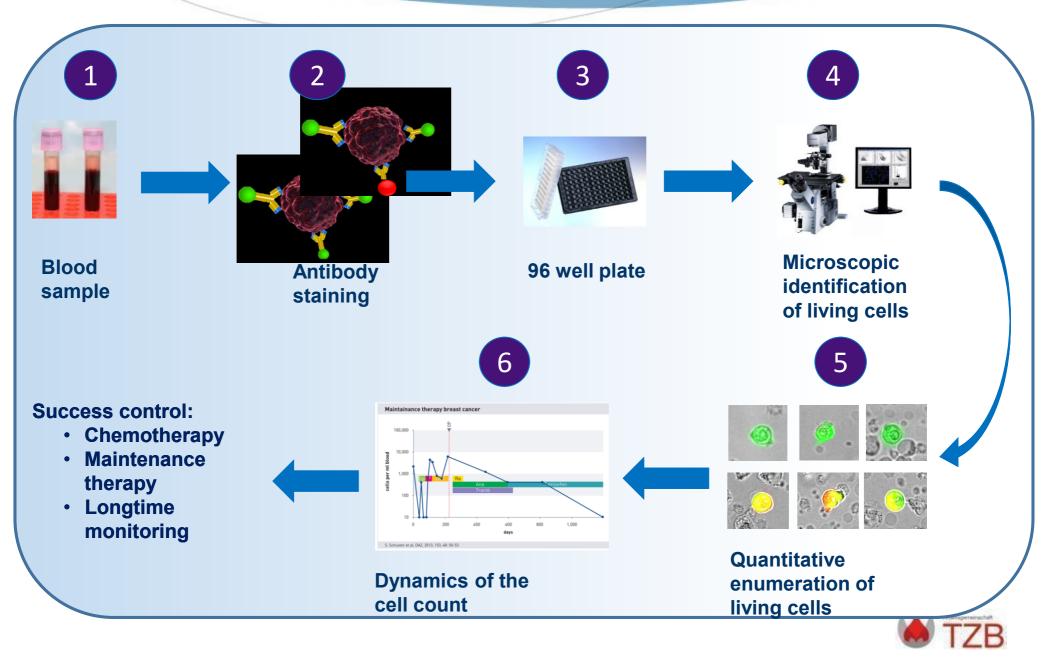
Personalised Testing of Natural Substances to Support Cancer Cell Apoptosis

Katharina Pachmann, SIMFO GmbH and Transfusion Medical Laboratory Bayreuth, Germany



Methodology



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



Chemosensitivity

J Cancer Therapy 2013, 4:597-605

Chemosensitivity Testing of Circulating Epithelial tumour 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)



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

Nadine Rüdiger¹, Ernst-Ludwig Stein², Erika Schill², Gabriele Spitz², Carola Rabenstein², Martina Stauch³, Matthias Rengsberger⁴, Ingo B. Runnebaum⁴, Ulrich Pachmanu², Katharina Pachmann¹²:

¹Clinic for Internal Medicine II, University Hospital, Friedrich Schiller University, Jena, Germany; ²Transfusionsmedizinisches Zentrum, Bayreuth, Germany; ³Onkologische Schwerpunktpraxis, Kronach, Germany; ⁴ Women's Hospital, University Hospital, Friedrich Schiller University, Jena, Germany. Email: ¹Yacahmann(Baborpathmann, de

Received February 25th, 2013; revised March 26th, 2013; accepted April 2nd, 2013

Copyright © 2013 Nadine Rüdiger et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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/Pincipal Findings: After only red blood cell lysis, omitting any emichment (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-epithelia 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 tratment success.

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

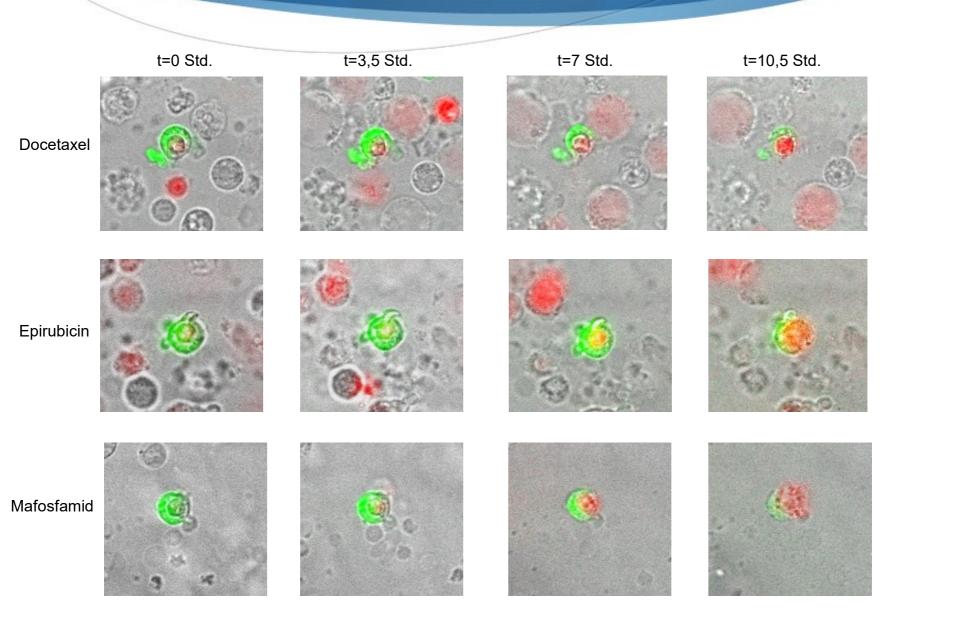
1. Introduction

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 'Corresponding autor. 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

Copyright © 2013 SciRes.

