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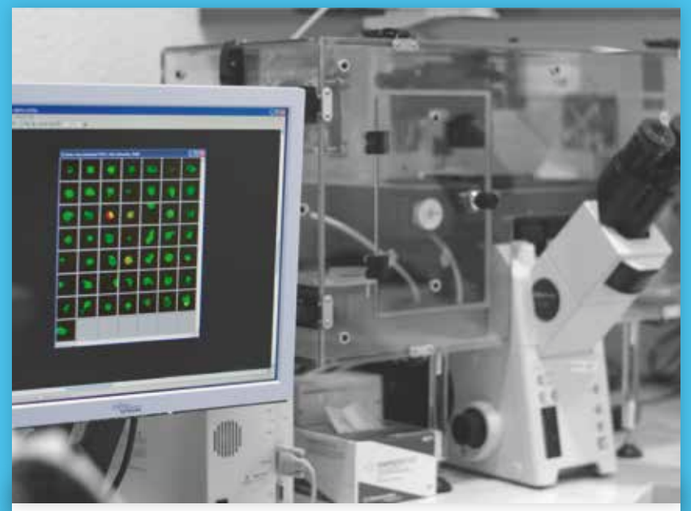

simfo

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maintrac has been performed by the DIN EN ISO 15189 accredited specialized medical laboratory Dr. Pachmann since 2005

170503-BRAS-GB-WM

maintrac

maintrac diagnostics –
determination and characterisation
of circulating epithelial tumour cells
prior to, during, and after
therapy.

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Contents

maintrac method

1. The maintrac method

Cancer is a highly complex, heterogeneous, and rapidly changing disease.

The diagnosis of cancer is a life-changing event for patients with multiple challenges on all fronts.

The challenge for physicians is to individually weigh up what action is required. Some tumours grow very slowly or remain dormant, while others are highly aggressive. Tumours can therefore vary greatly.

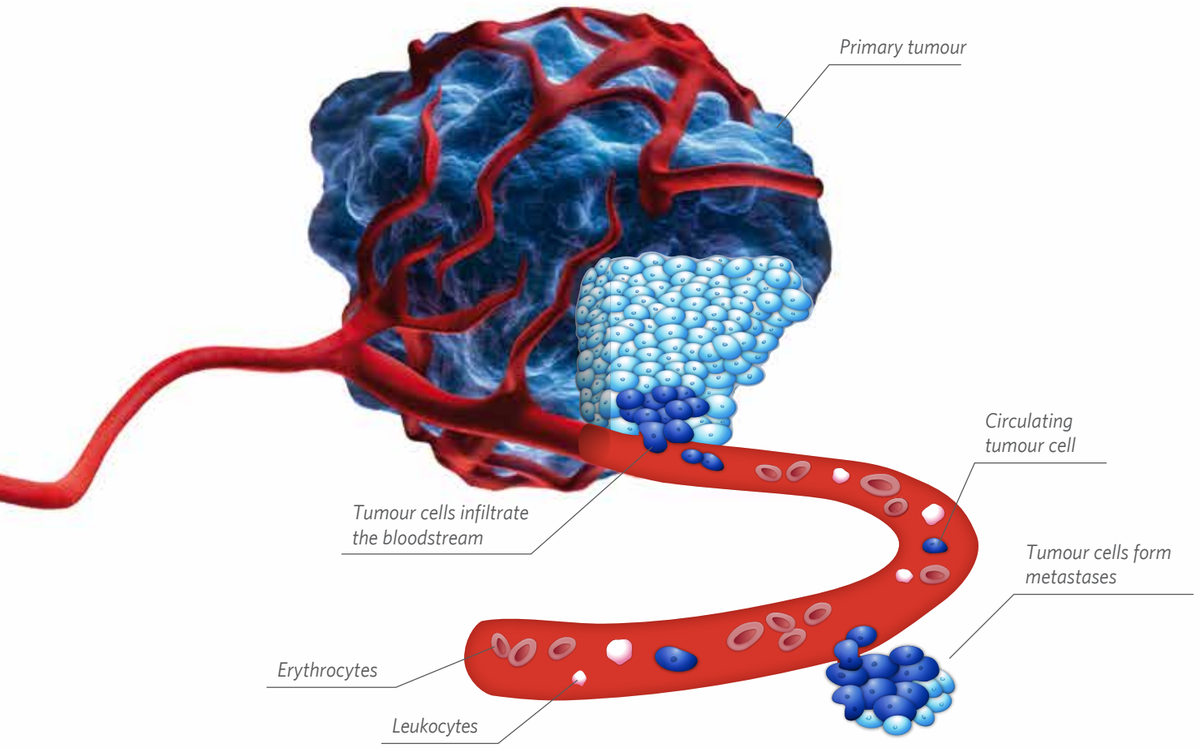
The aim is to prevent disease progression. Tailored treatment strategies are required, and it is vital to monitor the success of therapy for each patient.

Crucial to the further progression of the disease is the risk of tumour metastases, which can lead to the failure of vital organs. Metastases are formed from cells that have left the tumour and are highly likely to reflect the heterogeneity of the cells within the tumour.

These cells - circulating epithelial tumour cells (CETCs) - can still be found in blood many years after surgery or therapy. A transformation of tumour cell characteristics is possible during cancer progression.

maintrac is a highly sensitive microscopic method that is used for identifying CETCs. maintrac precision diagnostics allows patients and physicians to use the dynamics and characteristics of tumour cells to make joint treatment decisions. maintrac can be a turning point in the decision made on a patient´s care.

