A Multi-center Clinical Study to Harvest and Characterize Circulating Tumor Cells from Patients with Metastatic Breast Cancer Using the Parsortix® PC1 System in support of FDA clearance

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INTRODUCTION

Circulating tumor cells (CTCs) captured from the blood of cancer patients may serve as a non-invasive surrogate source of tumor material to investigate tumor characteristics in real-time. However, the only FDA-cleared CTC assay is limited to the enumeration of surface marker-defined epithelial cells and not designed for further characterization of the CTCs identified. The Parsortix® PC1 system is a semi-automated microfluidic device capable of capturing and harvesting CTCs from peripheral blood based on cell size and deformability, making it cell-surface marker agnostic. Here, we demonstrate that the Parsortix® PC1 system enables the enrichment and capture of CTCs from the blood of patients with metastatic breast cancer (MBC) and their interrogation using evaluation techniques commonly available in clinical laboratories.

MATERIALS AND METHODS

The ANG-002 HOMING study was a prospective clinical trial (identifier: NCT03427450) sponsored by ANGLE Europe Limited (Guildford, UK), the manufacturer of the Parsortix® PC1 System. Enrollment included patients with MBC (either newly diagnosed about to start a new line of therapy of any type for MBC or progressive/recurrent MBC) as well as a control population of healthy female volunteers (HVs) who self-declared no prior/current history of cancer. Participants were enrolled, and samples were collected and processed from:

- The University of Texas MD Anderson Cancer Center, Houston, TX
- University of Southern California, Los Angeles, CA
- University of Rochester Medical Center, Rochester, NY
- Northwestern University, Chicago, IL

Each participant provided 2 tubes of EDTA blood. CTCs were enriched using the Parsortix System and a Parsortix GEN3D6.5 Cell Separation Cassette (Angle, Europe, Ltd). The cells enriched by the system from the tube containing the largest volume of blood were deposited onto a glass slide using a CytoSpin and subsequently stained with Wright-Giemsa on an automated stainer prior to cytology review by a board-certified pathologist to identify and enumerate CTCs. As proof of principle, cells harvested from the second blood sample were evaluated using one of three additional techniques: molecular profiling by qRT-PCR, RNA sequencing, or cytogenetic analysis of HER2 amplification by FISH.

OBJECTIVES

Demonstrate capture and harvest of MBC CTCs by Parsortix PC1 system and ability to evaluate harvested cells using multiple downstream evaluation methods.

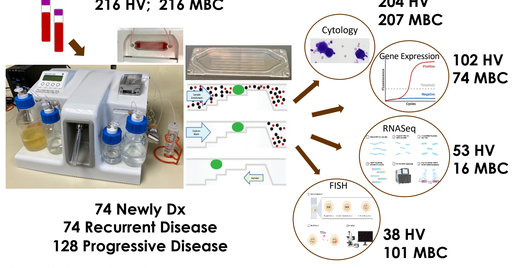

Figure 1. Study Overview.

REFERENCE

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Acknowledgements

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Some figures created in Biorender.