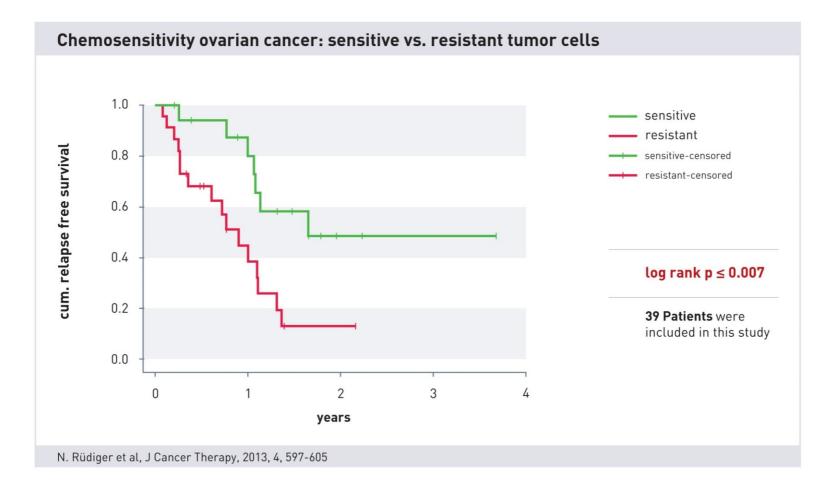
JCT





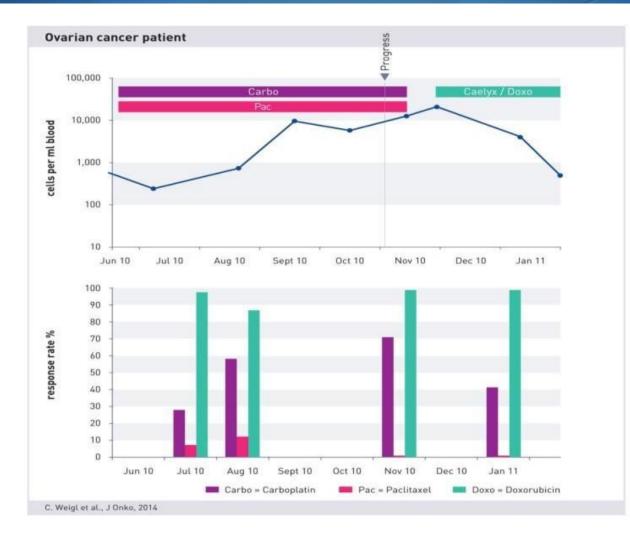


Pilot Study: Relapse free survival of patients with ovarian carcinoma patients with sensitive vs. resistant CETCs





Case report: Ovarian carcinoma Resistance to guideline drugs with progress, sensitivity to second line drug





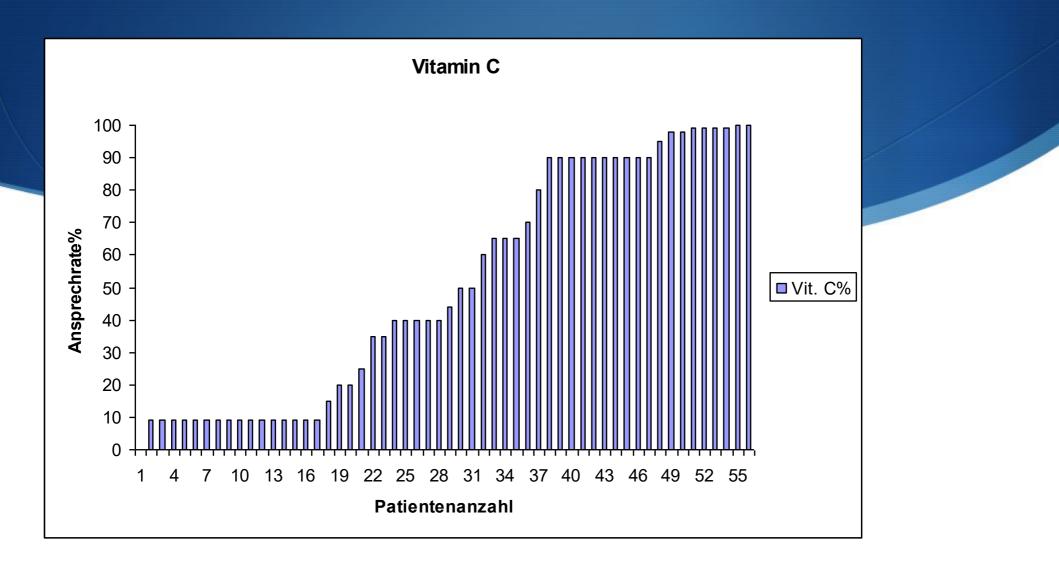
Case report breast cancer increasing resistance to drugs





