EpCAM-Independent Isolation of Circulating Tumor Cells with EMT Phenotype in Patients with Primary Breast Cancer Treated with Primary Systemic Therapy

Fanny Le Du[1,2], Takeo Fujii[1], Darren Davis[3], Weiguo Wu[3], Jackson A Summer[1], Mariana Chavez-MacGregor[1], Carlos H. Barcenas[1], Abenaa M. Brewster[1], James L. Murray[1], Ricardo H. Alvarez[1], Vicente Valero[1], Debu Tripathy[1], James M. Reuben[4], and Naoto T. Ueno[1]

[1] Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [2] Department of Medical Oncology, Eugène Marquis Cancer Center, Rennes, France; [3] ApoCell, Inc., Houston, TX, USA; [4] Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

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Introduction

- Tumor cells with a mesenchymal phenotype, including cancer stem cells (CSCs), are known to contribute to metastasis.
- Circulating tumor cells (CTCs) with epithelial phenotypes in peripheral blood can be detected using an anti-EpCAM antibody for capture, which may not detect CTCs undergoing epithelial-mesenchymal transition (EMT).
- We have developed an antibody-independent CTC enrichment platform, Apostream®, which does not rely on EpCAM-based capture.

Objectives

Determine the clinical relevance and feasibility of measuring EMT CTCs in breast cancer patients.

Methods

- Blood samples from newly diagnosed breast cancer patients were prospectively collected before neoadjuvant systemic treatment [NST] (T^0), after NST (T^1), and after definitive surgery (T^2) and processed using the Apostream® system.
- Isolated cells were stained with antibodies to leukocytes (anti-CD45) and the DAPI nuclear stain to exclude leukocytes.
- The residual cells were stained with the following additional antibodies and examined on a laser scanning cytometer to identify 4 CTC subsets based on protein expression levels of various markers:

✓ **Epithelial** (CK+, EpCAM+, or E-cadherin+)
✓ **EMT** (β-catenin+ or vimentin+)
✓ **Combined epithelial or EMT** (CK+, EpCAM+, E-cadherin+, vimentin+ or β-catenin+)
✓ **CSC** (CD44+ and $CD24^{low}$).

- Pathological complete response (pCR) to preoperative chemotherapy was correlated to CTC levels and marker expression.

Results

Fig. 1 Trial Design

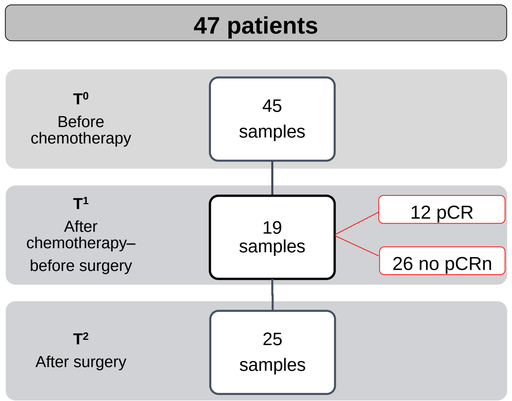


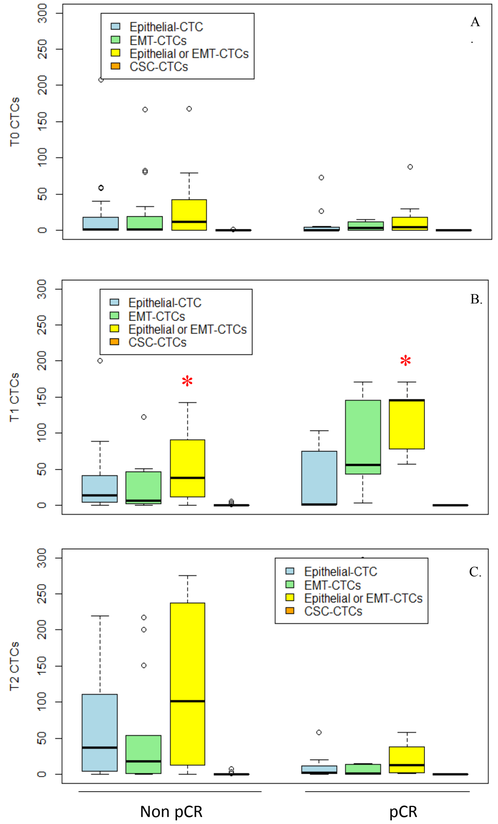
Table. 1 Baseline characteristics of the patients.

No of patients	47
Mean age [range]	51.7 [31-79]
Stage	
• II	12
• III	29
• IV	6
Tumor staging	
• T1	1
• T2	14
• T3	5
• T4	1
• T4d	26
Nodal staging	
• N0	7
• N1	16
• N2	6
• N3	18
ER status	
• Negative	24
• Positive	23
HER2 status	
• Normal	34
• Positive	13
Nottingham grading index	
• 1	1
• 2	14
• 3	30
• NA	2
Proliferation index (Ki67)	53 [10-99]

Table. 2 Detection rate (≥ 1 cell) **and mean number** (range) **of CTCs detected for each CTC phenotype.**

	Epithelial CTCs	EMT CTCs	Epithelial or EMT CTCs	CSC CTCs
T^0 No of patients (%) mean (range)	**24 (53)** 34 (0-512)	**24 (53)** 12 (0-167)	**28 (62)** 48 (0-559)	**2 (4)** 0.2 (0-7)
T^1 No of patients (%) mean (range)	**15 (79)** 36 (0-201)	**17 (90)** 54 (0-327)	**17 (90)** 90 (0-528)	**3 (16)** 0.5 (0-6)
T^2 No of patients (%) mean (range)	**21 (84)** 63 (0-637)	**18 (72)** 70 (0-645)	**22 (88)** 133 (0-697)	**4 (16)** 0.5 (0-8)

Fig. 2 CTC levels according to pCR status at baseline (A)**, after chemotherapy** (B)**, and after surgery** (C)**.**
* Significant difference between pCR and non-pCR groups.



- β-catenin+ EMT-CTCs at T^0 are more likely to be detected at higher clinical stage (p=.014).
- Vimentin+ EMT-CTCs at T^0 are more likely to be detected in HER2-negative breast cancers (p=.016)
- Patients with a higher level of combined epithelial or EMT CTCs before surgery (T^1) are more likely to achieve pCR (p=.038).

Conclusion & Future Perspective

- Preliminary results indicate that Apostream® was successful in detecting EMT-CTCs in this ongoing prospective study. CTC (epithelial and EMT-CTCs) levels after chemotherapy predict pCR.
- We need to await for enrollment and follow-up data of 50 patients to be more conclusive.
- Changes in EMT CTC levels during treatment will be explored in all cohorts.

Contact

E-mail address: nueno@mdanderson.org