Over 650 patients have so far been analysed in numerous clinical trials involving maintrac, and the results have been published in high-ranking journals worldwide. Since 2005, the method has been performed in a laboratory with DIN EN ISO 15189 accreditation – the most rigorous possible standards for laboratory testing of this field. maintrac diagnostics is fully validated and subject to continuous quality assurance.



2. Fundamentals of maintrac diagnostics

2.1 Methodology

Using maintrac, real-time activity and properties of the tumour cells can be determined in blood prior to, during, and after any therapeutic initiatives are taken.

The maintrac procedure

In blood, CETCs are found amongst leukocytes. After erythrocyte lysis, the total cell population is analysed for the presence of epithelial cells. maintrac marks EpCAM, located on the cells, using a fluorescent antibody (1). The cells are simultaneously treated with propidium iodide to differentiate between living and dead cells (2). Using a fluorescence microscope, the EpCAM-positive cells are automatically identified and counted (3). A follow-up of serial values allows an assessment of disease activity (4).

- ***maintrac provides an indication of the success of chemotherapy during treatment via the reduction of living tumour cells in blood.[1,4]***
- ***The efficacy of a drug can be determined before and during therapy.[5]***
- ***If therapy does not appear to be sufficiently effective, further drugs can be examined.***
- ***The tumour characteristics can be continuously monitored, and therapy can be adjusted swiftly as appropriate.***
- ***A relapse can be detected early by an increase in CETCs during long-term monitoring.[2,3]***

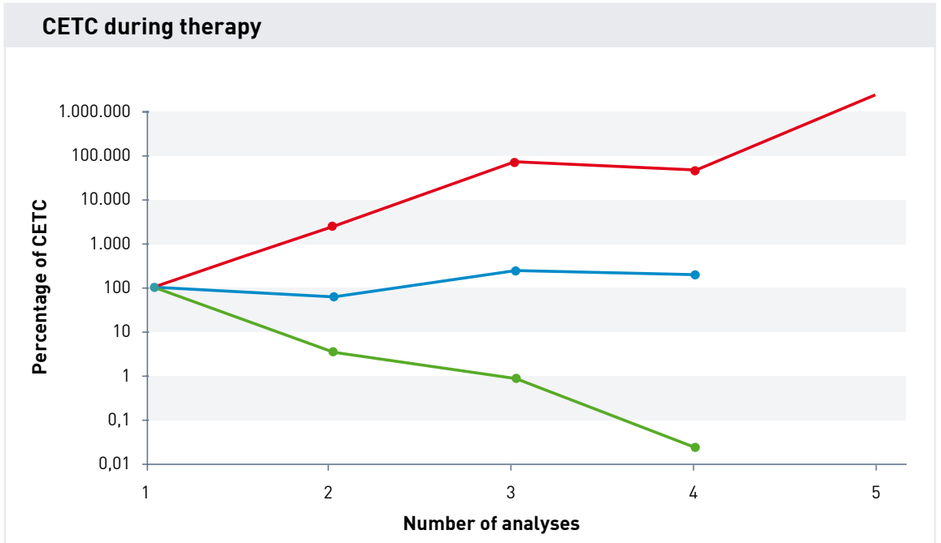
Interventions / further diagnostic steps can be agreed with the patient´s doctors based on the results.

maintrac diagnostics

2.2 Dynamics of the maintrac cell count

For disease progression, it is the dynamics of the cell numbers that are crucial, not individual values. The absolute CETC figure cannot be regarded as a metric for the stage of disease, but progression or degression of cell numbers reflect the course of disease or the effect of treatment.

Knowing the dynamics of the cell count can help to optimally adjust therapy. In patients, this may reduce the fear of an undetected relapse and improve the quality of life.



The cell count is repeatedly determined.

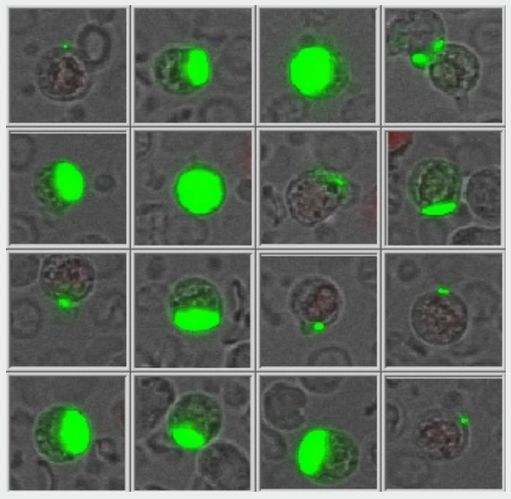
Increase in cell numbers	=	*therapy optimisation required*
Constant cell numbers	=	*positive course*
Decrease in cell numbers	=	*effective therapy*

2.3 Sensitive detection of epithelial cells

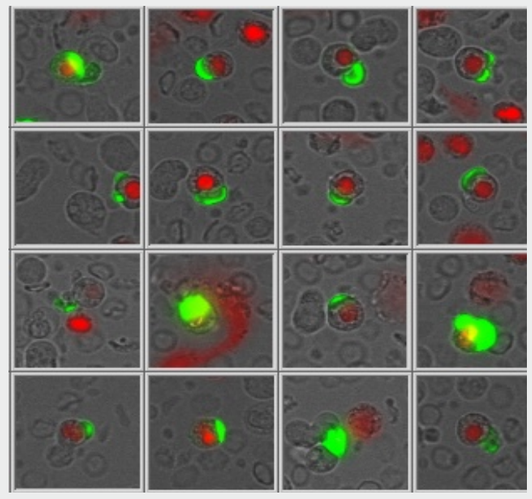
The intensity of the EpCAM signal on CETCs is highly variable, with strong and weakly labelled cells. With maintrac, CETCs that only weakly express EpCAM are also detected - they are assumed to be in the epithelial-mesenchymal transition (EMT). EMT is considered an important step towards the development of metastases.