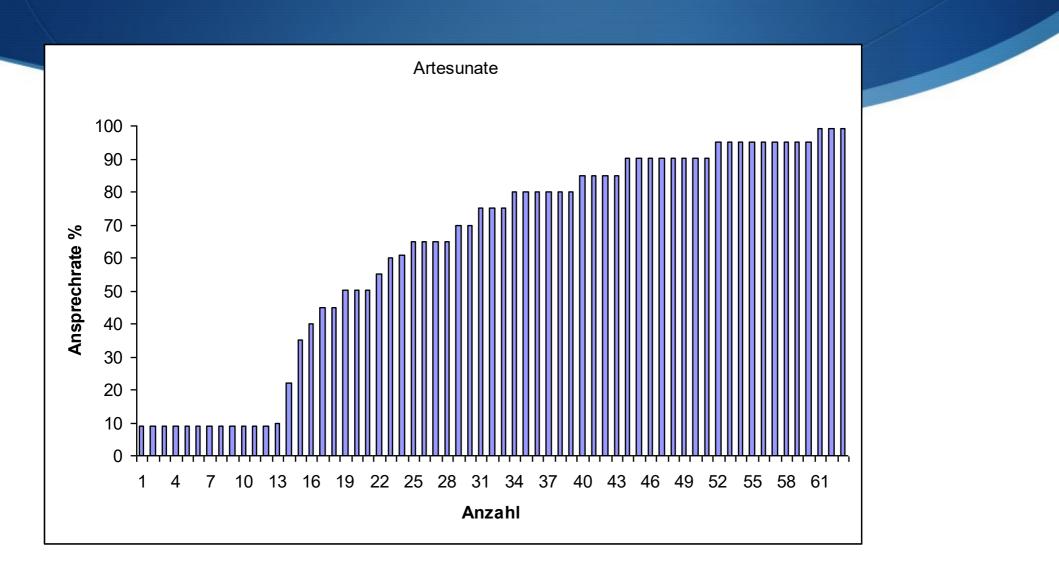
Adjuvant therapy using natural agents





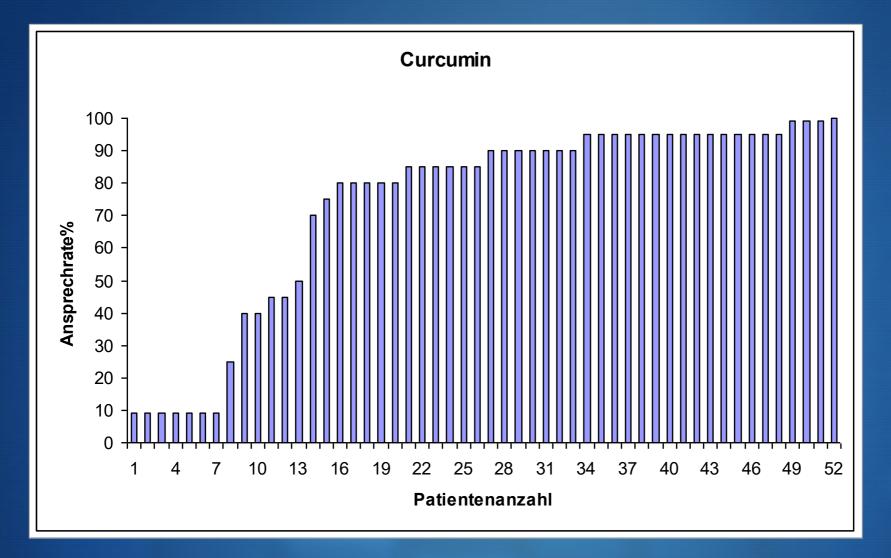
Patients total: 56		
Sensitivity > 50%	25 Patients	45%
Sensitivity < 50%	31 Patients	55%





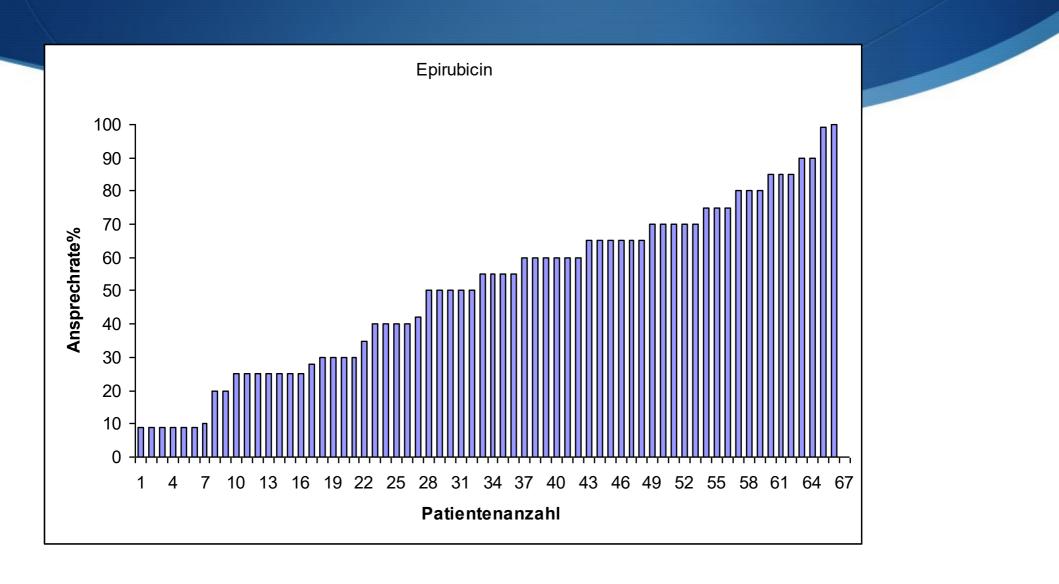
Patients total: 63		
Sensitivity > 50%	42 Patients	67%
Sensitivity < 50%	21 Patients	33%





