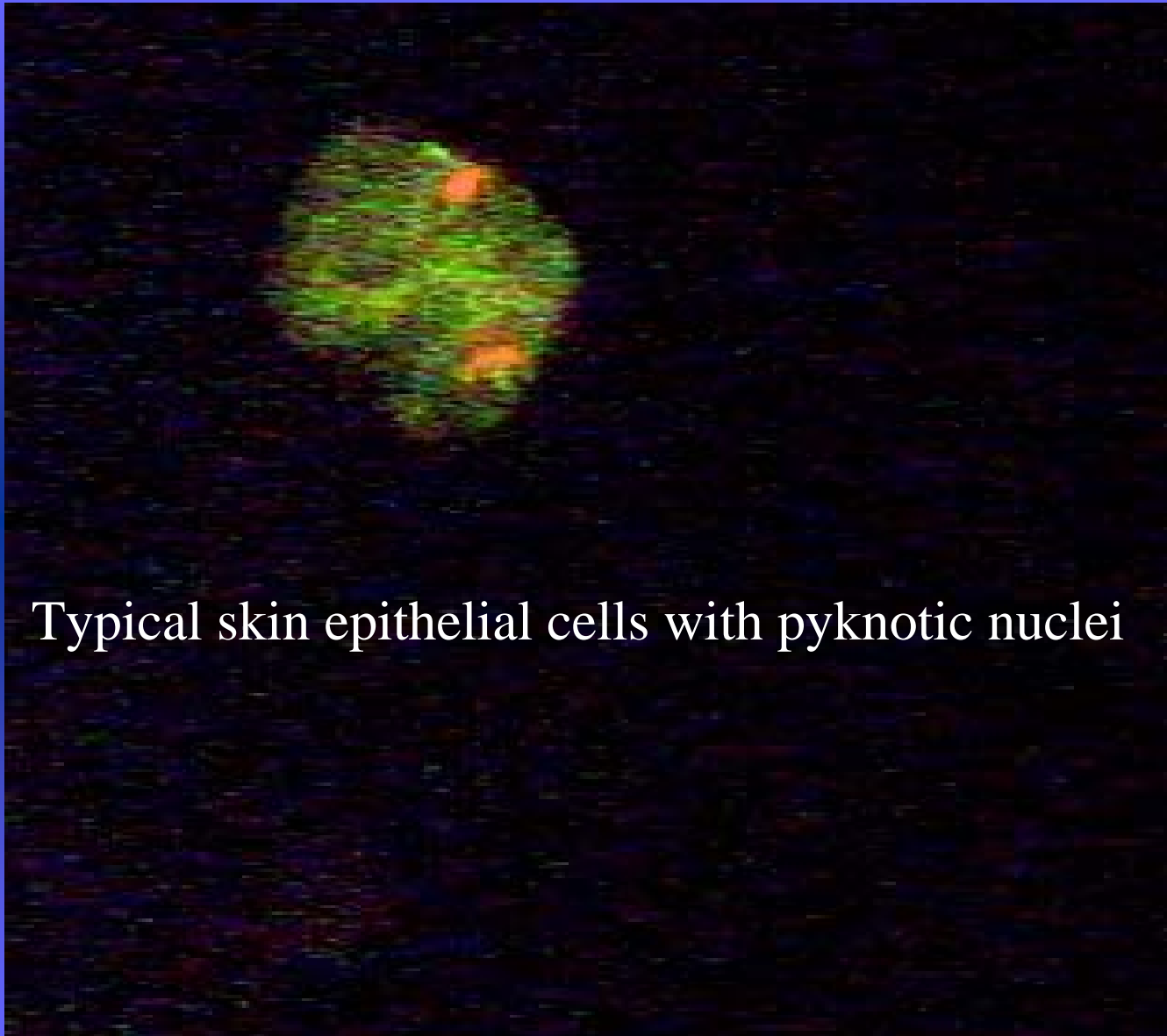


Relocalisation of positive cells



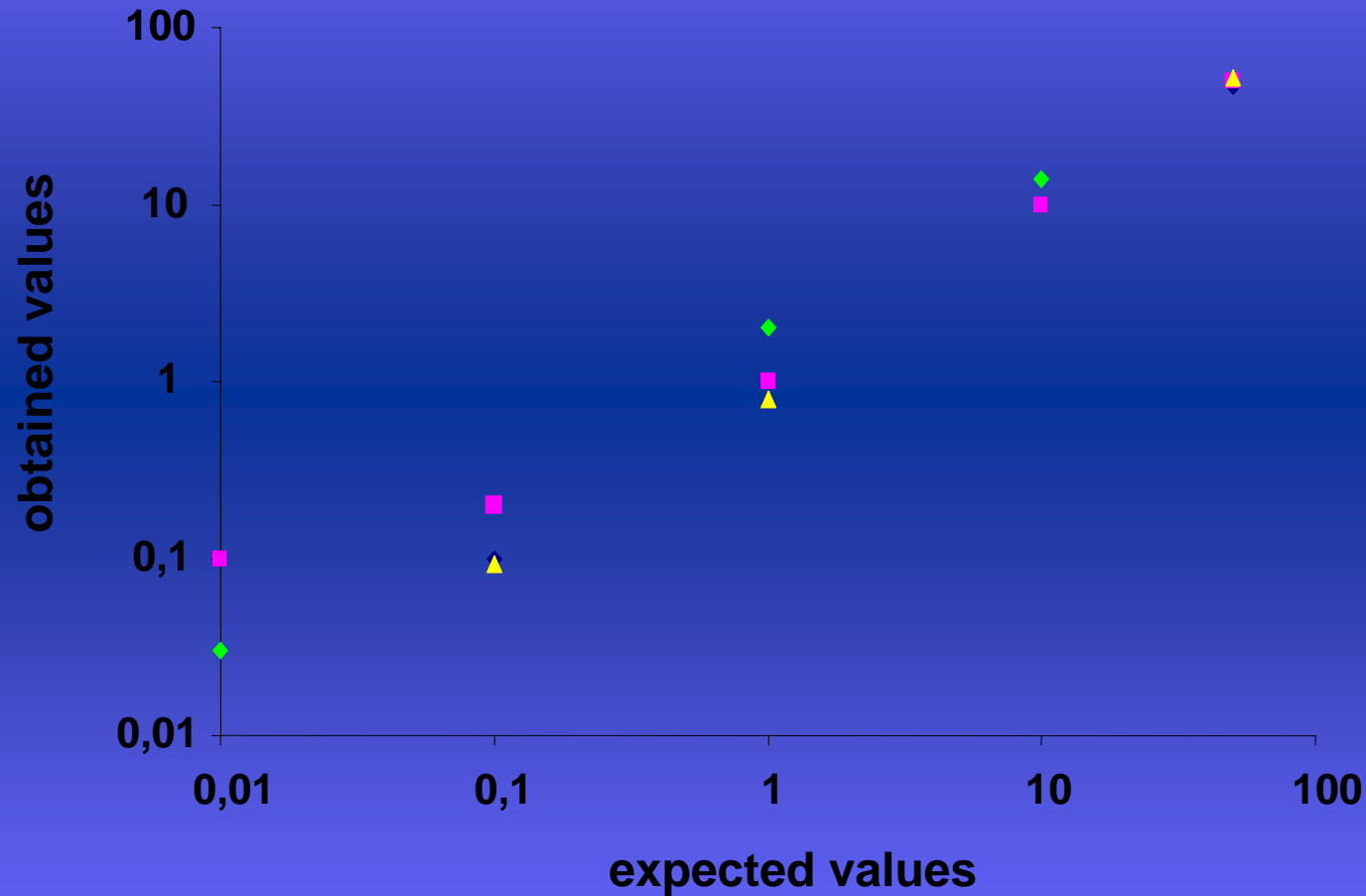






Typical skin epithelial cells with pyknotic nuclei

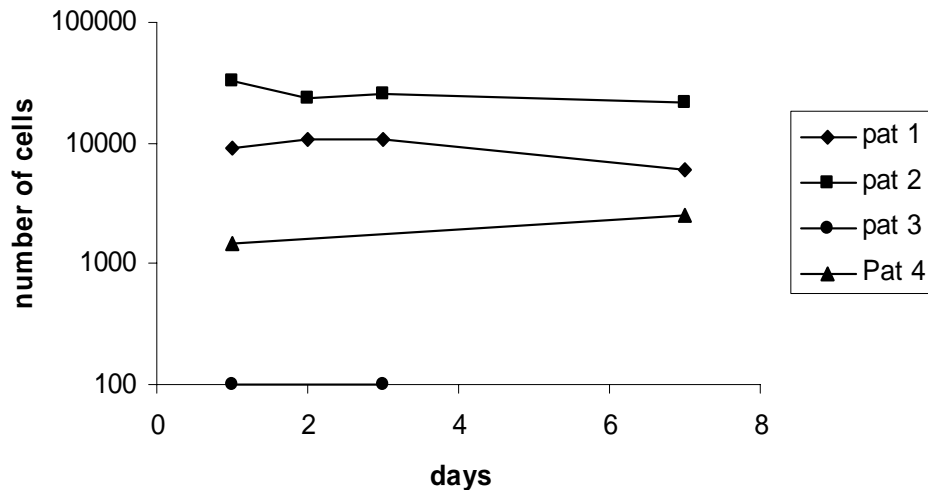
Expected vs. obtained numbers of positive cells



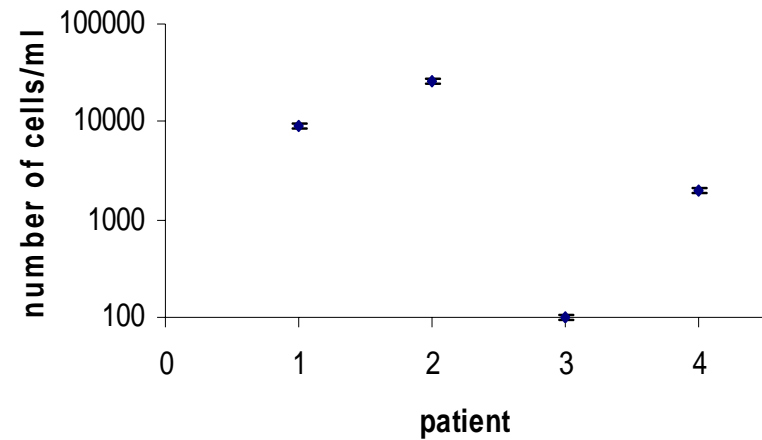
Pachmann et al 2001

Reproducibility of repeated analyses

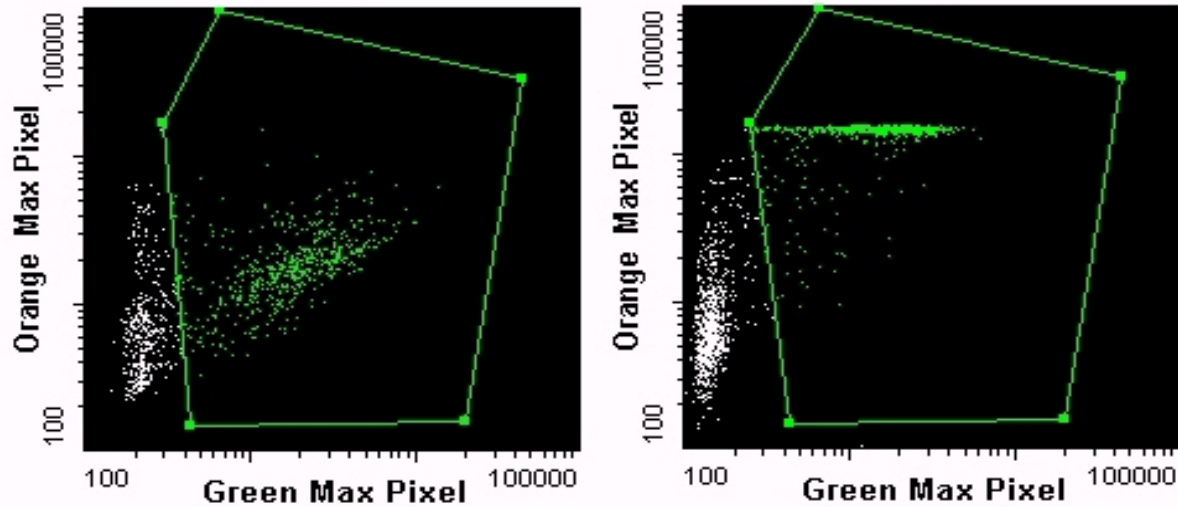
Numbers of circulating epithelial cells recovered over time



mean and standard deviation in 4 patients with repeated analysis



Comparison of cells staining with anti-HEA and anti-Cytokeratin



Pachmann et al 2005a

Specificity and sensitivity

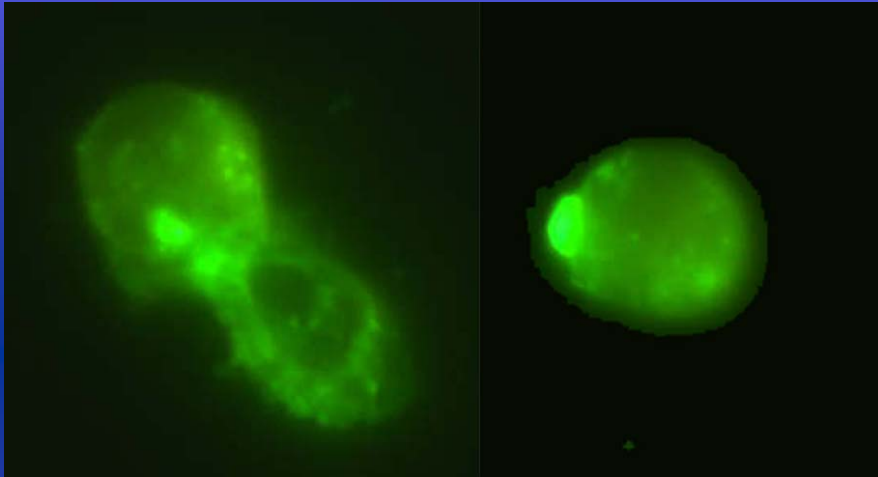
$$DS = \frac{\textit{true negatives}}{\textit{true negatives} + \textit{false positives}} = \frac{97}{100}$$

$$DE = \frac{\textit{true positives}}{\textit{true positives} + \textit{false negatives}} = \frac{92}{100}$$

Further characterization of circulating tumor cells

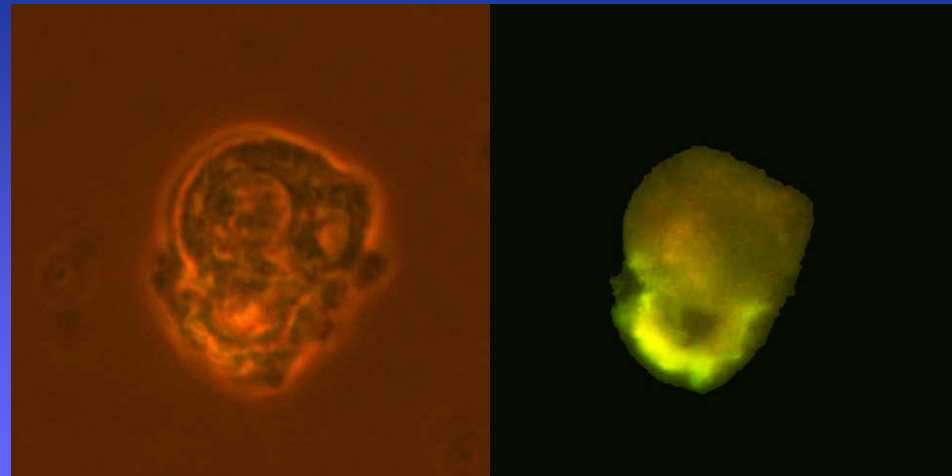
- **Tissue specific markers** **ER, PR, PSA, MeIA, PLAP**
- **Early response to therapy** **Apoptosis, fragmentation**
- **Growth behavior** **Her2/neu gene amplification**

Typical epithelial cells



staining for
HEA - receptor

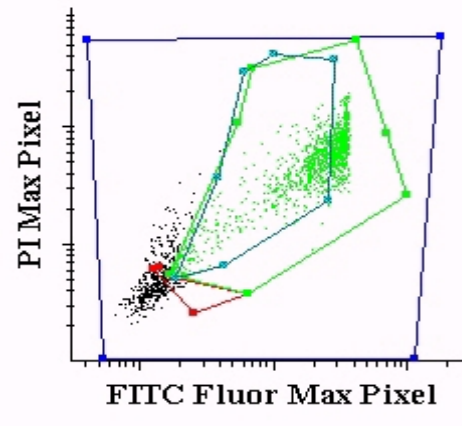
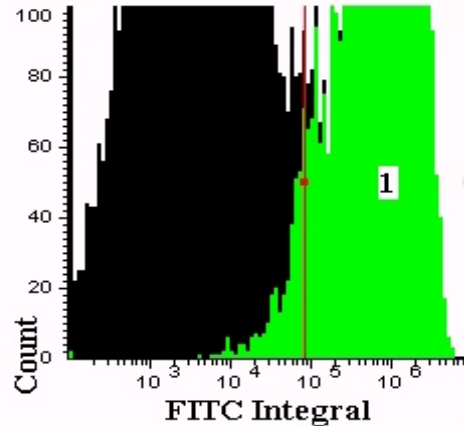
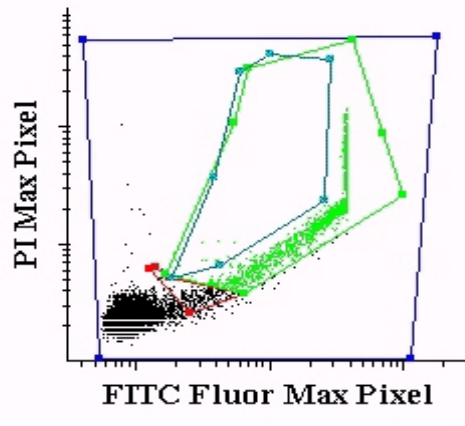
staining for the
estrogen receptor



