Circulating tumor cell enumeration using immunofluorescence staining following cell retrieval on the ClearCell® FX1 system

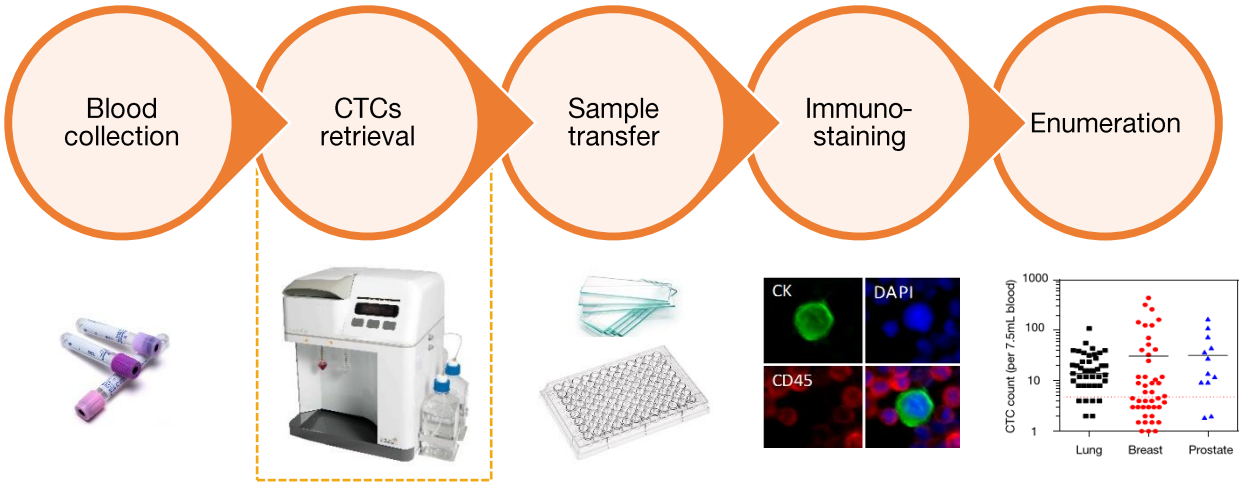

Figure 1. Workflow integrating ClearCell® FX1 system to retrieve CTCs for enumeration and biomarker characterisation using immunofluorescence assays.

Introduction

Liquid biopsy is a non-invasive procedure to detect signs of cancer using a blood sample, avoiding the need for conventional, invasive surgical procedures. One approach includes the isolation and detection of tumor cells that have detached from the primary tumor into the bloodstream of patients. These circulating tumor cells (CTCs) play an important role in real-time monitoring for patient prognostication, assessment of therapeutic response and evaluation of predictive biomarkers that can aid treatment decisions. The ability of the ClearCell® FX1 system to retrieve these CTCs from a single blood draw provides an invaluable opportunity to advance the understanding of cancer metastasis.

Linear recovery demonstrated

To demonstrate the advantages and consistency of the label-free CTCs isolation approach of the ClearCell® FX1 system, cancer samples were mimicked by spiking different concentrations (ranging from 50 to 2000 cells) of human lung adenocarcinoma tumor cells (H1975 cell line) into 7.5 mL of healthy donor blood. The results indicated a linear recovery of H1975 tumor cells across the wide dynamic range of spiked cells (Figure 2).

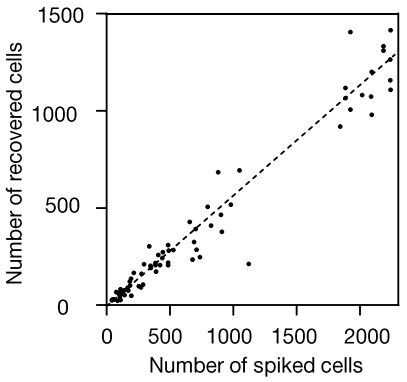

Figure 2. Validation of system efficiency and precision of the ClearCell® FX1 system. Linear recovery of H1975 tumor cells by the ClearCell® FX1 system was demonstrated with R^2=0.9537. A series of cell concentration ranging from 50-2,000 cells pre-labelled with CellTracker™ dyes were spiked into 7.5 mL of healthy donor blood.

Unbiased label-free CTCs isolation

To circumvent the bias of using epithelial biomarkers to isolate CTCs, the ClearCell® FX1 system uses an inertial focusing microfluidics technology that isolates CTCs based on their size and deformity. The label-free isolation of CTCs allows the retrieval of both EpCAM-low expressing and EpCAM-high expressing cells.

