Next Gen Liquid Biopsy: Comprehensive Analysis from a Single Tube of Blood

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RARECYTE® Precision Biology for Life Sciences

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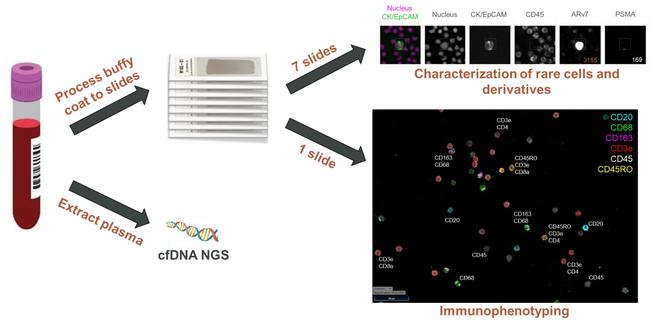
BACKGROUND

In this study, we utilize the high-multiplex protein quantitation capability of Orion™, and cell picking capability of CyteFinder®, to perform a comprehensive liquid biopsy analysis of whole blood to enumerate circulating tumor cells (CTCs), large extracellular vesicles (LgEVs), and CTC-white blood cell hybrids; quantify protein biomarker expression on CTCs; perform immune profiling on white blood cells (WBCs); and perform targeted mutation profiling on CTCs and cell-free DNA (cfDNA) from plasma, using 7.5 mL of blood. This array of tests was applied to cancer samples to demonstrate clinical feasibility.

METHODS



Multi-analyte workflow. Cells from liquid biopsy samples were processed to microscope slides using AccuCyte® blood processing kits. Slides were fixed and stained with either a 5-plex CTC assay or an 18-plex immune-oncology panel, imaged using CyteFinder® or the Orion Spatial Biology platform to evaluate biomarker expression on CTCs, LgEVs, and hybrid cells, and to profile WBCs. Individual CTCs and WBCs from clinical samples were isolated using CytePicker® for downstream DNA analysis. cfDNA was isolated from plasma removed during the AccuCyte process. Isolated CTCs, WBCs, and cfDNA were sequenced using a cancer hotspot targeted NGS panel to detect molecular abnormalities.

IMMUNE CELL PROFILING

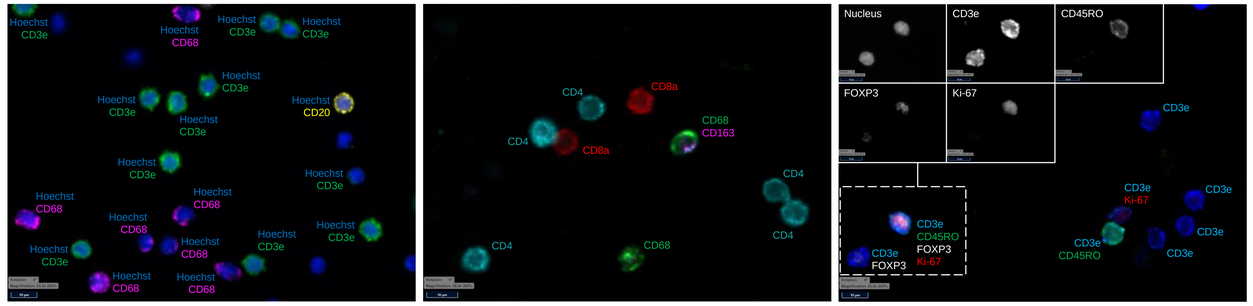
Example 18-plex Staining Panel

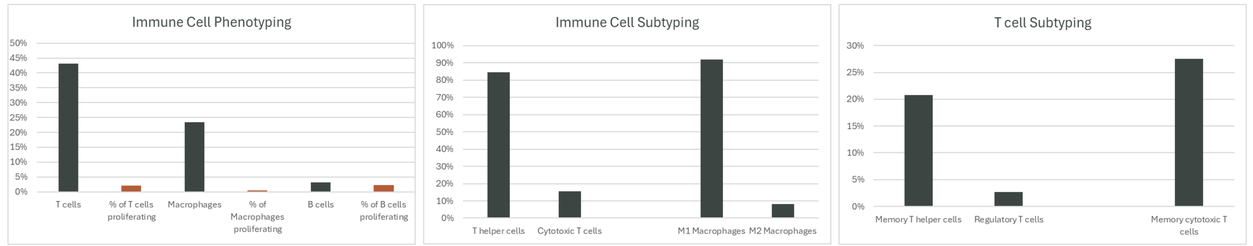
Hoechst
CD45
Ki-67
CD3e
CD20
CD4
CD163
CD8a
E-cadherin
PD-L1
PD-1
CD45RO
FOXP3
CD68
CD31
SMA
Pan-CK
Vimentin

Cell Type / Cell State	Biomarkers
T cells	CD3e
T helper	CD3e, CD4
T cytotoxic	CD3e, CD8
B cells	CD20
Macrophage (CD68)	CD68
M2 Macrophage (CD163)	CD163, CD68
T reg	CD3e, CD4, FOXP3
Immune checkpoint T cell	PD-1, CD3e, CD4
Immune checkpoint macrophage	PD-L1, CD163
Immune checkpoint (tumor)	PD-L1, Pan-CK
Memory T helper	CD45RO, CD3e, CD4
Memory T cytotoxic	CD45RO, CD3e, CD8a
T cell proliferation	CD3e, Ki-67
Tumor proliferation	Pan-CK, Ki-67

Immune cell profiling through customizable panel design. Staining panels of 17 biomarkers plus Hoechst (nuclear dye) are fully customizable to targets of interest. Panels include a broad array of markers to characterize many different cell types (B cells, T cells, Macrophages, Granulocytes, Monocytes, etc.) and/or add depth to the phenotyping through inclusion of proliferation markers (e.g. Ki-67), activation markers (e.g. CD45RO, Granzyme B, HLA-DR, etc.), or checkpoint markers (e.g. PD-1, PD-L1, etc.).

