Characterization of PD-L1 expression on circulating tumor cells (CTCs) isolated with a label-free inertial microfluidic system from advanced non-small cell lung cancer patients (NSCLC pts)

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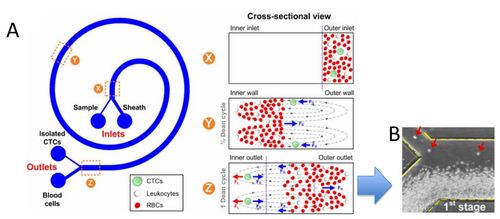
Introduction

NSCLC exhibits intratumor heterogeneity, with subpopulations of cells undergoing epithelial-mesenchymal transition. Such CTCs from NSCLC pts may be missed by the EpCAM-based CELLSEARCH® system (CS). The label-free ClearCell® FX inertial microfluidic system (FX) isolates CTCs based on size and inertia and may lead to more accurate CTC capture. PD-1/PD-L1 inhibitors have shown benefit in PD-L1+ NSCLC pts, but responses are still seen in PD-L1- pts, suggesting limitations in tumor PD-L1 testing. Also, PD-L1 testing on CTCs may be more practical than tumor rebiopsies and may provide insights into cancer heterogeneity.

ClearCell® FX System Mechanism of Isolation



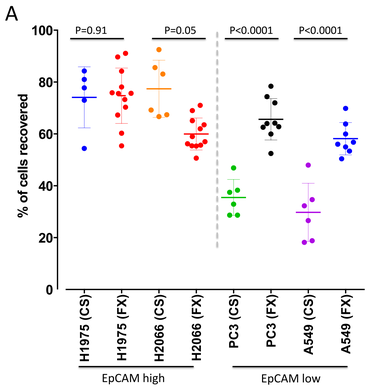
CTCs are enriched from blood components using Dean Flow Fractionation. A) Patient blood and sheath fluid are pumped in and are separated by a density gradient (X). The tube curvature cause shear and lift forces that cause cell migration across the density gradient (Y). Rate of movement is based on cell 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).

Method

FX was validated with EpCAM-high and EpCAM-low cancer cell lines labeled with CellTracker dyes spiked into healthy volunteer (HV) blood for repeatability and reliability. Enriched cells were detected with the automated Bioview Duet imaging system for recovery (%). Identical spiking studies were conducted on CS for comparison. Antibodies for 5-color immunofluorescence (IF) (CK, CD45, DAPI, TTF1 [to detect lung adenocarcinoma cells], PD-L1) were optimized on EpCAM-high and EpCAM-low cell lines. 8ml of blood from NSCLC pts were enriched with FX for 5-color IF characterization. 7.5ml of blood from the same pts taken at identical timepoints were enumerated with CS for comparison. Blood was obtained from HV for CTC enumeration on FX and CS as controls.

System Validation Results

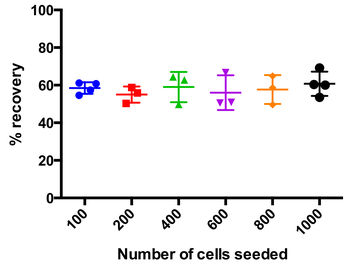
A



B

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

C



System validation of the FX platform using cell tracker labelled cell lines: (A&B) Cell recovery of EpCAM-high cancer cell lines using FX produced similar counts to CS. In contrast, for EpCAM-low cells, a significant difference in cell recovery between FX and CS was seen. The FX platform also exhibited a consistent recovery of 60-80% irrespective of seeding density (C)

NSCLC Patient Samples Results

Baseline Patient Information

A

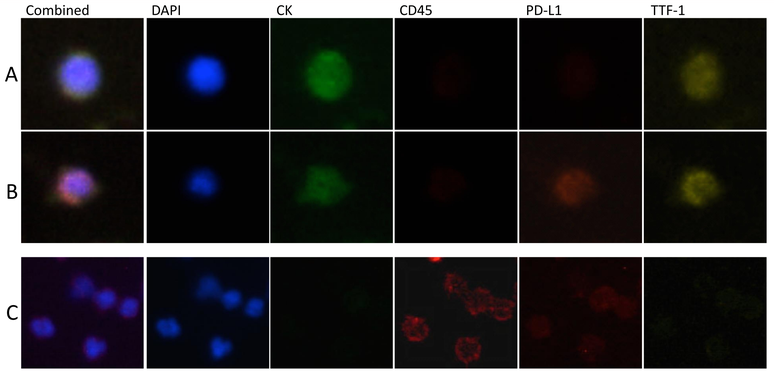
Patient no.	Age at consent	Gender	Number of prior chemotherapy lines	EGFR	ALK	KRAS	PD-L1
MC01417	78	M	2	No	No	No	UNK
MC01596	62	F	1	No	No	No	UNK
MC01623	59	F	2	No	No	No	UNK
MC01681	69	F	1	Yes	No	UNK	UNK
MC01677	64	F	0	Yes	UNK	UNK	UNK
MC01679	51	M	1	Yes	No	UNK	UNK
MC01664	56	M	1	Yes	No	No	UNK
MC01700	71	F	1	Yes	No	No	UNK
MC01711	52	M	1	No	No	UNK	UNK
MC01755	67	F	3	No	No	UNK	UNK
MC01859	82	M	1	No	No	No	UNK
MC01844	75	F	1	No	Yes	No	UNK
MC01879	64	F	3	No	No	UNK	UNK
MC01880	68	F	1	UNK	UNK	UNK	UNK
MC01882	81	F	1	No	UNK	UNK	UNK
MC01884	62	F	5	No	No	No	UNK
MC01885	53	F	2	No	No	No	UNK
MC01886	69	F	1	No	No	UNK	UNK
MC01878	39	M	1	No	Yes	No	Yes
MC01887	51	F	3	No	No	UNK	UNK
MC01952	72	M	2	No	No	UNK	UNK

