

Isolation of ovarian cancer circulating tumor cells using an epitope independent microfluidic cell capture device and their interrogation using a multiplex gene expression assay or immunofluorescence

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Introduction

Emerging technologies for the isolation and interrogation of rare circulating cells, such as circulating tumor cells (CTCs), present an opportunity for the detection of cancer with a simple blood test, or liquid biopsy.

The present study was designed to evaluate the ability of an epitope independent microfluidic cell capture device (Parsortix[®] System, Figure 1) to isolate circulating tumor cells (CTCs) from the blood of women with stage III-IV high grade serous ovarian cancer (HGSOC), followed by evaluation using either a highly multiplexed gene expression assay (HyCEAD/Ziplex; Figure 2) or by immunofluorescence (IF) staining for epithelial specific markers and ovarian related markers.

This was an IRB approved prospective clinical trial conducted through the Wilmot Cancer Institute Gynecologic Oncology Division.

Study Design / Methods

Patients: A total of 41 women diagnosed with HGSOC and 39 healthy women were enrolled into the study. Four (4) of the HGSOC patients and one (1) of the healthy women were non-evaluable for the study. In the remaining 75 evaluable subjects, the first set of subjects enrolled in each group (26 HGSOC and 27 healthy women) had their blood samples evaluated using HyCEAD/Ziplex molecular analysis while the final 11 subjects enrolled in each group had their blood samples evaluated using IF on cytology slides for detection of tumor cells (Fig. 3).

Blood Sample Processing: Blood collected into the K₂EDTA tubes (~9mL per tube) were either processed within 8hr of collection or stored at room temperature for ~96hr before processing. Cells captured by the Parsortix[®] system were either eluted directly into HyCEAD lysis buffer and stored frozen for subsequent molecular evaluation or immediately deposited into special cell capture chambers or onto glass slides using a cytocentrifuge for subsequent IF staining and cytological evaluation. HyCEAD lysates were processed using the HyCEAD/Ziplex assay to simultaneously assess the expression level of 48 different genes.

Data Analysis: Gene expression data was evaluated using multivariate logistic regression (MLR) and ROC curve analysis. CTCs deposited on cytology slides or captured in special chambers were visualized by 4-color IF staining using a combination of epithelial specific, ovarian cancer related, and blood cell related markers as well as DAPI and automatically imaged using the BioView Allegro Plus imaging system.