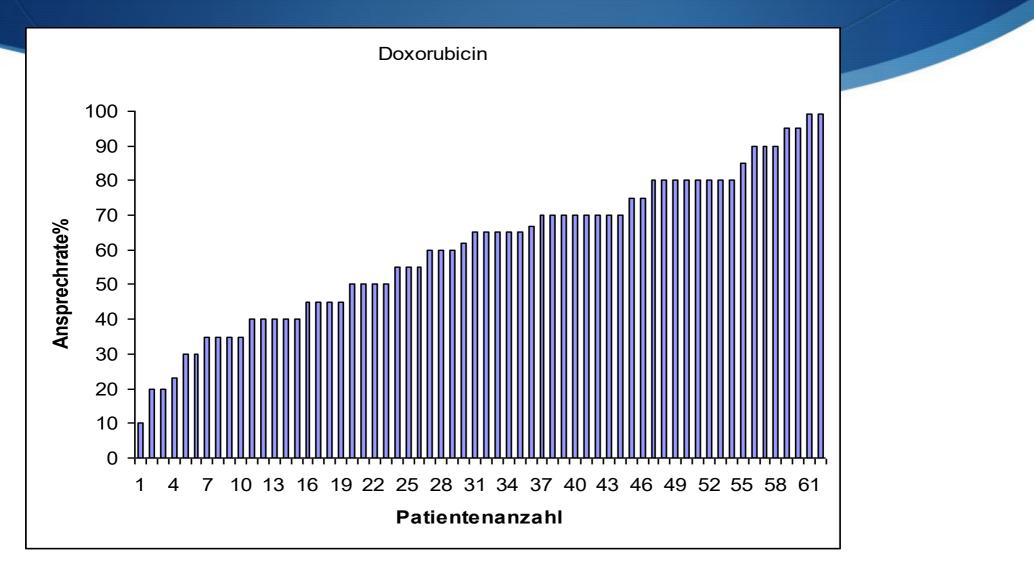
Patients total: 52		
Sensitivity > 50%	39 Patients	75%
Sensitivity < 50%	13 Patients	25%





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%



Natural agents suggested by maintrac

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





Dr. med. Ulrich Pachmann Tel.: +49 921 / 85 02 00 Fax +49 921 / 85 02 03 Email: mail@laborpachmann.de



Labor Dr. mod. Ulrich Pachmann. Kutpromenade 2. 95448 Baywuth Therapist 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, I	nitial 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):

	Nur	nber of potential t	umor cells	
Examination parameter	In the sample (1ml)	In circulation (51) (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	
Amygdalin	70	Curcuma*	40	The ideal is a reduction by 100% in
Artesunat	95	Prosanalin*	85	short-term cell culture
Boswellia*	60			1



*provided by the patient

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):

	Nur	nber of potential t	umor cells	
Examination parameter	In the sample (1ml)	In circulation (51) (in millions)	In addit examination: % of EpCAM-pos cells	Cell fragments
EpCAM	500	2,5		numerous

in-y	itro-vitality	reduction in relation with eutherapeuti		entration and time (in%) rations of
Vitamin C	70	DCA	60	
Amygdalin	70	Curcuma*	40	The ideal is a reduction by 100% in
Artesunat	95	Prosanalin*	85	short-term cell culture
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):

	Nu			
Examination parameter	In the sample (1ml)	Incirculation(51) (inmillions)	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	The ideal is a reduction by 100% in	
Cisplatin	65	Vitamin C	40	short-term cell culture
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):

	Nu	Number of potential tumor cells				
Examination parameter	In the sample (1ml)	In circulation (51) (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.



Maintrac sensitivity to natural agents is available in three levels of concentration

	with	tion in relation to c eutherapeutic cond eduction by 100% in	centration	s of	1%)
Quercetin	85	Quercetin	90	Quercetin	99
0,1-fold		1-fold		10-fold	
Vitamin C		Vitamin C		Vitamin C	
30g	55	30g	75	30g	90
0,1-fold		1-fold		10-fold	
Artesmisinin		Artesmisinin		Artesmisinin	
250mg	25	250mg	90	250mg	98
0,1-fold		1-fold		10-fold	
Curcumin		Curcumin		Curcumin	
450mg	n.a.	450mg	90	450mg	n.a.
0,1-fold		1-fold		10-fold	



This is how the circulating epithelial cancer count presented

Diagnosis:	
Adenocarcinoma	a of the caecum (initial diagnosis: 12/2015)
TNM:	T3 N2 M1, KRAS Exon 2 Codon 12 Mutation (Gly12Val)
-	Liver, lung and omental metastases
-12/15:	right hemicolectomy
- 03-08/16:	Chemotherapy with Capecitabine and Avastin
- 13.09.16:	left lung ablation
- 28.11.16:	left and right hemihepatectomy and non anatomical liver resection in S6 right
	hemihepatectomy

- current : ProCurcumin, ProSirtusan, Supplements (Tisso)

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):

	Nu	Number of potential tumor cells				
Examination parameter	In the sample (1ml)	In circulation (51) (in millions)	In addit examination: % of EpCAM-pos. cells	Cell fragments		
EpCAM	250	1,25		some		

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 addition, there were some 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.



Testing the sensitivity of chemotherapy vs. natural agents

	Nu	Number of potential tumor cells				
Examination parameter	In the sample (1ml)	In circulation (51) (in millions)	In addit. examination: % of EpCAM-pos. cells	Cell fragments		
EpCAM	200	1		some		

in-v	vitro-vitality	v reduction in relat with eutherapeu		entration and time (in%) rations 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.