Cytology

Parameter and Categories	Eligible HV Subjects and MBC Patients with Evaluable Cytology Slides from Cytology Evaluation											
	Evaluable HV Subjects			All Evaluable MBC Patients			Newly Diagnosed MBC Patients			Progression/Recurring MBC Patients		
	N	>=1 CTC	>=5 CTC	N	>=1 CTC	>=5 CTC	N	>=1 CTC	>=5 CTC	N	>=1 CTC	>=5 CTC
All Subjects / Patients	192	19 (9.9%)	2 (1.0%)	194	94 (48.5%)	44 (22.7%)	69	23 (33.3%)	9 (13.0%)	125	71 (56.8%)	35 (28.0%)
Age at the time of the blood collection												
<55 Years Old	159	15 (9.4%)	1 (0.6%)	96	41 (42.7%)	17 (17.7%)	33	6 (18.2%)	3 (9.1%)	63	35 (55.6%)	14 (22.2%)
>=55 Years Old	33	4 (12.1%)	1 (3.0%)	98	53 (54.1%)	27 (27.6%)	36	17 (47.2%)	6 (16.7%)	62	36 (58.1%)	21 (33.9%)
Fisher's Exact Test p-value		0.748	0.315		0.117	0.123		0.022	0.481		0.857	0.167
Blood Collection Method												
via Venipuncture	192	19 (9.9%)	2 (1.0%)	159	61 (38.4%)	22 (13.8%)	65	19 (29.2%)	7 (10.8%)	94	42 (44.7%)	15 (16.0%)
via Port	0	0 (0.0%)	0 (0.0%)	35	33 (94.3%)	22 (62.9%)	4	4 (100.0%)	2 (50.0%)	31	29 (93.5%)	20 (64.5%)
Fisher's Exact Test p-value					<0.001	<0.001		0.012	0.060		<0.001	<0.001
Race												
White	102	7 (6.9%)	1 (1.0%)	141	71 (50.4%)	31 (22.0%)	51	19 (37.3%)	7 (13.7%)	90	52 (57.8%)	24 (26.7%)
Black	20	5 (25.0%)	0 (0.0%)	21	13 (61.9%)	9 (42.9%)	7	2 (28.6%)	2 (28.6%)	14	11 (78.6%)	7 (50.0%)
Hispanic	37	4 (10.8%)	1 (2.7%)	21	4 (19.0%)	1 (4.8%)	8	2 (25.0%)	0 (0.0%)	13	2 (15.4%)	1 (7.7%)
Other / Unknown	33	3 (9.1%)	0 (0.0%)	11	6 (54.5%)	3 (27.3%)	3	0 (0.0%)	0 (0.0%)	8	6 (75.0%)	3 (37.5%)
Fisher's Exact Test p-value		0.114	0.710		0.022	0.026		0.740	0.388		0.008	0.084
Previous History of Cancer?												
Yes	0	0 (0.0%)	0 (0.0%)	13	11 (84.6%)	4 (30.8%)	4	3 (75.0%)	0 (0.0%)	9	8 (88.9%)	4 (44.4%)
No	192	19 (9.9%)	2 (1.0%)	181	83 (45.9%)	40 (22.1%)	65	20 (30.8%)	9 (13.8%)	116	63 (54.3%)	31 (26.7%)
Fisher's Exact Test p-value					0.008	0.496		0.104	1.000		0.076	0.265
Breast Cancer ER Status												
Positive	0	0 (0.0%)	0 (0.0%)	155	70 (46.4%)	35 (23.2%)	52	18 (34.6%)	7 (13.5%)	99	52 (52.5%)	28 (28.3%)
Negative	0	0 (0.0%)	0 (0.0%)	42	24 (57.1%)	9 (21.4%)	16	5 (31.3%)	2 (12.5%)	26	19 (73.1%)	7 (26.9%)
Fisher's Exact Test p-value					0.727	1.000		1.000	1.000		0.076	1.000
Breast Cancer PR Status												
Positive	0	0 (0.0%)	0 (0.0%)	121	57 (47.1%)	26 (21.5%)	42	13 (31.0%)	6 (14.3%)	79	44 (55.7%)	20 (25.3%)
Negative	0	0 (0.0%)	0 (0.0%)	67	33 (49.3%)	15 (22.4%)	24	9 (37.5%)	3 (12.5%)	43	24 (55.8%)	12 (27.9%)
Unknown	0	0 (0.0%)	0 (0.0%)	6	4 (66.7%)	3 (50.0%)	3	1 (33.3%)	0 (0.0%)	3	3 (100.0%)	3 (100.0%)
Fisher's Exact Test p-value					0.879	1.000		0.599	1.000		1.000	0.830
Breast Cancer Her2-neu Status												
Negative	0	0 (0.0%)	0 (0.0%)	156	70 (44.9%)	33 (21.2%)	62	21 (33.9%)	8 (12.9%)	94	49 (52.1%)	25 (26.6%)
Positive	0	0 (0.0%)	0 (0.0%)	24	17 (70.8%)	8 (33.3%)	3	1 (33.3%)	1 (33.3%)	21	16 (76.2%)	7 (33.3%)
Equivocal	0	0 (0.0%)	0 (0.0%)	8	4 (50.0%)	2 (25.0%)	1	1 (100.0%)	0 (0.0%)	7	3 (42.9%)	2 (28.6%)
Unknown	0	0 (0.0%)	0 (0.0%)	6	3 (50.0%)	1 (16.7%)	3	0 (0.0%)	0 (0.0%)	3	3 (100.0%)	1 (33.3%)
Fisher's Exact Test p-value					0.061	0.778		0.533	0.452		0.098	0.809
Sites of Metastasis (more than one may apply)												
Abdomen	0	0 (0.0%)	0 (0.0%)	6	3 (50.0%)	2 (33.3%)	3	1 (33.3%)	1 (33.3%)	3	2 (66.7%)	1 (33.3%)
Adrenal Gland	0	0 (0.0%)	0 (0.0%)	3	2 (66.7%)	1 (33.3%)	0	0 (0.0%)	0 (0.0%)	3	2 (66.7%)	1 (33.3%)
Ascites	0	0 (0.0%)	0 (0.0%)	1	0 (0.0%)	0 (0.0%)	1	0 (0.0%)	0 (0.0%)	0	0 (0.0%)	0 (0.0%)
Bone	0	0 (0.0%)	0 (0.0%)	132	72 (54.5%)	40 (30.3%)	39	14 (35.9%)	9 (23.1%)	93	58 (62.4%)	31 (33.3%)
Brain	0	0 (0.0%)	0 (0.0%)	26	17 (65.4%)	9 (34.6%)	4	2 (50.0%)	1 (25.0%)	22	15 (68.2%)	8 (36.4%)
Chest Wall	0	0 (0.0%)	0 (0.0%)	16	11 (68.8%)	4 (25.0%)	4	3 (75.0%)	2 (50.0%)	12	8 (66.7%)	2 (16.7%)
Kidney	0	0 (0.0%)	0 (0.0%)	0	0 (0.0%)	0 (0.0%)	0	0 (0.0%)	0 (0.0%)	0	0 (0.0%)	0 (0.0%)
Liver	0	0 (0.0%)	0 (0.0%)	73	41 (56.2%)	19 (26.0%)	9	2 (22.2%)	2 (22.2%)	64	39 (60.9%)	17 (26.6%)
Lung	0	0 (0.0%)	0 (0.0%)	70	35 (50.0%)	19 (27.1%)	22	6 (27.3%)	1 (4.5%)	48	29 (60.4%)	18 (37.5%)
Lymph Nodes	0	0 (0.0%)	0 (0.0%)	101	48 (47.5%)	19 (18.8%)	31	10 (32.3%)	3 (9.7%)	70	38 (54.3%)	16 (22.9%)
Other Site(s)	0	0 (0.0%)	0 (0.0%)	33	15 (45.5%)	4 (12.1%)	9	4 (44.4%)	1 (11.1%)	24	11 (45.8%)	3 (12.5%)