The high sensitivity of maintrac allows for the almost complete and reproducible detection of CETCs. The cells detected are a representative sample of the overall CETCs in the blood.

Typical living and dead cells from a patient sample:



Living tumour cells are stained green.



Dead tumour cells are additionally stained red.

2.4 Specificity – Are all EpCAM-positive cells tumour cells?

The dynamics of the overall population of epithelial cells are crucial for the evaluation using maintrac as this alone can provide information about tumour activity.

Epithelial cells are not usually present in circulation. However, in addition to cancer, epithelial cells can also enter the bloodstream due to various other events such as:

- *Injuries*
- *Burns*
- *Inflammatory diseases*
- *Surgery*

In contrast to these cells, which are usually rapidly removed from the circulation within days to weeks, epithelial tumour cells can survive in tissue for years and recirculate in the bloodstream. These "dormant cells" are not eliminated by the immune system nor by most cytotoxic agents, and can also lead to a renewed breakout of the disease even after several decades.

A complete medical history allows better evaluation of the results.

2.5 Is there a cut-off or threshold level?

No threshold level is specified when determining the number of CETCs. Studies have shown that even small tumours may have a high number of CETCs that are released into the bloodstream, and vice versa. The dynamics of the cell count play the main role in the aggressiveness of the tumour.

Adjuvant therapy

During therapy, the number of CETCs in blood may fall below the limit of detection. This does not necessarily imply that there are no more tumour cells in the body. Therefore, treatment or monitoring should not be discontinued. Tumour cells can also survive in tissue. After the completion of therapy, these cells may reenter the bloodstream recirculate and the number of CETCs may once again multiply. maintrac is able to detect this increase early on.

Treatment of metastases

If the tumour has already metastasised and not yet been treated, usually a lot of cells are found in the blood. Effective therapy leads to an initial decrease in tumour cells. During the course of therapy, tumour tissue in the metastases dissolves. As a result, accompanying the reduction of metastases, cells may be released from the tumour. This may lead to a temporary increase in CETCs that should not result in the discontinuation of therapy. A change in therapy should only be considered if cell numbers continue to rise despite continuation of the therapy.

3. Use of maintrac diagnostics

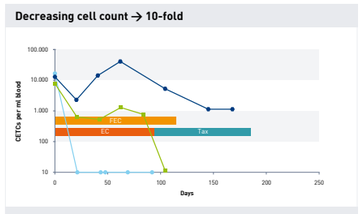
After diagnosis (e.g., of breast cancer), the established sequence in therapy usually comprises surgery, adjuvant chemotherapy, radiotherapy (if required), and, in hormone receptor-positive tumours, maintenance therapy with hormone blockers.

3.1 Monitoring during adjuvant chemotherapy

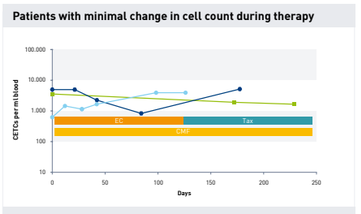
Various studies have shown a significant survival benefit from adjuvant chemotherapy. Adjuvant chemotherapy is now an integral part of current guidelines. It is aimed at destroying any remaining cells that left the tumour before or during surgery.

Despite adjuvant chemotherapy, 20-25% of breast cancer patients develop distant metastases within the first five years. It remains unclear which patients actually benefit from this kind of therapy.

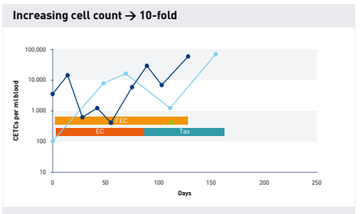
Characteristics of the tumour that has been removed are meant to predict the extent of which chemotherapy could be successful. The size of the tumour, lymph node involvement, degree of malignancy and molecular genetic characteristics of the tumour are prognostic or predictive markers that forecast the course of disease or prospective effectiveness of treatment with a degree of probability.



Three patients with decreasing cell count during therapy



Patients with mimimal change in cell count during therapy



Patients with increasing cell count by the end of therapy.

maintrac makes use of the remaining CETCs to monitor the success of chemotherapy.

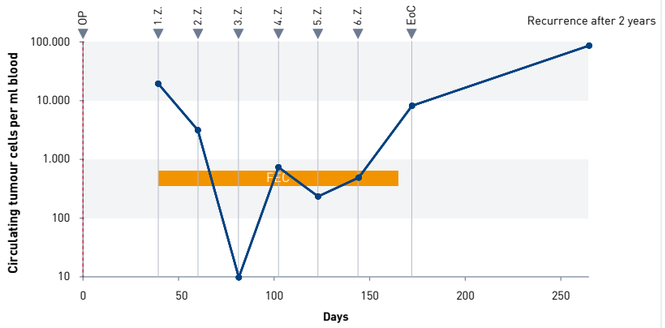
With maintrac the response to various therapeutic measures can be monitored in realtime since the dynamics of the cell count significantly correlate with tumour activity.