Further characterisation of circulating tumor cells

- tissue specific antigen ER, PR, PSA, MeIA, PLAP
- response to therapy Apoptosis, Fragmentation
- Growth potential Her2/neu Genamplification

Further characterization of circulating tumor cells: apoptosis

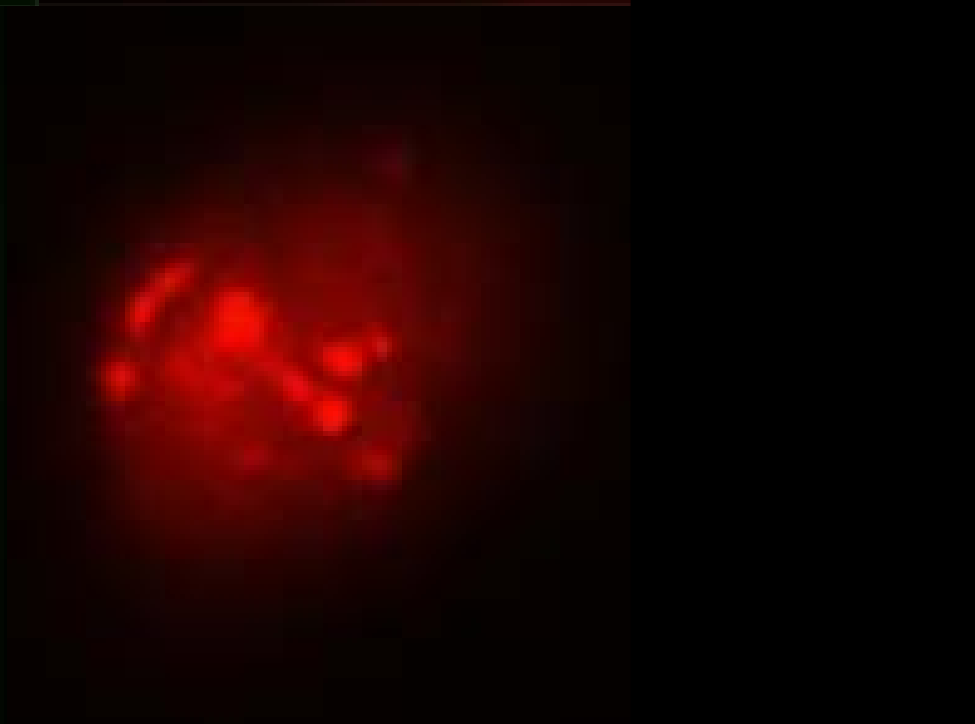


FITC Fluor Max Pixel

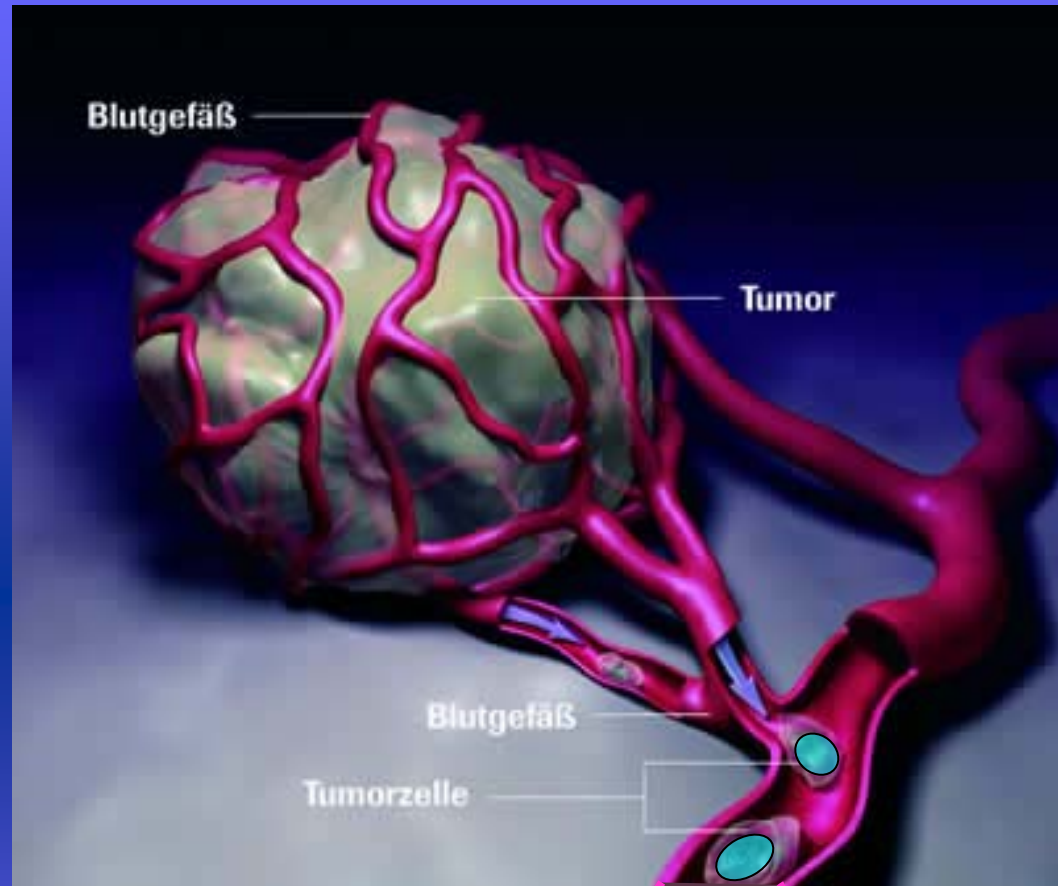
Rgn. #	Count	Pct.	Mean
1	29195	100.0%	744
2	615	2.1%	303
3	6672	22.9%	2909
4	37	0.1%	481
Total	29195		744

FITC Fluor Max Pixel

Rgn. #	Count	Pct.	Mean
1	1704	99.9%	1702
2	71	4.2%	161
3	1230	72.1%	2304
4	587	34.4%	1589
Total	1705		-2115



Most of the cancer patients do not die from the primary tumor but from the distant metastases. Once the primary tumor is formed, cells begin to dissociate from the tumor and spread to other parts of the body via the circulatory or lymph system. These disseminated tumor cells are able to form metastases.



**Distant
metastasis**

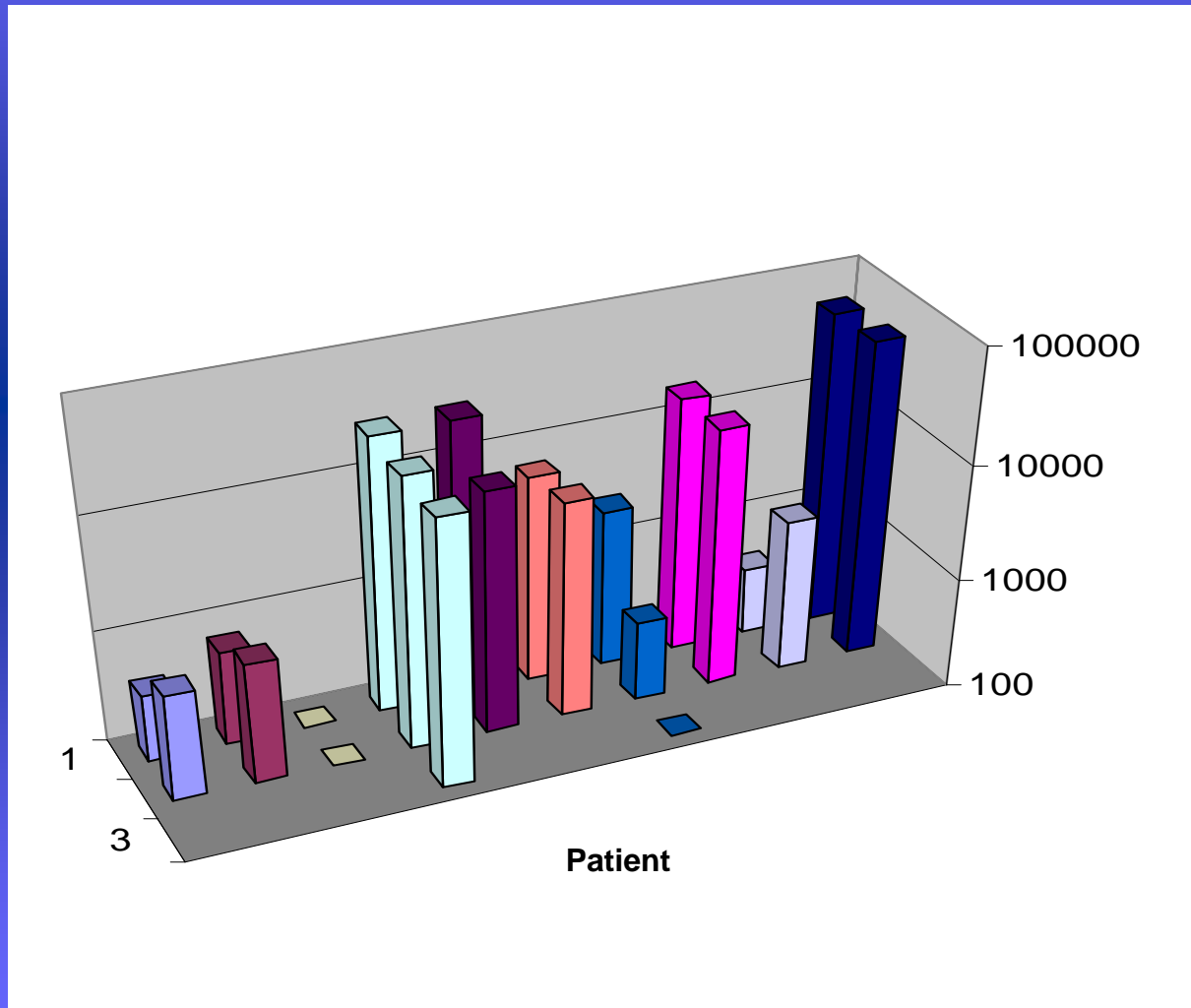
**What influences the number of
CETC in blood**

A thick, wavy, orange-to-red gradient line curves across the frame from the bottom left towards the top right. The background is a solid blue color.

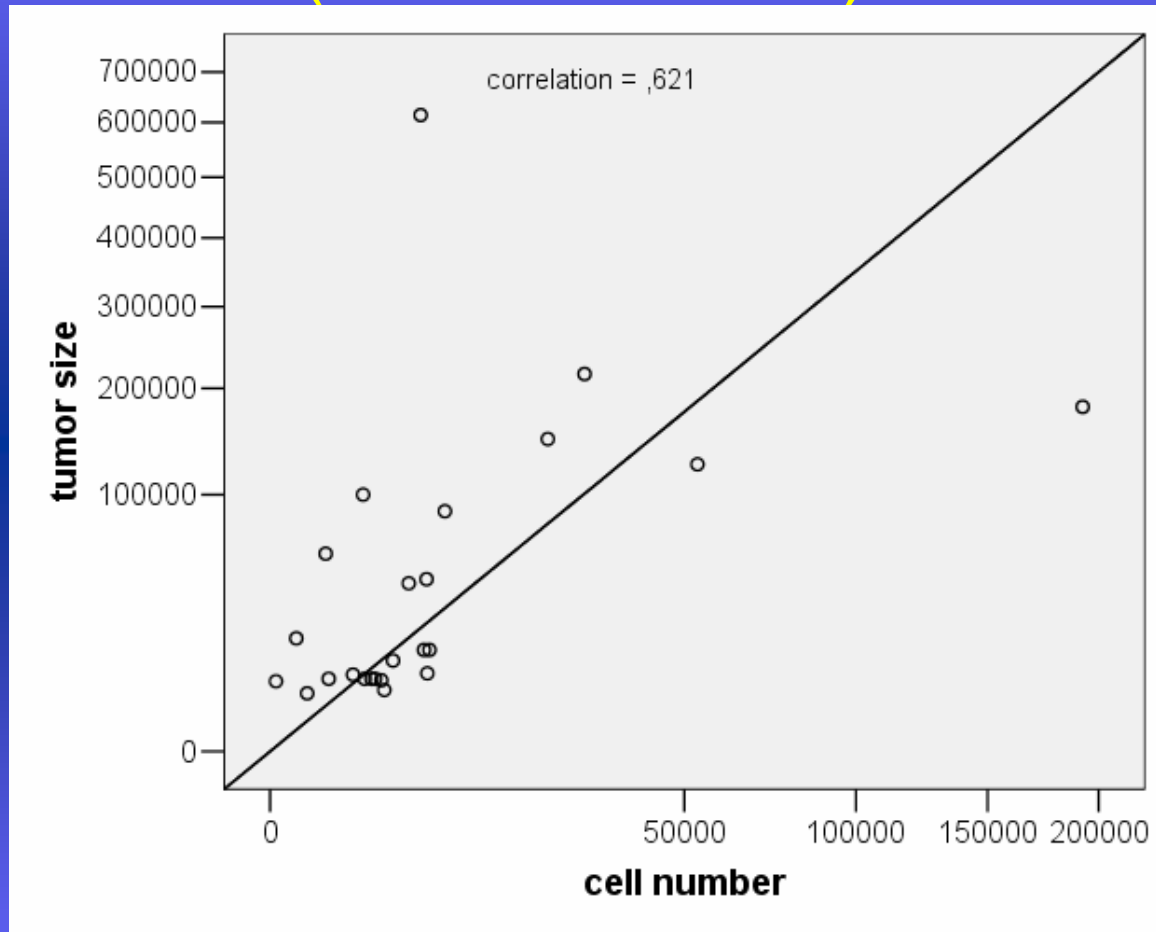
blood vessels are the „highways“

the number of cars on a highway does not
tell anything about where they come from
or where they go to

Changes in numbers of CETC before and after core biopsy



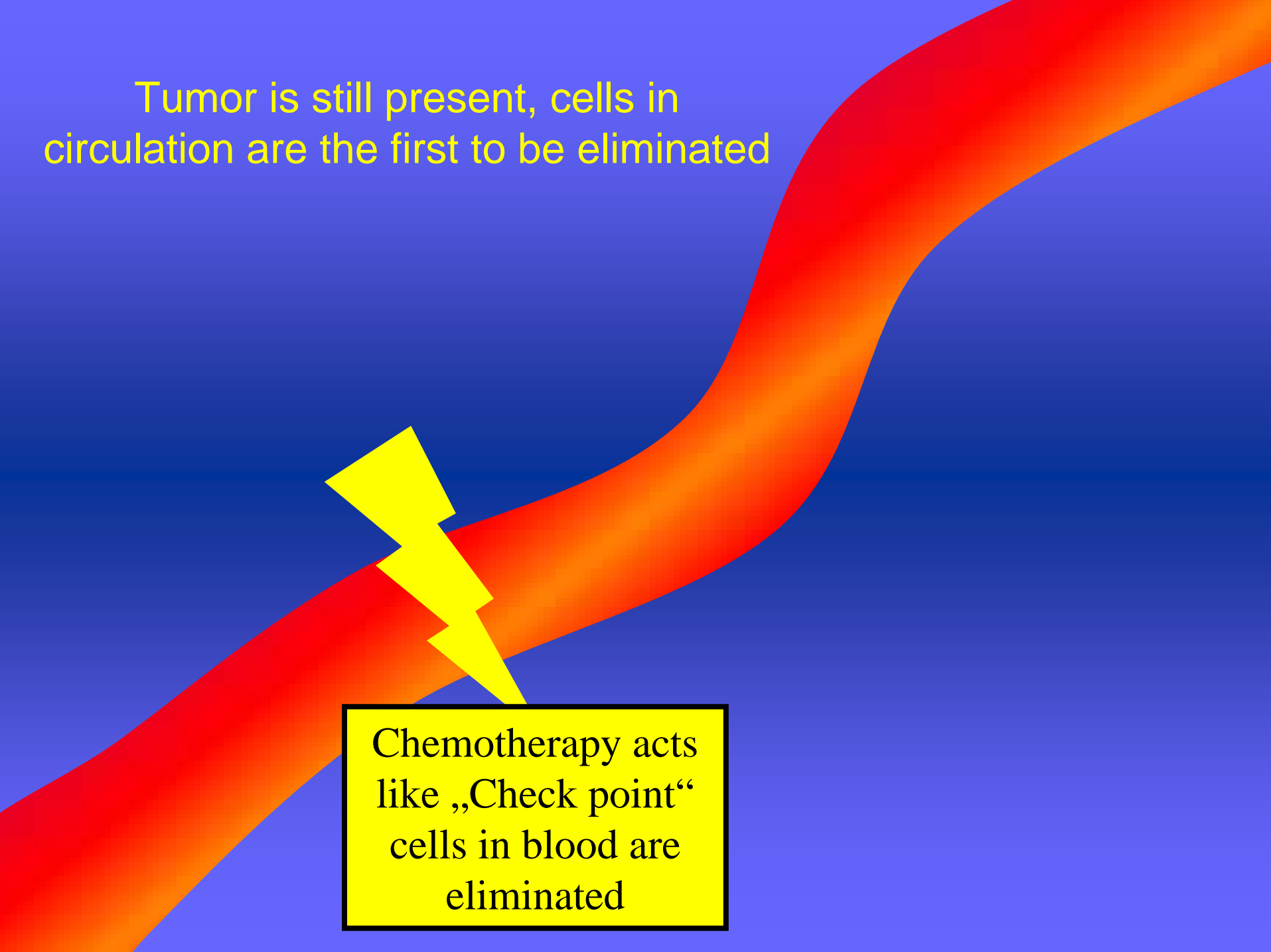
Seeding of cells during tumor growth (breast cancer)