Table 1. CTC prevalence rates from cytology review in MBC and HVs by demographic and clinical characteristics.

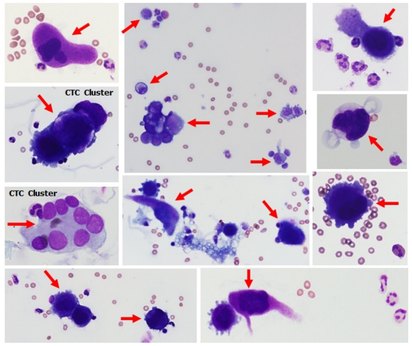


Figure 2. Representative images of cells classified as CTCs (red arrows) from MBC patients that were harvested by the Parsortix PC1 system and deposited onto cytology slides by cytocentrifugation (images not to same scale) and Wright-Giemsa stained.

RESULTS

Cytology

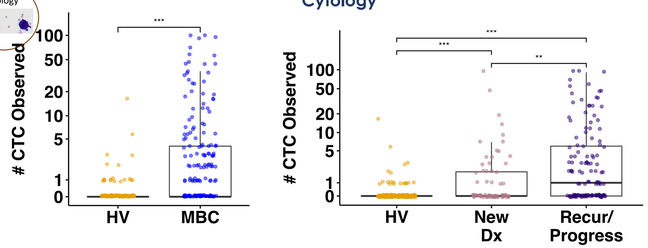


Figure 3. CTC numbers from the review of evaluable Wright-Giemsa-stained cytology slides.

Gene Expression by qPCR

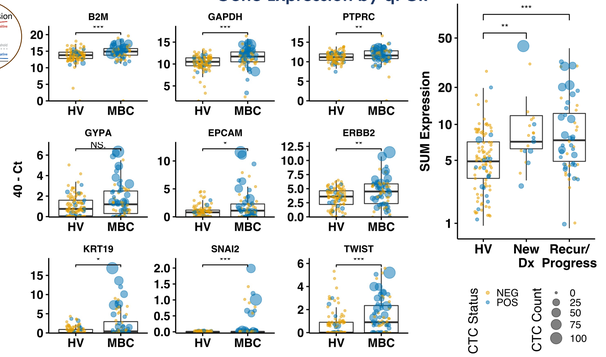


Figure 4. Expression of CTC related genes is higher in MBC compared to HV. Smaller plots show individual genes while larger plot to right shows total CTC-related gene expression, where "SUM Expression" = Sum of 40-Ct values for KRT19, EPCAM, ERBB2, TWIST, and SNAI2.