In addition, there were some specific cell fragments detected.



Curcumin in three levels of concentration

	Nu	Number of potential tumor cells				
Examination	In the sample	In circulation (51)	In addit. examination:	Cell		
parameter	(1ml)	(in millions)	% of EpCAM-pos. cells	fragments		
EpCAM	1 250	6,25		numerous		

	with	ction in relation to e eutherapeutic con- eduction by 100% in	centration	sof	1%)	
450mg 0,1-fold	70	450mg 1-fold	90	Curcumin 450mg 10-fold	95	

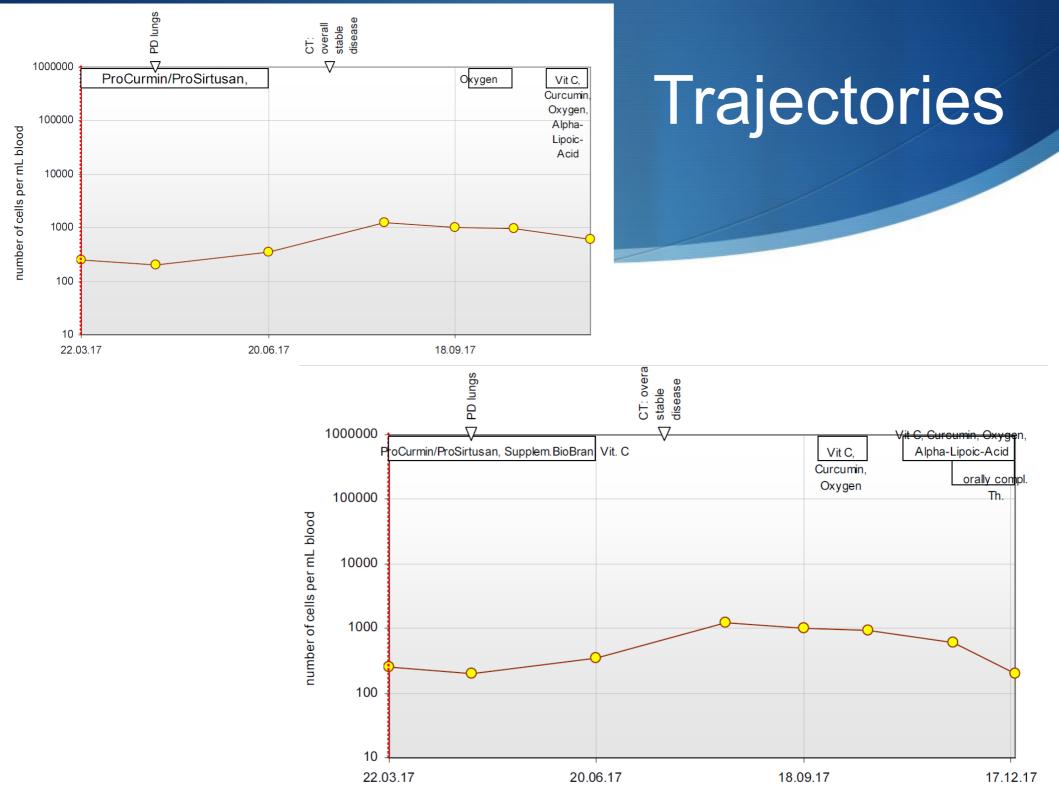


Vitamin C in three levels of concentration

	Nu	Number of potential tumor cells				
Examination	In the sample	In circulation (51)	In addit. examination:	Cell		
parameter	(1ml)	(in millions)	% of EpCAM-pos. cells	fragments		
EpCAM	1 000	5		numerous		
1						

	with	ction in relation to eutherapeutic con reduction by 100% in	centratio	ns of	1%)	
Vitamin C 0,1-fold 3g	30	Vitamin C 1-fold 30g	45	Vitamin C 10-fold 300g	45	





Strength of different combinations

- 06/17:	Therapy with ProCurcumin, ProSirtusan, Supplements (Tisso), Biobran, Vit.C
- 20.07.17:	CT-Scan: number of small lesions in the lungs including the right upper lobe
	(6mm) and right lower lobe (9mm). The liver remains clear. Overall her disease
	has been classed as stable
25.00 16.10	

- 25.09.-16.10.17: Therapy with 3 x Vitamin C Infusions (25g), 2 x Curcumin Infusions (150mg), 3 x Oxygen Therapy
- 01.11.-21.11.17: Therapy with 3 x Vitamin C Infusions (25g), 2 x Curcumin Infusions (150mg), 3 x Oxygen Therapy, Alpha-Lipoic-Acid
 since 21.11.17: Therapy with 2x Vitamin C Infusions (25g), 2x Curcumin Infusions (200mg), 2x Alpha Lipoic, Gluthation, Oxgen therapy, daily ProSirtusan, Curcumin longvida, orally Vitamin C and Vitamin D Tisso

	Nu			
Examination parameter	In the sample (1ml)	In circulation (51) (in millions)	In addit. examination: % of EpCAM-pos. cells	Cell fragments
EpCAM	550	2,75		numerous

in-vitro-vitality reduction in relation to concentration and time (in%)					
with eutherapeutic concentrations of					
Quercetin +Artesunate	85	Vitamin C +Curcumin	60	The ideal is a reduction by 100% in short-term cell culture	