- *Patients whose CETCs considerably decrease or are completely eliminated have significantly higher chances of remaining relapse-free.[1]*
- *A decline of CETCs within 1-3 cycles is observed in patients that respond well to treatment.*
- *An increase during the course of therapy, even after an initial response, may indicate that tumour cells are still active.*

Swift treatment decisions can therefore be made that are only possible due to the quantitative detection of CETCs using maintrac.

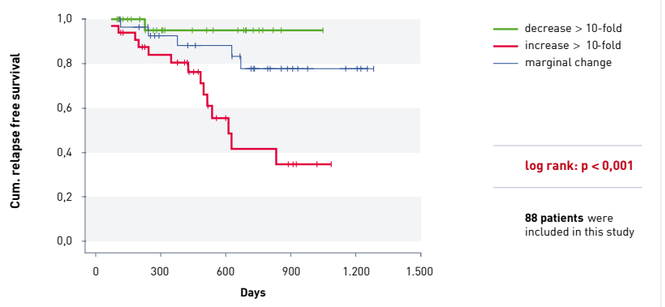
use of maintrac diagnostics

Example from a breast cancer patient



Typical progression of the cell count in a breast cancer patient with an initial response to therapy, followed by an increase in cell numbers and subsequent relapse.
In some patients, the first cycles of chemotherapy lead to a significant reduction of tumour cells, but the cell count increases again in the course of further therapy. Studies have shown that patients whose cells demonstrate these dynamics are most likely to relapse.

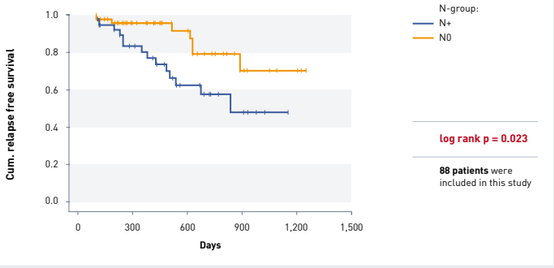
Adjuvant therapy in breast cancer



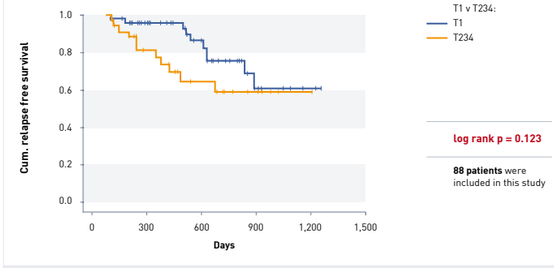
Kaplan-Meier plot: Relapse-free survival of patients whose cell numbers were reduced by chemotherapy (green curve: more than 90% of the patients survived without relapse for at least 4 years), of patients whose cell numbers were not affected by treatment (blue curve: approx. 80% survived for more than 4 years), and of patients whose cell count increased at the end of chemotherapy (red curve: less than 40% survived for 4 years without a relapse)[1].

The behaviour of CETCs outperforms the nodal status and the tumour size in significance[1]

Adjuvant therapy in breast cancer nodal status



Adjuvant therapy breast cancer tumour size



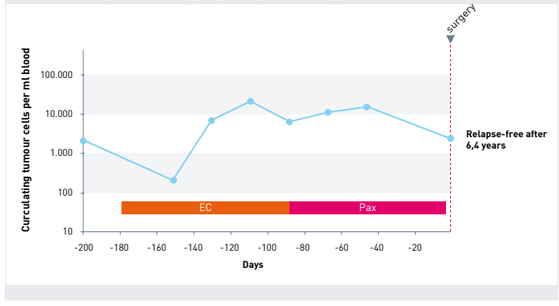
3.2 CETCs during neoadjuvant chemotherapy

A form of therapy currently preferred is neoadjuvant chemotherapy, in which an attempt is made to reduce tumour size before surgery. In addition to the positive news for the patient that the tumour has decreased, a complete disappearance is sometimes observed (pathologic complete remission - pCR). pCR is a favorable prognostic factor for individual patients undergoing treatment.

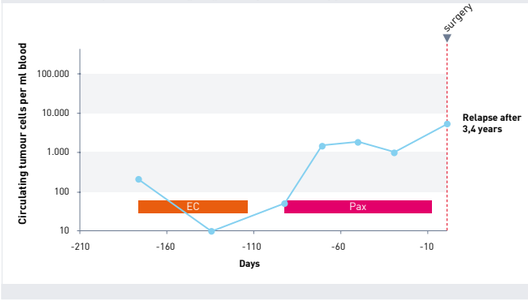
However, relapse is also observed in patients who initially achieve pCR. This may be explained by surviving cells that are released during neoadjuvant therapy for tumour reduction into peripheral blood. The result is an increase in CETCs.

