

MP Ward^{1,2,3}, SE Lochrin^{3,4}, C Ovaere^{3,5}, C O'Gorman^{3,5}, BM Mohamed^{1,2,3}, M Bates^{1,2,3}, TE Kelly^{1,2,3}, R O'Connor^{1,2,3}, J Felce⁵, E Kilgour⁵, J Kennedy^{3,4}, K Cadoo^{3,4}, W Kamran^{3,6}, J Beirne^{3,6}, FA Saadeh^{3,6,7}, LA Norris^{3,7}, CM Martin^{1,2,3}, SA O'Toole^{1,2,3,7*}, JJ O'Leary^{1,2,3*}

¹ Emer Casey Molecular Pathology Research Lab, Coombe Women and Infants University Hospital, Dublin 8, Ireland. ² Department of Histopathology, Trinity College Dublin, Ireland. ³ Trinity St James's Cancer Institute, St James's Hospital, Dublin 8, Ireland. ⁴ The Haematology, Oncology and Palliative Care (HOPE) Directorate, St James's Hospital, Dublin 8, Ireland. ⁵ CRUK Manchester Institute Cancer Biomarker Centre, University of Manchester, Manchester, UK. ⁶ Division of Gynaecological Oncology, St James's Hospital, Dublin 8, Ireland. ⁷ Department of Obstetrics and Gynaecology, Trinity College Dublin, Ireland. *joint Senior Authors

Introduction

CTCs are rare pre-metastatic precursor cells that transit from primary tumours into the blood circulatory system.

CTC counts have been found to be a reliable biomarker for guiding therapy choices in metastatic breast cancer [MBC] and are indicative of a poorer prognosis.

However, research has shown difficulties exist with classical CTC detection methods in ovarian cancer [OC].

This study aims to improve the isolation and characterisation of CTCs and CTC clusters from both high and low CTC trafficking models.

Patient cohort and methods

Metastatic breast cancer [MBC] patients n=20

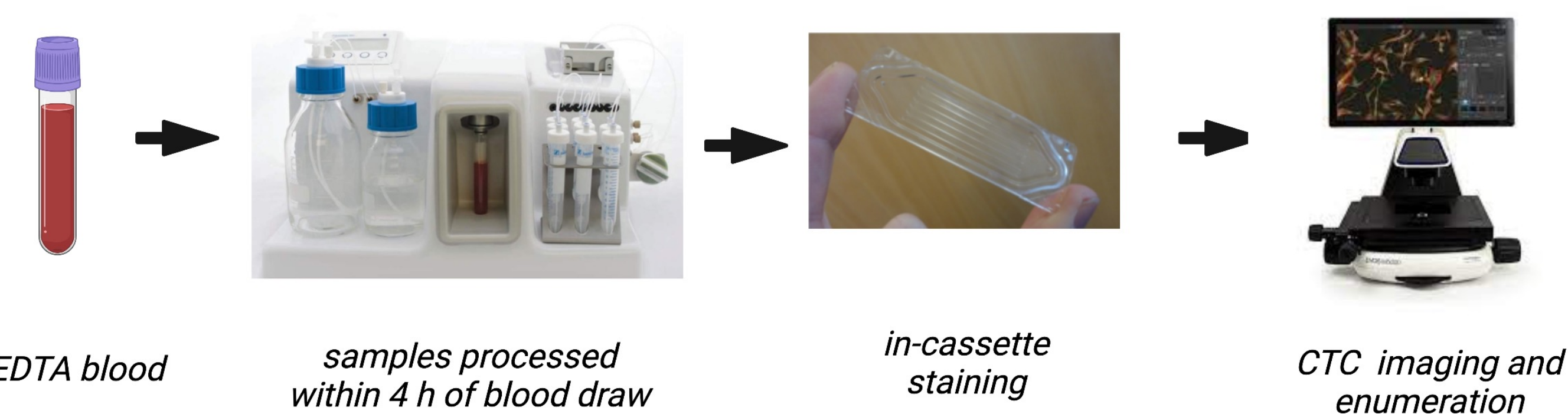
Single blood sample prior to treatment

n=10 patients matched samples compared using Parsortix and CellSearch enumeration (CRUK Manchester).

