EVALUATION OF A NOVEL MICROFLUIDIC DEVICE FOR EPITOPE-INDEPENDENT ENRICHMENT OF CIRCULATING TUMOUR CELLS.

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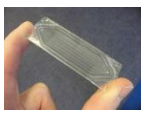
Background

- Circulating tumour cells (CTCs) have potential utility as minimally-invasive biomarkers to aid treatment decision making.
- Many CTC technologies (including the gold standard, CellSearch®) that enrich CTCs based on expression of specific epitopes may miss CTC heterogeneity and new epitope-independent methods are needed.
- The Parsortix system from ANGLE uses a patented micro-fluidic technology in the form of a disposable cassette to capture and then harvest circulating tumour cells (CTCs) from blood.

Parsortix system



Parsortix cassette

- The cassette is designed to capture CTCs based on their less deformable nature and larger size as compared to other blood components.
- Avoiding the use of antibodies for capture has a number of potential advantages including ability to capture CTCs with weak cell marker expression as well as mesenchymal cells and clusters of cells.
- Captured cells can be fixed and stained in the cassette to allow in-cassette identification and enumeration or alternatively can be recovered (harvested) to allow external staining and/or genetic analysis.

Objective

- Evaluation of the capture and harvest efficiency of the Parsortix system using normal healthy volunteer blood spiked with cultured cells.
- Establish approach with stabilised blood – 4 days kept at RT.
- Comparison of the capture efficiency of the Parsortix system with CellSearch®.

Methods

- All blood samples were collected into 10mL CellSave preservative tubes, allowing up to 96 hours for sample processing.

CellSave preservative tube

- For evaluation of the system, blood samples from normal healthy volunteers were spiked with cultured cells (HT29) pre-labelled with a fluorescent compound (CellTracker™ Green).
- Prior to enrichment, the plasma component was removed from the blood and stored for subsequent analysis (e.g. cfDNA, miRNA).
- Remaining blood was resuspended in HBS (HEPES Buffered Saline) and then enriched using the Parsortix system.
- Downstream techniques (e.g. DEPArray™ isolation, whole genome amplification) were carried out in accordance with manufacturer's instructions.

High Capture Efficiency

- Samples of 7.5 mL of HNV blood were spiked with 1000 HT29 cells (133 cells/mL) pre-labelled with CellTracker ™ Green.
- One of the samples was enriched using the Parsortix device, with the captured cells being counted following visualisation with a fluorescence microscope.
- The second sample was enriched using CellSearch®, with the captured cells being scored and counted by a qualified CellSearch® analyst.

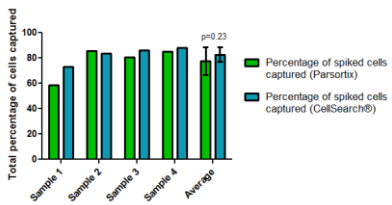


Figure 1. Comparison of capture efficiency of Parsortix and CellSearch®

- The capture efficiency of the Parsortix system is not significantly different from that of the CellSearch® system when tested using samples spiked with a cell line expressing high levels of EpCAM (Figure 1).

Effective Leukocyte Removal

- Duplicate samples of 0.5 mL and 4 mL of HNV blood spiked with CellTracker ™ Green positive HT29 cells (50 cells / mL spiked in) were separated using Parsortix, stained with DAPI and harvested.
- Cells were then visualised using a fluorescence microscope and counted.

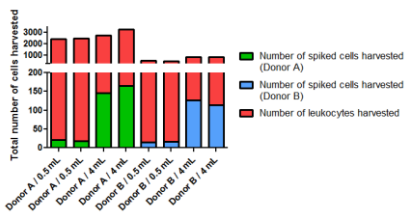


Figure 2. Impact of donor variability on number of harvested leukocytes

- An 8-fold increase in volume of enriched blood (0.5 mL to 4mL), resulted in an 8-fold increase in spiked cell recovery, without a significant increase in contaminating leukocytes (Figure 2).
- The number of contaminating leukocytes appears to be donor dependent, but independent of sample volume enriched.

Optimised Recovery

- Optimising enrichment was undertaken to reduce absolute leukocyte levels in order to simplify direct CTC molecular analysis.
- Duplicate samples of 1 mL of HNV spiked with CellTracker ™ Green labelled cells (100 cells / mL) were run through Parsortix using the optimised protocol, with both spiked cells and leukocytes being counted after harvest.

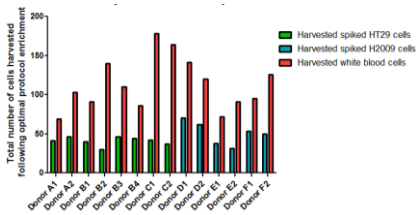


Figure 3. Evaluation of optimised enrichment protocol

- The harvest of HT29 cells ranged between 30% and 46%, whilst the harvest of H2009 cells ranged between 31% and 70% (Figure 3).
- The number of contaminating leukocytes was donor dependent and ranged between 69 and 178.

Downstream processing

- Following enrichment by the Parsortix device, cells may be subjected to further downstream processing for single cell isolation and molecular analysis.
- One of the options is the isolation of single cells from the enriched population using technologies such as the DEPArray (Figure 4a).
- Single cells or groups of cells may be subjected to whole genome amplification in order to perform mutational analysis or copy number aberration analysis (Figure 4b).

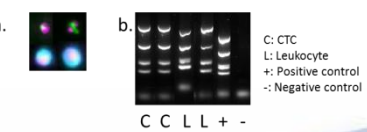


Figure 4. Single cell isolation and molecular analysis

a. Single cells isolated from the blood of a NSCLC patient using the Parsortix system, followed by immunofluorescent labelling (Green – Cytokeratin, Blue – CD45, Pink – DAPI), and DEPArray isolation.

b. Quality control gel following whole genome amplification of isolated cells using Ampli1 Kit.

Conclusions

- The Parsortix system does not require red cell lysis is compatible with blood preservation collection tubes, allows plasma collection from the same sample and is straight-forward to use with minimal user intervention.
- Spiked cell analysis indicates that the system provides a good level of capture (>80%), comparable to the reference CellSearch® system .
- Since the system is marker independent it does not require the use of capture antibodies to enrich CTCs, facilitating capture of CTCs with weak cell marker expression or cells lacking the targeted epitope, e.g. mesenchymal cells.
- Using an optimised enrichment protocol, the system delivers high harvest efficiency (c.45%) with very low levels of background leukocyte contamination (< 200 cells).
- Enriched samples provide an ideal starting point for both single cell isolation and batch molecular analysis.