Accordingly, neoadjuvant therapy should be continued until a reduction in peripheral circulating tumour cells is achieved.[4]

Breast cancer patients undergoing neoadjuvant therapy

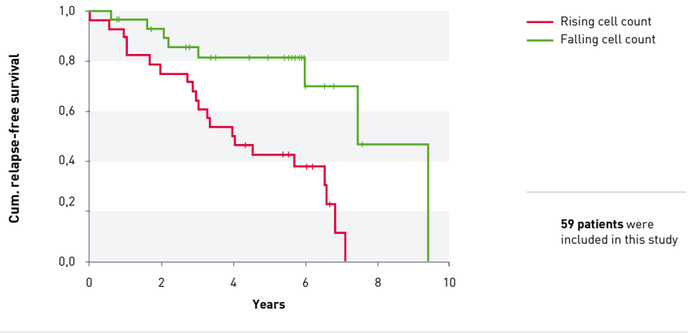


Breast cancer patients undergoing neoadjuvant therapy



Typical progression of the cell count in neoadjuvant therapy. Therapy initially effectively eliminates circulating cells. However, additional cells from the disintegrating tumour may be flushed into the bloodstream again.

Relapse-free survival - neoadjuvant therapy



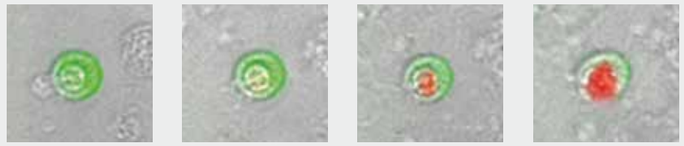
Kaplan-Meier estimator: Relapse-free survival of patients whose cell count decreased at the end of neoadjuvant therapy (green curve), and of patients whose cell numbers remained elevated at the end of chemotherapy (red curve).

use of maintrac diagnostics

3.3 Analysis of drugs before chemotherapy

maintrac identifies living CETCs; direct analyses can therefore be made of the cytotoxic effect of medication on CETCs.

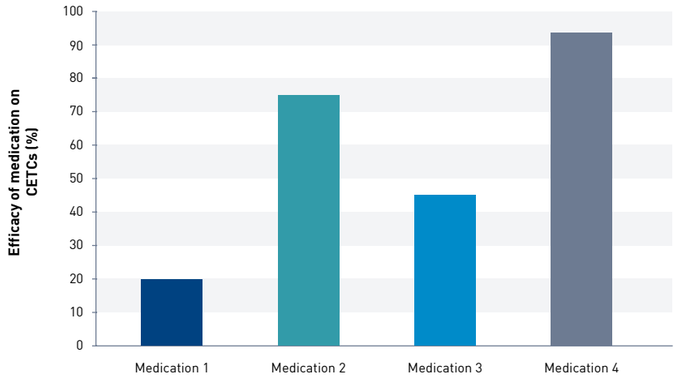
CETCs are exposed to various concentrations of the substances to be tested in vitro. At different points in time the death rate of the CETCs is calculated in comparison to a sample without the respective drug.



Example of a dying cell in the presence of a cytotoxic substance over time.

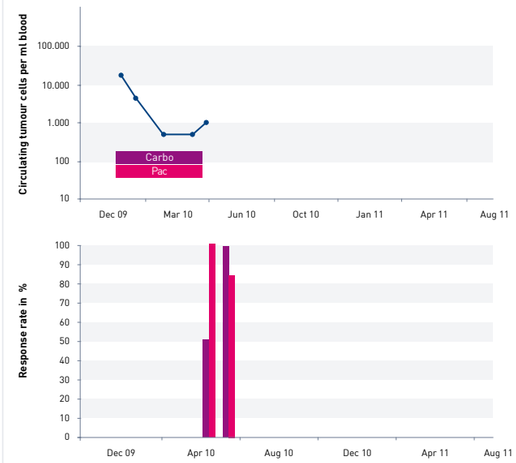
The degree of effectiveness of the drugs administered varies between patients. maintrac can identify the therapeutic agent with the highest individual probability of effectiveness.

Influence of different medications on CETCs

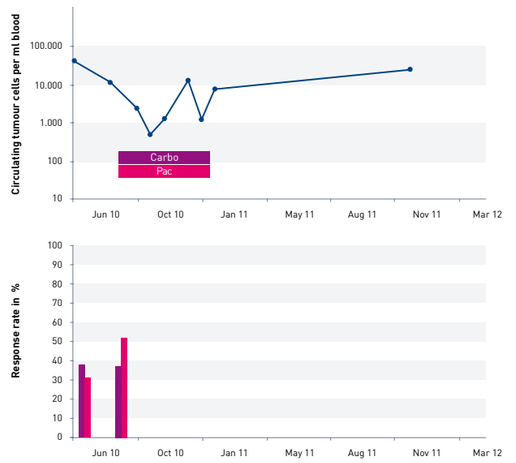


Medication 4 shows the highest efficacy on CETCs in this patient blood sample. This increases the chances for a sustainable and successful therapy.

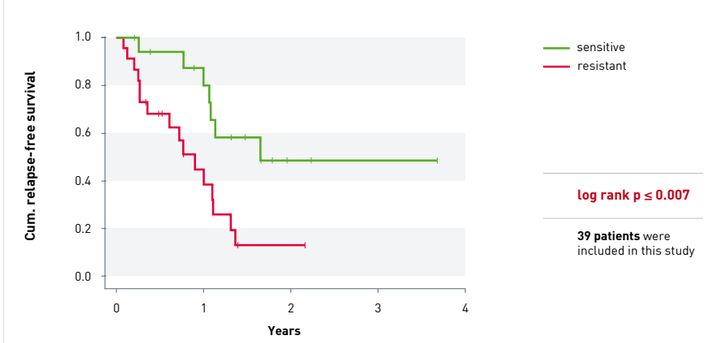
Therapy in ovarian cancer



Therapy in ovarian cancer



Chemosensitivity in ovarian cancer: sensitive vs. resistant tumour cells



Patients whose CETCs have a death rate of less than 50% before commencing therapy, have a significantly poorer prognosis.[5]

3.4 Therapy-related properties of tumour cells

Certain therapies are only useful if the respective tumour cells are susceptible to it (targeted therapy). So far, such analyses have mainly been performed on primary tumours. Changes due to therapy and recurrent tumour activity due to spontaneous events such as mutations or alterations of the phenotype have not been taken into consideration. Therapy-relevant properties of tumour cells may particularly change in the later stages of treatment.