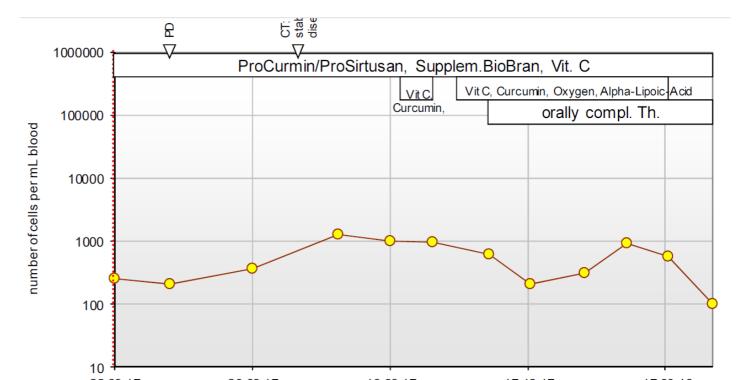


recommendations produces a

- 21.11.17-03/18: Therapy with 2x Vitamin C Infusions (25g), 2x Curcumin Infusions (200mg),
- since 21.11.17: Alpha Lipoic, Gluthation, Oxgen therapy, daily ProSirtusan, Curcumin longvida, orally Vitamin C and Vitamin D Tisso, Artesimin, Quercetin, Astaxanthin, Selenium, Biobran, Faty acids

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):

	Nui			
Examination parameter	In the sample (1ml)	1		
EpCAM	100	0,5		numerous



Evidencing the effectiveness of curcumin

	Nu			
Examination	In the sample	In circulation (51)	In addit. examination:	Cell
parameter	(1ml) (in millions) % of EpCAM-pos. cells			fragments
EpCAM	200 1			numerous

	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					
Curcumin 450mg 0,1-fold	30 450mg 80 450mg 95				95	

The material for examination could be thoroughly evaluated.

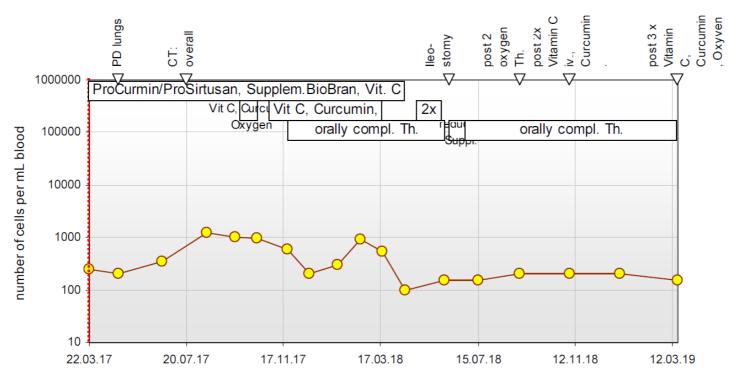
Under ongoing complementary therapies we again found a slightly increased number of live, potentially malignant tumor cells circulating in the blood. In comparison to the previous findings from September 2018 the number of potential tumor cells has not changed.



Therapy effectiveness evidenced in patient stability

June 11th and under ongoing complementary therapies, cells numbers remained relatively stable.

We recommend a control in 3 months.





Effectiveness of Hypericin in a specific case

	Nu	Number of potential tumor cells				
Examination	In the sample	In circulation (51)	In addit. examination:	Cell		
parameter	(1ml)	(in millions)	% of EpCAM-pos. cells	fragments		
EpCAM	300	1,5		numerous		

	with	tion in relation to eutherapeutic con eduction by 100% in	centration	s of	1%)	
Hypericin 0,1-fold15Hypericin 1-fold80Hypericin 10-fold90						

The material for examination could be thoroughly evaluated.

Under ongoing complementary therapies, we found a slightly increased number of live, potentially malignant tumor cells circulating in the blood.

In comparison to the previous findings from March 2019, the number of potential tumor cells has doubled.



Capecitabine declines in effectiveness over time ...

	Nui	Number of potential tumor cells			
Examination parameter	In the sample In circulation (51) In addit examination: (1ml) (in millions) % of EpCAM-pos. cells			Cell fragments	
EpCAM	200	1		some	

2017

in-v	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					

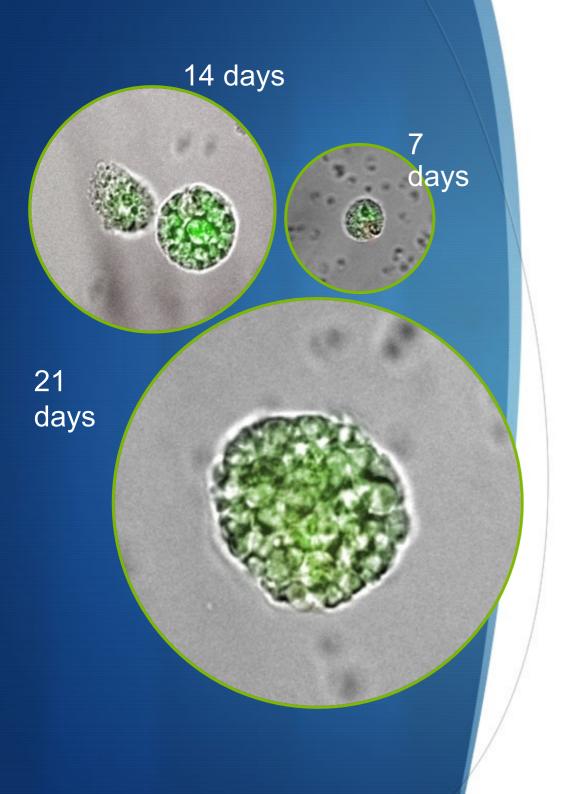
2019

in-vitro-vitality reduction in relation to concentration and time (in%) with eutherapeutic concentrations of					
5FU205FU/ Honokiol25The ideal is a reduction by 1 short-term cell culture					
5FU/ Curcumin	80				