the number of CETC increases with tumor size

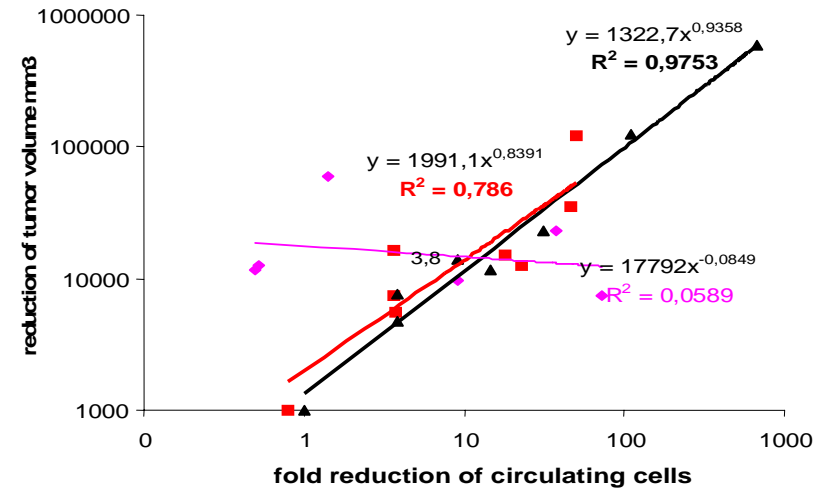
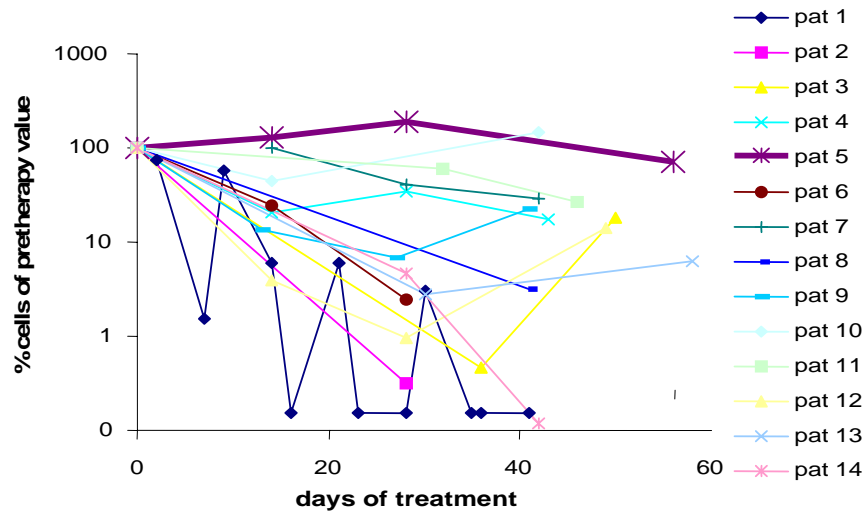
Influence of systemic chemotherapy
on the number of CETC in blood

Tumor is still present, cells in circulation are the first to be eliminated



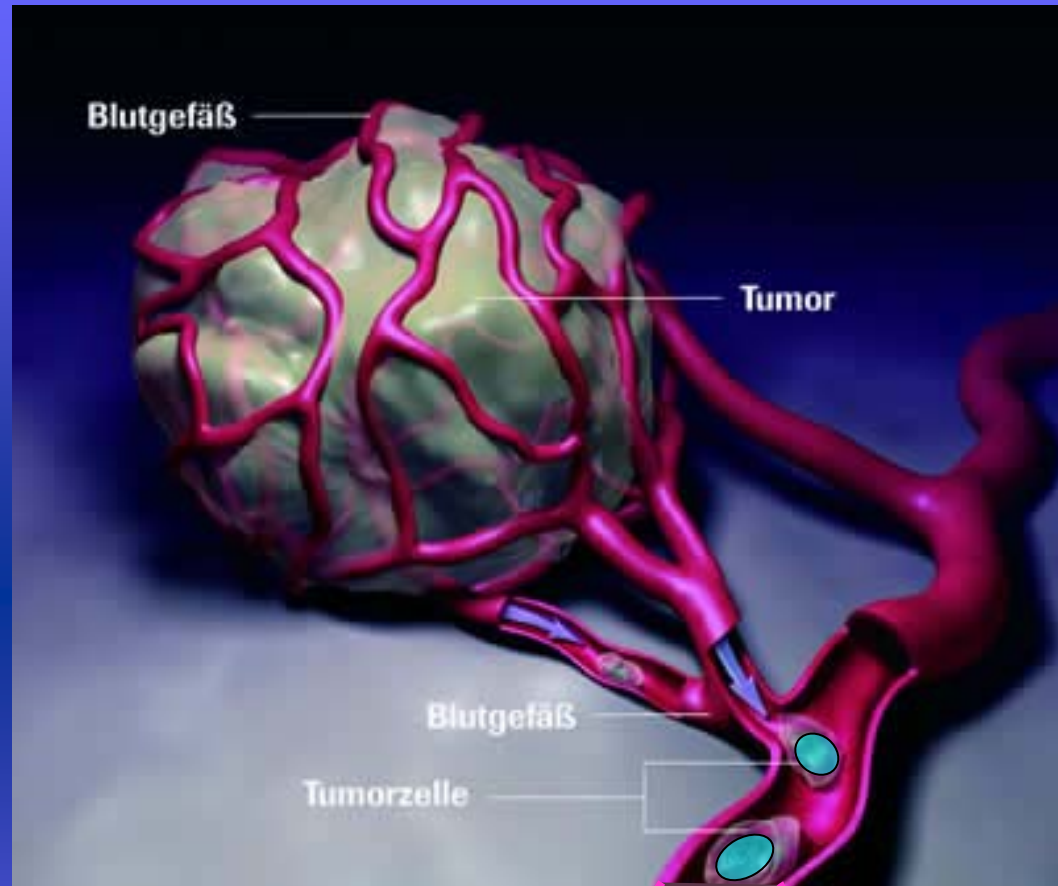
Chemotherapy acts like „Check point“ cells in blood are eliminated

Reduction in numbers of circulating cells vs. reduction in tumor size

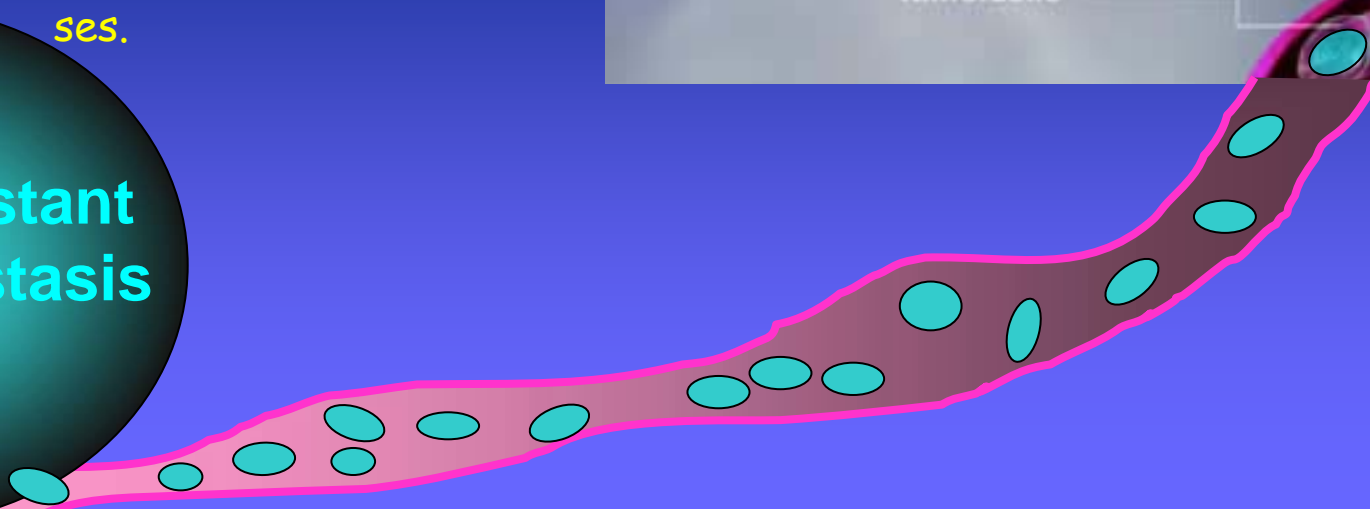


Pachmann et al 2005b

The primary tumor forms a tight mass. When the tumor shrinks during primary chemotherapy cells can again dissociate from the tumor and spread to other parts of the body via the circulatory or lymph system. These disseminated tumor cells are able to form metastases.



Distant metastasis



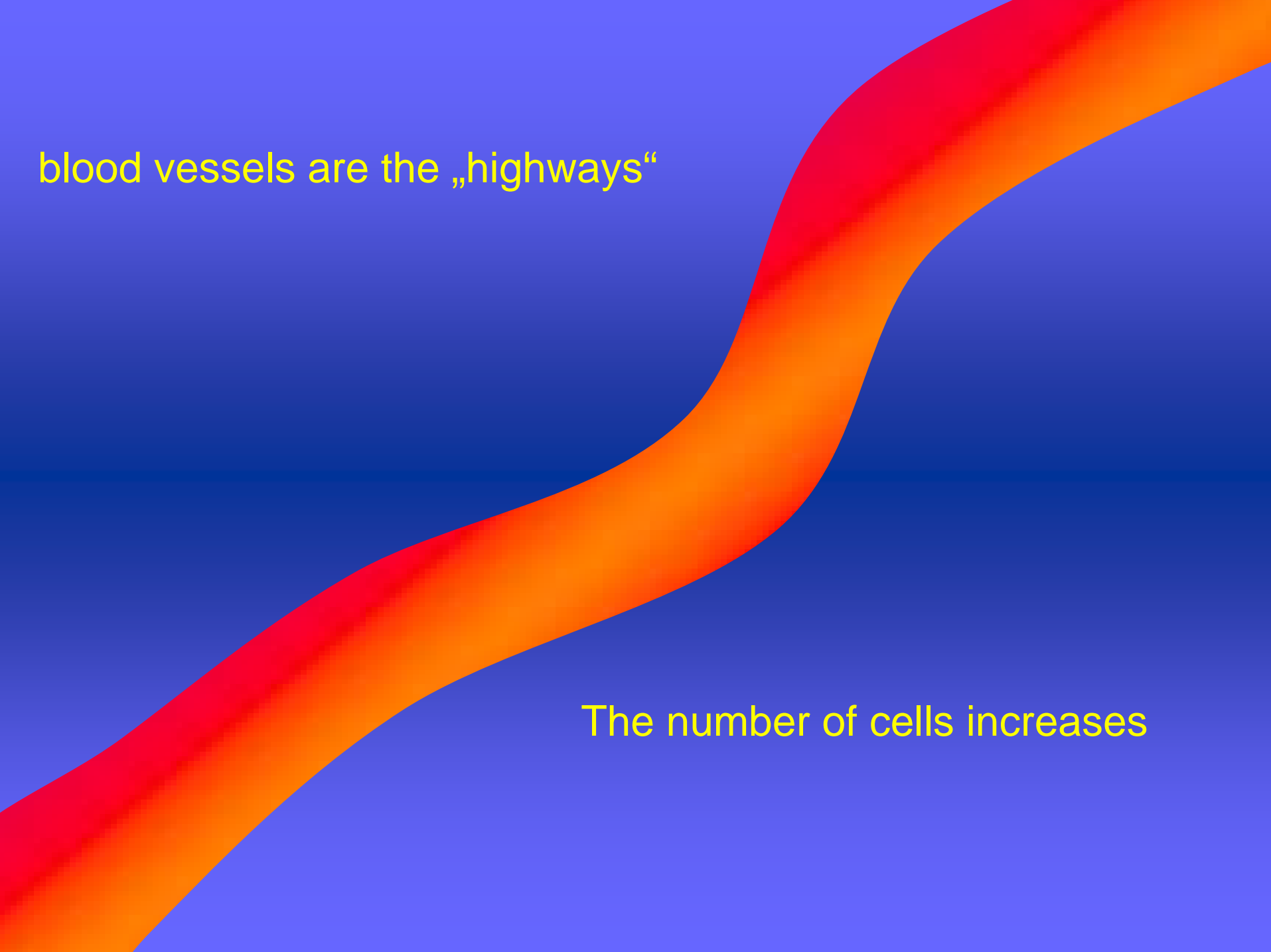
The primary tumor can be compared to an arena packed with visitors



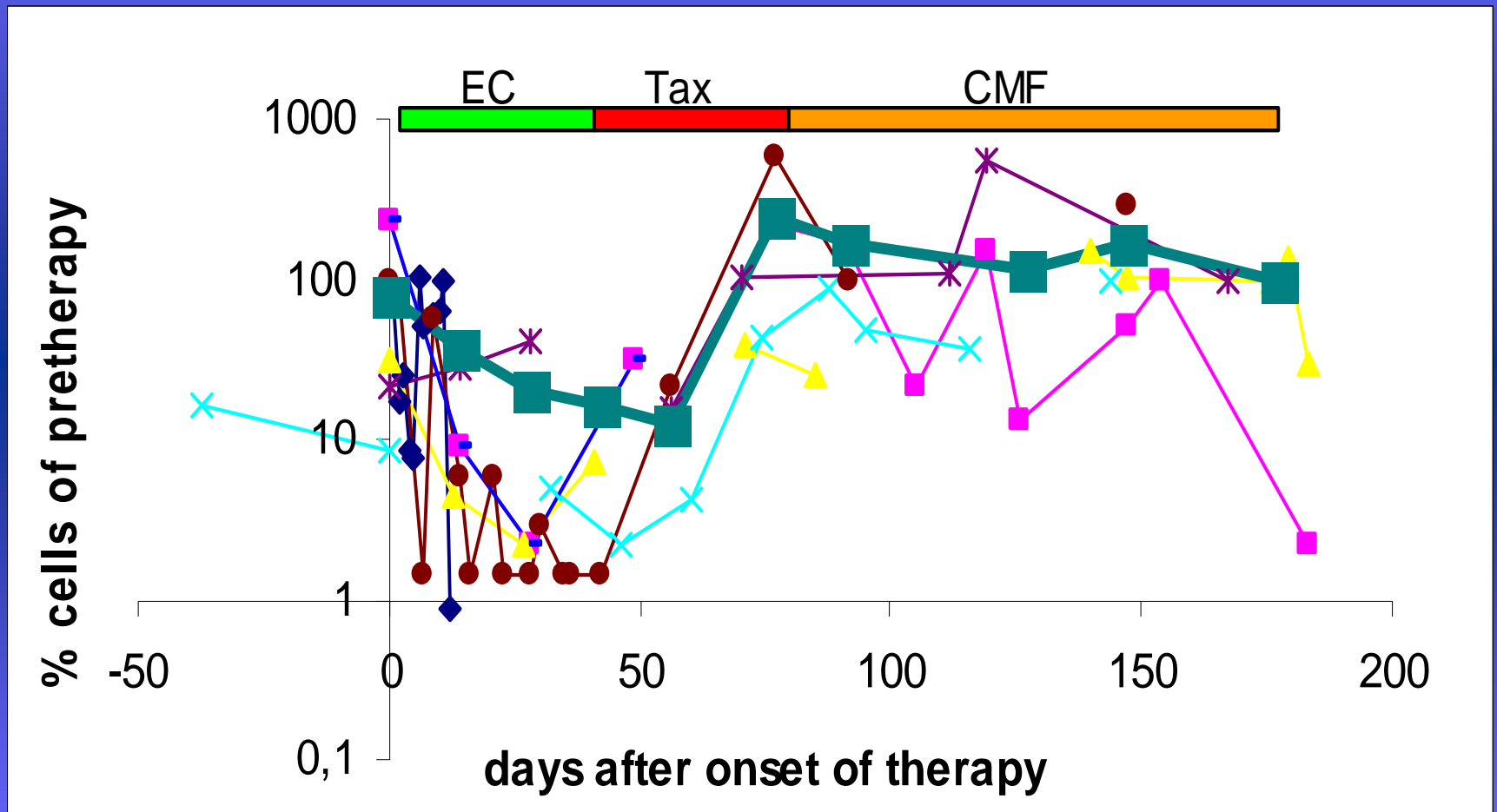
When the game is over (the tumor shrinks) there will be lots of cars on the highway (lots of cells in the blood).