Changes may affect the surface properties, gene expression, or DNA of the tumour cells.

These changes can be monitored on the CETCs, and hormone receptors such as oestrogen, progesterone, and androgen receptors or EGFR and HER2/neu can be determined. Gene mutations such as the HER2/neu amplification can be visualised using FISH (fluorescence in situ hybridisation).

Similar to tumour material, indications about the organ of origin of the metastases can be obtained based on the characterisation of CETCs in carcinomas of unknown origin.

maintrac enables the characterisation of various cell properties that are important for treatment decisions, and allows targeted adjustment of therapy:

Hormone receptors

AR *(androgen receptor)*
ER *(oestrogen receptor)*
PR *(progesterone receptor)*

Amplifications

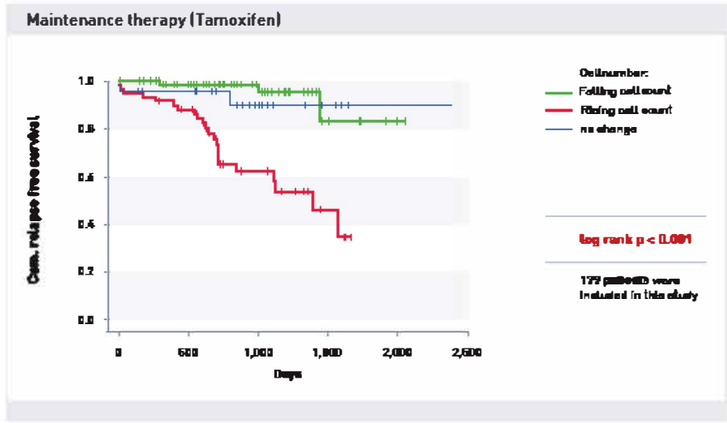
HER2/neu amplification
EGFR amplification

Others

B7-H3 / CD 276 *(inhibitor of T-cell activation)*
c-Kit *(stem cell factor receptor)*
EGFR *(epidermal growth factor receptor)*
IGF1R *(insulin-like growth factor 1 receptor)*
Ki67 *(growth fraction)*
Melan A *(melanoma antigen recognised by T-cells)*
PDL-1 *(programmed death ligand 1)*
PLAP *(placental alkaline phosphatase)*
PSA *(prostate specific antigen)*
PSMA *(prostate specific membrane antigen)*
Thomsen Friedenreich antigen
Tissue Factor
TUNEL *(detection of apoptosis)*
VEGFR2 *(vascular endothelial growth factor receptor 2)*

3.5 Monitoring during endocrine therapy

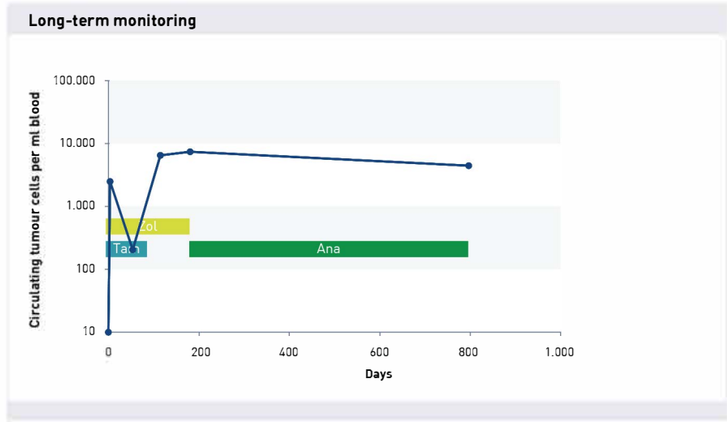
Long-term endocrine treatment with either the selective oestrogen receptor modulator Tamoxifen or aromatase inhibitors is of particular value in adjuvant therapy.



The dynamics of CETCs showed a high correlation with the clinical outcome. An increase in the cell count by a factor of 10 was significantly associated with relapse.

The decrease or increase in circulating epithelial cells during endocrine therapy is prognostically relevant for the assessment of therapeutic success.

In a study, 179 patients with primary breast cancer undergoing adjuvant endocrine therapy were monitored using maintrac and followed for up to five years.[2]



Case studies in which therapy was switched from Tamoxifen to aromatase inhibitors due to increasing CETC numbers, were extremely promising. The number of CETC decreased or often stabilised after a change in medication.

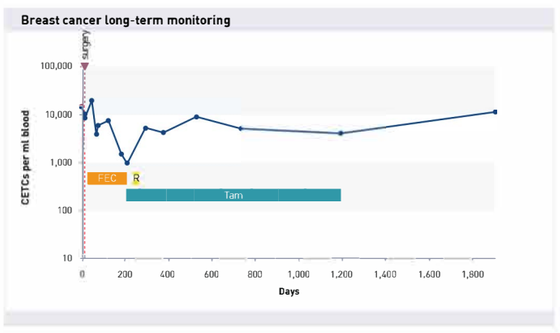
maintrac is ideally suited monitoring endocrine therapy in oestrogen receptor-positive patients, and taking appropriate action if increasing cell numbers are detected.

