# ClearCell FX®, a marker-independent process for enriching viable circulating tumour cells (CTCs) from melanoma patients' blood.

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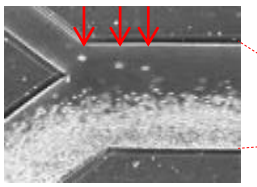
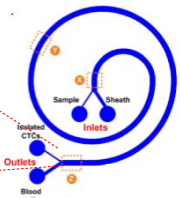
## Introduction

- Melanoma, a highly aggressive form of skin cancer, affects around 12,000 individuals annually in the UK. With a dismal 5-year survival rate for stage IV melanoma, novel insights into early detection and resistance emergence are vital.
- Circulating tumour cells (CTCs) represent a rare population of cells which have shed from a primary tumour into the bloodstream and are responsible for initiating metastases at distant sites. CTCs can be obtained via a minimally invasive 'liquid biopsy' to aid the diagnosis, prognosis and monitoring of disease, as well as to study resistance to treatment.
- The phenotypic heterogeneity of melanoma CTCs poses difficulties in isolating these cells using marker-dependent approaches and therefore other methods were sought.
- ClearCell FX® is an automated microfluidic-based system which enriches for CTCs based on size, deformability and inertia in fluid flow. ClearCell FX® exploits the impact of inherent hydrodynamic forces, present in curvilinear microchannels, on CTCs.
- In this system, a sample of cells is pumped alongside a sheath fluid in a spiral biochip at optimal flow rates (x). Particles in the sample are subjected to inertial focusing forces - namely dean drag fractionation (DDF) and inertial lift focusing (y).
- The combination of these forces separates larger cells (directed to the inner wall of the spiral) from the smaller cells (directed towards outer wall) in the sample (z).

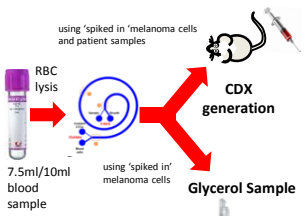
**ClearCell FX®**

**CTC chip® FR1**






## Methods




**Markers in Assay:**
HMB45
Tyrosinase
MART-1
CSPG4
CD45 (Negative excluder)
DAPI (Nuclear marker)

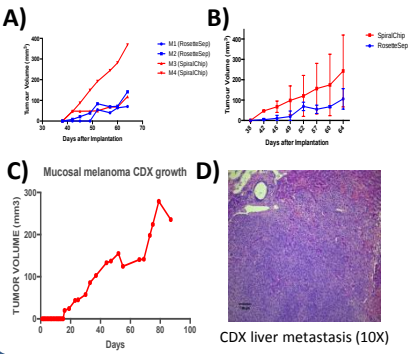
### CDX

- Enriched samples are centrifuged at 250 x G for 5 minutes.
- Cells are then resuspended in 100µl matrigel.
- This is injected subcutaneously into immunocompromised mice to generate CTC derived explants (CDX).

### Glycerol Sample

- Aliquots of enriched samples are stored in glycerol at -20°C.
- A minimal step staining assay is used to evaluate melanoma cell recovery. Sample is plated into a 96-well plate and imaged on the Opera Phenix.
- Cells are taken out of the plate and put onto DEPArray to isolate single cells.
- Whole Genome Amplification (WGA) of single cells, followed by a quality control (QC) check of the WGA process to assess DNA integrity.

## Cell Viability and CDX Model

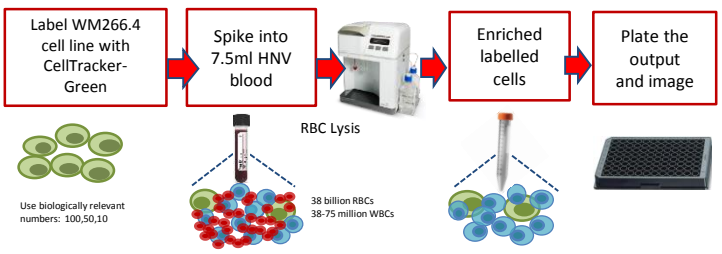



CDX liver metastasis (10X)

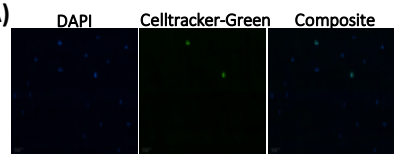
**Using 'spiked in' SKMEL13 cells we compared the ability of ClearCell FX® enriched cells to generate a CDX model in comparison to an established method - Rosette Sep.**
**A:** Paired blood samples were processed using either ClearCell FX® or RosetteSep (N=2).
**B:** Combined data from both mice.

**ClearCell FX® enriches CTCs capable of generating tumours when transplanted into immunocompromised mice.**
**C:** Patient CTCs generated a tumour in mice (F1)
**D:** H&E staining of liver tissue.

## System Validation




A)




B)

| Time Point | % Yield | WBC Count |
|---|---|---|
| Control: 91 | | |
| 24 hr | 67 | 45,869 |
| 48 hr | 61 | 94,318 |
| 72 hr | 58 | 61,285 |
| Control: 47 | | |
| 24 hr | 53 | 53,839 |
| 48 hr | 85 | 54,488 |
| 72 hr | 55 | 48,641 |
| Control: 8 | | |
| 24 hr | 75 | 30,350 |
| 48 hr | 50 | 50,973 |
| 72 hr | 75 | 84,190 |

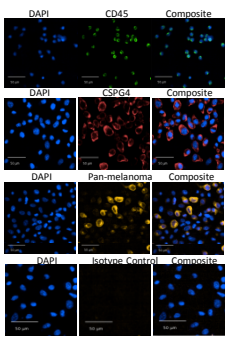
Average Yield: 66% (N=23)

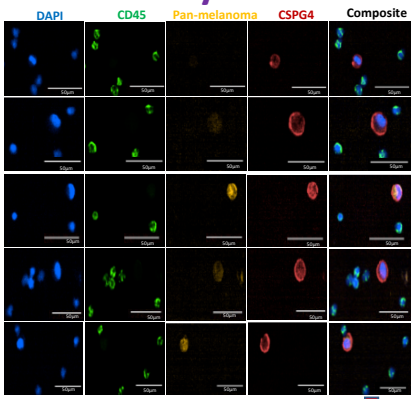
*A: A single field in a well containing the enriched output. Images were taken on the Opera Phenix. Green: labelled WM266.4 cells Blue: DAPI (nuclear marker).*

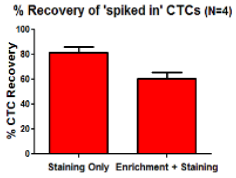
*B: A representative validation table displaying a time course from 24 - 72 hour sample storage. Table displays the percentage recovery of 'spiked in' melanoma cells along with respective WBC contamination levels from each run.*

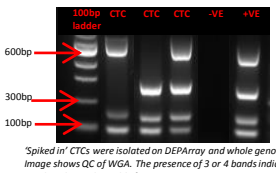
## Melanoma Assay

*Single stain controls*




DEPArray

WGA

*'Spiked in' CTCs were isolated on DEPArray and whole genome amplified. Image shows QC of WGA. The presence of 3 or 4 bands indicate DNA is of good quality and suitable for sequencing.*

## Conclusions

ClearCell FX® provides a robust and efficient method for enriching CTCs from patients' blood without affecting cell viability, and downstream functional analysis is uncompromised. Enriched cells can be assessed for the expression of melanoma markers and subsequently sequenced for confirming mutational status. We have also demonstrated the ability of ClearCell FX® enriched cells to generate CDX models which can be used to assess potential therapies.

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