Single cell molecular characterization and PD-L1 expression analysis of model CTC using the RareCyte platform

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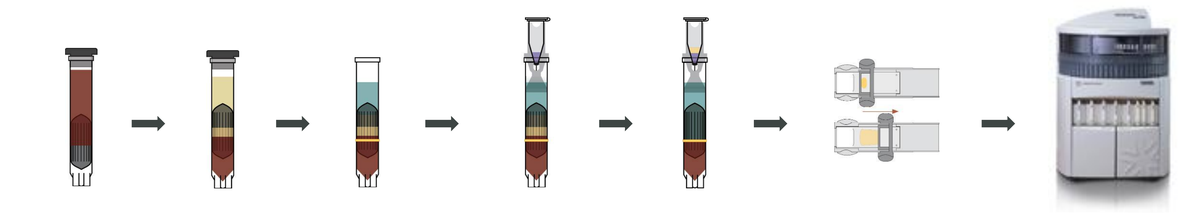
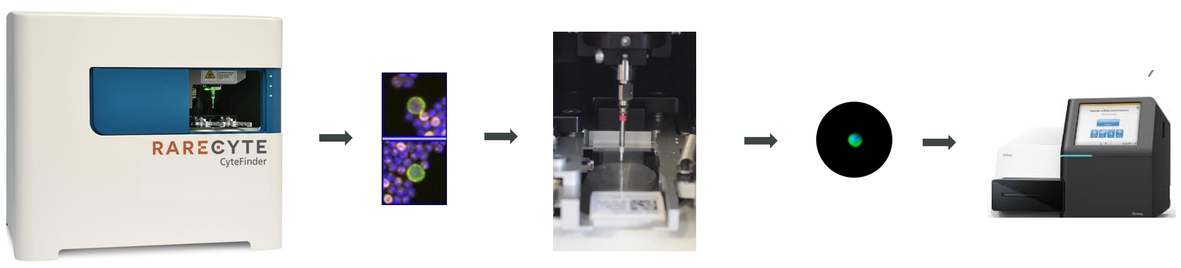
Background

Circulating tumor cells (CTC) are seeds for metastasis, the mechanism most responsible for cancer-related deaths. CTCs can be detected in peripheral blood, affording oncologists the attractive potential to phenotype a patient's cancer and to monitor changes in disease over time using a non-invasive procedure. Evaluation of CTCs are thus an attractive approach to understanding the potential response or non-response to anti-PD-1/PD-L1 therapies. CTCs are challenging to identify and characterize because they are exceedingly rare events within blood. RareCyte has developed the AccuCyte® - CyteFinder® system, which provides an exquisitely sensitive and repeatable workflow from blood collection to single CTC isolation. In a blinded fashion, we evaluated the sensitivity of RareCyte platform to detect model spike-in CTCs and correctly phenotype them based on PD-L1 expression and molecular characterization.

Sample preparation and analysis workflow



Transfer blood to Separation Tube | Centrifuge | Apply CyteSeal, remove plasma & add Displacement Fluid | Add Collector with Isolation Tube | Centrifuge to capture nucleated cells | Spread onto slides | Stain cells with immunofluorescent antibodies on automated slide stainer



CyteFinder Imaging System with CyteMapper® analysis tools | Retrieve cells with CytePicker® module | Deposit cell into PCR imaging tube | Ampliseq for Illumina Cancer HotSpot Panel sequencing with MiSeq