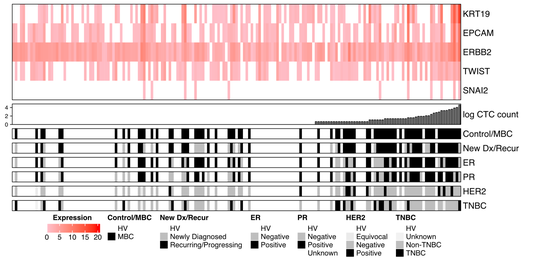
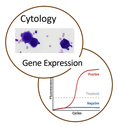
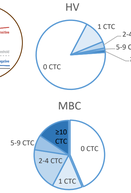


Figure 5. Gene expression and matched CTC count overview. Heat map of CTC gene expression shows correlation of gene expression with the number of CTCs observed

Gene Expression

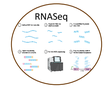




Group	N	KRT19	EpCAM	TWIST1	SNAI2	KRT19, EpCAM, TWIST &/or SNAI2
Negative Controls	18	0.0%	0.0%	0.0%	0.0%	0.0%
Positive Controls	18	100.0%	100.0%	100.0%	100.0%	100.0%
All HV Subjects	102	0.0%	1.0%	1.0%	0.0%	1.0%
with evaluable CTC Count	99 (97.1%)	0.0%	1.0%	1.0%	0.0%	1.0%
with 0 CTC	83 (83.8%)	0.0%	1.2%	1.2%	0.0%	1.2%
with 1 CTC	11 (11.1%)	0.0%	0.0%	0.0%	0.0%	0.0%
with 2 – 4 CTCs	3 (3.1%)	0.0%	0.0%	0.0%	0.0%	0.0%
with 5 – 9 CTCs	1 (1.0%)	0.0%	0.0%	0.0%	0.0%	0.0%
with >=10 CTCs	1 (1.0%)	0.0%	0.0%	0.0%	0.0%	0.0%
All MBC Patients	74	21.6%	13.5%	5.4%	0.0%	24.3%
with evaluable CTC Count	71 (95.9%)	22.5%	14.1%	5.6%	0.0%	25.4%
with 0 CTC	31 (43.7%)	22.6%	9.7%	6.5%	0.0%	25.8%
with 1 CTC	10 (14.1%)	0.0%	0.0%	0.0%	0.0%	0.0%
with 2 – 4 CTCs	14 (19.7%)	28.6%	21.4%	7.1%	0.0%	35.7%
with 5 – 9 CTCs	5 (7.0%)	0.0%	0.0%	0.0%	0.0%	0.0%
with >=10 CTCs	11 (15.5%)	45.5%	36.4%	9.1%	0.0%	45.5%

Table 2. Correlation of individual CTC related genes with CTC counts. Percent of samples with positive gene expression (Ct ≤ 35.0). Positive gene expression by HV samples was rare and the dual tests of cytology and gene expression improved specificity.

RNASeq



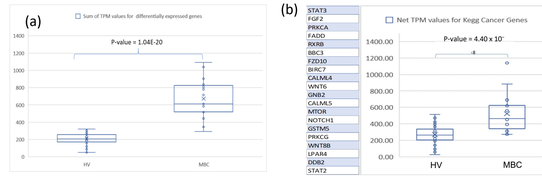


Figure 6. (a) Sum of TPM values for all genes differentially expressed ($p < 0.001$) between Parsortix PC1 harvests from HV and MBC patients. (b) Genes from the KEGG Cancer Pathway were differentially expressed between the HV and MBC harvests ($p < 0.05$).

HER2 FISH

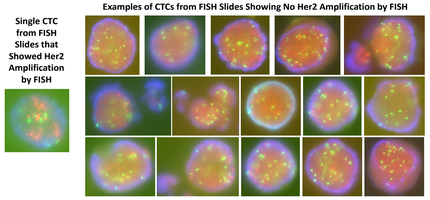


Figure 7. Example images of HER2 FISH-stained CTCs from MBC patients that were deposited onto cytology slides (images not to scale).

CONCLUSION

Cells harvested from peripheral blood of HVs and patients with MBC using the Parsortix® PC1 System can be successfully evaluated using currently available laboratory methods for the identification and characterization of CTCs: cytology (i.e., Wright-Giemsa staining), qRT-PCR, RNA-sequencing, and FISH. Data generated from this study was used to support a *De Novo* classification of the Parsortix® PC1 system (DEN200062) as a Class II prescription device that was granted by the FDA on 24 May 2022.