# A novel *ex vivo* culture workflow to enrich and expand circulating tumor cells (CTCs) from patients with Stage III/IV Breast Cancer (BCa) (LB-370)

Qiang Zhang[1], Youbin Zhang[1], Lisa Flaum[1], Lorenzo Gerratana[1], Brian Helfand[2] William Gradishar[1], Leon Platanias[1], and Massimo Cristofanilli[1]*

1. Departments of Medicine, Division of Hematology/Oncology, Robert H Lurie Comprehensive Cancer Center, Feinberg School of Medicine, Northwestern University, Chicago, IL ; 2. Northshore University Heathsystem, Evanston, IL.

## Introduction

Circulating tumor cells (CTCs) play a critical role in BCa metastases. It has been proposed that the isolation, ex vivo culture, and characterization of CTCs may provide an opportunity to noninvasively monitor the changing patterns of drug susceptibility in individual patients as their tumors acquire new mutations. Molecular and genomic characterization of CTCs may help to predict BCa prognosis and identify which patients may derive treatment benefit, especially for those with metastatic or recurrent disease.

However, CTCs are present at very low concentrations in the peripheral blood of patients with solid tumors, and their enrichment and expansion is technically challenging. Most methods yield low numbers of partially purified CTCs that are fixed before isolation, damaged during the cell purification process, or irreversibly immobilized on an adherent matrix. Here we describe an ex vivo CTC culture workflow to expand CTCs for patients with Stage III/IV BCa. These CTCs can be genotyped and functionally characterized over the course of therapy and they have the potential to identify treatments that most effectively target the evolving mutational profile of the primary tumor.

## FDA Approved CellSearch System

We evaluated cases using the FDA approved semi-automated fluorescence CELLTRACKS ANALYZERII® System (Menarini). We use CTC Kit with antibodies targeting EpCAM for capturing CTCs, Anti-CK-PE for the epithelial cells, DAPI stains the cell nucleus, anti-CD45-APC for leukocytes. The CTCs were classified based on phenotype as $CK^+$, $EpCAM^+$, $DAPI^+$ and $CD45^-$ (Figure 1)

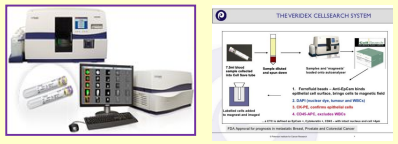

Figure 1: Procedure of CTC Enumeration by CellSearch System

## Materials and Methods

**Blood Samples:** Duplicate whole blood samples (7.5ml/each) were collected in EDTA tubes from 16 patients with stage III/IV BCa patients before or after systemic therapy and stored at 15-30 ℃ until processing.

**CTC Enumeration:** CTC Enumeration was performed on one of the samples from each patient using the CELLSEARCH® System to confirm the presence of >=5 CTCs per 7.5mL of blood.

**CTCs Enrichment:** CTCs enrichment was performed on the second sample from each patient using the Parsortix™ System (ANGLE PLC), a microfluidic based technology that captures rare cells based on size and deformability. Using a proprietary Cell Separation Cassette with a critical gap size of 6.5µm, the Parsortix System captured viable CTCs from the blood inside the cassette. The CTCs were then harvested by inverting the flow direction and flushing the CTCs from the cassette into 200µL of PBS (Figure 2).

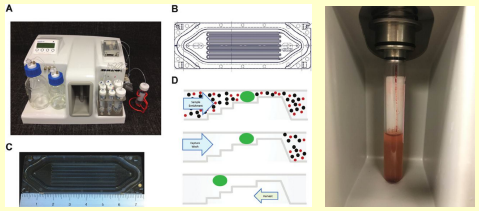

Figure 2. Procedure of CTCs sorting and enrichment by Parsortix System

**Tissue Culture:** The harvested CTCs were transferred and grown in ultralow attachment plates containing RPMI-1640 medium supplemented with EGF (20ng/ml), basic FGF (20ng/ml), B27 (10ml), and 1×Antibiotic-antimycotic. CTCs were cultured in a humid 37ºC incubator with 5% $CO_2$ and 4% $O_2$. CTC counting was performed every week using a hemocytometer. DNA isolation of the CTC culture was performed on Day 21 using DNAzol.

## Results

• 16 patients had >=5 CTCs as defined by the CELLSEARCH System at the time of the blood collection.

•Using the Parsortix System, highly purified CTCs (ranging in number from 300 to 17,250 cells) were isolated from the blood samples for each patient and placed into culture (day 0).

•During the first week, the CTCs could be expanded to 3.5 - 5.5 fold, and then to 9.5 - 22.5 fold during the second week, to maximum amount of 112,500 within 14 days.

•The isolated CTCs were maintained without altered morphology at the same concentrations until Day 21 (Figure 3). An average of 186ng and 1200ng of DNA could be isolated and purified from ~10,000 and ~110,000 cultured CTCs, respectively.

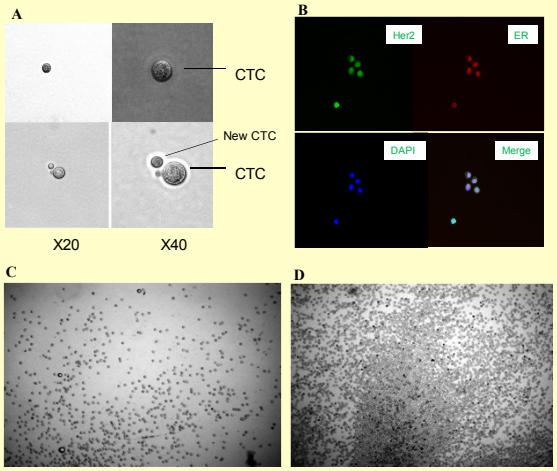


**Figure 3: Expansion of CTCs. A.** CTCs collected by Parsortix system. **B.** CTCs were confirmed to be HER2 and ER positive by immunofluorescence staining. **C. D.** CTCs could be expanded to 3.5 - 5.5 fold in first week, and then to 9.5 - 22.5 fold during the second week, to maximum amount of 112,500 within 14 days.

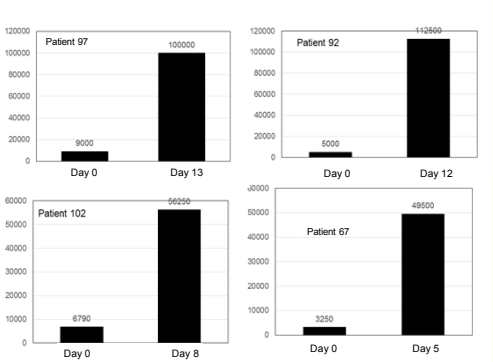


**Figure 4. Expansion of CTCs from individual patient**

## Conclusions

Using a combination of standard immune magnetic selection and size-dependent isolation:

- We identified and optimized a workflow for the recovery and culturing of CTCs from Stage III/IV BCa patients
- This approach allows for effective *ex vivo* culture.
- With further optimization, this strategy may be utilized for organoids development, in-vtiro drug testing, representing an important tool for personalized precision therapy.

## Acknowledgement

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