High-grade serious ovarian cancer [OC] patients n=29

Neoadjuvant cohort: blood sample pre and post chemotherapy.

Primary surgery: peripheral sample and ovarian vein blood sampling at the time of surgery.



Results

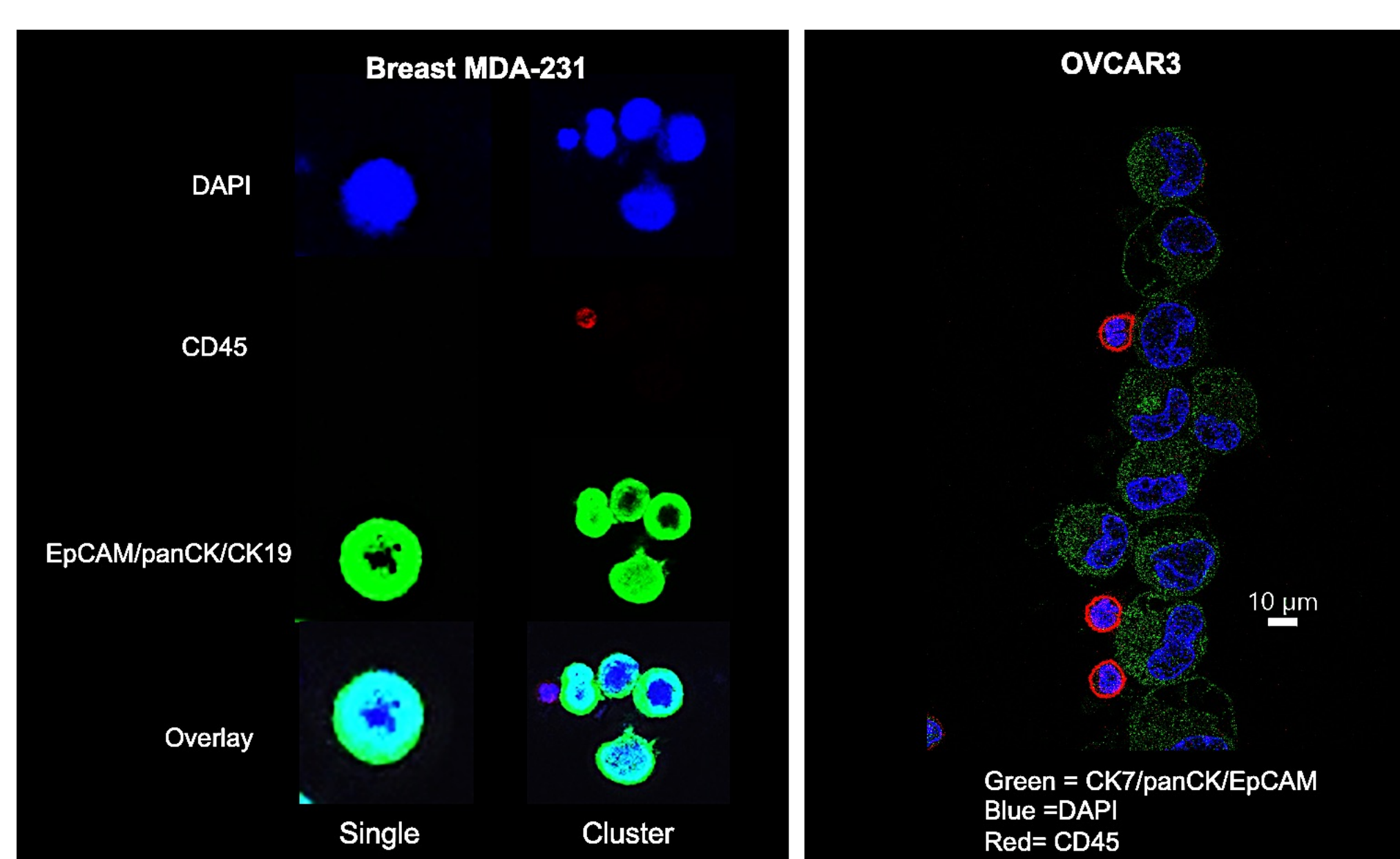
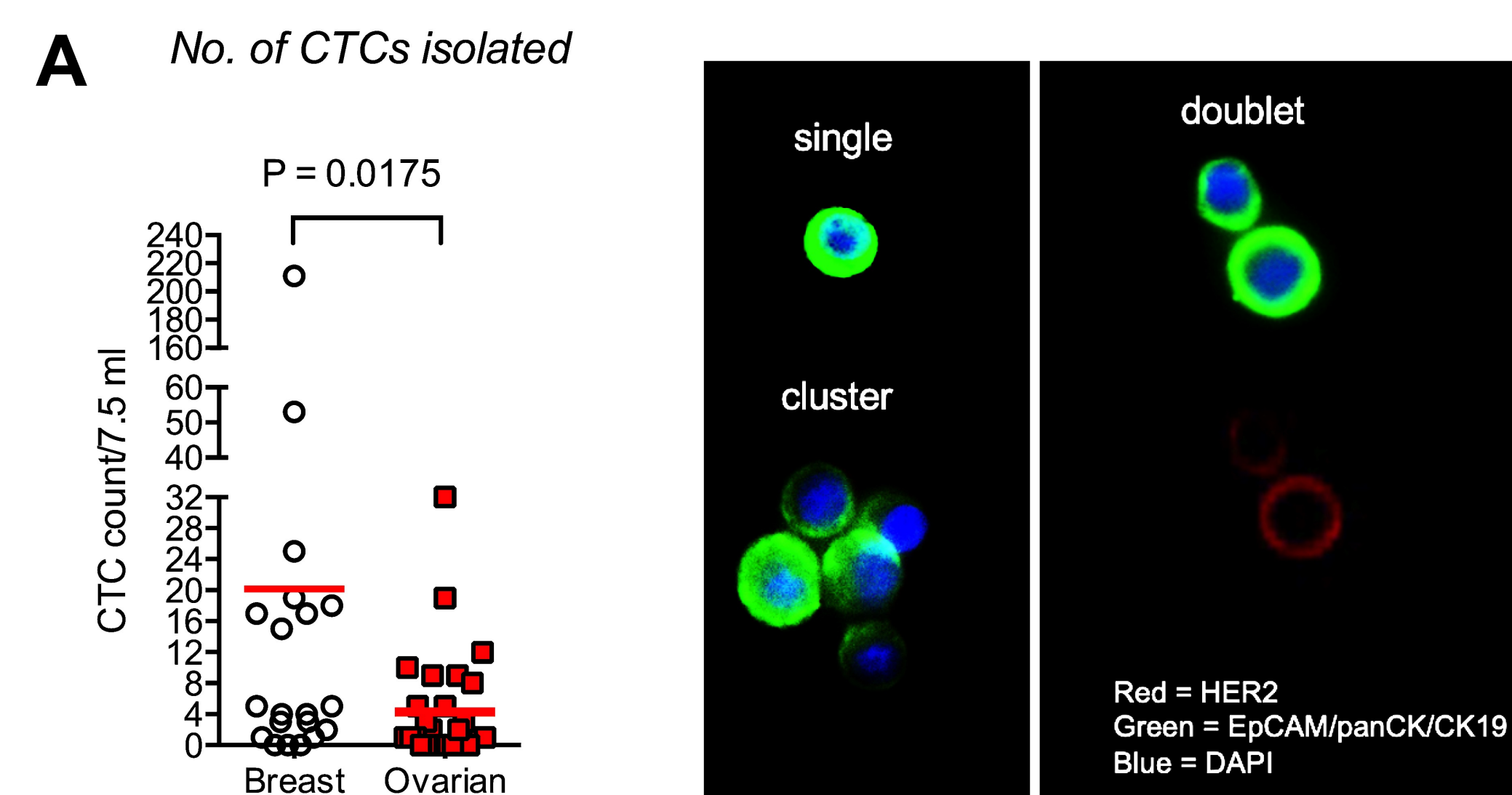


Figure 1: Optimization of in-cassette staining for breast and ovarian cell lines recovered using Parsortix.