Clonal expansion of circulating tumour cells





Tumour spheres from CETC

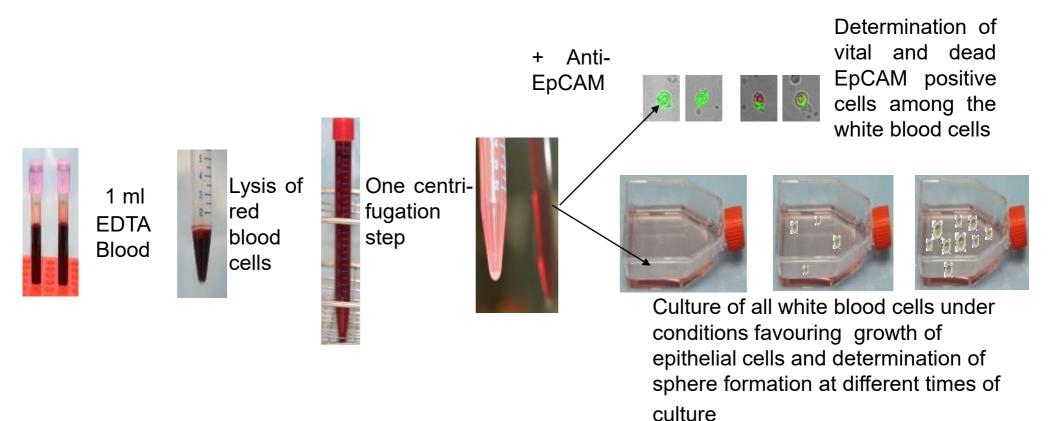
Spheres were detected in 86 out of 109 patients (78.9%);

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

All spheres detected are positive for EpCAM.

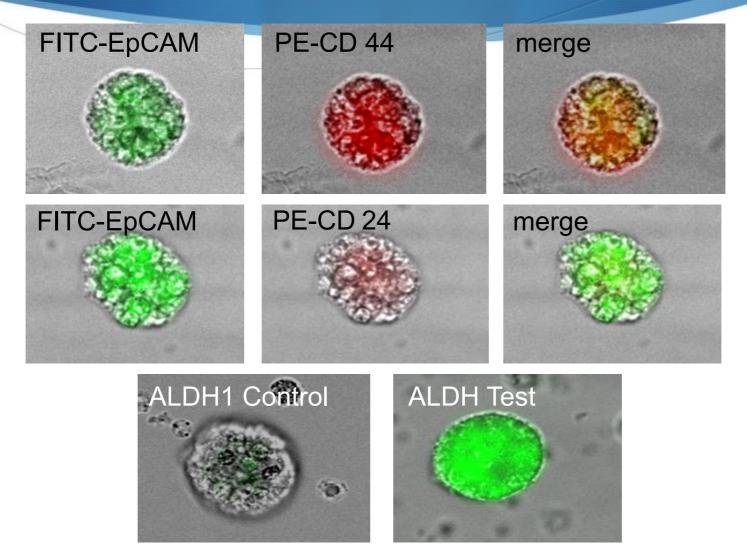


San Antonio Breast Cancer Symposium - Cancer Therapy and Research Center at UT Health Science Center – December 10-14, 2013





Stem cell marker expression in tumour spheres

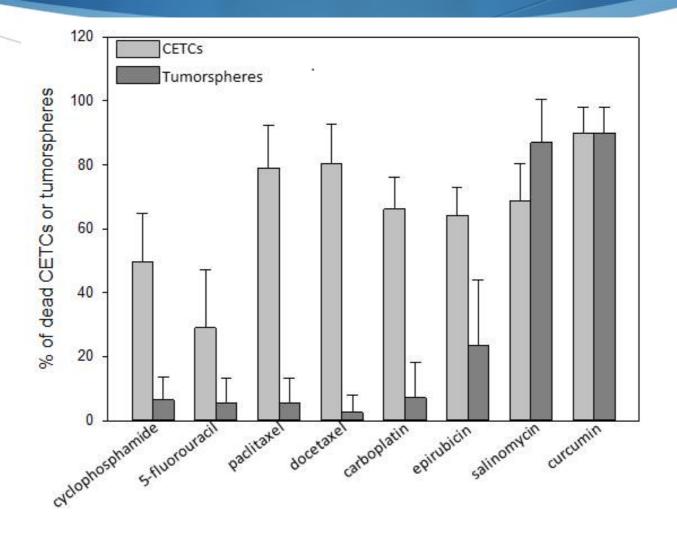




Chemosensitivity of tumour spheroids



Chemosensitivity of tumour spheroids vs. CETC





Selected agents not so effective against circulating stem cells

	Nui			
Examination parameter EpCAM	In the sample (1ml) 100	In circulation (51) (in millions) 0,5	In addit examination: % of EpCAM-pos. cells	Cell fragments some
Circulating Cancer Stem Cells	<u>250</u>			

in-vitro-vitality reduction in relation to concentration and time (in%) with eutherapeutic concentrations of						
Salinomycin Acid	40	Shogaol	60	The ideal is a reduction by 100% in short-term cell culture		

in-vitro-vitality reduction on <u>Circulating Cancer Stem Cells</u> in relation to concentration and time (in%) with eutherapeutic concentrations of							
Salinomycin acid	<10	Shogaol	<10	The ideal is a reduction by 100% in short-term cell culture			

The material for examination could be thoroughly evaluated.

