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Circulating Tumor Cell Detection

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Clinical Reference Manual for Circulating Tumor Cell Detection in Breast Cancer

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Preface

Circulating Tumor Cells (CTCs) are detached from a primary tumor and entered the bloodstream. These cells, which can survive in the circulatory system and proliferate in the appropriate environment, have the potential to lead to tumor recurrence and metastasis. CTCs can also exist in the form of cell clusters in the blood circulation system, known as Circulating Tumor Microemboli (CTM). CTM, possessing a strong potential for deterioration, is considered an independent risk factor for prognosis, recurrence, and metastasis. The detection of CTCs and CTMs has significant application for tumor screening, treatment, and prognosis.

The application of CTCs in breast cancer started relatively early. Breast cancer is the first cancer that showed clear benefits from the results of CTC detection. In 2004, the US FDA approved the application of CTC detection in breast cancer, and in 2012, China's NMPA also approved the application of CTC detection in breast cancer. As a convenient blood test, CTC detection can capture and evaluate circulating tumor cells at any time to determine the prognosis of breast cancer patients.

In 2010, the AJCC Cancer Staging Guidelines included CTCs in the TNM staging system of breast cancer as a new M stage (distant metastasis) standard, listed as cMo (i+) stage. In 2018, CTC was listed as another breast cancer prognosis evaluation indicator after ER/PR, HER2, Ki67, and tumor histological grading: late-stage clinical breast cancer peripheral blood CTC>5/7.5ml, early-stage clinical breast cancer peripheral blood CTC≥1/7.5ml suggests poor prognosis. CTC has officially entered the tumor staging system, providing comprehensive guidance for diagnosis and prognosis evaluation. In 2017, the NCCN Breast Cancer Clinical Practice Guidelines introduced the cMO (i+) stage, confirming the importance of CTCs. In 2019, China's CSCO Breast Cancer Clinical Diagnosis and Treatment Guidelines stated that CTCs can reflect the situation of tumor tissue and replace tissue samples for pathological diagnosis, disease monitoring, etc., and can be used to judge the prognosis. This highlights the importance of CTCs in managing breast cancer patients.

Application of Circulating Tumor Cells (CTC) Detection in the Diagnosis and Treatment of breast cancer

Assessment on the risk of recurrence after breast cancer surgery.

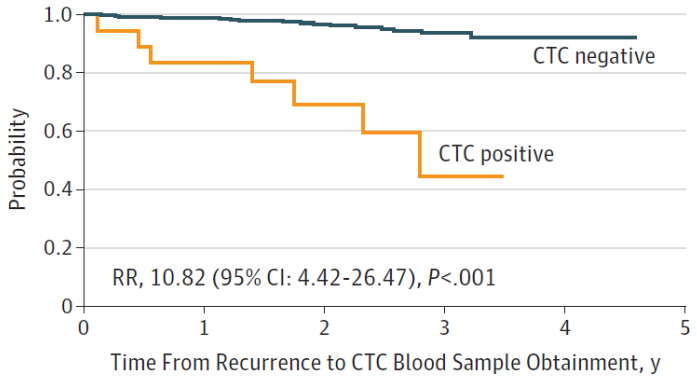
The recommended testing timing and frequency for CTC as follows:
1.The first CTC test within one week before surgery.
2.The second CTC test from one week to one month after surgery.
3.After the surgery and adjuvant therapy, consider conducting a CTC test every 6-12 months. After 5 years post-surgery, consider conducting a CTC test every 2-3 years.
4.If CTCs counts detected after surgery exceeds the minimum value, consider new or adjuvant therapy.
5.If new or adjuvant therapy is conducted after surgery, please refer to the following text for the detection frequency during the adjuvant therapy.
6.If CTCs counts during the follow-up period after surgery exceeds the minimum value, consider strengthening the follow-up frequency and retesting CTC. If CTCs counts of two consecutive tests is higher than the threshold, it suggests that the patient has a higher risk of recurrence and should consider more detailed examinations or preventive interventions to reduce the risk of recurrence/metastasis.