blood vessels are the „highways“

The number of cells increases

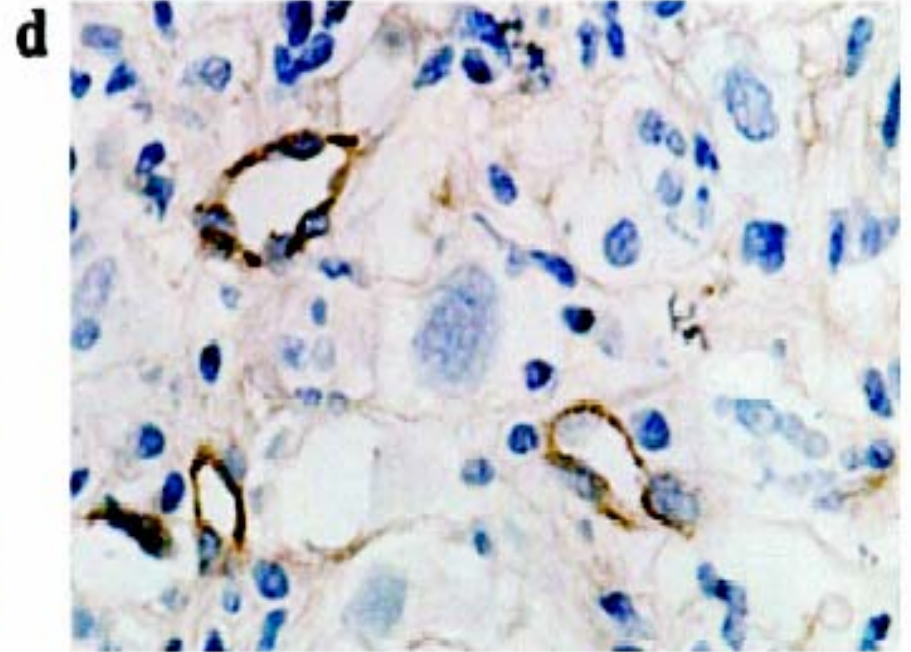
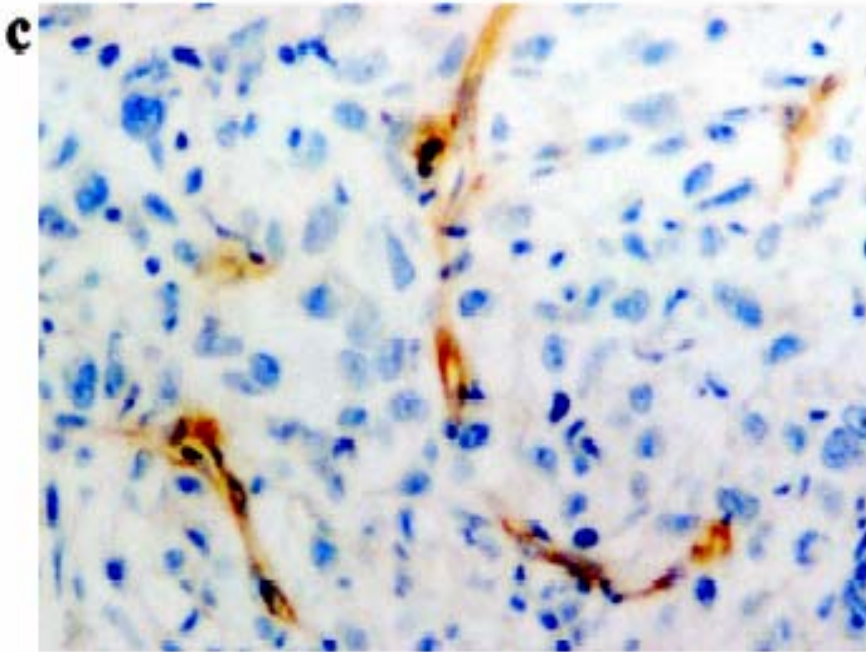


Influence of neoadjuvant therapy on circulating cells (breast cancer)

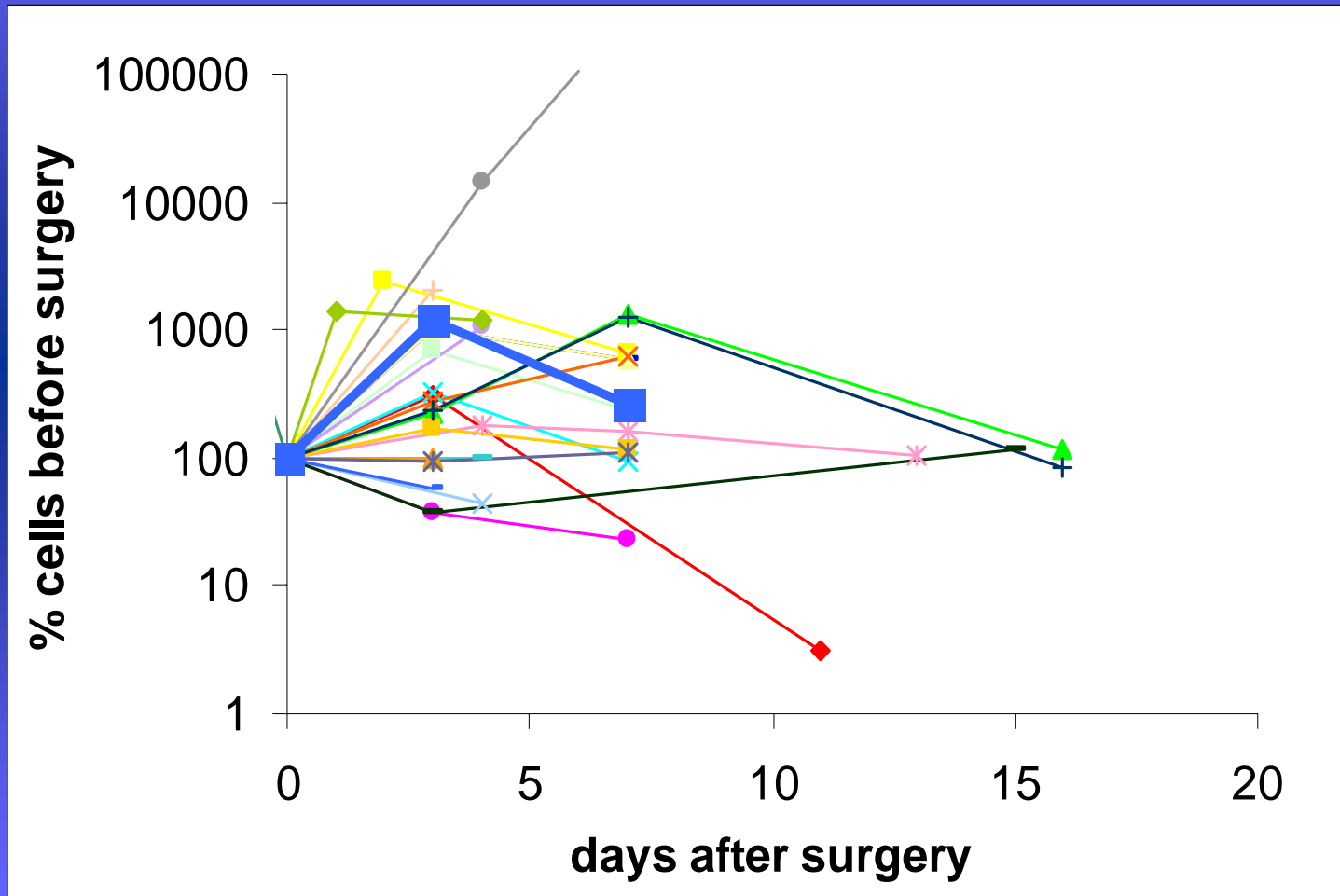


An increase in circulating epithelial cell numbers occurs in 50/50 patients

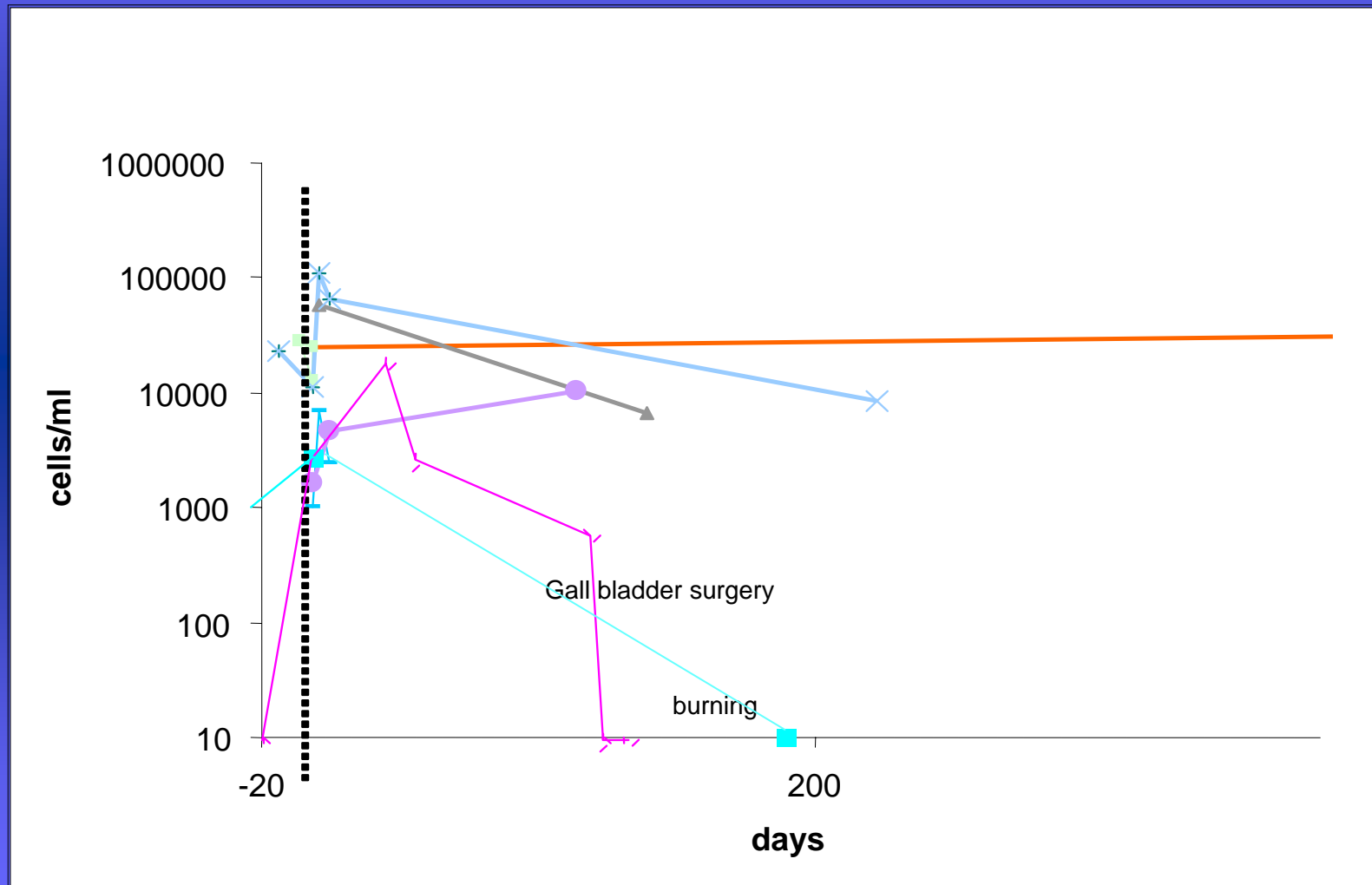
Taxol: Influence on cell density and intratumoral pressure (Jain 2003)



Influence of surgery on circulating cells in breast cancer



Remaining cells after surgery of breast cancer (Pachmann 2005c)

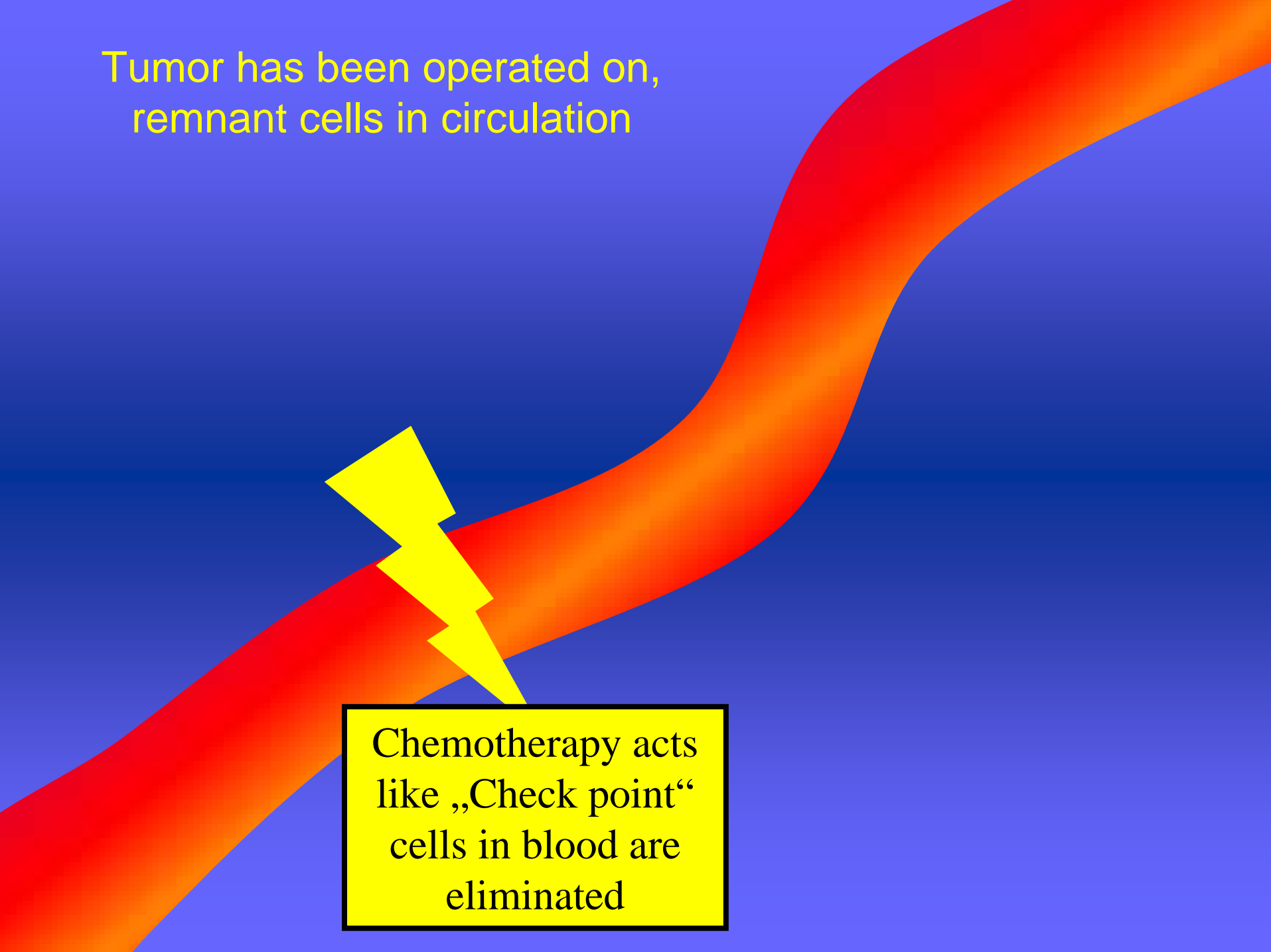


CETC can remain for long times in the blood. The development of metastases depends on additional properties of these cells.

According to current international guidelines, approximately 80% of all patients with breast carcinoma will receive systemic adjuvant therapy.

Minimal residual disease, which is believed to be the origin of subsequent disease recurrence and, thus, is the target for adjuvant treatment, cannot be detected and monitored by conventional screening methods

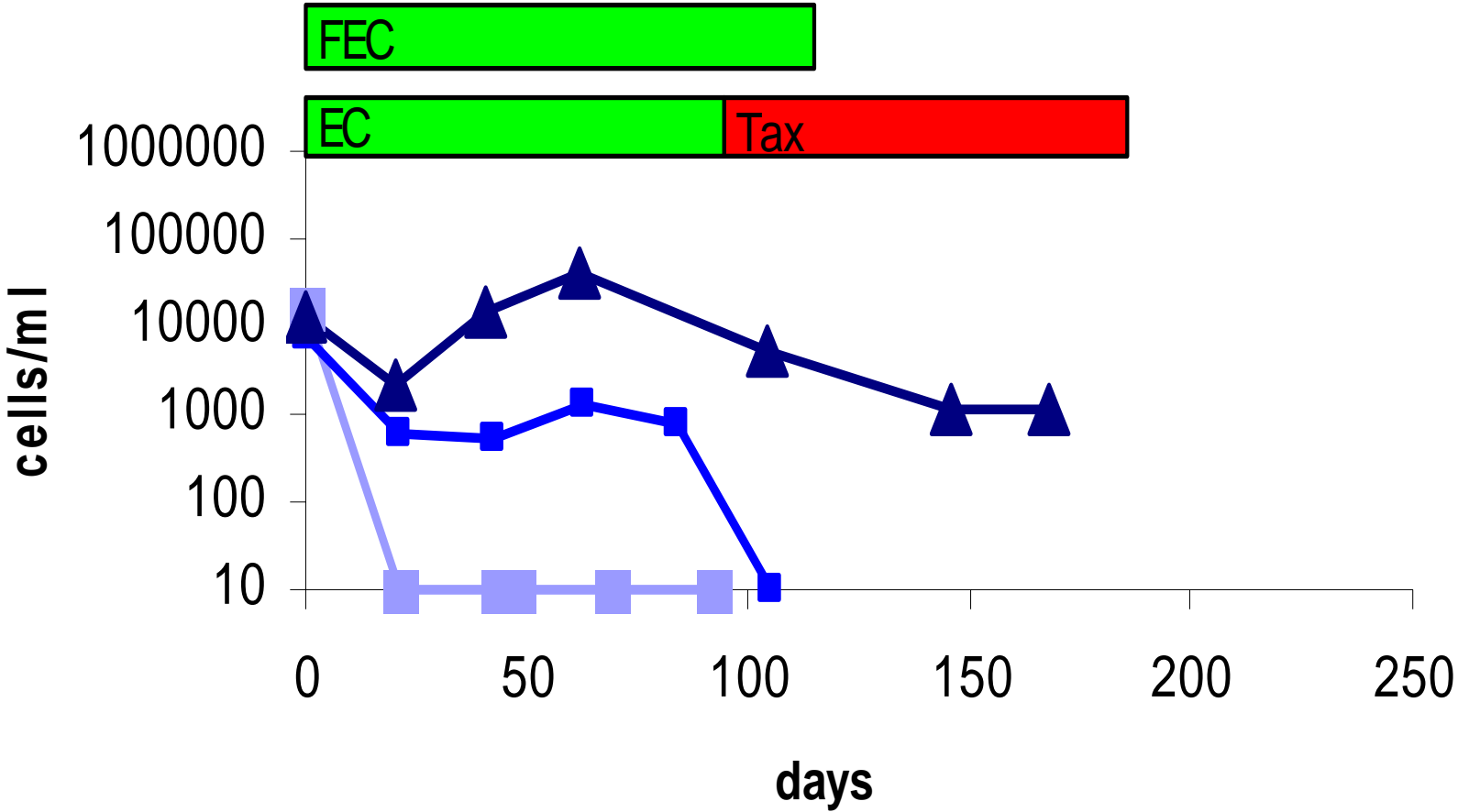
Tumor has been operated on,
remnant cells in circulation