We now found a minimally to slightly increased number of live, potentially malignant tumor cells circulating in the blood.

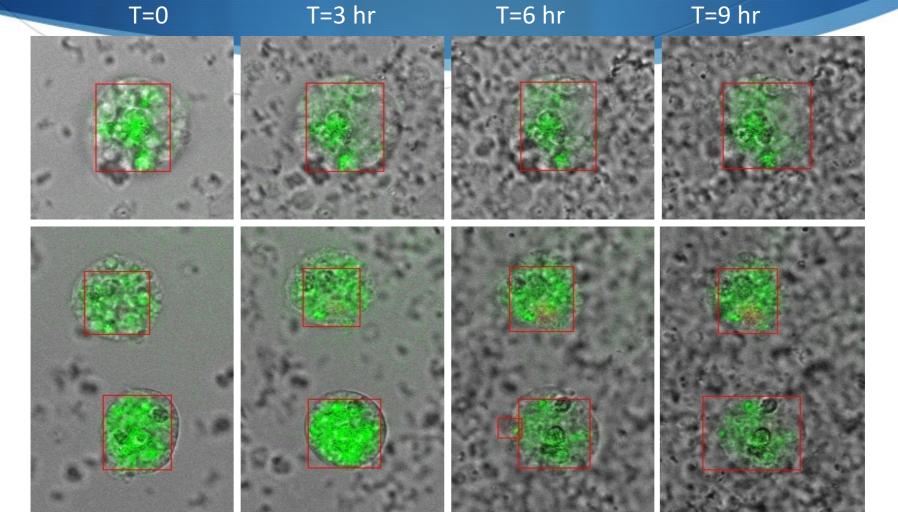
In comparison to the previous findings from August 27th, 2019 the number of potential tumor cells has doubled.

In addition, there were some specific cell fragments detected.

Specific cell fragments occur, for example as part of an immune response and indicate damaged cells.



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 lifethreatening 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.

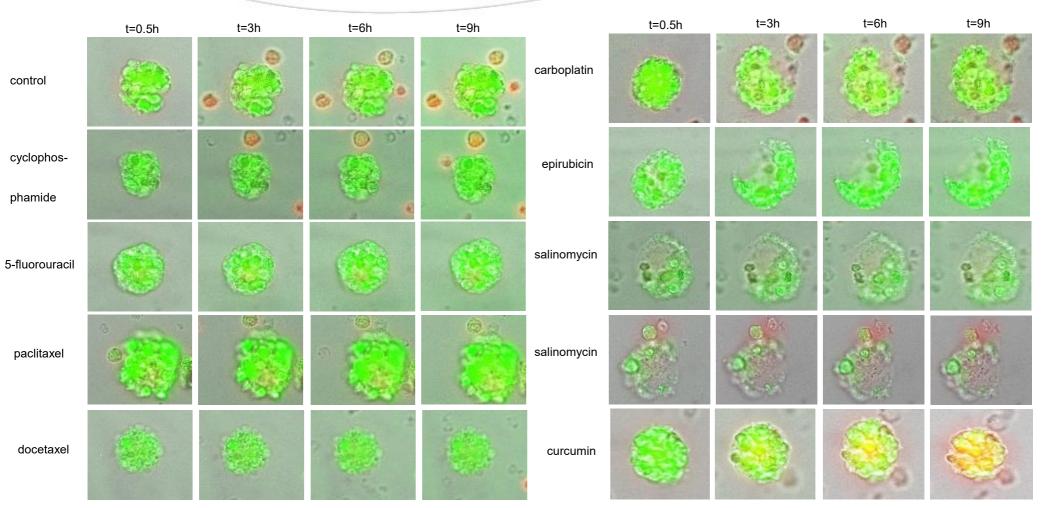
Results: Peripherally circulating cells from patients with malignant tumours in different stages of disease were analyzed for the presence of circulating epithelial tumour suspect cells and the frequencies of tumourspheres. tumourspheres could so far be grown from 79% of 36 patients in whom more than 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



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.



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



Fully accredited laboratory



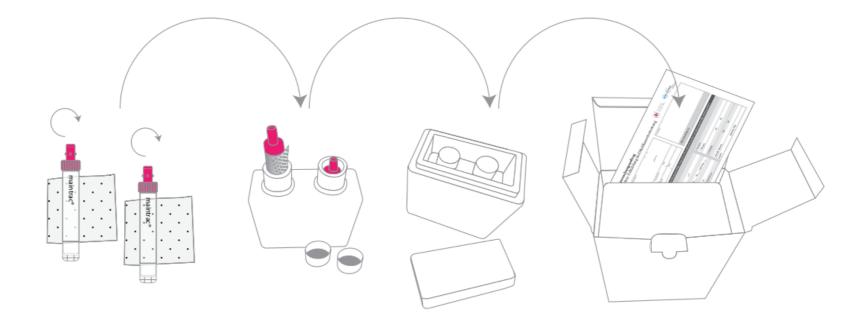


Blood collection kit





Sample packaging





Shipping and results

Within 48 to max. 72 h at room temperature



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





Thank you for your attention