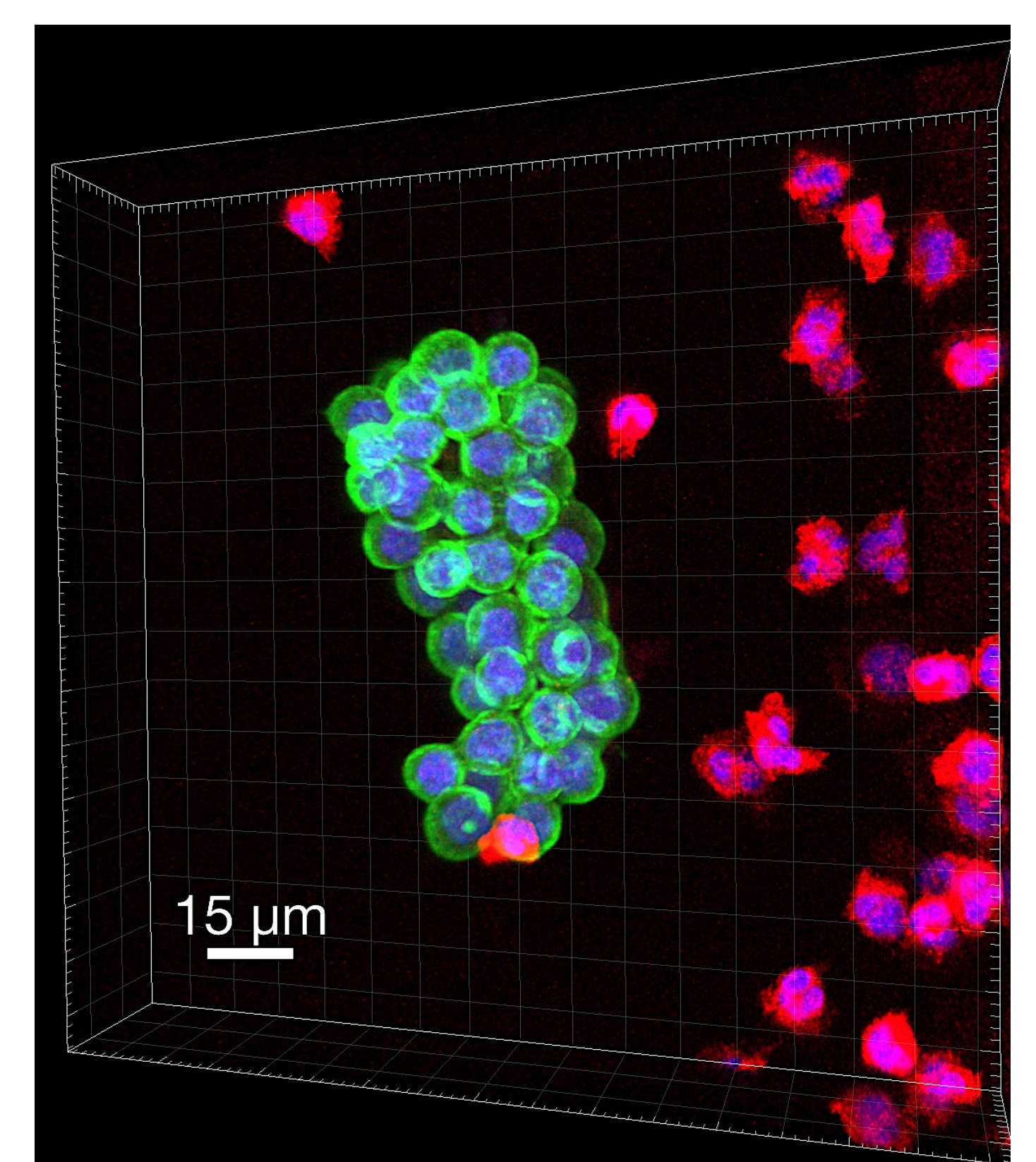
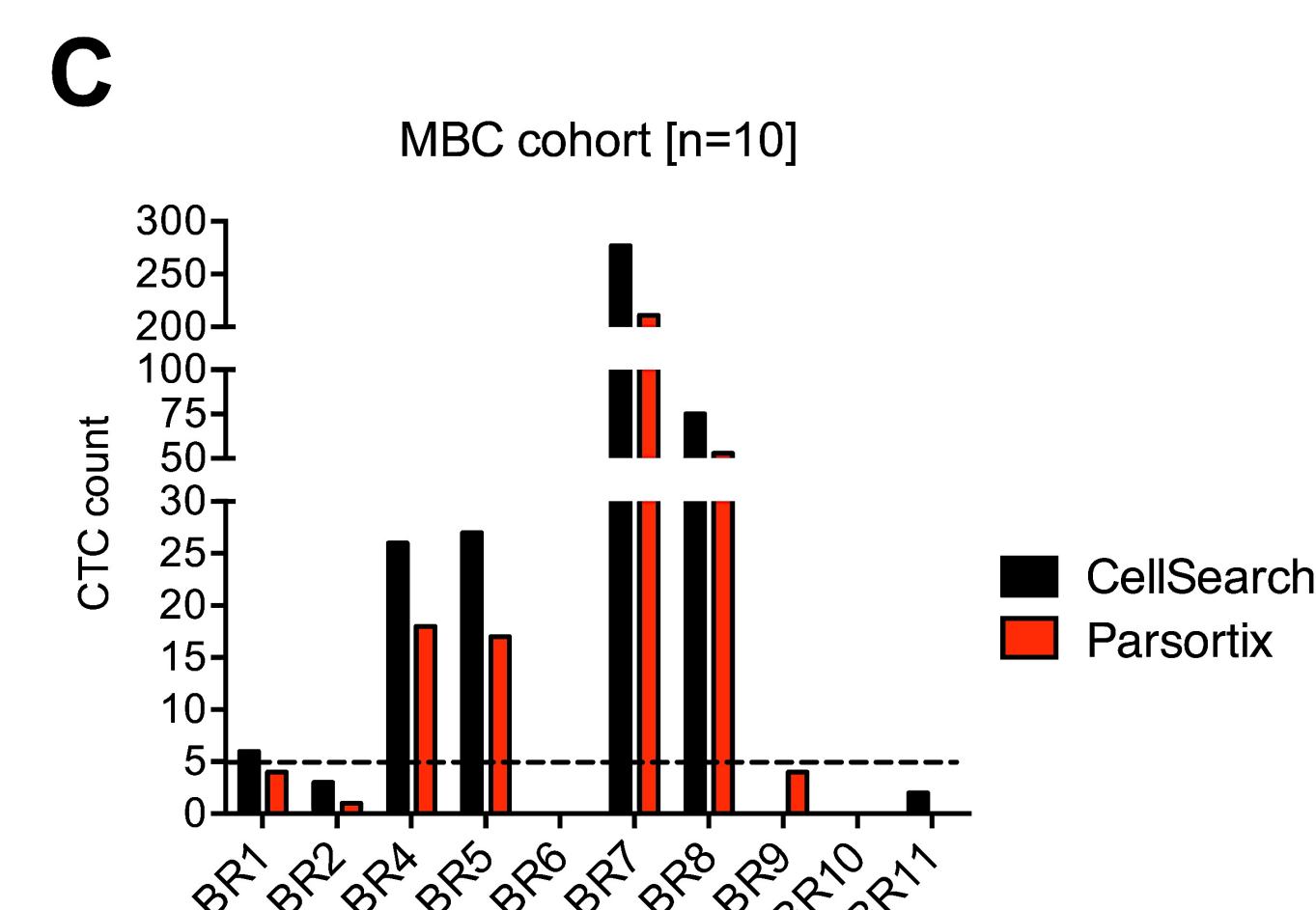
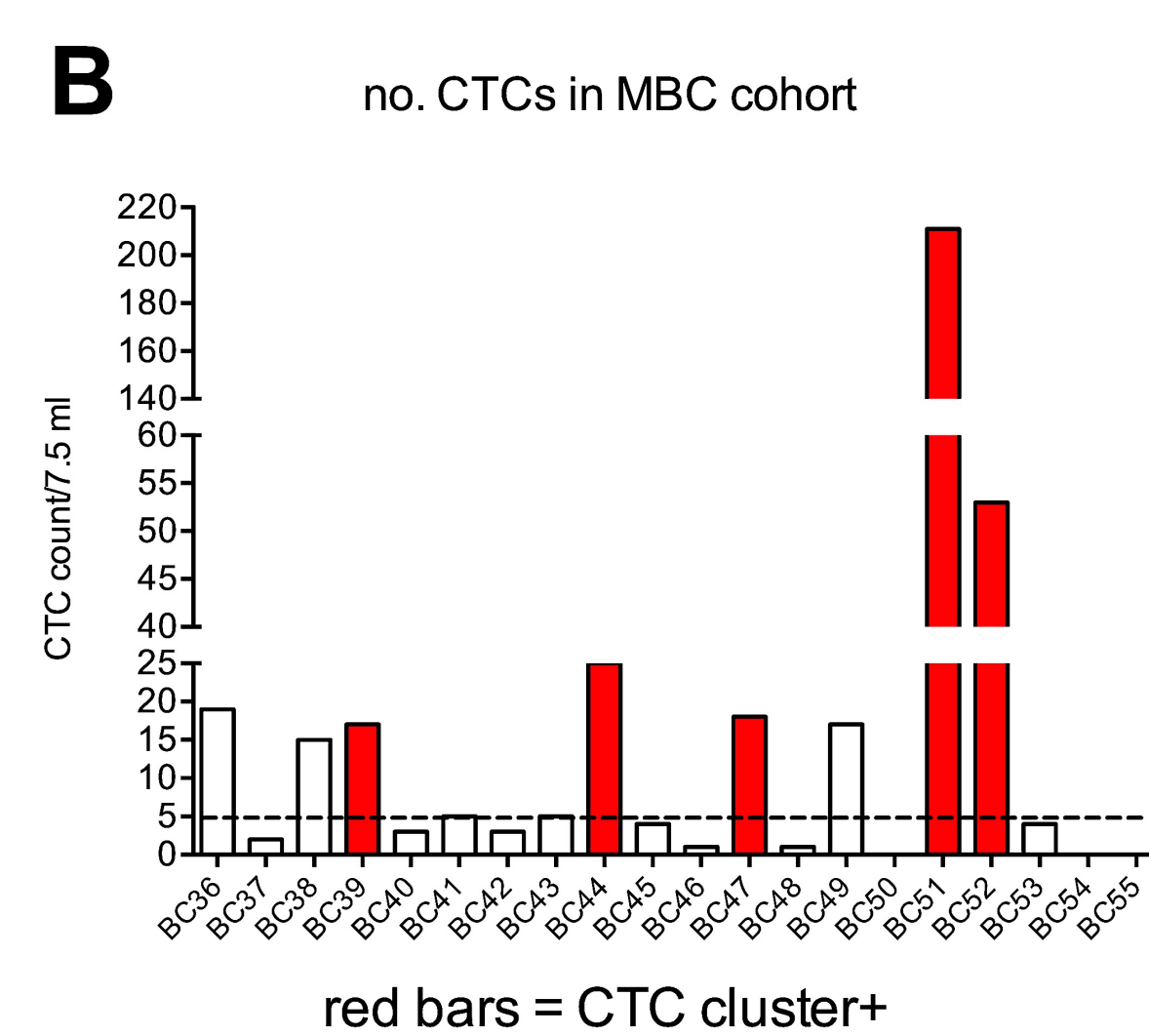
- Breast staining antibody cocktail : DAPI [blue], EpCAM/panCK/CK19 [488 green], HER2 [555] and CD45 [647].
- Ovarian staining antibody cocktail : DAPI [blue], EpCAM/panCK/CK7 [488 green], CD42b [555] and CD45 [647].