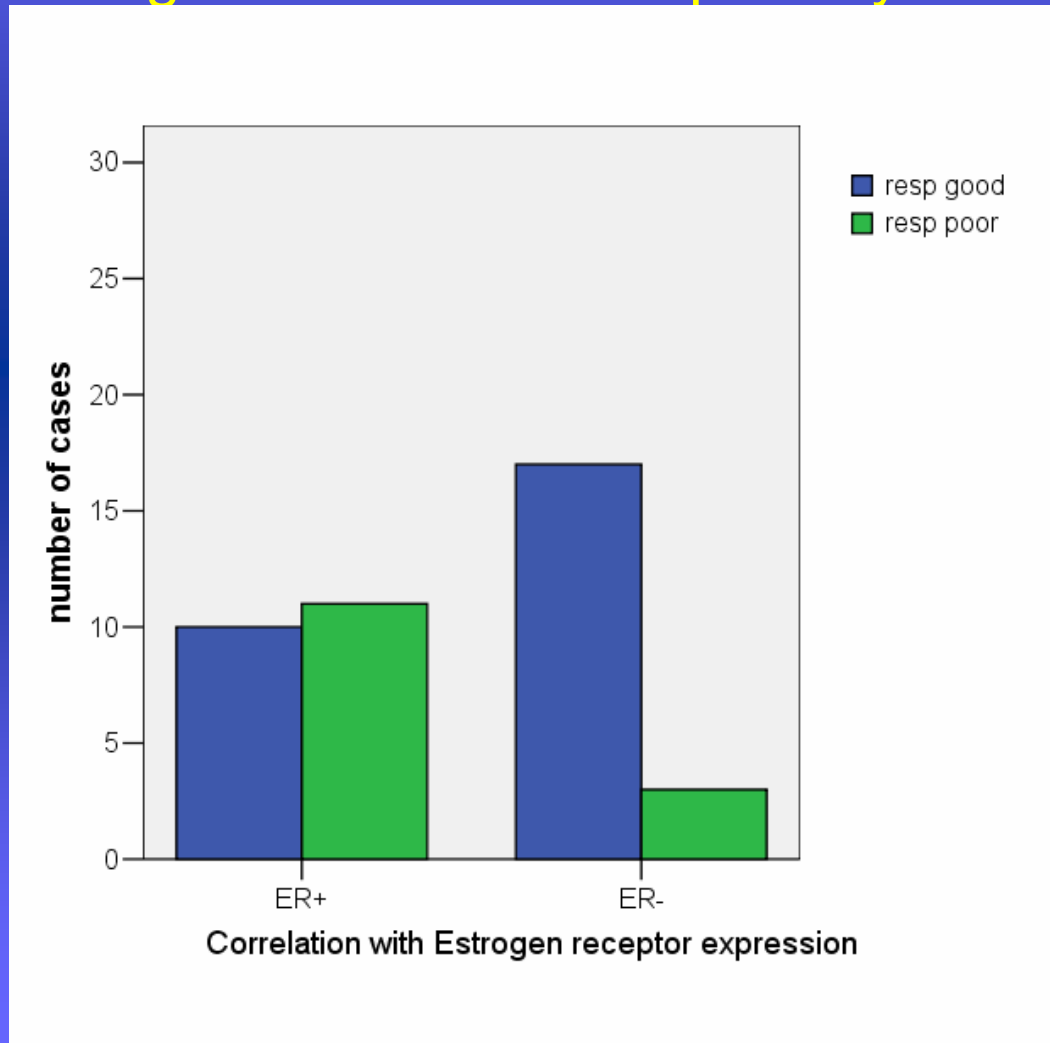
Chemotherapy acts
like „Check point“
cells in blood are
eliminated

**Adjuvant therapy can reduce
CETC in blood**

decrease in cell numbers more than tenfold

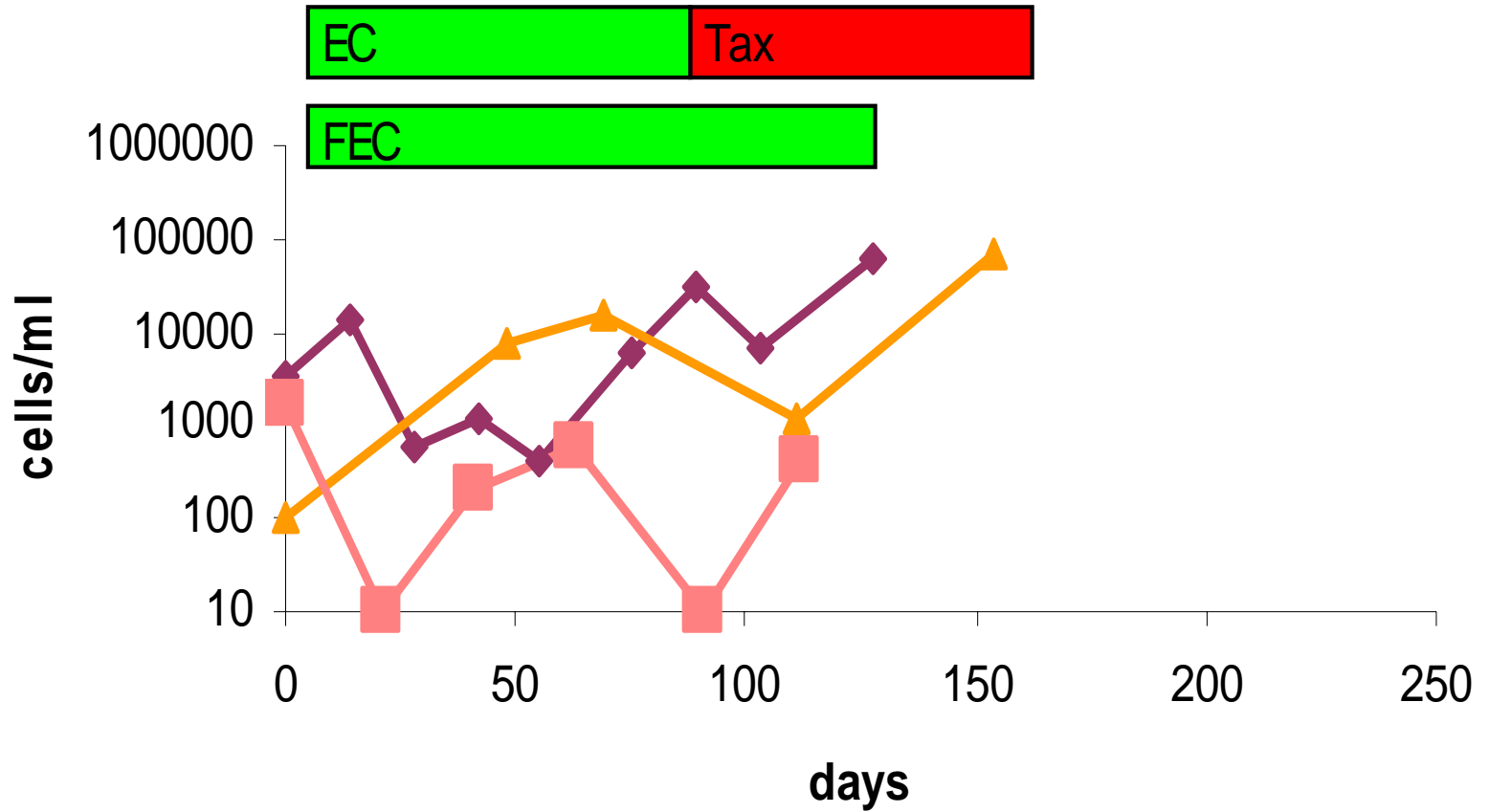


CETC from ER- tumors respond better to therapy than those from ER+ tumors (confirming known data from primary tumors)

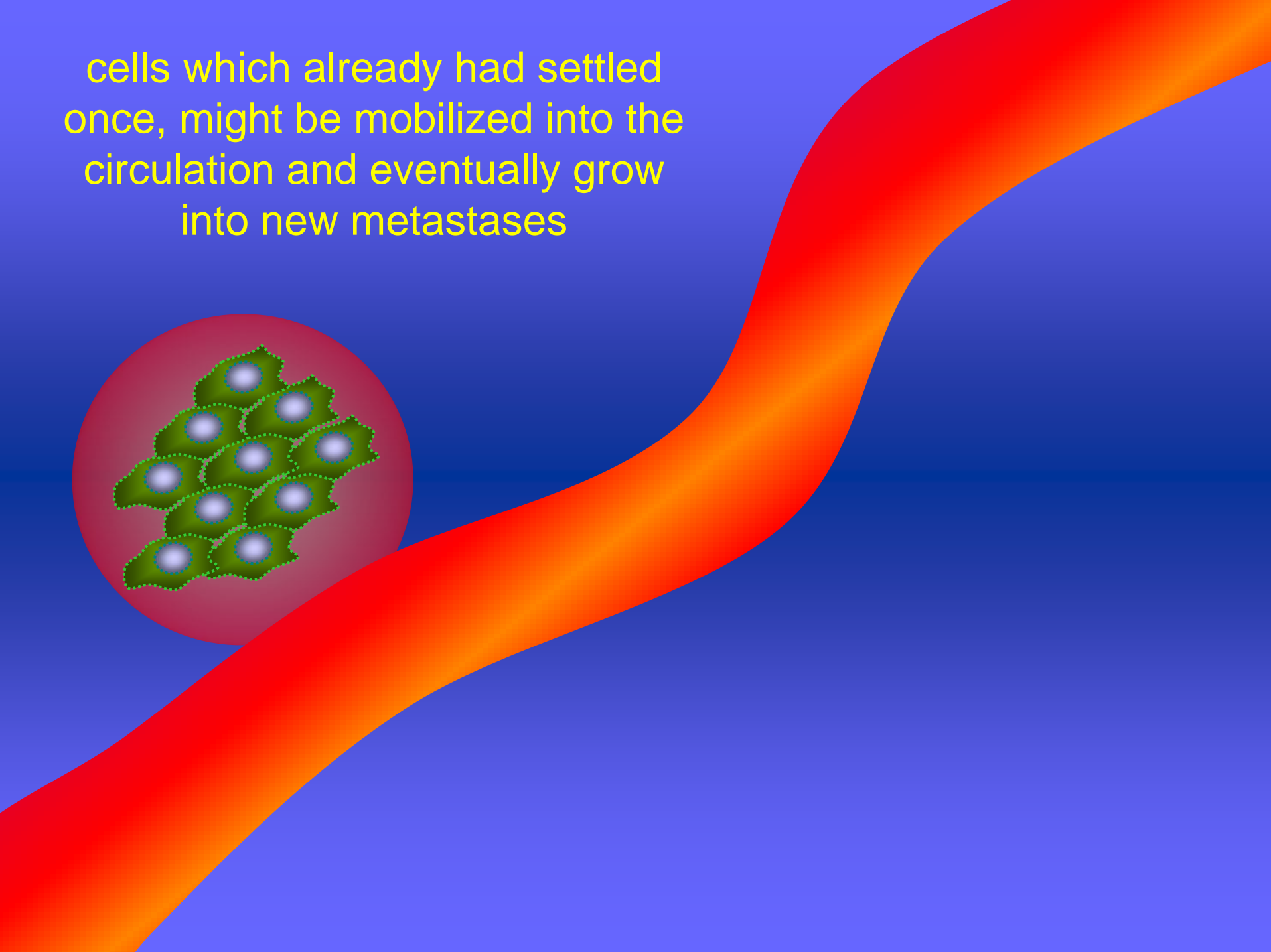
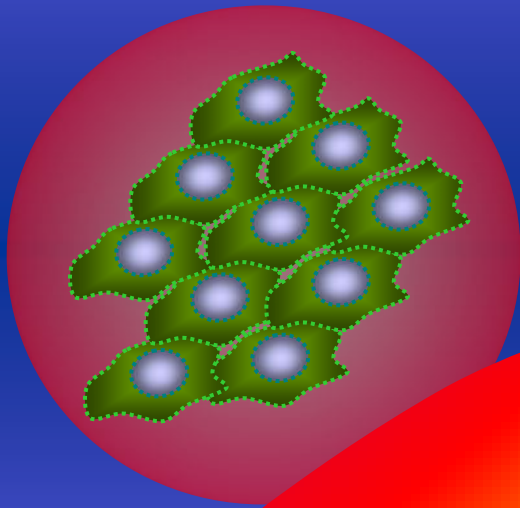


In some patients CETC do not respond to adjuvant therapy or even re-increase in blood

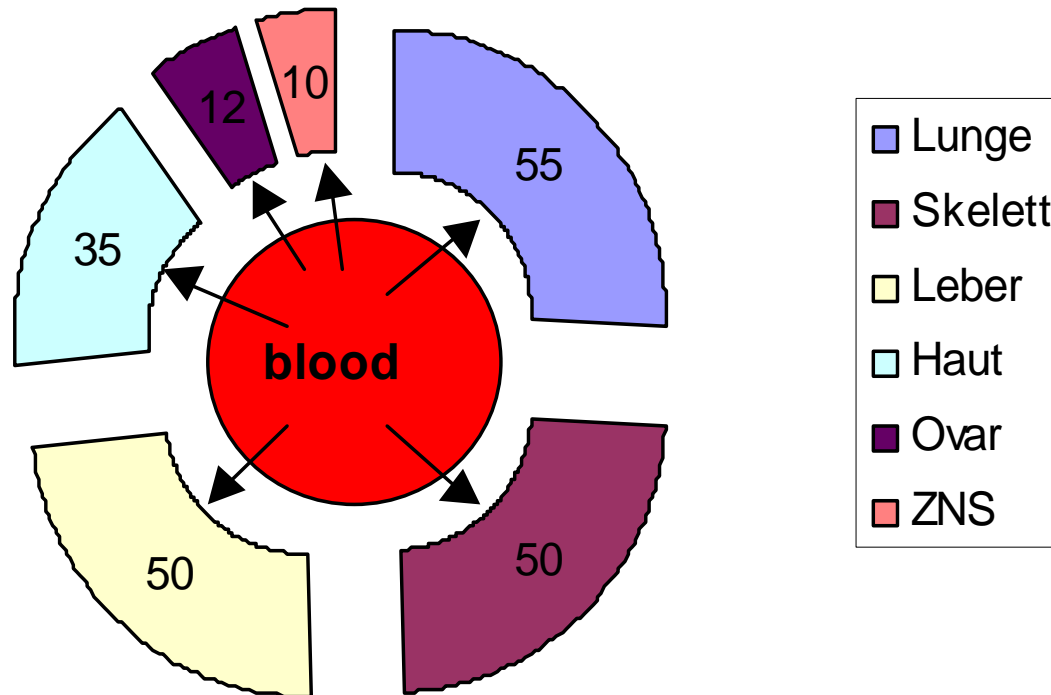
increase in cell numbers more than tenfold



cells which already had settled once, might be mobilized into the circulation and eventually grow into new metastases

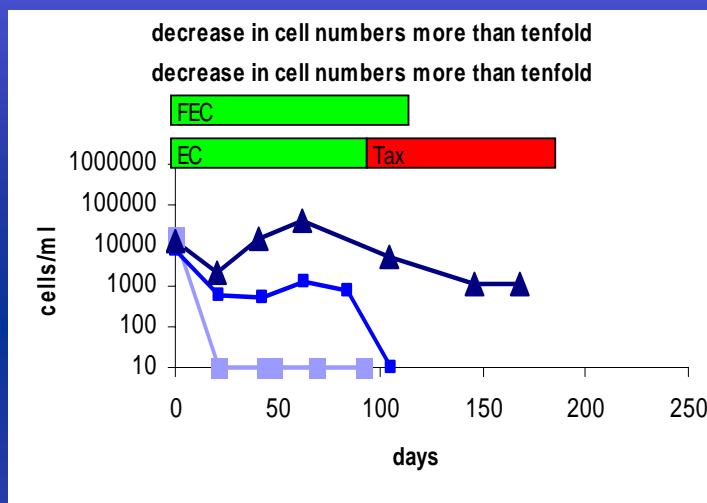


% blood borne Metastases

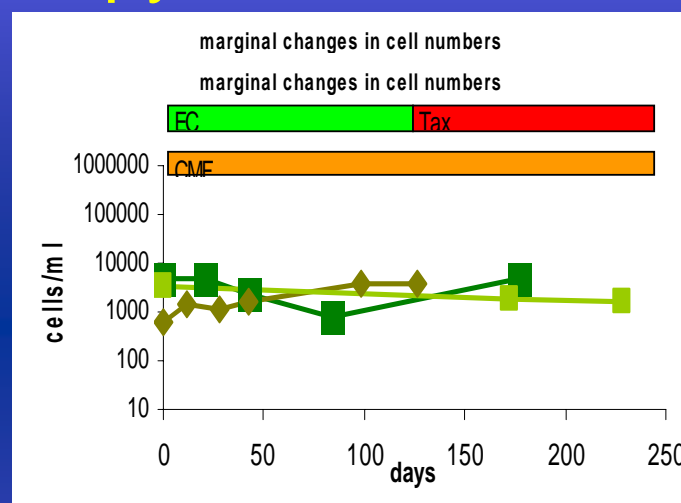


In some patients adjuvant chemotherapy reduces the numbers of CETC, in some patients CETC do hardly respond to therapy and in some patients numbers of CETC even increase during therapy