Clearbridge BioMedics
Bringing clarity to cancer

In these experiments, known EpCAM-low (A549 and PC3) and EpCAM-high (H2066 and H1975) expressing tumor cells were labelled with CellTracker™ dyes prior to spiking into healthy donor blood. The isolated cells were detected with the automated Bioview Duet imaging system and percent recovery was calculated. Percent recovery for EpCAM-high cell lines H2066 and H1975 were 64.9 ± 9.2% and 74.1 ± 10.7% respectively. Percent recovery for EpCAM-low expressing cell lines A549 and PC3 were in a similar range with 59.3 ± 7.6% and 68.6 ± 6.9% respectively (Figure 3).

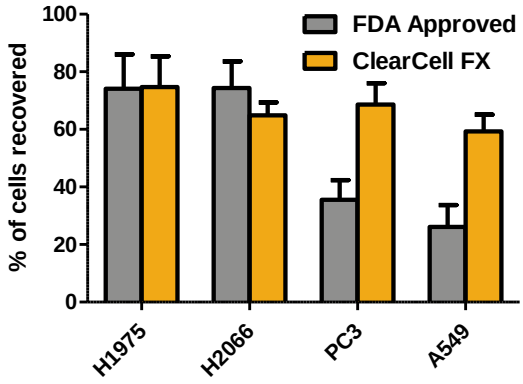

Figure 3. Validation of system efficiency and precision of the ClearCell® FX1 system. The ClearCell® FX1 system is able to isolate tumor cells from different cancer types with low and high EpCAM expression levels. Average recovery rates of EpCAM-low expressing A549 and PC3, compared with EpCAM-high expressing H1975 and H2066 cell lines.

To validate the applicability of the ClearCell® FX1 system across different cancer types, three different cancer cell lines representing lung (H1975), prostate (PC3) and breast (SKBR3) cancers were processed and successfully identified by immunofluorescence staining of pan-Cytokeratin, CD45, and DAPI (Figure 4).

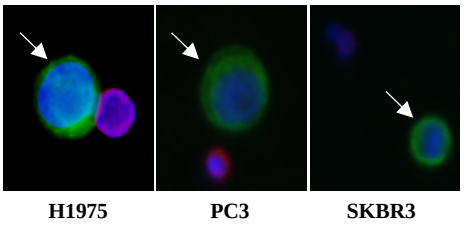

Figure 4. Validation of system efficiency and precision of the ClearCell® FX1 system. Representative images of tumor cells (CK^+ shown in green, $DAPI^+$ shown in blue and $CD45^-$ shown in red) identified with immunofluorescence staining. Arrows identify the CTCs.

The use of a label-free isolation system such as the ClearCell® FX1 system allows for the recovery of a heterogeneous population of tumor cells that can provide additional biological information about the diseased state.

Intact CTCs and CTMs isolated from breast, lung and prostate cancer patients

The enumeration and characterization of CTCs from cancer patients can lead to better informed treatment options, prognosis, and patient treatment response monitoring. Supporting studies have shown that the significant presence of CTCs (≥5 CTCs for metastatic breast and prostate, and ≥3 CTCs for colorectal cancer) is inversely associated with increased progression-free survival and overall survival.[1–3]

To further validate the capability of the ClearCell® FX1 system to isolate CTCs from different cancer types, 60 samples comprising metastatic lung, breast, and prostate cancer were studied. As the ClearCell® FX1 system obviates the need of a single biomarker and recovers a heterogeneous CTC population without bias, the number of CTC counts has a larger dynamic range from 0 to 382 as compared to EpCAM+ based assays. Immunofluorescence labelling of CK+, DAPI+, and CD133+ in metastatic lung, prostate, and breast tumor cells indicated the presence of both single CTCs and micro-emboli after cell retrieval using the ClearCell® FX1 system.