Patients undergoing breast tumor resection, whether it is total mastectomy or breast-conserving surgery, have a risk of metastasis after surgery. Clinical doctors comprehensively evaluate the risk of recurrence or metastasis of patients through multiple factors such as pathological staging, surgical margin, lymph node infiltration, tissue typing, and age of disease. However, the clinical research results show that these evaluation indicators are insufficient, especially most of the indicators are detected during surgery, and there is a lack of effective biomarkers to monitor the metastasis of tumors for patients who have been followed up for several years or even decades after surgery. At the same time, the current international and domestic guidelines for the monitoring and follow-up of breast cancer patients after surgery include 6-12 months of medical history inquiry and routine physical examination, as well as possible breast imaging examination. These routine examinations have a weak detection capabilities for small recurrent or metastatic lesions. Therefore, patients are often diagnosed as postoperative recurrence/metastasis, which is already in the late stage and has lost the possibility of radical cure.

Circulating Tumor Cells (CTC) are a new type of biomarker that reflects the proliferation status, invasion ability, and the ability to escape apoptosis and immune killing of tumors. Detection of CTCs in the blood of breast cancer patients indicates that the patient has had a trace amount of hematogenous metastasis, and the risk of recurrence or metastasis is higher than that of patients who have not detected CTCs. For these patients, more close observation, lifestyle changes, and possible endocrine therapy should be considered.

In 2018, a retrospective study was published in 《JAMA Oncology》. They selected 547 stage II-II breast cancer patients with lymph node infiltration or other high-risk factors. CTC detection was performed within 4.5-7.5 years after chemoradiotherapy. The study found that the recurrence risk of CTC-positive patients is 13.1 times that of CTC-negative patients. Therefore, for HR-positive high-risk breast cancer patients after 5 years of surgery, CTC detection can provide an independent prognosis evaluation for clinical recurrence after surgery.

Figure 2. Time to Recurrence by Circulating Tumor Cell (CTC) Assay Result Among Patients With Hormone Receptor–Positive Breast Cancer



No. at risk

CTC negative	335	306	211	102	16	0
CTC positive	18	13	7	3	0	0

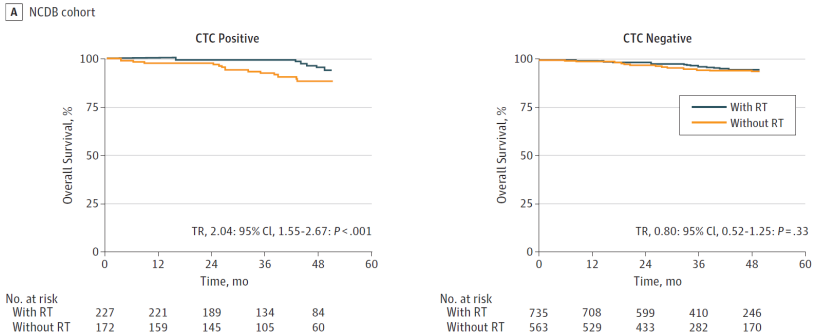
RR indicates relative risk.

The necessity and treatment effect evaluation of postoperative radiotherapy for breast cancer

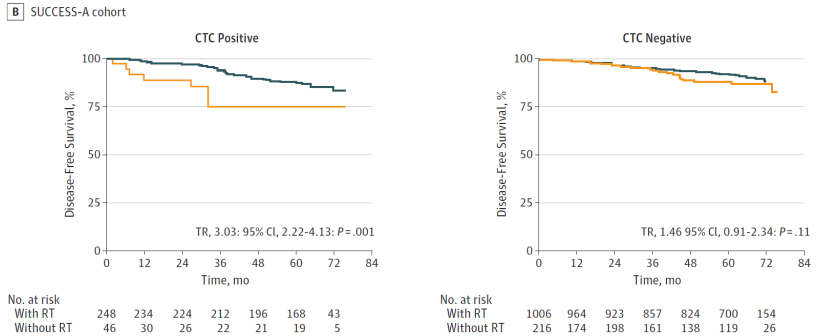
Clinical CTC detection time point and frequency recommendation:
1.The first CTC detection within one week before surgery.
2.The second CTC detection one week to one month after surgery, before radiotherapy to assess the patient's risk.
3.After radiotherapy, consider performing a CTC detection every 6-12 months: After 5 years after surgery, consider performing a CTC detection every 2-3 years.
4.If adjuvant therapy is performed after surgery, please refer to the following text for the detection time point and frequency during the adjuvant therapy period:
5. After surgery, if CTCs counts detected exceeds the minimum value, recommend to perform whole breast radiotherapy. After radiotherapy, CTC is recommended to be use. If CTCs counts are still higher than the minimum value, consider preventive intervention to reduce the risk of recurrence/metastasis.
6. If CTCs counts during the follow-up period after surgery exceeds the minimum value, consider strengthening the follow-up frequency and retesting CTC. If CTCs counts in two consecutive tests is higher than the threshold, it suggests that the patient has a higher risk of recurrence and consider more detailed examinations or preventive interventions.

