Molecular characterization of PD-L1 status of circulating tumor cells (CTCs) isolated with a novel label-free inertial microfluidic system from patients (pts) with advanced cancers

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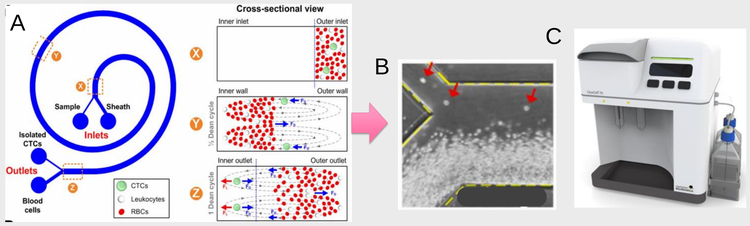
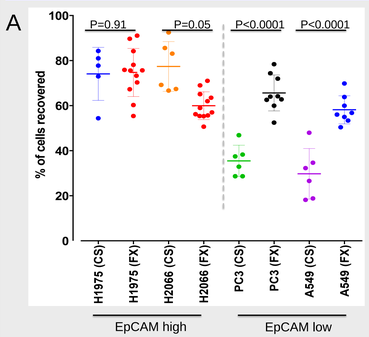
Introduction

The ClearCell® FX system (FX) is a novel label-free platform, which enriches CTCs through inertial microfluidics in comparison to the FDA-approved CELLSEARCH® system (CS) which uses EpCAM-immunomagnetic beads to isolate CTCs.
We hypothesise that size-based CTC capture will lead to more accurate assessment of CTCs, including tumour heterogeneity and PD-L1 expression. FX may also capture CTCs that have undergone epithelial-mesenchymal transition, resulting in loss of EpCAM expression and potentially being missed by CS, which are prevalent in advanced cancers.



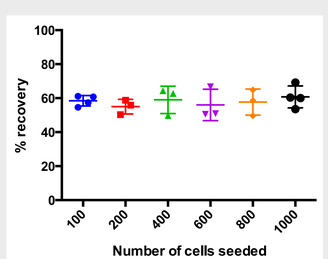
CTCs are enriched from blood components using Dean Flow Fractionation. **A**) Patient blood and sheath fluid are pumped in and are separated by a density gradient (X). The tube curvature cause shear and lift forces that cause cell migration across the density gradient (Y). Rate of movement is based on cell size with smaller cells travelling faster. (Z) At 1 dean cycle the larger CTCs are most separated from the smaller blood cells and are drawn off. **B**) A representative image of point Z using tumour cells spiked into whole blood (Hou et. al. 2015 Scientific Reports). **C**) Representative image of the ClearCell® FX platform.

System Validation Results



B

Cell line	EpCam Level	Platform	Tube	Recovery Results % (± SD)
H1975	High	CS	CellSave	74.1 ± 11.9
		FX	STRECK	74.7 ± 10.7
PC3	Low	CS	CellSave	35.5 ± 6.9
		FX	STRECK	68.6 ± 7.4
A549	Low	CS	CellSave	26.1 ±7.6
		FX	STRECK	59.3 ± 5.8
NCI-H2066	High	CS	CellSave	74.4 ± 9.2
		FX	STRECK	64.9 ± 4.5



Validation of the FX platform using CellTracker labelled cell lines spiked into HV blood: (**A**&**B**) Comparison of EpCAM-high and EpCAM-low cell lines enumerated on both FX and CS. Although similar counts were observed with EpCAM-high cell lines (FX 67%±11 vs CS 74%±10 [p=0.11]), a significantly higher recovery of EpCAM-low cell lines was seen with FX compared to CS 62%±8 vs 32%±9 [p<0.0001]). . (**C**) Consistent recovery of 60-70% seen regardless of seeding density on FX.

Patient Sample Results

A Non-Small Cell Lung Cancer

Patient no.	CellSearch Count	FX Count	TTF-1+ PD-L1 -	TTF-1+ PD-L1+
1	0	26	0	26
2	0	30	2	10
3	0	90	4	56
4	0	16	8	4
5	1	6	2	4
6	3	4	0	4
7	0	80	0	0
8	0	40	0	12
9	0	4	0	0
10	1	28	0	0
11	0	20	2	2
12	0	24	0	10
13	0	30	0	10
14	38	44	0	10
15	0	92	4	8
16	0	28	0	2
17	5	40	10	6
18	0	304	10	190
19	0	10	0	6
20	0	90	0	88
21	0	66	4	22

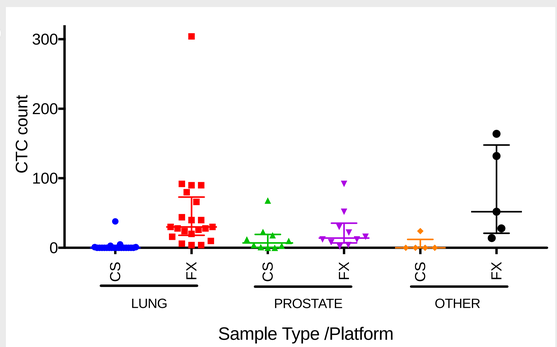
B Castration-Resistant Prostate Cancer

Patient no.	CellSearch Count	FX Count	AR+ PD-L1 -	AR+ PD-L1+
1	0	22	2	6
2	18	4	0	0
3	10	30	0	6
4	23	2	0	2
5	3	52	0	8
6	0	16	0	0
7	1	12	0	8
8	12	8	0	0
9	4	12	0	0
10	68	92	0	0