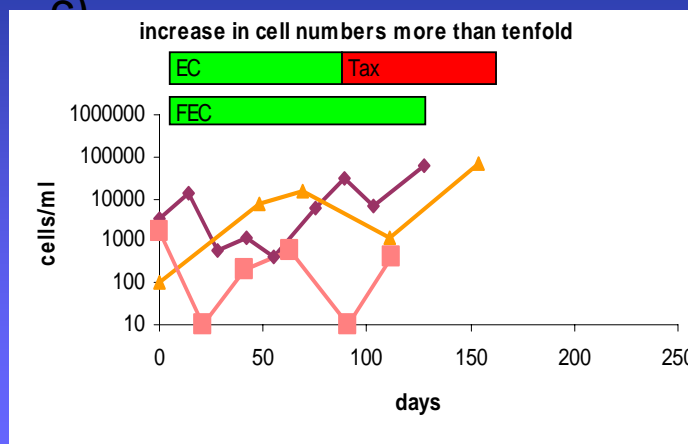
a)



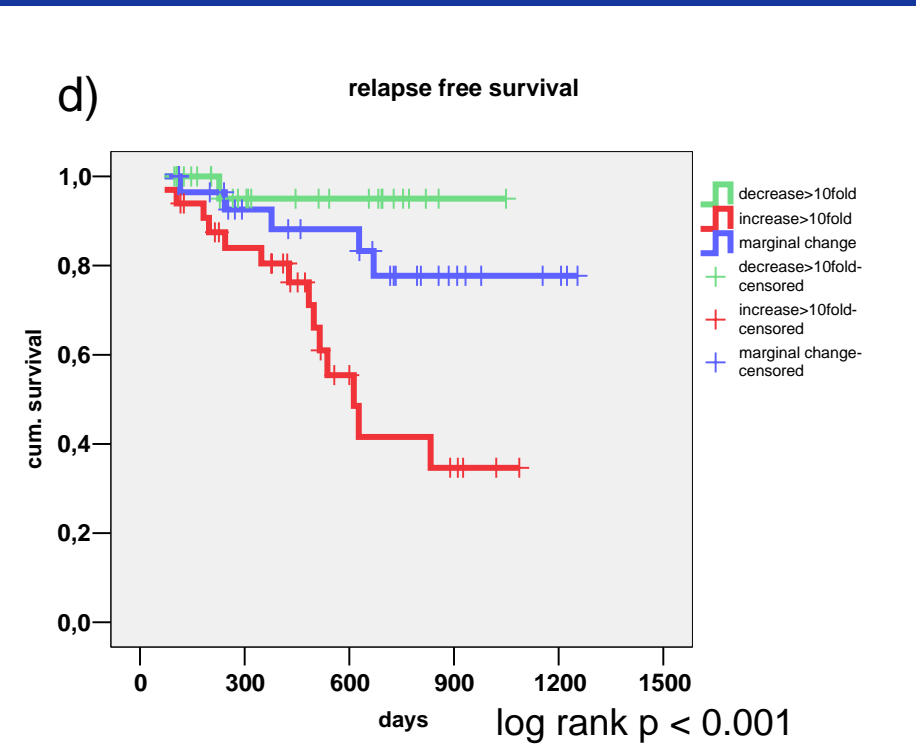
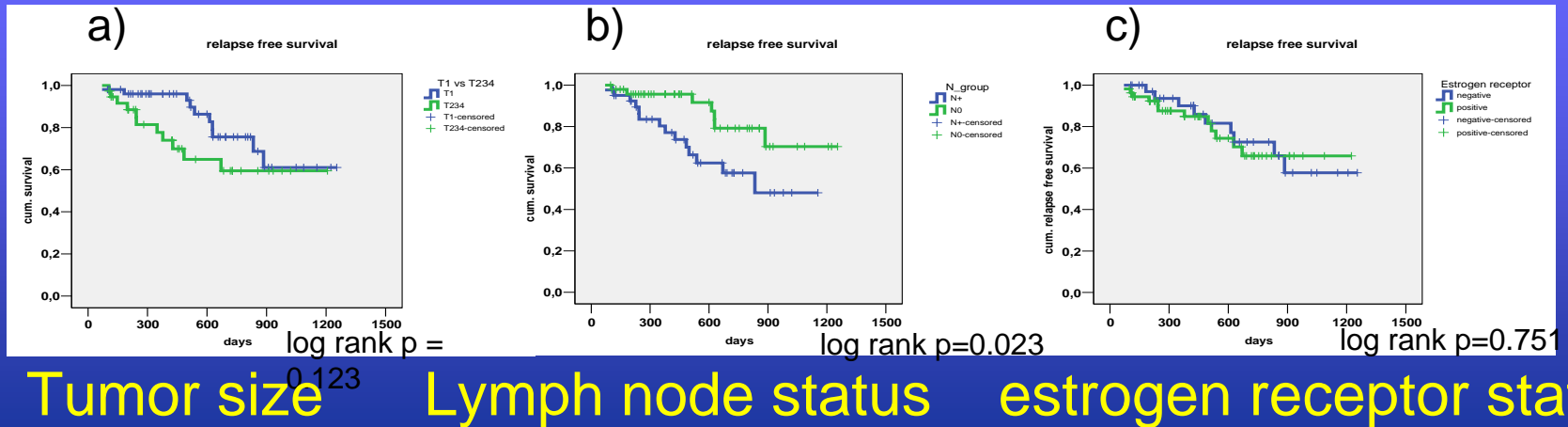
b)



c)



prognostic relevance



days	0	300	600	900	1200
Pat. at risk	28	16	10	1	0
relapses	0	1	1	1	1

Pat. at risk	30	21	18	7	3
relapses	0	2	3	5	5

Pat. at risk	33	24	9	4	0
relapses	0	5	11	14	14

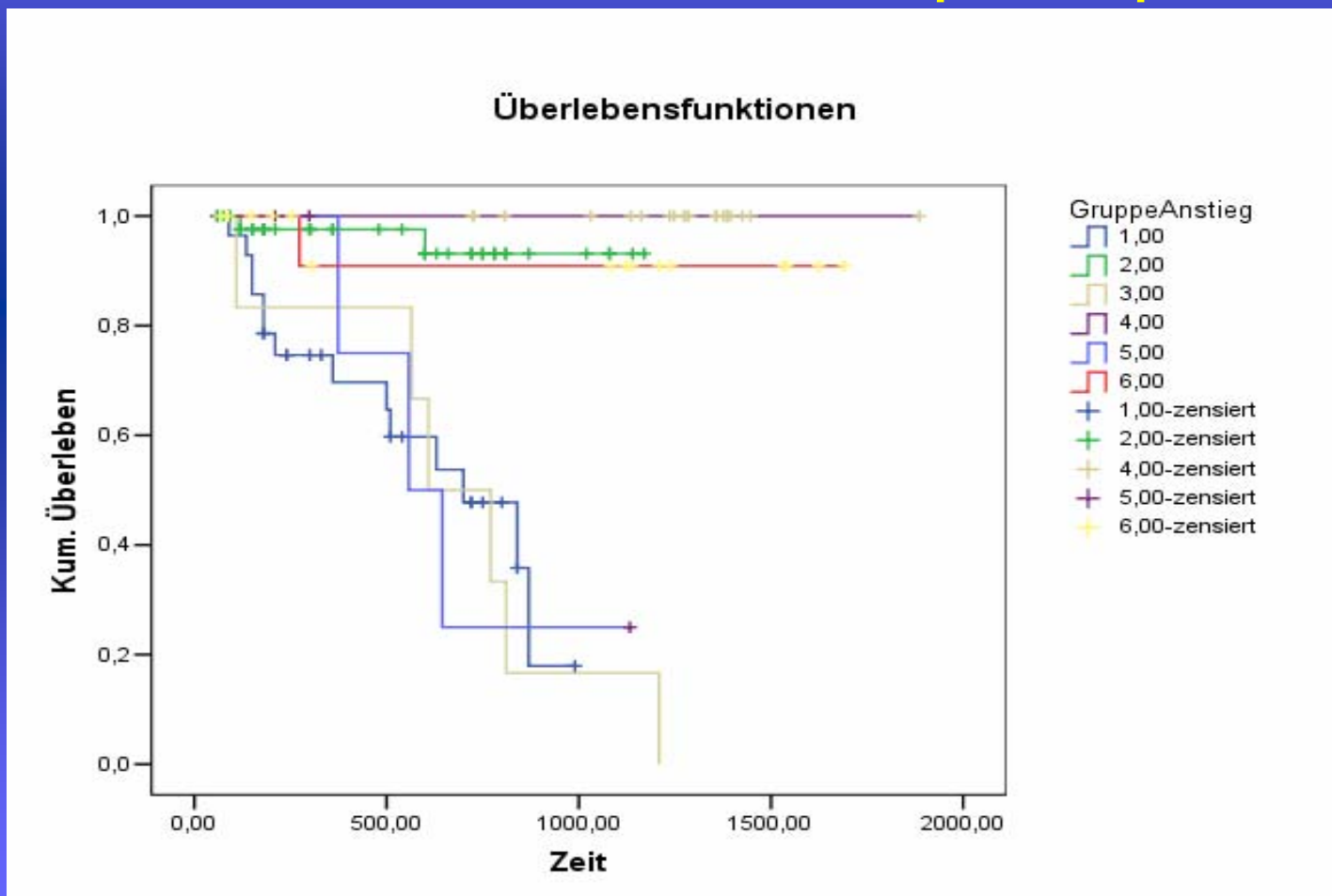
increase in CETC

A more than tenfold increase in CETC towards the end of adjuvant therapy highly significantly ($p < 0.001$) correlates with a 11-16 fold risk for early relapse (in the next 5 years).

	Good prognosis N0/ER+/Her2-			Poor prognosis N1>/ER+/-		
Cell number	Total	CR	Rel	total	CR	Rel
Reduction at end of therapy	9	9	0	19	18	1
Marginal changes at end of therapy	7	7	0	23	18	5
Increase at end of therapy	9	6	3	24	13	11

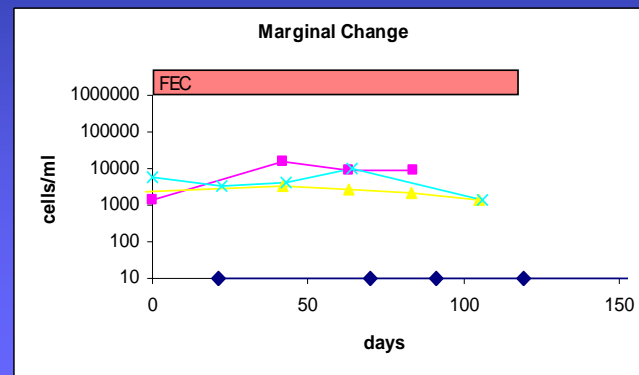
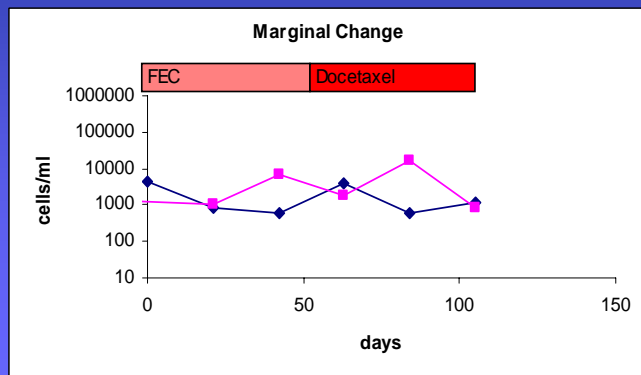
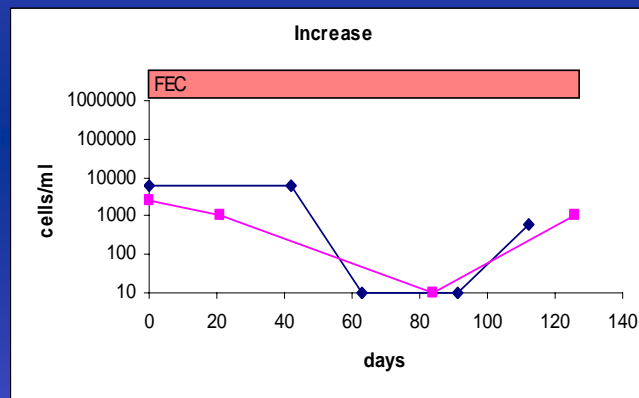
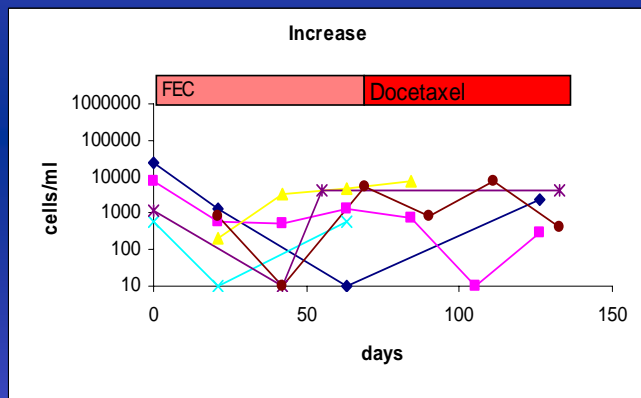
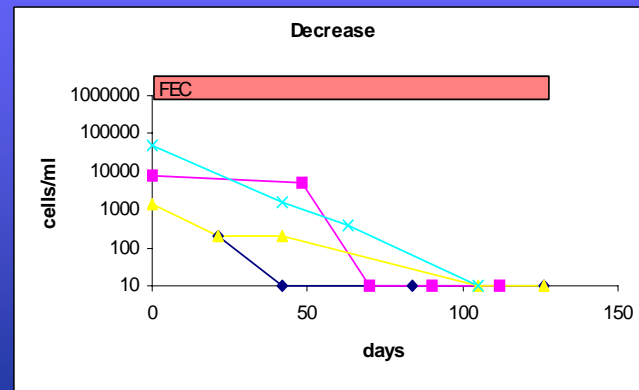
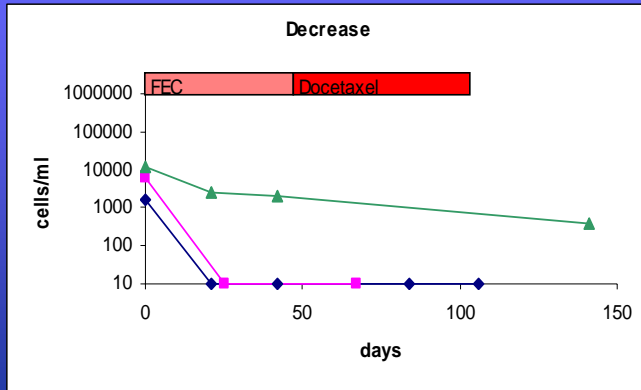
increasing cell numbers are seen most frequently in poor prognosis patients

For patients with increasing numbers of CETC results for relapse free survival from three different centers are superimposeable

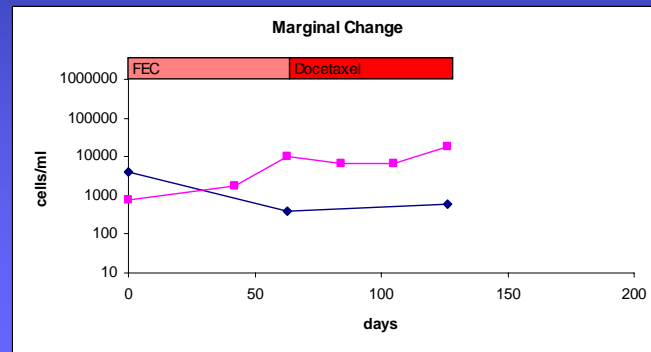
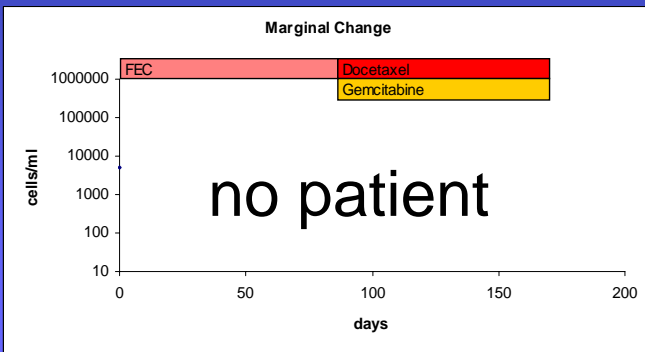
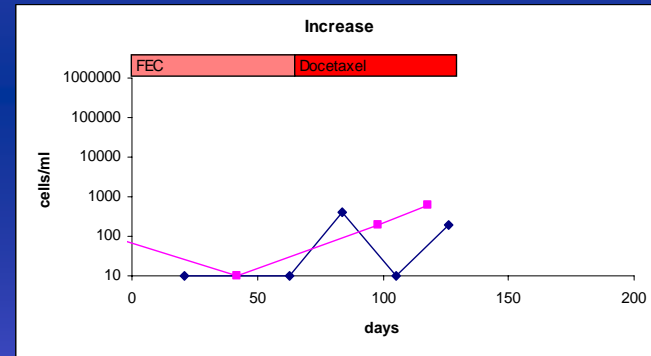
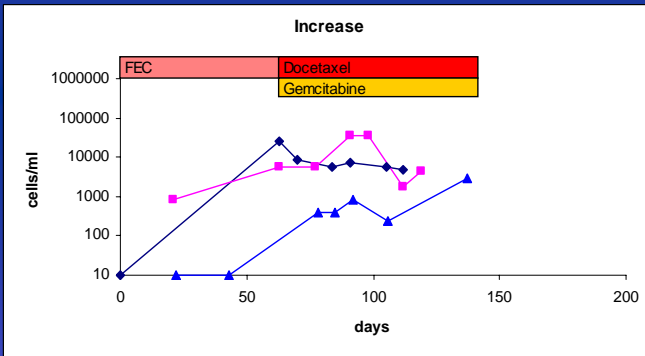
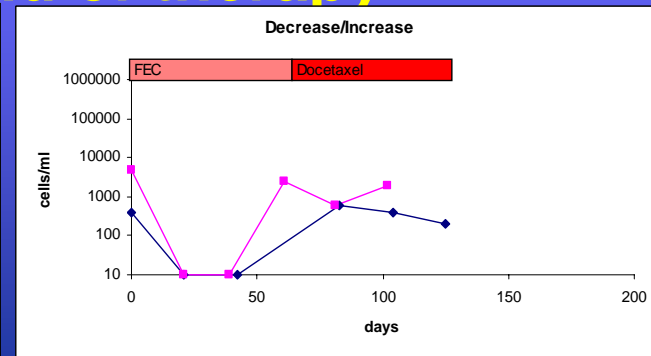
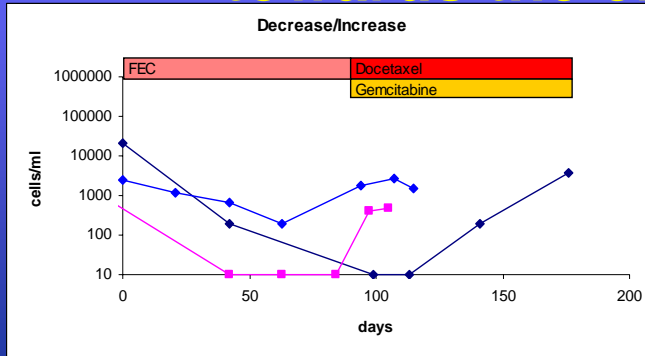