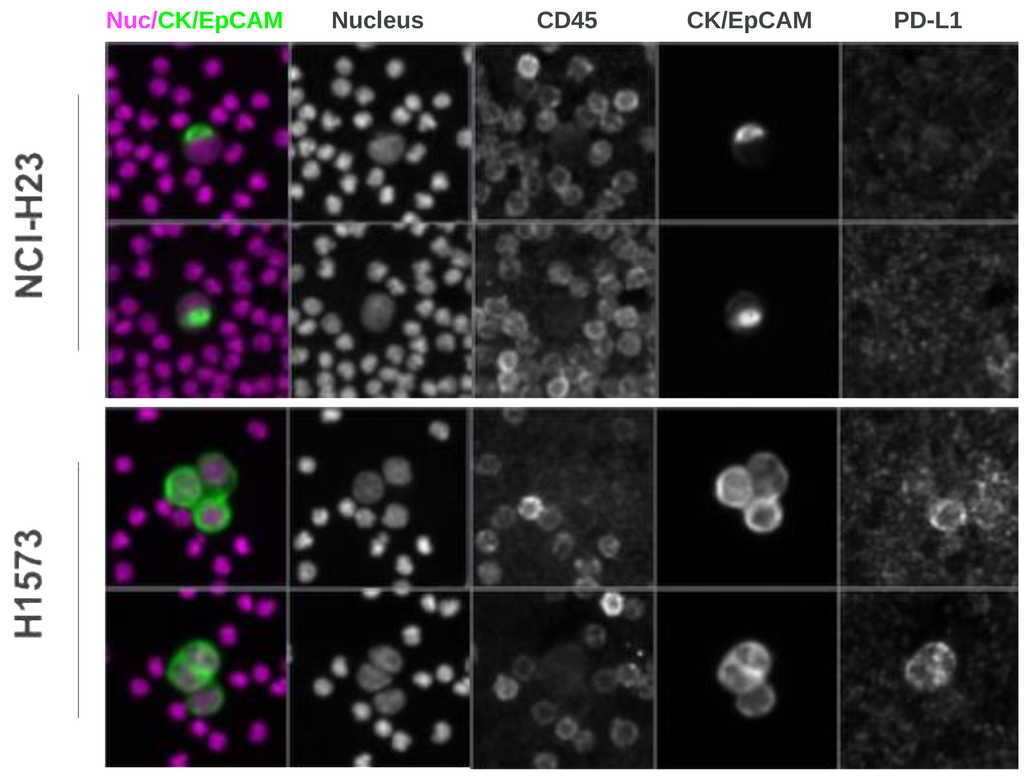


Figure 1: PD-L1 CTC spike-in models. 4-parameter analysis of cells detected using CyteFinder Imaging System

Sample	Cell Line	CTC Count@ RareCyte	CTC Count@ Pharma Partner	PDL1 MFI: Mean ± Std Dev	PDL1 MFI: Range	PDL1 MFI: Med (IQ Range)
1A	A549	81	106 - ND	154 ± 25	17 - 1597	84 (48 – 195)
2A	NCI-H358	86	118 - ND	364 ± 24	63 - 1327	322 (207 – 461)
3A	None	0	N/A	NA	NA	NA
4A*	NCI-H441	**4	23 - 5	898 ± 187	455 - 1362	887 (455 – 1260)
5A	NCI-H23	**16	26 - 11	148 ± 40	20 - 578	66 (53 – 227)
6A 6B	MCF7	**7 **4	21 - 16	60 ± 20	28 – 156	44 (31 – 83)
7A	SK-MEL-28^	0	16 - 9	NA	NA	NA
8A 8B	H1573^^	3 85	22 - 18	167 ± 53	129 – 228	144 (129 – 228)
9A	MDA-MB-231	7	12 - 9	312 ± 148	73 - 534	323 (202 – 400)
10A	NCI-H441	46	80 - ND	930 ± 405	67 - 1994	957 (661 – 1110)
11A	NCI-H23	7	9 - 9	92 ± 72	16 - 194	57 (34 – 154)
12A	SKBR3	14	17-10	887 ± 424	18 - 1636	979 (675 – 1098)

Table 1: PD-L1 CTC summary
@ Cell count per 7.5 mL; RareCyte = CyteFinder; Pharma Partner = ViCell (pre-spike) – Calcein staining (post-spike)
* 4 of a total of 8 slides were stained/enumerated
** Clots visible around float for all samples processed on 180613; may affect CTC recovery
^ melanoma cell line that does not express EpCAM or CK and therefore not detected by the RareCyte panel used
^^ highly clumpy cell line

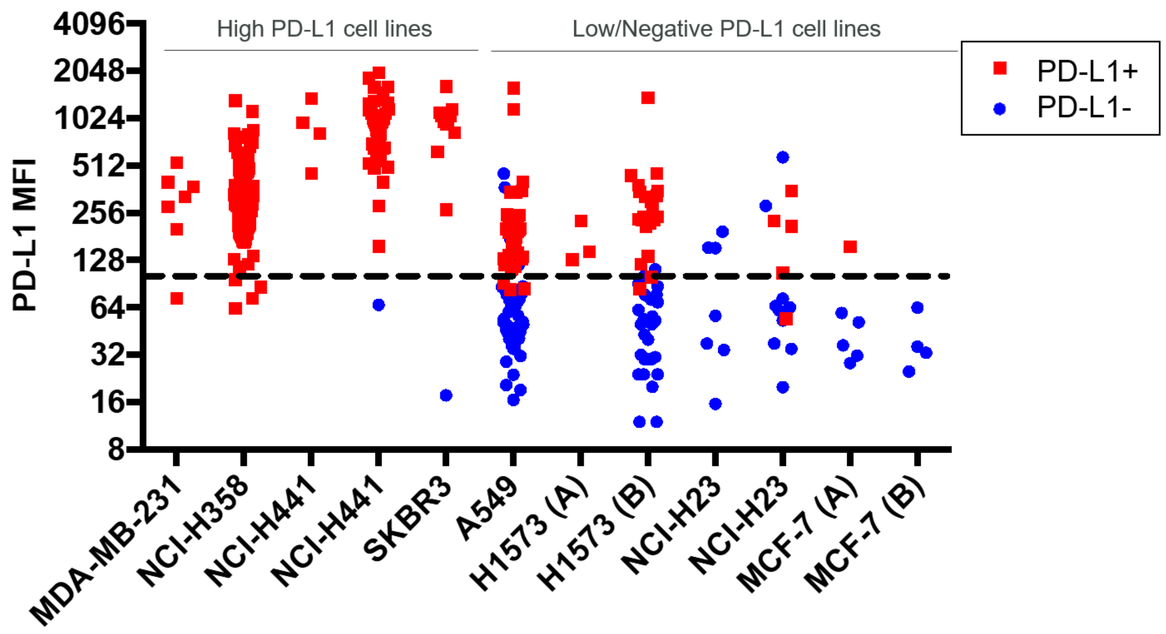


Figure 2: PD-L1 expression on CTC spike-in models. PD-L1 MFI (Mean Fluorescent Intensity) per pixel per cell using CyteFinder. CTC were visually stratified as PD-L1+ (■) or PD-L1- (●). Dashed line represents the 75% interquartile of all PD-L1- cells.