Breast MDA-231 and ovarian OVCAR3 cells were spiked into healthy blood at different cell numbers. 74% breast and 67% ovarian cell line recovery was reported using Parsortix.

Email: wardm6@tcd.ie
Twitter: @wardm6TCD



CTCs isolated from MBC patients are mainly single cells, with 25% of patients having CTC clusters present. A subset of CTCs are also HER2+ (Red).

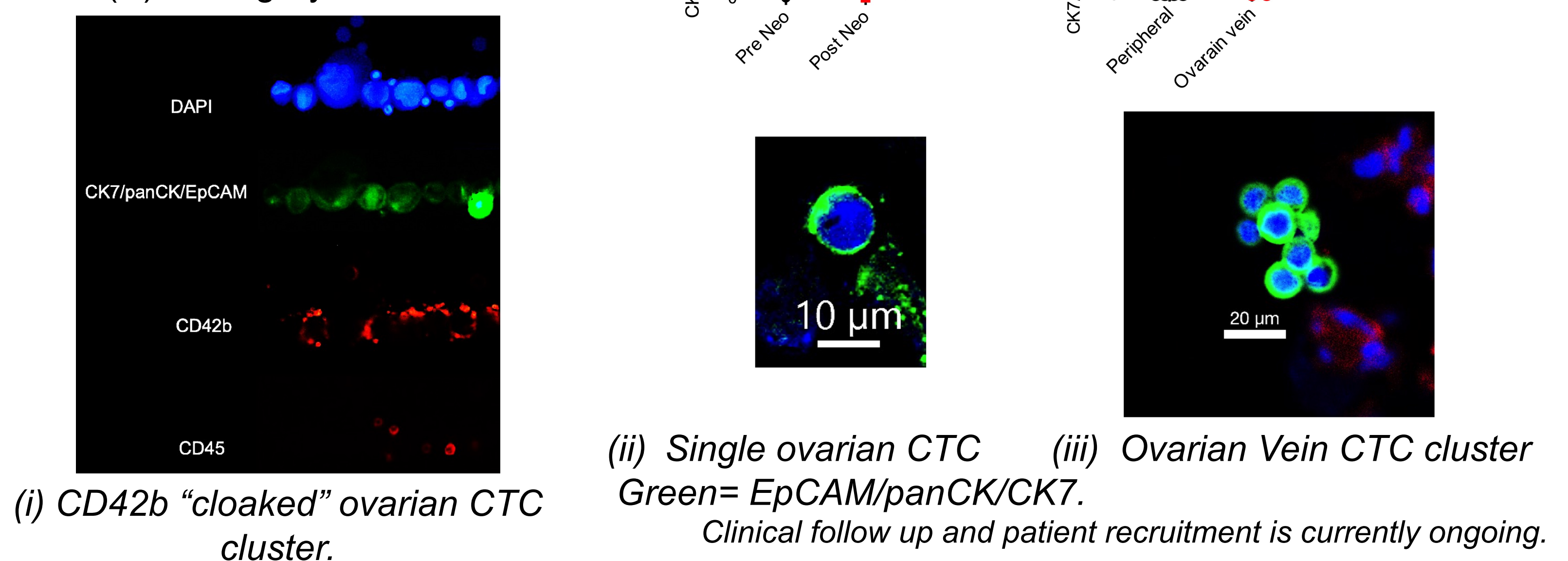


3D confocal imaging of a large CTC cluster isolated from MBC patient with immune cell infiltration. Green = EpCAM/panCK/CK19, red = CD45

Figure 2: (A) Isolation of CTCs from MC and OC patients. (B) Frequency of CTC clusters isolated using Parsortix in MBC. (C) Comparison between CTCs isolated from Parsortix and CellSearch in MBC.

Single, doublet and clusters of CTCs can be isolated from both MBC and OC patients. MBC patients have a higher incident rate of CTCs compared to OC patients. HER2+ CTCs were detected in patients with HER2+ tumours.

Figure 3: (A) (i) Ovarian CTCs isolated pre and post neoadjuvant chemotherapy. Ovarian CTC clusters cloaked with CD42b (B) Ovarian CTCs isolated from peripheral blood (ii) and ovarian vein (iii) at surgery.



Clinical follow up and patient recruitment is currently ongoing.

Conclusions

- Using TCD-ID and Parsortix CTC enrichment, CTC singlets, doublets and clusters can be isolated from MBC and OC patients.
- Concordance between CellSearch and Parsortix in MBC patients.
- Detection of CTC clusters in both cohorts and increased CTC detection frequencies in OC which has traditionally low numbers of CTCs.
- Clinical follow-up of our findings and further patient recruitment is on-going.
- Single-cell genomics is needed to interrogate the biology of ovarian vein isolated CTC clusters.