Ongoing studies

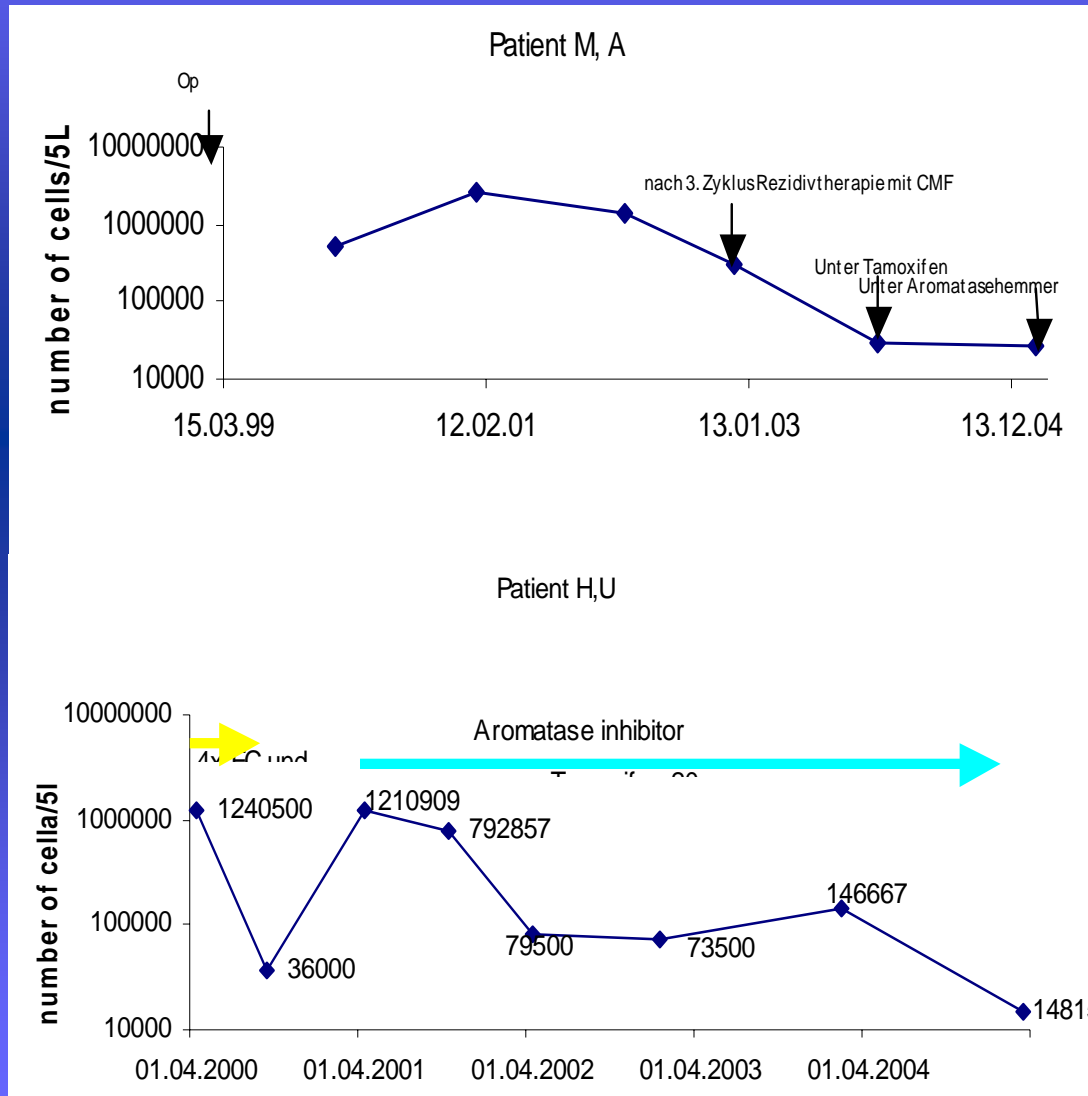
Patients from the NNBC study show a reduction in CETC in 58% and in an increase 24% in CETC



No patient from the Success study shows a reduction in CETC and 72% show an increase in CETC towards the end of therapy



Longitudinal monitoring during hormone treatment

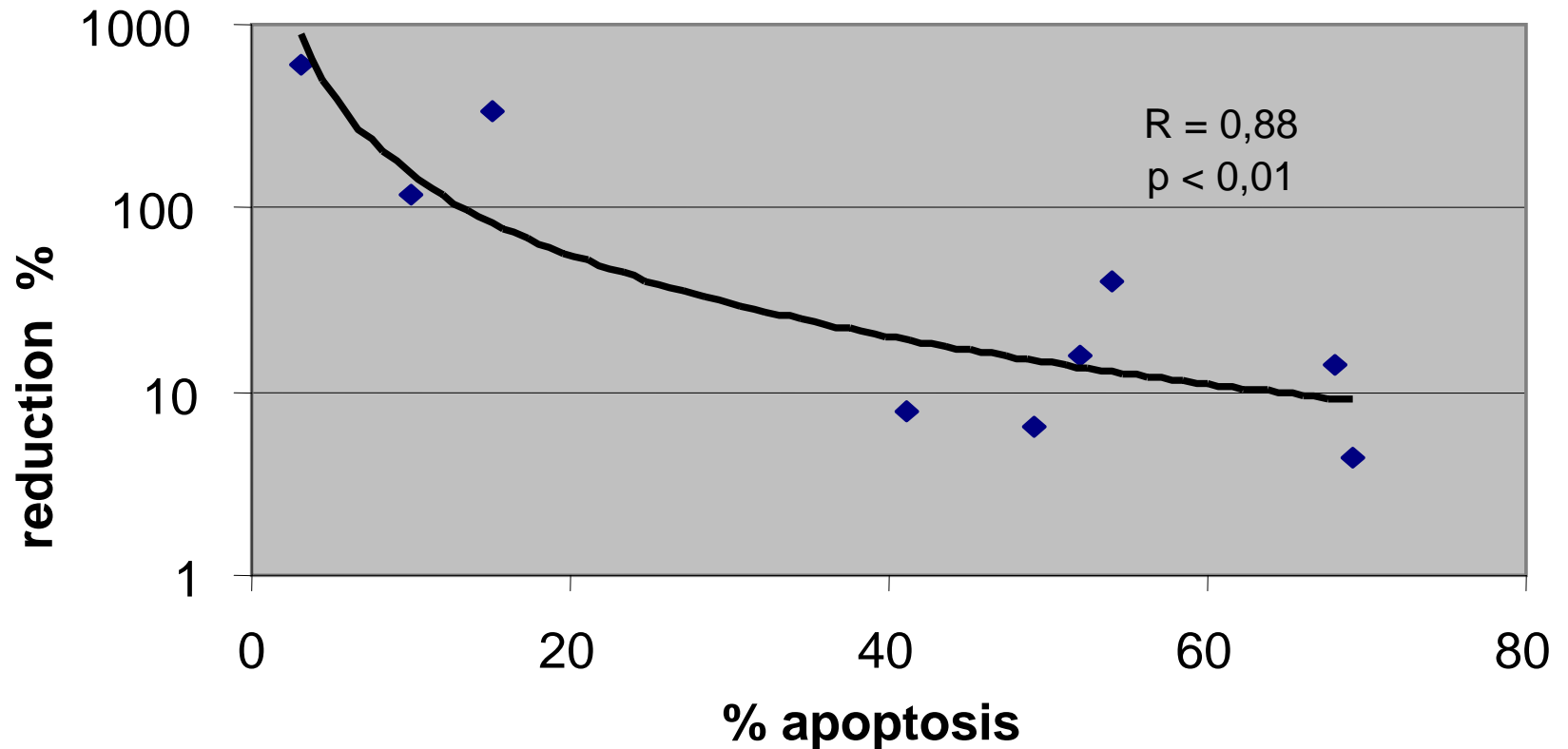


Analysis of apoptotic cells

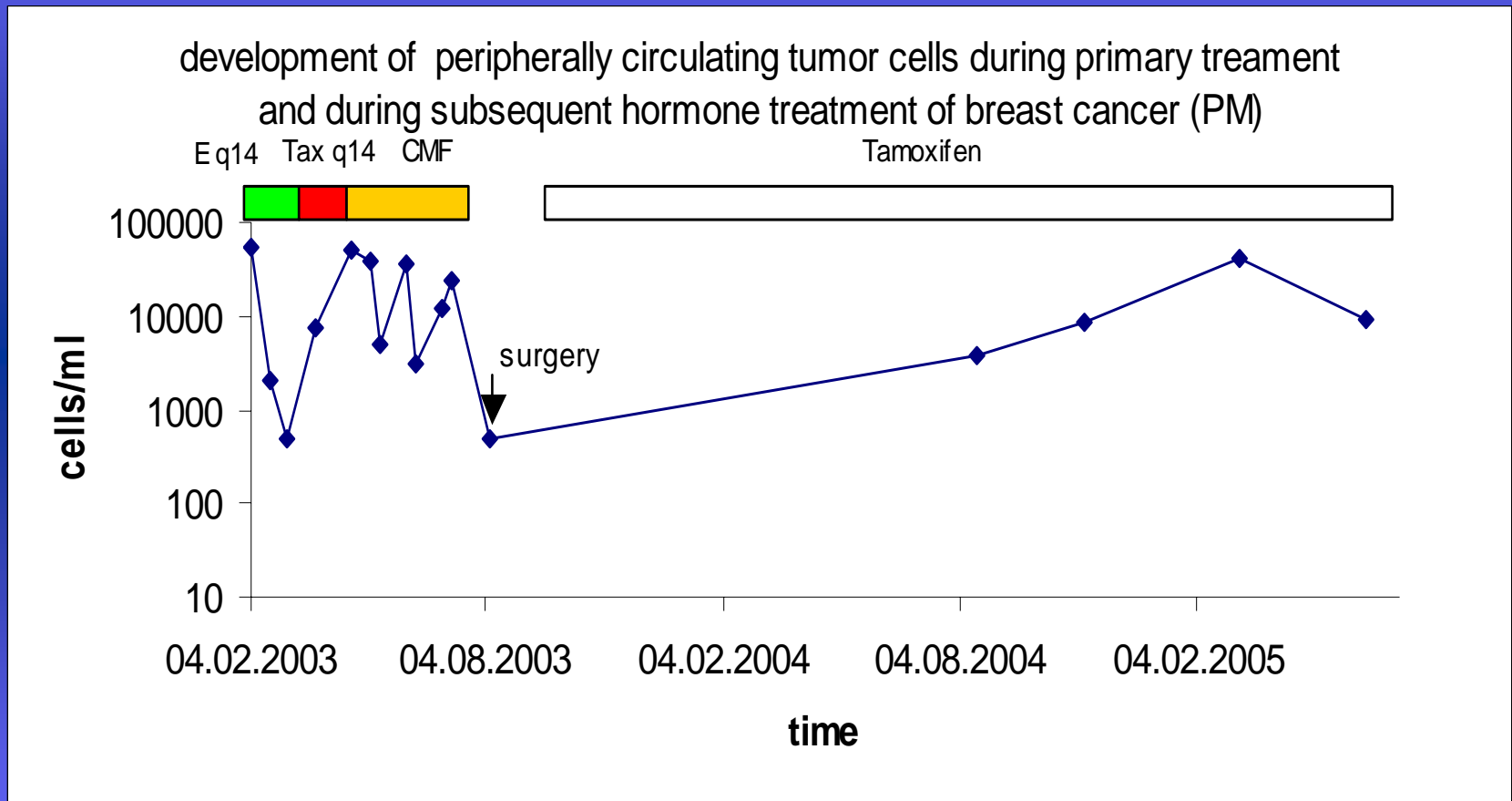


Further characterization of circulating tumor cells: apoptosis

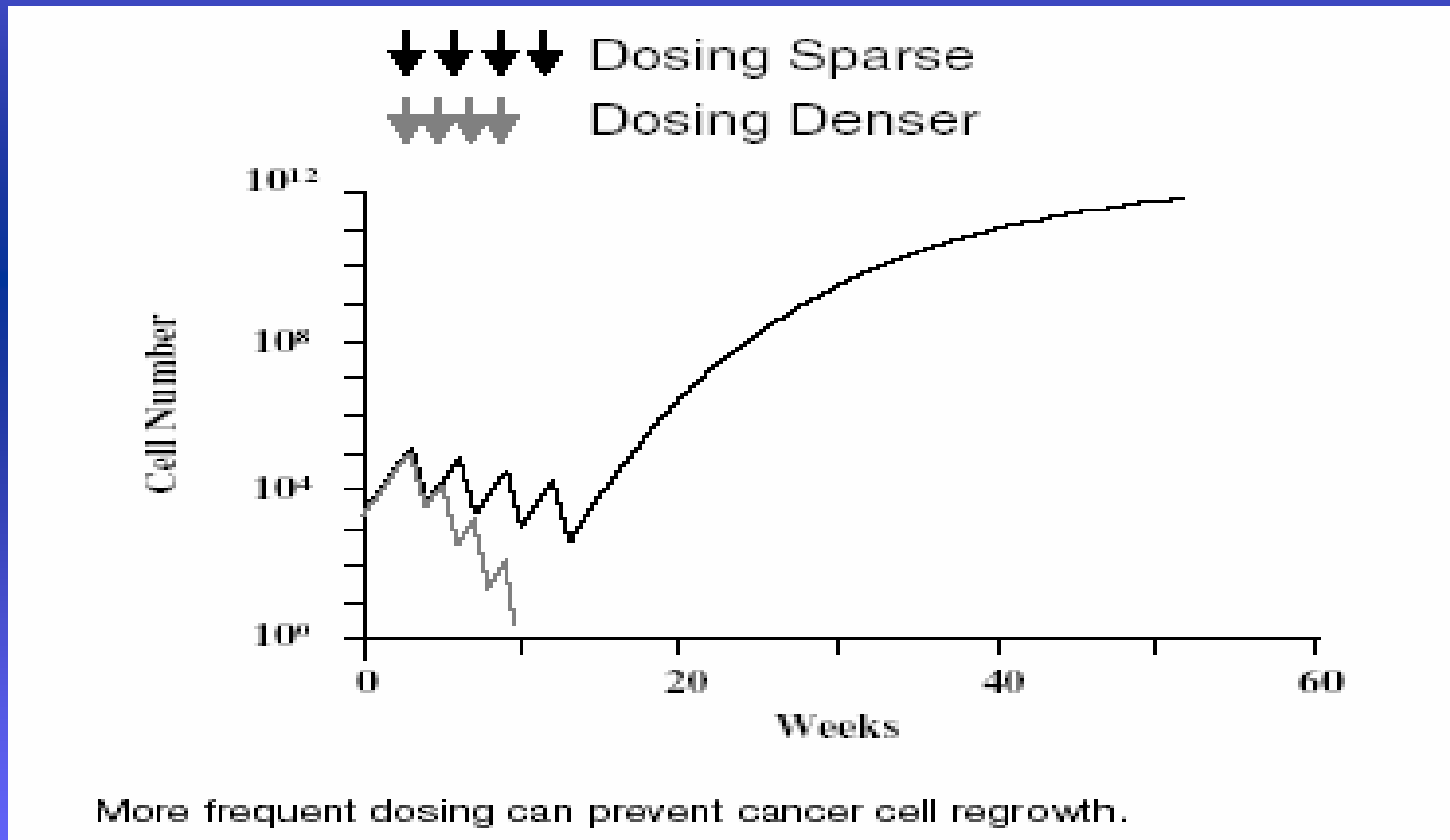
Relationship between apoptosis and
Reduction in cell number