Clinical doctors need more clinical evidence to decide whether to perform radiotherapy. In August 2018, scientists from the United States and Germany published a new study in the authoritative journal JAMA Oncology in the field of tumors. The research results show that the state of CTC can be used to accurately predict the radiotherapy effect of early breast cancer, especially patients with breast-conserving surgery. The study included 1697 cases from the National Cancer Database (NCDB) and 1516 cases of breast cancer patients from the 3-phase multicenter SUCCESS clinical trial (pT1-pT2 stage and pNO-pN1 stage CTC status is known), a total of 3,213 cases. The accelerated failure time model of multivariate parameters is used to evaluate the relationship between CTC status and radiotherapy (RT) and survival rate. Among them, the research results of the American NCDB team are that among CTC-positive patients, the overall survival rate of the radiotherapy group is significantly better

than the non-radiotherapy group (TR, 2.04, 95%; p＜0.001), while among CTC-negative patients, receiving radiotherapy or not is not significantly correlative (TR，0.80,0.95%; p=0.33) (Figure below).



The results of the German SUCCESS cohort study confirmed similar findings. CTC-positive patients receiving radiotherapy demonstrated not only significantly improved DFS (TR, 3.03; p<0.001) but also improved LRFS (TR, 2.73; p<0.001) and OS (TR, 1.83; p=0.003) compared to the non-radiotherapy group. Conversely, there was no significant correlation between radiotherapy in CTC-negative patients and DFS, LRFS, or OS. Moreover, CTC-negative patients exhibited significantly extended DFS (TR, 2.18; p<0.001), LRFS (TR, 2.12; p=0.008), and OS (TR, 1.97; p=0.007) compared to CTC-positive patients. Patients who received radiotherapy experienced significantly prolonged DFS (TR, 2.89; p<0.001), LRFS (TR, 4.35; p<0.001), and OS (TR, 1.95; p<0.001) (Figure below).



This study marks the first confirmation that CTCs can predict the local treatment response in early breast cancer. Incorporating CTC detection for personalized assessment of patients' recurrence risks could better serve "precision medicine," reducing treatment side effects for low-risk patients while ensuring efficacy for high-risk patients.

Prediction and evaluation of neoadjuvant therapy (NCT) in breast cancer:

Recommended clinical CTC testing timing and frequency:

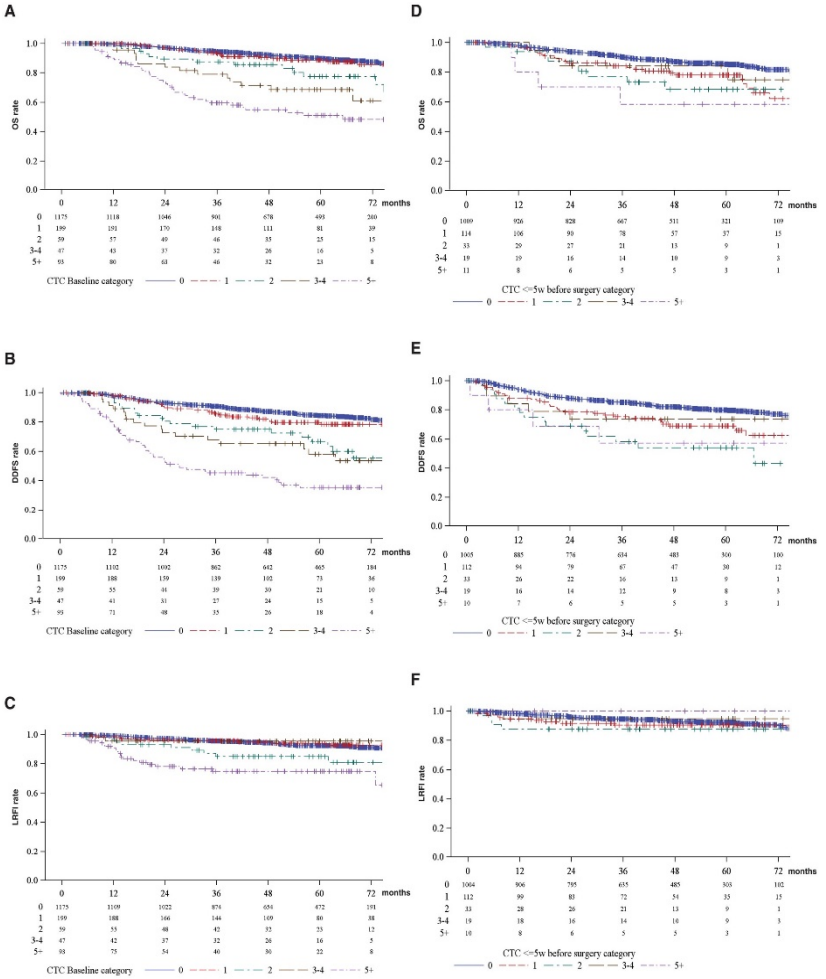
1.Baseline CTC detection within one week before commencing NCT.

2.Second CTC detection between one week after completing NCT and one day before surgery.

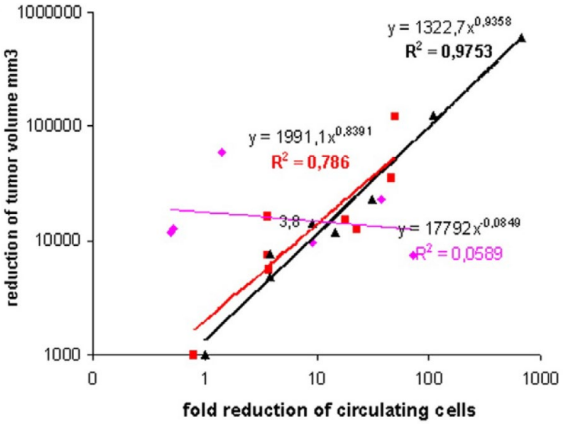
3.When deciding the necessity of NCT treatment for patients, recommend to consider CTC test results. If exceeding the threshold, NCT treatment is recommended. Evaluating NCT efficacy and predicting patient prognosis also rely on CTC test results.

CTC test results can assess recurrence risk in breast cancer, notably for higher-risk patients. For example, those with larger tumors, axillary lymph node metastasis, HER2-positive, or triple-negative breast cancer should consider neoadjuvant drug therapy before surgery to reduce postoperative risks. Additionally, patients considering breast-conserving surgery may also contemplate neoadjuvant therapy based on tumor size. However, in cases of HER2-positive or triple-negative tumors, determining the tumor size threshold for benefiting from neoadjuvant therapy becomes essential. (This might include tumor sizes larger than 3 cm but smaller than 5 cm, or situations where imaging suggests axillary lymph node metastasis, but the biopsy results are inconclusive, or where certain reasons prevent biopsy for qualitative determination of the axillary lymph nodes.) In such cases, clinical practitioners might require other novel biomarkers to assess high-risk factors and determine whether neoadjuvant therapy rather than direct surgery is more appropriate. As previously mentioned, detecting CTCs in peripheral blood objectively reflects tumor micro-metastasis and serves as a key predictive factor.