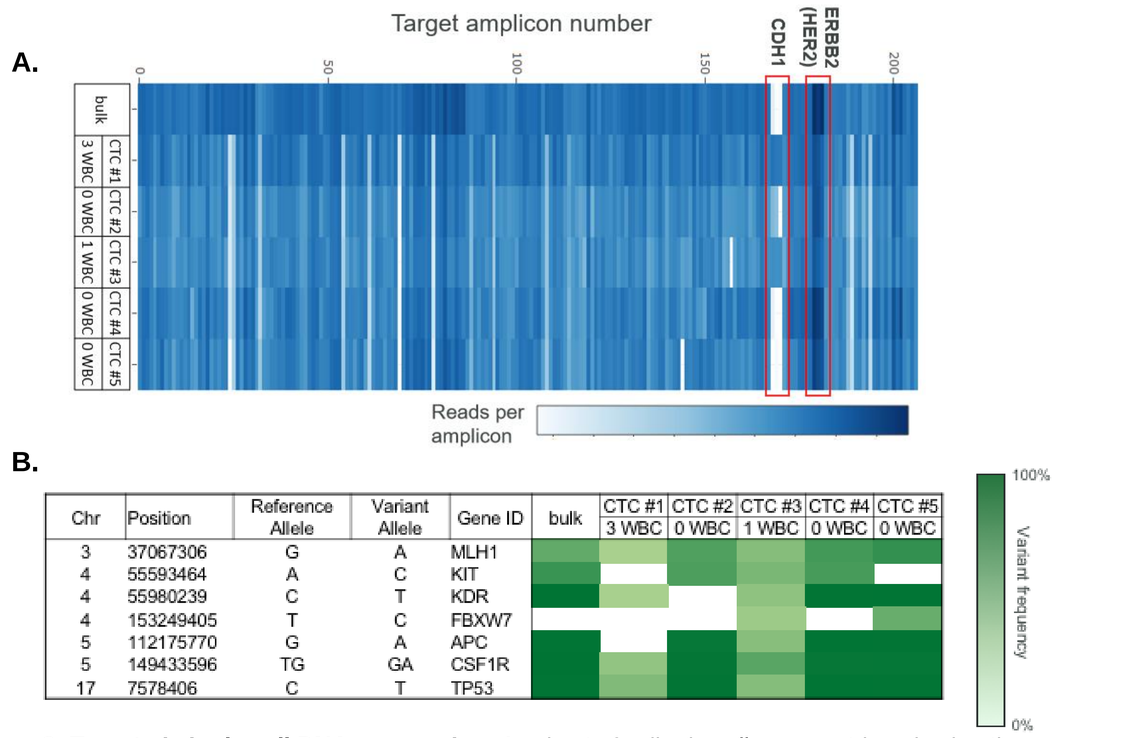


Figure 3: Targeted single cell DNA sequencing. A subset of spike-in cells were retrieved using the CytePicker Module and single-cell DNA sequencing was performed on a MiSeq (Illumina) using AmpliSeq™ Cancer Hotspot Panel (Illumina). A representative coverage heatmap displays the number of reads per target amplicon (A). Variant analysis was performed to identify cell line-specific mutations in individual cells (B).

Cell line	Expected mutation(s)@	Identified by single cell sequencing
A549	KRAS G12S STK11 Q37stop	✓ ✓
NCI-H358	TP53 deletion KRAS G12C	✓ ✓
MCF-7	PIK3CA E545K	✓
MDA-MB-231	TP53 R280K BRAF G464V* KRAS G13D*	✓
SKBR3	HER2 amplification CDH1 deletion MLH1 S406N KIT M541L TP53 R175H FBXW7 Y458C	✓ ✓ ✓ ✓ ✓ ✓

Table 2: Single cell targeted DNA NGS variant analysis summary.
@ as determined by sequencing bulk genomic DNA isolated from cultured cells
* variants did not meet the detection threshold in single cells due to high numbers of co-retrieved white blood cells

Results

Upon sample unblinding, it was revealed that no CTCs were identified in no spike controls nor spikes of non-epithelial lines. Of the 8 epithelial lines tested, recovery frequencies of 7 were closely correlated with expected values, with the exception of the H1573 line, likely due to large cell clusters. PD-L1 expression correlated with expected results and mutations identified by sequencing matched published reports.

Conclusions

In a blinded study using 8 different cell lines as model CTC the RareCyte platform produced expected results for recoveries, PD-L1 phenotype and mutational analysis of retrieved CTCs. These results provide confidence in the utility of the RareCyte platform for clinical CTC enumeration, PD-L1 assessment, and single cell molecular characterization.