B

Patient no.	Female	Male	Total
Age years : median (range)	65 (51-81)	61 (39-82)	64 (39-82)
Gender	14	7	21
Prior Surgery	0	0	0
ECOG at consent			
0	3	1	4
1	11	6	17
Number of prior chemotherapy lines			
0	0		0
1	7	5	12
2	4	4	8
3	9		9
5	5		5
Prior PD-1/PD-L1 directed therapy	0	0	0
Smoking status			
Ex smoker	8	5	13
Never	5	2	7
Smoker	1		1
Survival Status			
alive	12	5	17
dead	2	2	4
CTC counts			
CS mean (range)	3 (0 - 38)	1 (0 - 3)	2 (0 - 38)
CC mean (range)	66 (0 - 304)	24 (4 – 76)	52 (0 - 304)

A population of stage IV progressing NSCLC (adenocarcinoma) patient of varied background and age was utilised for this study (A&B).

5 Color IF on patient samples identifying CTCs

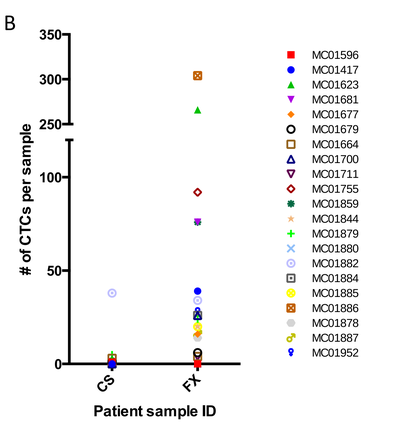


CTCs in NSCLC patient samples identified using 5 colour Immuno-fluorescence (A) a CTC positive for TTF-1 and negative for PD-L1 (B) a CTC positive for both TTF-1 and PD-L1 and (C) leukocytes that are identified by CD45

A

Patient no.	Cellsearch count	Clearbridge Streck 1 Total Count	TTF-1+ PD-L1-	TTF-1+ PD-L1+
MC01417	0	39		
MC01596	1	0		
MC01623	3	266		
MC01681	0	76	0	2
MC01677	0	16	4	2
MC01679	1	6	1	2
MC01664	3	4	0	2
MC01700	0	26	2	6
MC01711	0	4		
MC01755	0	92	2	4
MC01859	0	76	2	11
MC01844	0	20	1	3
MC01879	5	24	5	3
MC01880	0	0	0	0
MC01882	38	34	0	5
MC01884	0	26	0	1
MC01885	0	20	0	5
MC01886	0	304	5	95
MC01878	0	14	0	5
MC01887	0	16	1	1
MC01952	1	28	0	0

B



CTCs were detected in 19/21 pts with progressing stage IV NSCLC (adenocarcinoma) (mean 57; range 4-304) using FX, vs 7/21 pts (mean 7; range 1-38) using CS. CTC counts were higher with FX vs CS in 19/21 (90%) NSCLC pts, p=0.012. No CTCs were detected in HV with FX (n=10) and CS (n=5). After FX enrichment, CTCs from 17/21 NSCLC pts were available for 5-color IF testing. Of these 17 pts, 15 had TTF1+ CTCs. All 15 pts with TTF1+ CTCs had ≥1 PD-L1+ CTC. 6/15 (40%) pts only had PD-L1+ TTF1+ CTCs, with no PD-L1- TTF1+ CTCs. PD-L1 CTC heterogeneity was seen in the other 9/15 pts, with co-existing PD-L1+ and PD-L1- TTF1+ CTCs. 6 of these 9 pts had more PD-L1+ TTF1+ CTCs than PD-L1- TTF1+ CTCs.

Conclusions

FX resulted in consistently high cell recovery rates regardless of EpCAM status. Higher CTC counts were isolated with FX vs CS in 90% of NSCLC pts. 40% of NSCLC adenocarcinoma pts had PD-L1+ TTF1+ CTCs, but PD-L1 CTC heterogeneity was seen in other pts, which may in part explain differences in responses to PD-1/PD-L1 inhibitors in NSCLC