

BACKGROUND

- The presence of circulating tumor cells (CTCs) among women before and/or after completion of neoadjuvant chemotherapy (NAC) for breast cancer may be associated with an increased risk of recurrence, but limited data is available.

OBJECTIVES

- To use the Epic Sciences platform to detect and enumerate CTCs in blood samples from women with a new diagnosis of non-metastatic breast cancer of any subtype both i) prior to commencing NAC, and ii) after completion of NAC and surgery.

METHODS

- Women of any age with non-metastatic breast cancer of any subtype who have not yet commenced NAC were included. Those diagnosed with prior invasive cancer (apart from non-melanoma skin cancer diagnosed more than five years prior to enrollment) were excluded.
- Blood samples were obtained to measure CTCs prior to NAC and after NAC and surgery, respectively. CTC identification was based on immunofluorescence analysis using Epic Sciences platform as previously described (Fujii et al, PLoS One, 2017; Scher et al, Eur J Cancer, 2021)
- The presence of CTCs was correlated with clinical/pathological data and treatment response, which were abstracted from patients' medical records.
- The association between the presence of CTCs and clinical/pathologic characteristics was tested using Fisher's exact test for categorical variables and t-test or Wilcoxon rank sum tests for numerical variables. All analyses were performed using the R software package. An ad-hoc preliminary analysis was conducted among the first 34 of 50 participants.

RESULTS

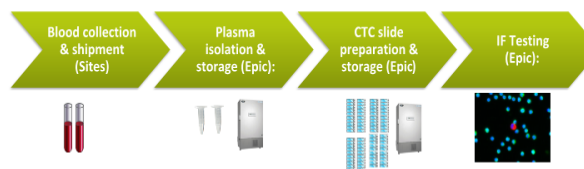
- 41 patients (out of an intended 50) have been recruited to-date.
- 34 participants have a pre- and/or post-treatment CTC measurement available; 1 was excluded due to a diagnosis of metastatic disease shortly after enrollment.
- Among 33 evaluable patients without metastatic disease, 6 (19%) had triple negative breast cancer (TNBC), 13 (39%) had HER2+ and 13 (39%) had hormone receptor (HR)+/HER2- breast cancer.
- Most (94%) received anthracycline and taxane-based NAC. The median age of breast cancer diagnosis was 50 (29-75).
- A total of 53 samples were tested for CTC enumeration (5 mL per sample) including 33 pre-treatment and 20 post-treatment samples.

Table 1. CTC distribution pre- versus post-NAC

Timepoint	# of Samples	# Samples with CTCs	% Samples with CTCs	Median CTCs/mL
Pre-Tx	33	24	73%	0.9
Post-Tx	20	8	40%	0.6
Total	53	32	60%	

- Among the 24 patients (73%) who had detectable CTCs pre-NAC, 10 had HR+/HER2- (41.7%), 9 had HER2+ (37.5%) and 4 (16.7%) had triple negative disease.
- Among the 20 patients for whom matched pre- and post-treatment CTC results were available, 16 (80%) had detectable CTCs pre-treatment and 8 (40%) had detectable levels post-NAC and surgery.
- Among the 8 patients (40%) for whom CTCs were detectable post NAC and surgery, 4 (50%) had HR+/HER2-, 2 had HER2+ (25%) and 2 (25%) had triple negative disease. 3 of these patients had numerically higher CTC levels after completion of NAC and surgery compared to pre-NAC levels, 2 of whom had HR+ breast cancer and one of whom had TNBC.

Figure 1. Workflow schematic



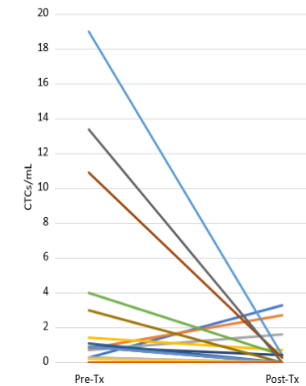
RESULTS

- A total of 7 of 33 patients achieved a pathological complete response (PCR) to NAC, among whom 3 had matched pre- and post-treatment CTC results available; none of these 3 patients had detectable CTCs post-treatment.

Table 2. CTC distribution by patient in pretreatment sample prior to NAC (Pre-Tx) versus post NAC (Post-Tx) as stratified by pathological complete response (PCR) and tumor subtype

Patient Sample	CTCs/mL		PCR status	Tumor subtype
	Pre-Tx	Post-Tx		
1	0.3	3.3	No	TNBC
2	0.8	2.7	No	HR+
3	0.7	1.6	No	HR+
4	1.4	0.7	No	HR+
5	19	0.4	No	TNBC
6	4	0.4	No	HR+
7	0.9	0.4	No	HR+/HER2+
8	10.9	0.3	No	HR+/HER2+
9	13.4	0	No	HR+
10	3	0	No	TNBC
11	1.1	0	No	HR+/HER2+
12	0.9	0	No	HR+/HER2+
13	0.9	0	No	TNBC
14	0.3	0	No	HR+
15	0.3	0	YES	HER2+
16	0.2	0	No	HR+
17	0	0	No	HR+/HER2+
18	0	0	YES	TNBC
19	0	0	No	HR+/HER2+
20	0	0	YES	HR+/HER2+

Figure 2. Distribution of CTC pre versus post NAC in patients



CTC monitoring of pre versus post NAC identifies patients with increased CTC as highlighted in red in the table (3/20, 15%), decreased CTC in blue (5/20, 25%) and undetectable CTC in purple (8/20, 40%).

CONCLUSIONS

- ~3 in 4 women with non-metastatic breast cancer who undergo NAC have detectable CTCs pre-treatment using the Epic Sciences Platform.
- Of 20 patients with matched pre-/post-treatment results, a high proportion (40%) have persistently detectable CTCs.
- Hence, CTCs may represent an additional measure of minimal residual disease for patients undergoing NAC for breast cancer.

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