C Other Cancer Types

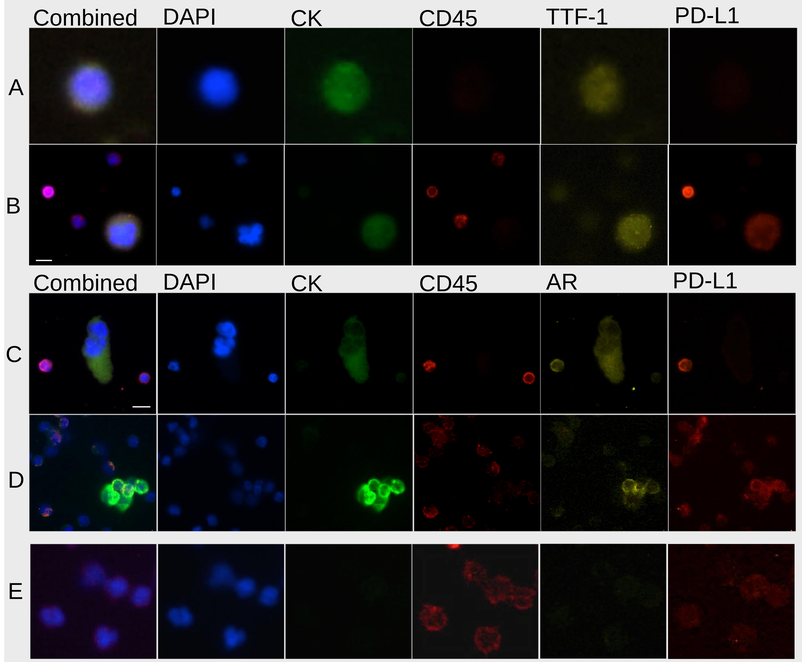
Patient no.	Tumour Type	CellSearch Count	FX Count	EpCAM+ PD-L1 -	EpCAM+ PD-L1+
1	Ovarian	0	28	0	4
2	Ovarian	0	14	0	14
3	Ovarian	0	164	0	76
4	Breast	24	132	0	20
5	Rectal	0	52	0	16

D



Of 36 pts, CTC counts were higher with FX vs CS in 33 (92%) pts: (**A**) 21/21 NSCLC, (**B**) 7/10 prostate, (**C**) 3/3 ovarian, 1/1 rectal and 1/1 breast cancer pts. Summarised in graph showing median and interquartile range (**D**). No CTCs were detected in HV blood (N=10) on FX and CS. 18/21 NSCLC, 5/10 prostate, 3/3 ovarian, 1/1 rectal and 1/1 breast cancer pts had ≥1 PDL1+ CTCs. Heterogeneity in PD-L1 expression was observed. While 18/21 NSCLC pts had ≥1 PD-L1+ TTF1+ CTCs, only 9 of these 18 pts had 100% PD-L1+ TTF-1+ CTCs. 5/10 prostate cancer pts had ≥1 PD-L1+ AR+ CTCs, but only 4 of these 5 pts had 100% PD-L1+ AR+ CTCs. All 3 ovarian cancer pts had ≥1 PD-L1+ EpCAM+ CTCs, with no PD-L1- EpCAM+ CTCs detected. 1/1 rectal and 1/1 breast cancer pts had 100% PD-L1+ EpCAM+ CTCs.

5 Colour IF



Five-colour immunofluorescence was used to identify CTCs; DAPI, CK (Cell Signaling), CD45 (Miltenyl), PD-L1 (AbCam) and either TTF-1 (Lung adenocarcinoma marker; Dako), AR (Prostate marker; AbCam) or EpCAM (other tumour types; Cell Signaling).
(**A**) CTC from an NSCLC patient, positive for TTF-1 and negative for PD-L1. (**B**) CTC from an NSCLC patient, positive for both TTF-1 and PD-L1. (**C**) CTC from a Prostate patient, positive for AR and negative for PD-L1. (**D**) CTC from a Prostate patient, positive for both AR and PD-L1. (**E**) WBCs identified by CD45 positivity.

Conclusions

Consistently high recovery rates of cell lines were seen in FX vs CS, regardless of EpCAM expression. Higher CTC counts were isolated with FX vs CS in 92% of pts across the different tumour types of NSCLC, Prostate, Rectal, Ovarian and Breast. CTC PD-L1 heterogeneity was observed and may in part explain differences in antitumor responses to PD-1/PD-L1 inhibitors. Clinical qualification of this 5-color IF PD-L1 CTC assay is ongoing in a PD-1 inhibitor NSCLC trial.