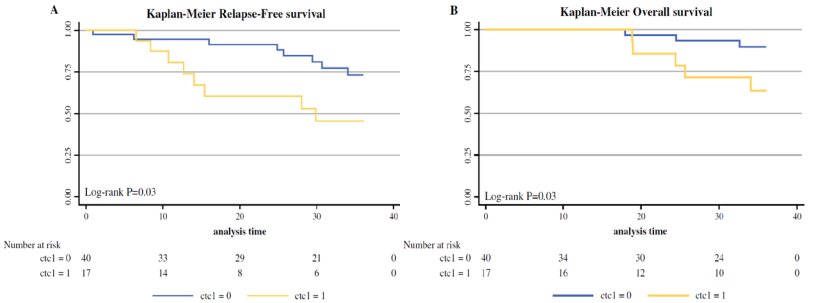
A meta-analysis in 2018 in 21 breast cancer patients undergoing neoadjuvant therapy. 1574 patients underwent CTC testing before NCT treatment, while 1200 patients were tested for CTCs before surgery following NCT completion. Patients with detected CTCs before NCT had shorter OS, DFS, and LRFS. Moreover, this risk significantly increased with higher detected CTC counts. The figures below show similar trends in CTC detection before surgery.



Evaluating neoadjuvant therapy effectiveness poses challenges, particularly for non-pCR patients. Recent studies on CTCs in neoadjuvant breast cancer therapy have emerged. In 2005, a research team from Jena University, Germany, conducted a study in BCR examining CTC count changes in 30 breast cancer patients before and after neoadjuvant chemotherapy (prior to treatment and after 3-4 cycles). They found that the reduction in CTC counts accurately predicted tumor shrinkage during subsequent surgery as shown in Figure below).



In 2015, a research team at MD Anderson Cancer Center tested 57 triple-negative breast cancer patient's post-neoadjuvant therapy. They found that those with positive CTC results had notably reduced RFS (recurrence-free survival) and OS (overall survival).



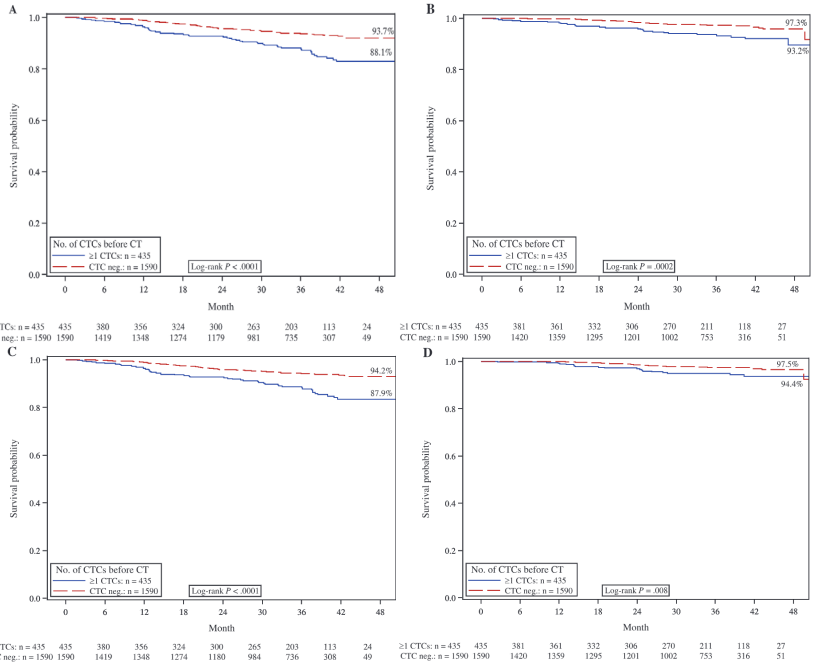
Breast Cancer Adjuvant Chemotherapy Efficacy Prediction and Evaluation

Clinical Recommendations for CTC Timing and Frequency:

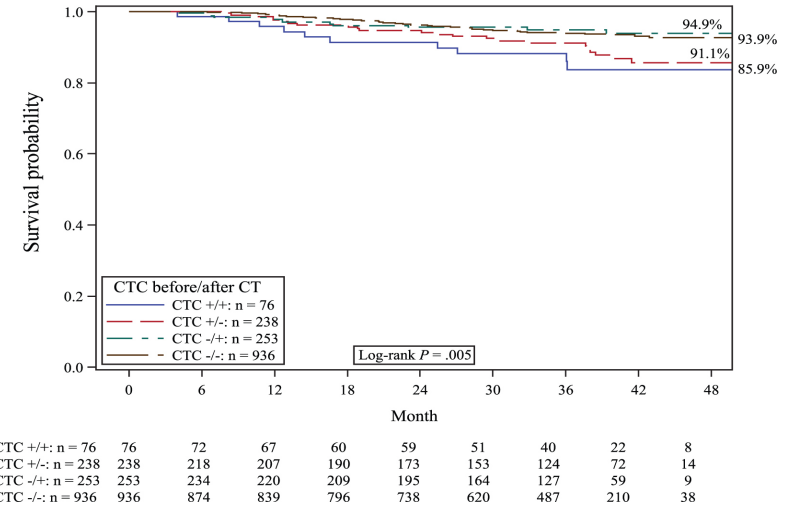
1. Perform the first CTC test between one week post-surgery and one day pre-adjuvant chemotherapy: Consider intensified treatment if CTC count exceeds the threshold.
2. Conduct a second CTC test after completing the same adjuvant chemotherapy regimen: Extend the treatment duration if CTC count remains above the threshold.
3. After completing all adjuvant therapies, monitor patients for detected CTC counts beyond the threshold during follow-up: Intensify the follow-up frequency and conduct CTC retesting. Consistent detection of CTC counts above the threshold indicates higher risk of recurrence, warranting detailed examinations or preventive interventions to reduce recurrence/metastasis risks.

Except for in situ carcinoma, most invasive breast cancer patients are recommended for postoperative adjuvant therapy. For those with HER2-positive tissue subtypes, considering postoperative chemotherapy combined with targeted therapy is advised. For patients with triple-negative breast cancer and those meeting specific criteria (positive axillary lymph nodes, tumor diameter >1cm, lymph node negative but with vascular or lymphatic infiltration, high Ki67 or low differentiation), postoperative adjuvant chemotherapy is recommended. HR-positive patients without the high-risk factors should consider adjuvant endocrine therapy. However, clinicians often encounter low-risk patients experiencing recurrence/metastasis post-adjuvant therapy, indicating that current risk prediction indicators are incomplete.

Clinical teams at the University of Munich analyzed partial data from the SUCCESS trial, including 2026 early breast cancer patients' pre-neoadjuvant therapy and 1492 early breast cancer patient's post-adjuvant chemotherapy. The study revealed that the presence of CTCs signifies shorter DFS, distant metastasis-free survival, and overall survival.



Patients with elevated CTC counts above the threshold pre- and post-treatment exhibited significantly worse prognosis (Figure below).



CTC +/+: n = 76	76	72	67	60	59	51	40	22	8
CTC +/-: n = 238	238	218	207	190	173	153	124	72	14
CTC -/+: n = 253	253	234	220	209	195	164	127	59	9
CTC -/-: n = 936	936	874	839	796	738	620	487	210	38

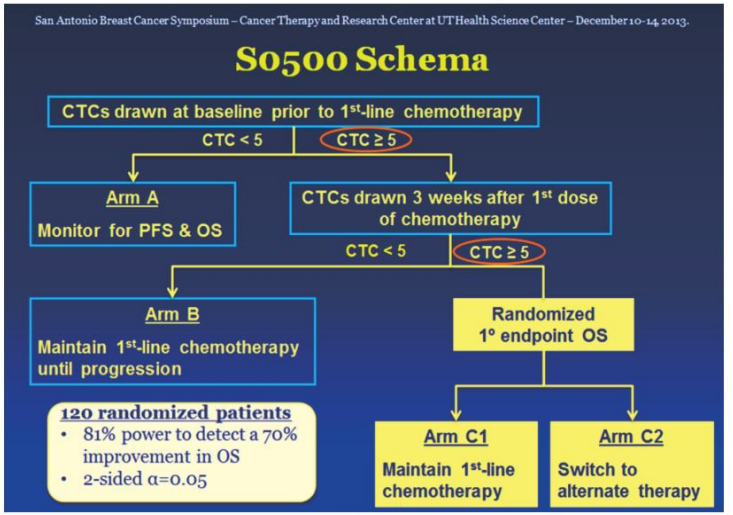
Prediction and Prognostic Evaluation of Advanced Breast Cancer Salvage Treatment Efficacy

Clinical Recommendations for CTC Timing and Frequency:

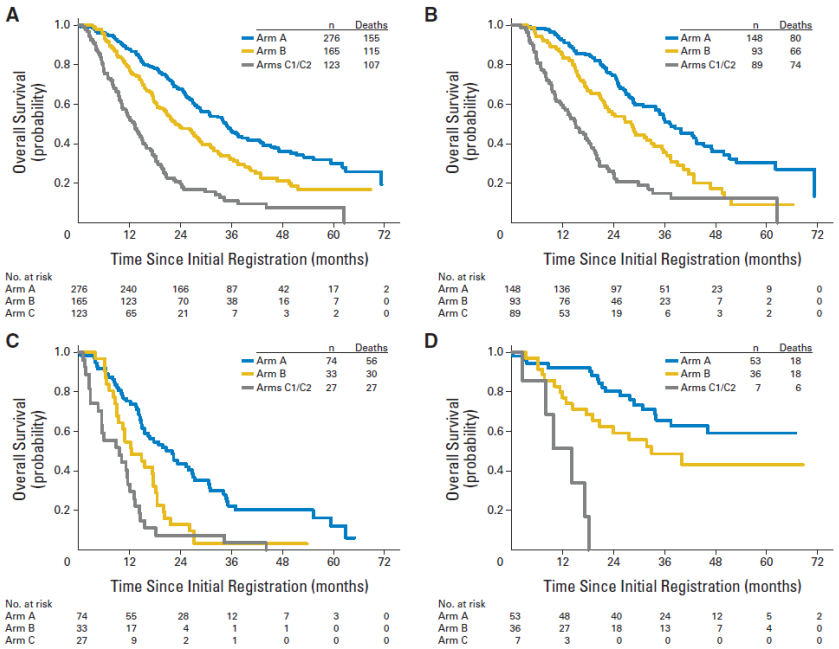
1. Baseline CTC testing one week before systemic therapy: Consider poor prognosis if CTC count exceeds the threshold. Evaluate the necessity for continued treatment or explore new drug clinical trials.
2. Second CTC test after completing two treatment cycles and before the third: If both CTC tests show counts above the threshold, it suggests poorer treatment efficacy, prompting consideration of treatment plan alteration. If the second CTC test, although still above the threshold, shows a significant decrease (over 50%) from baseline, it indicates the current treatment's effectiveness. Consider maintaining the treatment plan and perform a third evaluation.
3. If changing the treatment plan, conduct a CTC test after two cycles under the new plan, comparing the results with the previous two tests. If CTC count remains above the threshold, reconsider the necessity of continued treatment or explore new drug clinical trials.
4. After completing all systemic therapies, if CTC count exceeds the threshold during follow-up, it indicates poor treatment efficacy and prognosis. Reevaluate the necessity for continued treatment or explore new drug clinical trials.

In China, the proportion of breast cancer patients diagnosed at an advanced stage exceeds that in Western populations. This includes patients with advanced-stage tumors at initial diagnosis and those with tumor recurrence/metastasis after surgery, many of whom have lost the chance for surgery and require systemic salvage treatments, including endocrine therapy, chemotherapy, and targeted therapy. When making decisions regarding clinical treatment and evaluating treatment efficacy, clinicians require more information to determine treatment plans, treatment intensity, and patient benefit.

2004 study with 177 metastatic breast cancer patients showed that pre-treatment CTC quantity predicted prognosis. Patients with <5 cells/7.5mL of blood had better PFS and survival than those ≥5 cells/7.5mL, indicating shorter DFS, metastasis, and survival.



A research team conducted a SWOG SO500 trial with 595 advanced breast cancer patients. Those below the CTC threshold pre-treatment fared best. Decrease below the threshold post one cycle indicated better prognosis, while persistent high counts indicated the worst prognosis (see figure below).



A: All enrolled patients; B: HR-positive patients; C: HER2-positive patients; D: Triple-negative breast cancer patients.

HER2 and Efficacy Prediction of Targeted Therapy in Advanced Breast Cancer

Clinical Recommendations for CTC Timing and Frequency:

1.Routine HER2 immunohistochemistry and fluorescence in situ hybridization (FISH) for patients with biopsy tissue. HER2-positive patients undergo comprehensive treatment involving HER2-targeted drugs.

2.For relapsed or progressing HER2-positive breast cancer, re-evaluate HER2 status via re-biopsy before reinitiating HER2-targeted treatment, especially for patients with recurrence after one year of treatment.