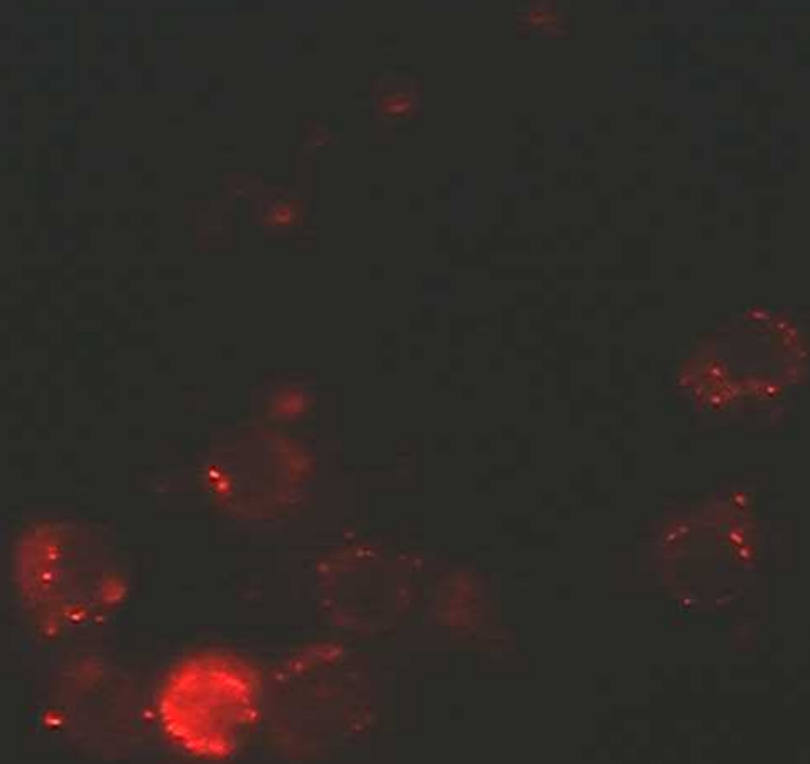
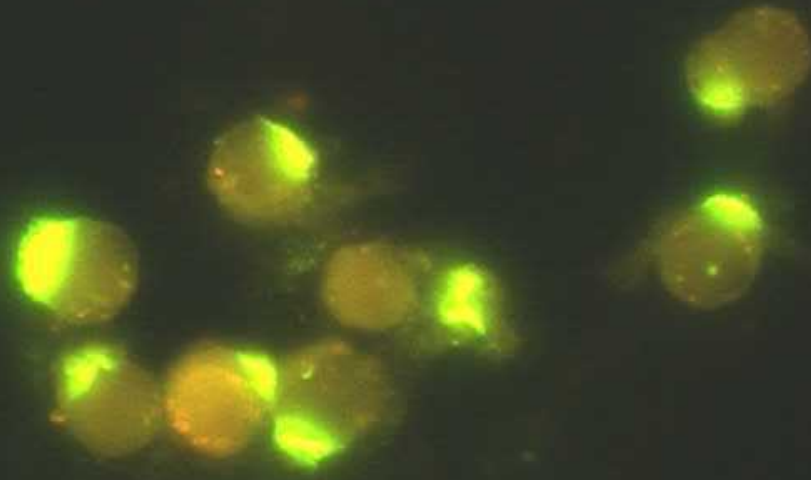
Course of disease subsequent systemic chemotherapy



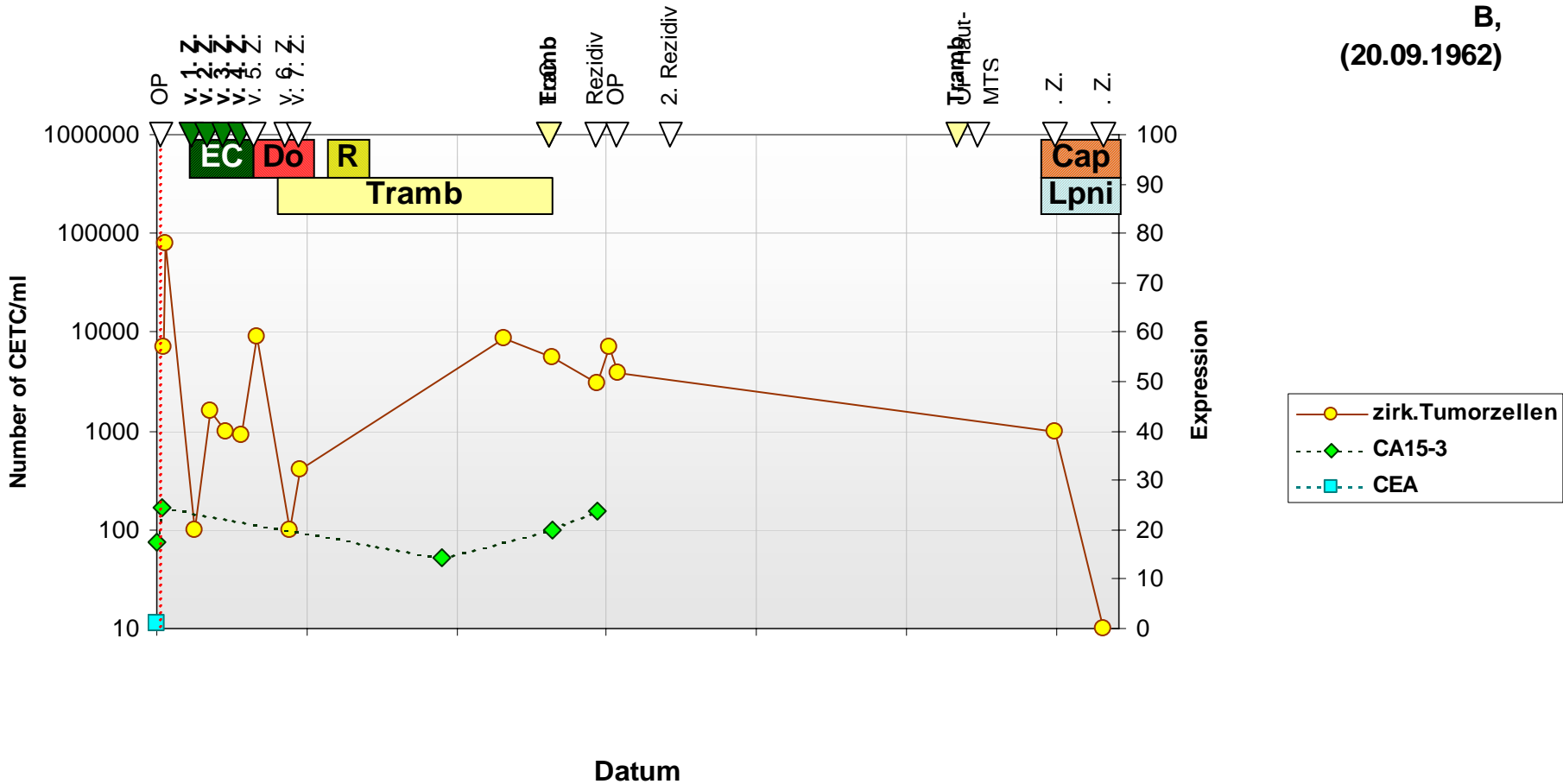
Tumor growth subsequent to systemic chemotherapy: the Norton-Simon Hypothesis



Her2/neu amplification



An increase in CETC during trastuzumab therapy predicts for early relapse, Lapatinib together with capecitabine can eliminate also these cells



Questions:

What does the increase in CETC come from?
Are these tumor cells resistant to therapy?
Is it release from occult metastases?

Obviously cells with the potential to settle and grow are present preferentially among the cells increasing towards the end of therapy.

Questions:

Might patient with such a profile during therapy benefit from additional therapy cycles?

Might we be able to fish such cells?

If we prevent such cells from resettling, can we prevent metastasis formation?

Future

Banking tumor cells

testing chemosensitivity

new therapeutic approaches

Future

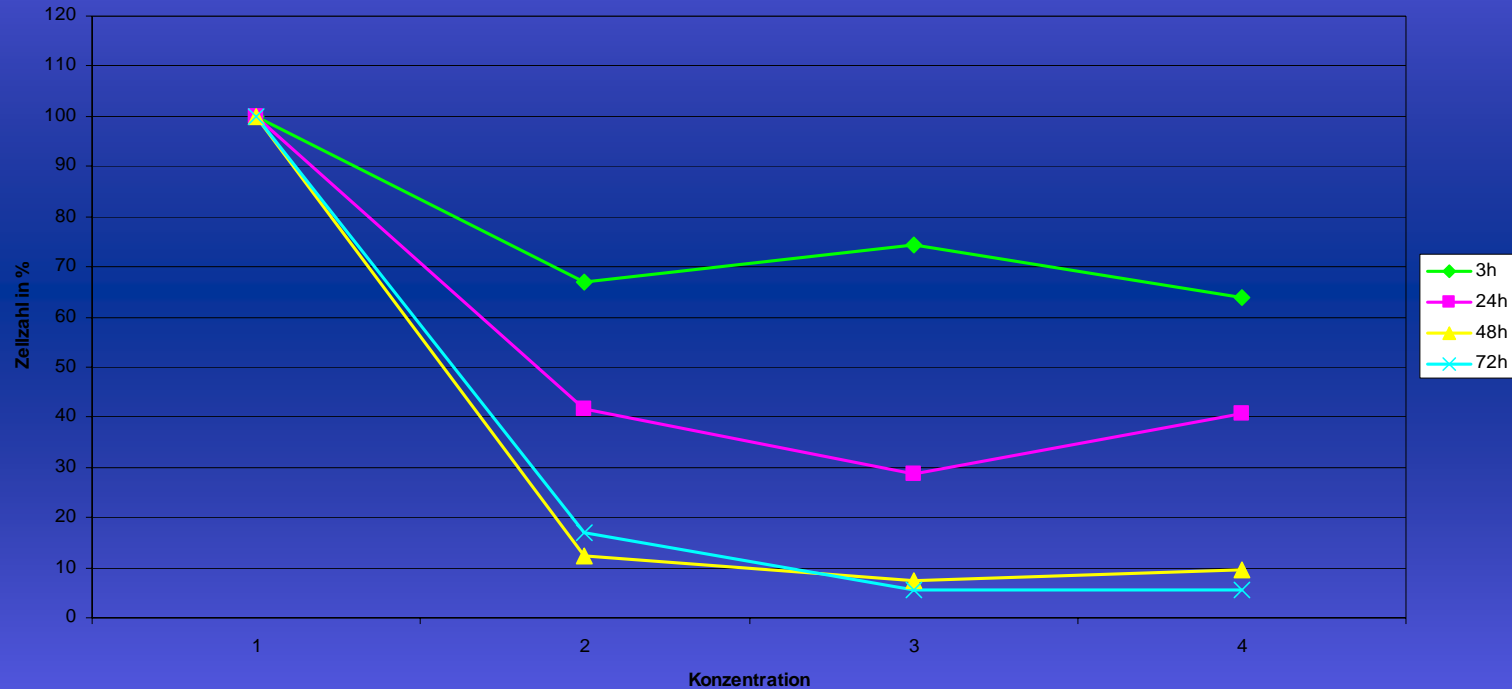
Banking tumor cells

testing chemosensitivity

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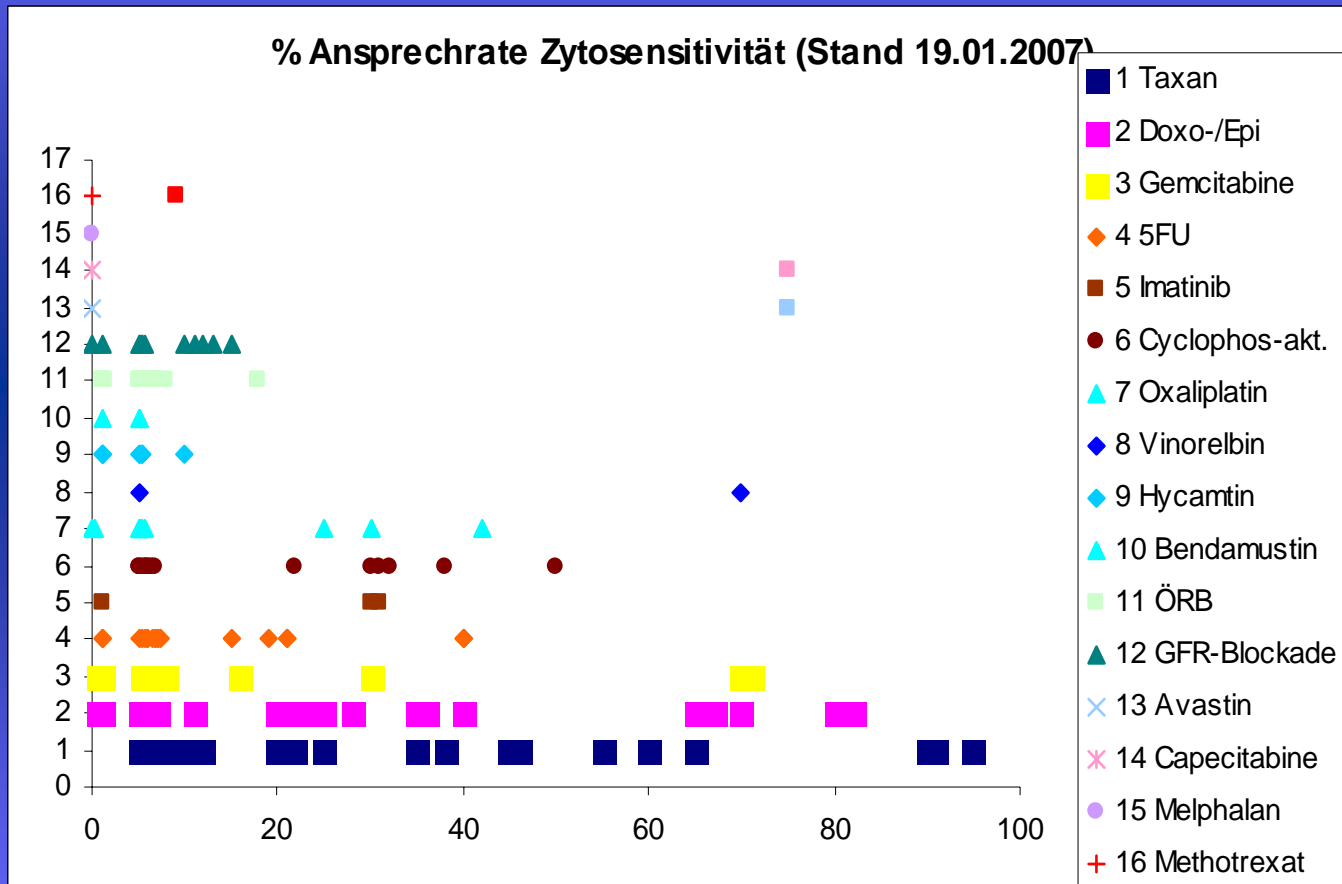
Chemosensitivity can be tested directly in the CETC

Lab. Nr: 5286 Zahl der HEA positiven Zellen unter Cyclophosphamid in %des Ausgangswertes
(M:\Chemosensitivität\Patienten\...)



In responsive cells the reaction is time and dose- dependent

Chemosensitivität: höchste Effektivität für Taxane und Anthrazycline



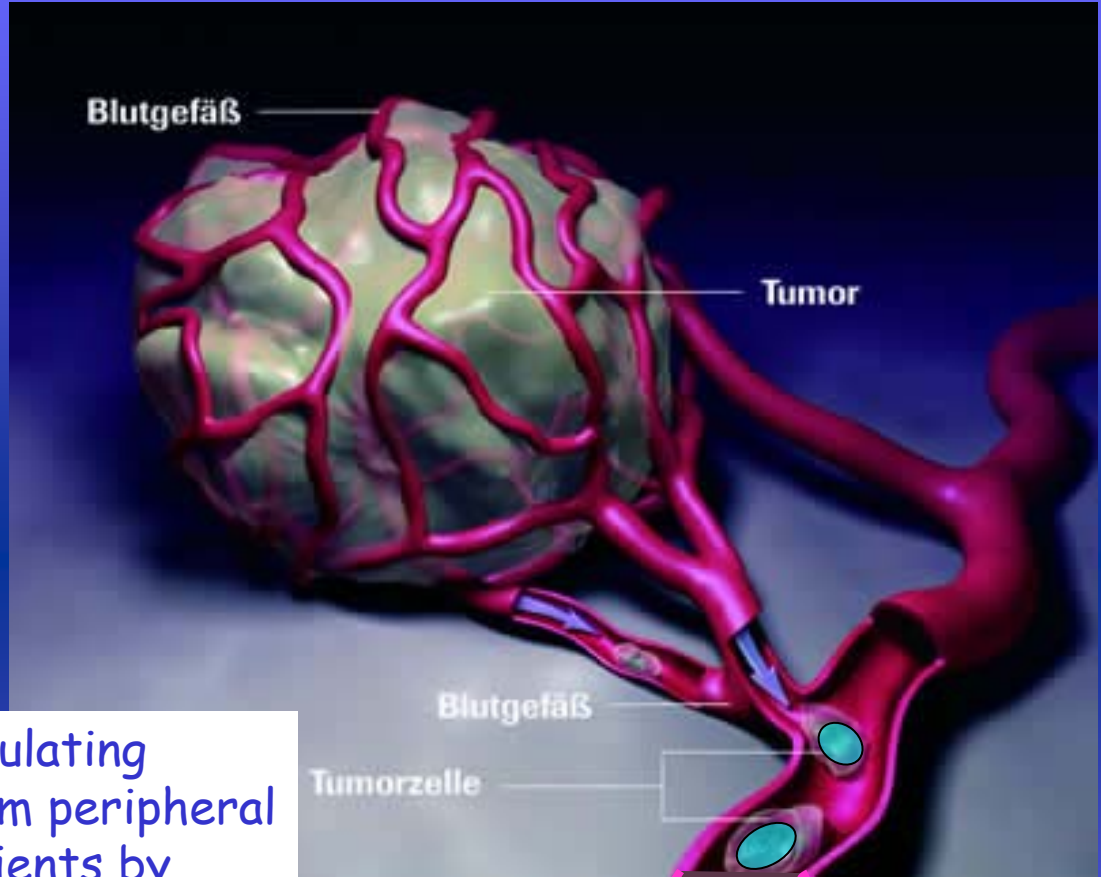
Future

Banking tumor cells

chemosensitivity

new therapeutic approaches

Our aim:



„Magnetic tumor cell depletion“

removal of circulating tumor cells from peripheral blood from patients by magnetic nanoparticles

→ protection from metastasis formation

~~Distant metastasis~~

Acknowledgement

KIM II, University of Jena:

Prof. K. Höffken

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Dr. Joachim Clement

Dana Hüttig

Conny Jörke

Bad Berka

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TZB, Bayreuth:

Dipl.Med. Babette Willen

Dr. Ernst-Ludwig Stein



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Thank you for your attention !