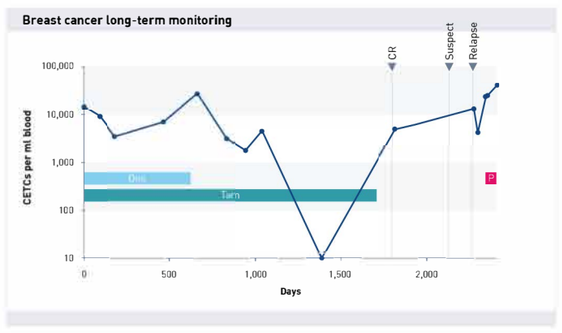
3.6 Decision-making aid at the end of endocrine therapy in breast cancer

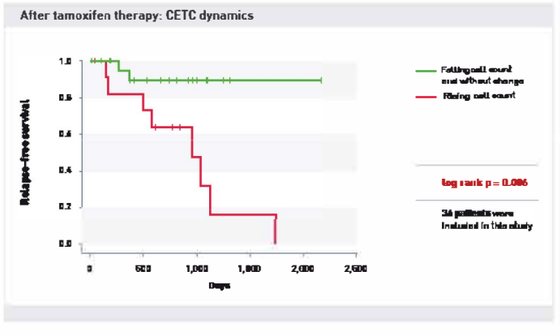
maintrac offers a basis for patients and therapists, for deciding whether endocrine therapy should be continued after five years.

After five years of endocrine therapy, breast cancer patients often question the benefical effects of a continuing treatment. Some of the side effects of endocrine therapy are serious and often lead to poor compliance. Many patients refuse to take the drug beyond five years. maintrac makes it possible to evaluate to what extent the patient may actually benefit from continuing endocrine treatment.

In hormone receptor-positive breast cancer patients who were followed up to seven years after discontinuation of maintenance therapy, an increase in CETCs after completion of therapy was found to be associated with a significantly higher relapse rate.[3]
Fluctuations in the number of CETCs have been detected in patients who take Tamoxifen irregularly. This indicates that resuming endocrine therapy if the number of CETCs goes up may be useful.







After completion of treatment, an increase in CETCs may suggest the wisdom of resuming therapy.

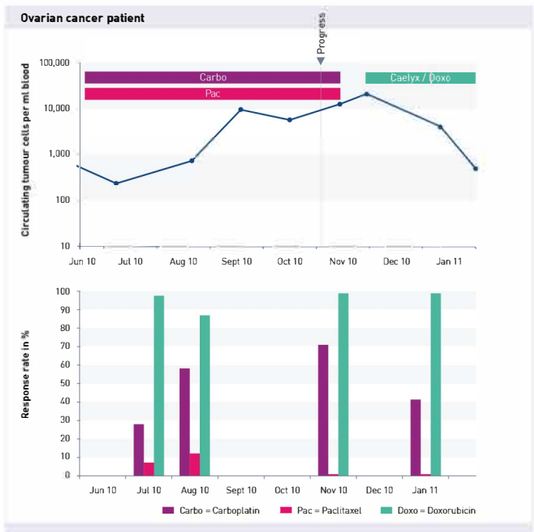
3.7 Therapy response if the tumour has metastasised

In cases of metastatic disease, maintrac allows you to identify the therapy most likely to be effective using circulating epithelial cells.

If metastases have developed systemic therapy is usually applied. The next question is which drug is the most effective to be administered. Drugs may not adequately reach the metastases as a result of a lack of functional lymphatic vessels and high intratumoural fluid pressure. The concentration of the drug achieved in the tumour is therefore often insufficient.

For the patient, it is beneficial if the most effective drug is individually administered instead of random by using less effective therapeutic agents.

With the help of maintrac, the degree of effectiveness of the proposed drugs can be analysed on the circulating tumour cells of patients before administration using maintrac. The therapeutic agent identified as most likely to be effective can then be administered.



Development of cell numbers of a female patient with ovarian cancer on conventional therapy using Carboplatin and Paclitaxel.

Despite therapy, there was an increase in cell numbers followed by the diagnosis of progression. A change in therapy to Caelyx resulted in a decrease in cell numbers. Simultaneously, the efficacy of the drug was also analysed in vitro, demonstrating Caelyx to be most effective.