3. Patients unable to undergo re-biopsy should consider CTC HER2 testing to assess HER2 status. Prefer CTC HER2 FISH first, followed by immunohistochemistry or immunofluorescence.

4. If primary HER2-positive breast cancer patients experience recurrence after treatment or if HER2-positive advanced breast cancer patients show progression after one year of HER2-targeted therapy, all negative CTC HER2 results suggest limited benefit from HER2-targeted treatment.

HER2-positive advanced breast cancer typically includes chemotherapy combined with HER2-targeted therapy (trastuzumab, pertuzumab). However, resistance often emerges during HER2-targeted therapy, leading to second-line options like lapatinib, neratinib, T-DM1, or chemotherapy. Reassessing HER2 status during treatment evolution is critical, especially if fresh tissue from re-biopsy is unattainable.

In 2016, a Chinese team at 307 Hospital published a study in BMC Cancer involving CTC testing and CTC HER2 status analysis in 101 HER2-positive metastatic breast cancer patients planning HER2-targeted therapy. They found CTC HER2 status accurately predicted the efficacy of HER2-targeted treatment, even more precisely than primary tissue samples of over a year. This study underscores the importance of re-evaluating HER2 status for HER2-positive patients experiencing recurrence after one year.

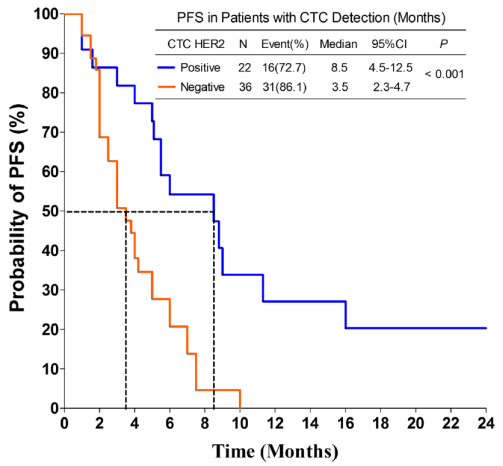


Fig. 2 Kaplan-Meier PFS plots of CTC HER2-positive and-negative patients. PFS was calculated from the time of the baseline blood draw. The coordinates of the dashed lines indicate the median survival time

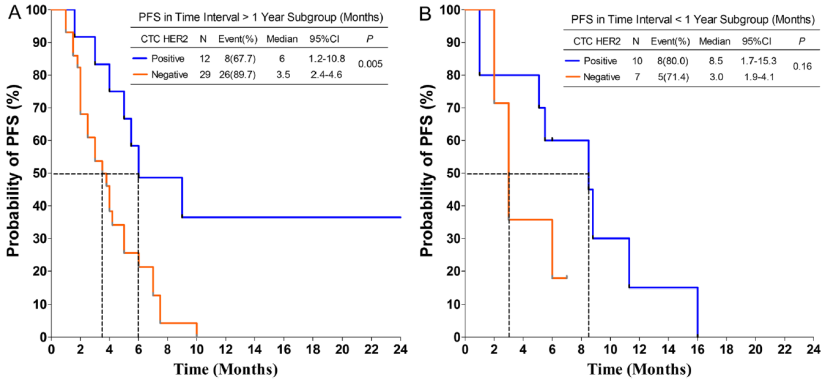


Fig. 3 Kaplan-Meier PFS plots of CTC HER2-positive and -negative patients in subgroups of time interval (between tissue and CTC HER2 testing) > 1 year (**a**) and < 1 year (**b**). PFS was calculated from the time of the baseline blood draw. The coordinates of the dashed lines indicate median survival time

Afterword

circulating tumor cell (CTC) detection plays a crucial role in various stages of breast cancer and during different treatments. Its applications and advantages include predicting progression-free survival and overall survival, earlier prediction of overall survival compared to imaging, real-time monitoring, rapid assessment of drug efficacy within 1-2 weeks, aiding prognosis and staging, facilitating individualized drug selection and treatment planning.

CTC detection seamlessly integrates with traditional pathology techniques like immunohistochemistry, immunofluorescence, and supports emerging diagnostic technologies (e.g., CNV, TMB, DGE) to acquire comprehensive tumor immunology and genetic information, advancing tumor diagnostics, and clinical research.

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