Figure 1: Parsortix[®] PR1 System

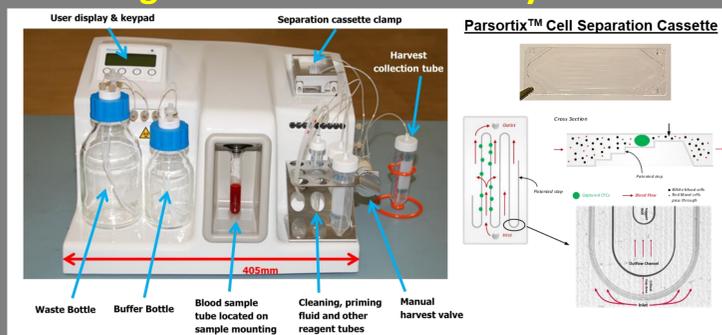


Figure 2: HyCEAD[™] Method and Ziplex[®] System

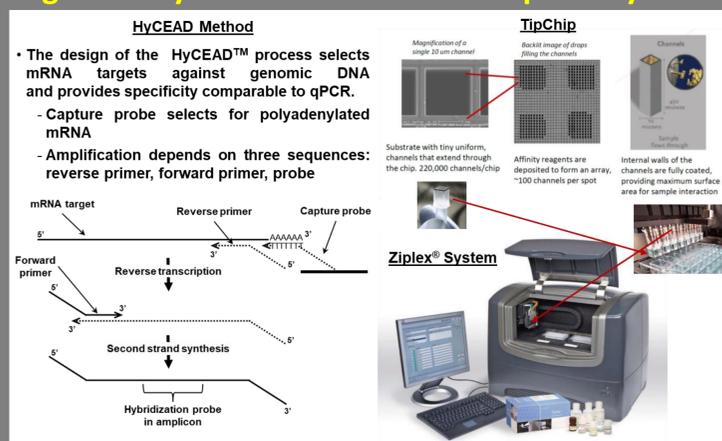


Figure 3: Study Schema

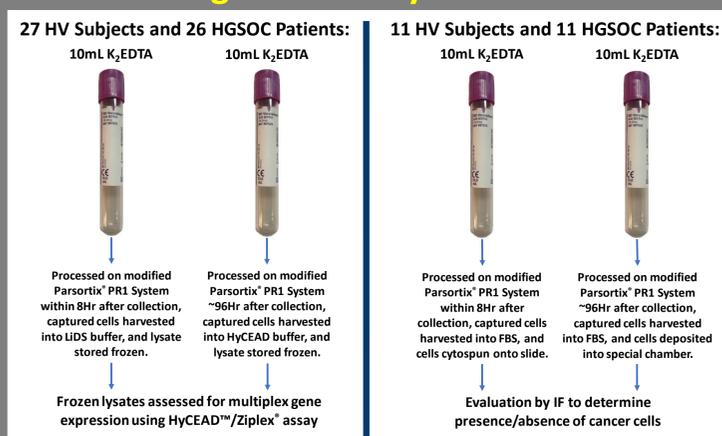


Table 1: Overview of IF Results in Evaluable HGSOC Patients

Evaluable HGSOC Patients	Cytospin Cytology Slide			Cell Capture Chamber			Cytology Slide and/or Chamber		
	Currently Receiving Chemotherapy? No	Yes	All HGSOC Patients	Currently Receiving Chemotherapy? No	Yes	All HGSOC Patients	Currently Receiving Chemotherapy? No	Yes	All HGSOC Patients
N	5 (50%)	5 (50%)	10	6 (55%)	5 (45%)	11 (100%)	6 (55%)	5 (45%)	11 (100%)
CTC Range	2 - 9	2 - 4	2 - 9	1 - 25	2 - 5	1 - 25	2 - 25	2 - 5	2 - 25
0 CTCs	2 (40%)	3 (60%)	5 (50%)	3 (50%)	2 (40%)	5 (45%)	2 (33%)	2 (40%)	4 (36%)
>=1 CTC	3 (60%)	2 (40%)	5 (50%)	3 (50%)	3 (60%)	6 (55%)	4 (67%)	3 (60%)	7 (64%)
>=3 CTC	1 (20%)	1 (20%)	2 (20%)	2 (33%)	2 (40%)	4 (36%)	2 (33%)	2 (40%)	4 (36%)

Figure 4: ROC Curves for Prediction of Healthy (N=27) vs. HGSOC (N=26)

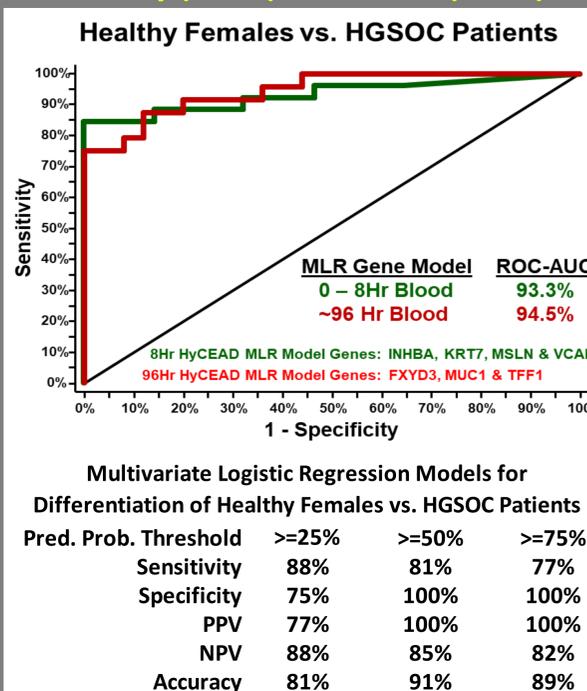
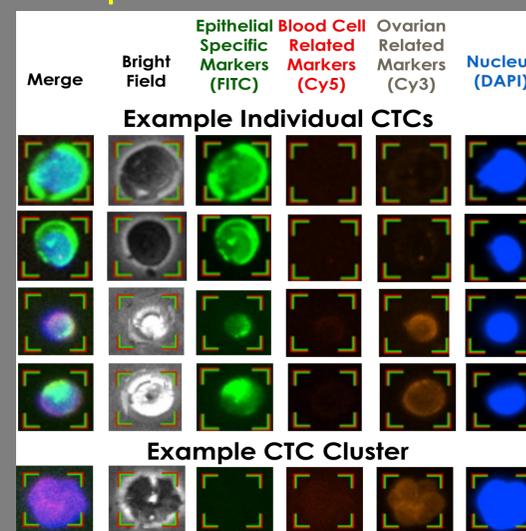


Figure 5: Examples of IF stained cells captured from HGSOC Patients



Results

- Blood from the HGSOC patients and healthy women (~9mL of blood per sample) was successfully processed through the Parsortix system up to 96hr post blood draw, with an average processing time of 71min for 0-8hr samples (average 2.4h post-draw) and 91min for 96hr samples (average 94hr post-draw).
- Multivariate logistic regression (MLR) evaluation of the multiplexed gene expression data identified small sets of genes in 8Hr and 96Hr HyCEAD lysates that demonstrated clear discrimination between the HGSOC patients and the healthy females (Figure 4).
 - For blood samples processed within 8hr after collection, MLR analysis resulted in a predictive model using information from 4 different genes that had a ROC curve AUC of 93.3%.
 - For blood samples processed within 96hr after collection, MLR analysis resulted in a predictive model using information from 3 different genes that had a ROC curve AUC of 94.5%.
- Four color IF evaluation identified CTCs (range: 1-25) in up to 64% of the HGSOC blood samples tested (Table 1). CTCs and CTC clusters were observed on the cytology slides and in the cell capture chambers for patients undergoing chemotherapy as well as in those not currently being treated with chemotherapy (Figure 5).

Conclusions

- Here we demonstrate the ability to isolate CTCs from blood of HGSOC patients up to 96hr after collection.
- Presence of CTCs could be identified by both the multiplexed gene expression assay and 4-color IF staining in HGSOC patients.
- A larger clinical study is currently ongoing to verify ability of multiplexed gene expression results to identify the presence of malignancy in women with a pelvic mass.



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