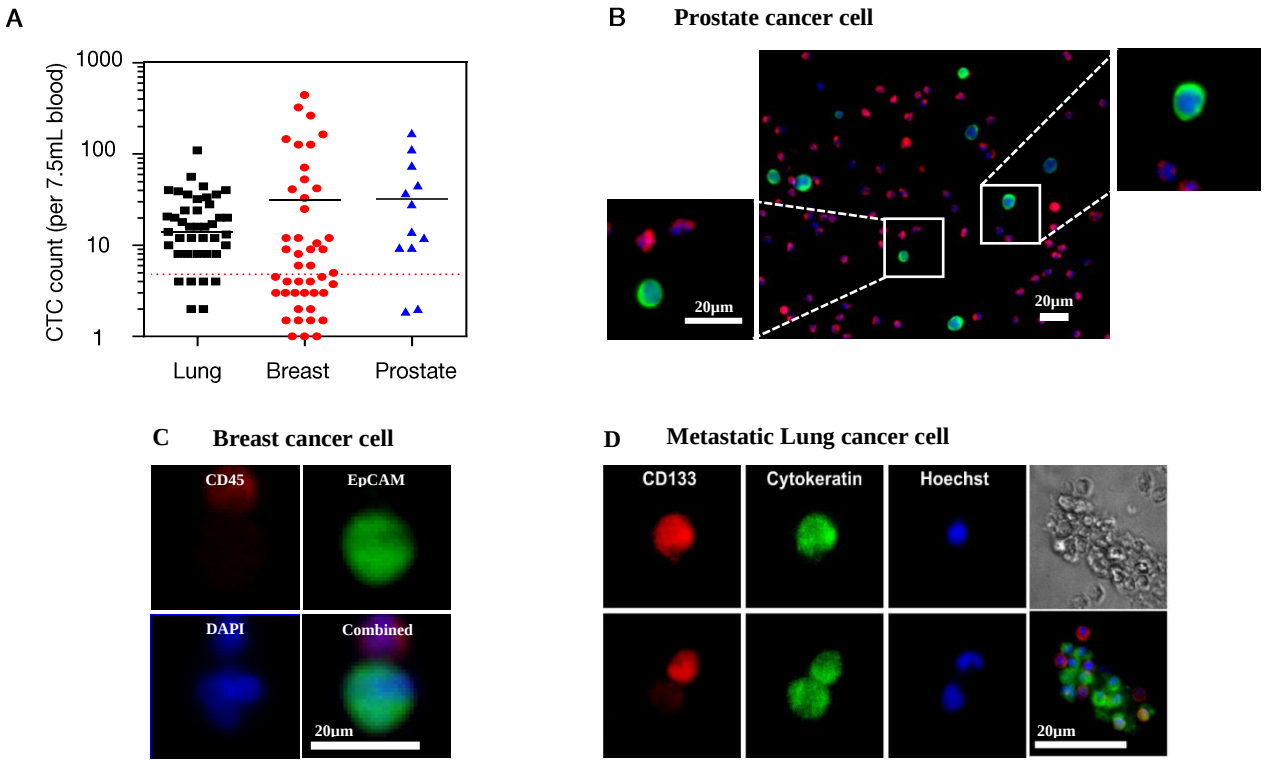

Figure 5. Clinical feasibility of using the ClearCell® FX1 system for the enumeration and characterisation of CTCs using immunofluorescence assays. (A) CTC counts of clinical blood samples from three different cancer types. Red dotted line indicates 5 CTCs count. (B) Representative images of CTCs fluorescently labelled for cytokeratin (Green), CD45 (Red), DAPI (Blue) after isolation from prostate cancer patient blood samples. Images on left and right show magnified view of cells identified as CK+/CD45-/DAPI+ indicated by the white boxes. (C) CTCs isolated from breast cancer patient fluorescently labelled for EpCAM, CD45, and DAPI. (D) CTCs isolated from metastatic lung cancer patient also show positive staining for CD133 stem cell marker. Both single CTCs and clusters can be isolated by ClearCell® FX1 system. The immunofluorescence assay demonstrates a heterogeneous population of cells within the micro-emboli.

Conclusion

The ClearCell® FX1 system enables the unbiased recovery of CTCs and CTMs through its label-free cell retrieval approach. The ClearCell® FX1 system has demonstrated consistent CTC recovery across different cancer cell lines of varying EpCAM expression and different cancer types in actual clinical samples. Wholly intact and viable CTCs collected in a liquid suspension can be transferred to various formats such as glass slides and microtiter plates for easy integration with routine pathology lab workflows including immunofluorescence staining. The immunofluorescence staining examples demonstrated the detection and characterization of the isolated CTCs and CTMs, offering a possible liquid biopsy solution. Circulating tumor cell enumeration has the potential to be used for patient stratification, treatment monitoring and personalized medicine.

References

1. Cristofanilli, M. et al. Circulating Tumor Cells, Disease Progression, and Survival in Metastatic Breast Cancer. 781–791 (2004).
2. De Bono, J. S. et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin. Cancer Res. 14, 6302–6309 (2008).
3. Cohen, S. J. et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J. Clin. Oncol. 26, 3213–3221 (2008).

Head Office
81 Science Park Drive
#02-03, The Chadwick
Singapore Science Park 1
Singapore 118257

USA
2075 Corte Del Nogal, Suite E
Carlsbad, CA 92011 USA

Japan
5-4-6 Kashiwanoha
Kashiwa City
Chiba Pref. 277-0882 Japan

For more information, email
support@clearbridgebiomedics.com