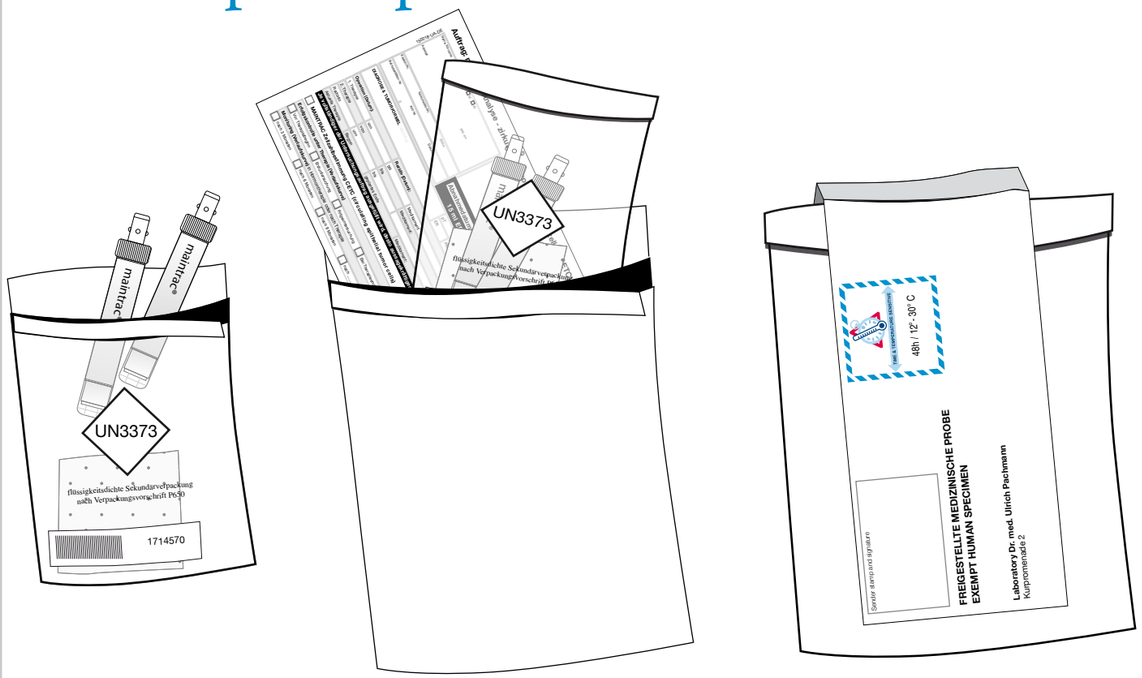
Reassesseing the efficacy, particularly with increasing cell numbers, is quite useful during the course of therapy. The development of resistance cannot be excluded.

Effective therapy is measured by a reduction in metastases. This may initially result in the release of tumour cells, as observed in neoadjuvant therapy.

While monitoring the circulating tumour cells in blood, an initial increase in cell numbers may be observed, comparable to that in neoadjuvant therapy, despite the approach chosen. This is not an indication that therapy should be discontinued. Therapy should be continued until a reduction in the number of peripheral circulating cells is observed.

If an increase in cell numbers is observed, the development of new metastases should always be considered, however, and further investigated.

4. Sample Shipment



Cell count and cell characterisation

- *Lab request form*

  (fully completed, signature of the patient)
- *15 ml of EDTA blood*

  (patient's name written on the tube as well as date of birth and sample collection date)

Shipment instructions

- *Shipment via courier (e.g., FedEx or DHL)*
- *Shipment has to arrive within 48 - 72 h*
- *Please send the blood sample together with the lab request form to the laboratory's address on the back of this brochure.*
- *Shipping at room temperature (do not refrigerate)*
- *Please mark the shipment as "**EXEMPT HUMAN SPECIMEN**".*

Drug testing

- *Please send a daily dose of the medication, or alternative remedies after consultation with our specialized medical laboratory.*
- *Lab request form*

  (fully completed, signature of the patient)
- *15 ml of EDTA blood*

  (sufficient for analysing up to 7 drugs; patient name written on the tube as well as date of birth and sample collection date)

Shipment information

- *Test results will usually be sent five days after receiving the sample.*
- *If you have any medical questions, please contact the specialized medical laboratory Dr. Pachmann. Phone: 0049 921 850 200 or email: maintrac@laborpachmann.de*

Further information is also available on the web: www.maintrac.com

5. Summary

maintrac detects and characterises CETCs, which serve as a success parameter and prognostic as well as predictive marker for personalised therapeutic decisions.

- ***Real-time monitoring using a 15 ml blood sample***
- ***Quantitative detection of CETCs***
- ***High sensitivity in patients with primary tumours***
- ***Monitoring of progress and efficacy during tumour therapy***
- ***Characterisation of therapy-relevant tumour properties***
- ***Identification of the most effective therapies***
- ***Long-term monitoring for up to 10 years and more after initial cancer diagnosis***
- ***Clinical benefit borne out by studies and experience.***

[1]. Pachmann, K. et al. Monitoring the response of circulating epithelial tumour cells to adjuvant chemotherapy in breast cancer allows detection of patients at risk of early relapse. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 26, 1208–1215 (2008).

[2]. Pachmann, K. et al. Assessing the efficacy of targeted therapy using circulating epithelial tumour cells (CETC): the example of SERM therapy monitoring as a unique tool to individualize therapy. J. Cancer Res. Clin. Oncol. 137, 821–828 (2011).

[3]. Pachmann Katharina & Schuster Stefan. Brustkrebs-Überwachung nach Ende der Hormontherapie: Bieten zirkulierende epitheliale Tumourzellen eine Entscheidungshilfe? gyne 36. Jahrgang, 28–32 (2015).

[4]. Camara, O. et al. The relevance of circulating epithelial tumour cells (CETC) for therapy monitoring during neoadjuvant (primary systemic) chemotherapy in breast cancer. Ann. Oncol. 18, 1484–1492 (2007).

[5]. Rudiger, N. et al. Chemosensitivity Testing of Circulating Epithelial Tumour Cells (CETC) in Correlation to Sensitivity and Clinical Outcome. J. Cancer Ther. 04, 597–605 (2013).