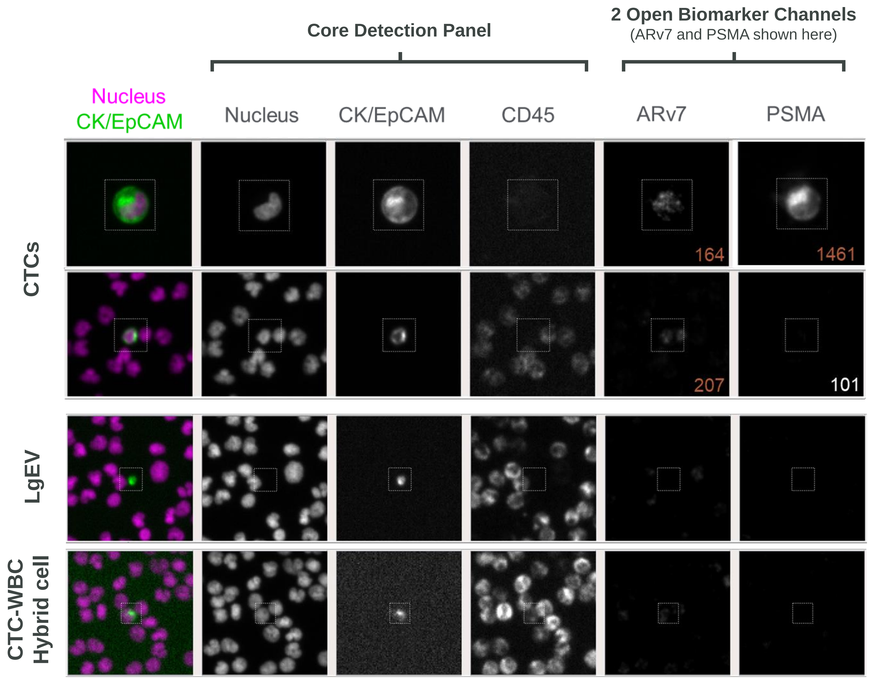
WBC PROFILING FOR REAL-TIME STUDY OF IMMUNE RESPONSE TO TUMOR





WBC profiling assesses changes in immune cell numbers and phenotypes in real-time. Immunophenotyping allows clinicians to monitor a patient's immune response to treatment at regular and frequent intervals via liquid biopsy. Immunophenotyping can also be used to determine mode of action through pharmacodynamic monitoring of patients on clinical trials. Images are representative of features characterized in bar charts. Bar charts show results from full scan area.

CHARACTERIZATION OF CTCs, LgEVs, AND HYBRID CELLS



Patient	#CTCs	ARv7 %+	PSMA %+
#1	1	100	0
#2	384	96.5	0
#3	3	33.3	33.3
#4	4	75	75
#5	2	100	0
#6	1	100	0
#7	4	75	25
#8	86	27.9	98.8
#9	9	77.8	11.1

Table of enumerated CTCs and biomarker positivity. Biomarker positivity is characterized on enumerated CTCs and cell derivatives. Data shown are from 9 Stage IV prostate cancer patient samples stained with an ARv7/PSMA dual biomarker assay.

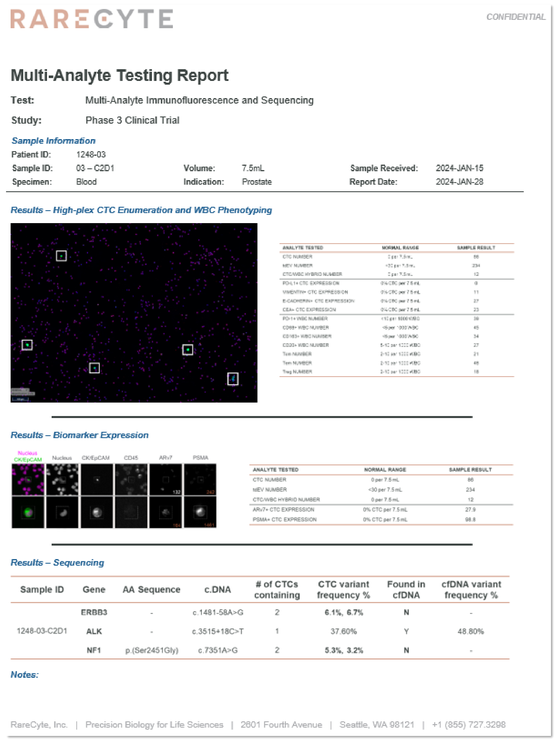
Example images of CTCs and cell derivatives. CTCs are characterized as nucleated, CK/EpCAM+, CD45- ; LgEVs are anucleated, CK/EpCAM+, CD45-; CTC-WBC hybrids are nucleated, CK/EpCAM+, CD45+. In this example, expression of two biomarkers germane to prostate cancer, ARv7 and PSMA, are quantified. These biomarkers can be replaced with others pertinent to study of interest.

GENOMIC PROFILING OF TUMOR

Sample ID	Gene	AA Sequence	c.DNA	# of CTCs containing	CTC variant frequency %	Found in cfDNA	cfDNA variant frequency %
#2	MLH1	p.(Pro536=)	c.1608T>C	2	9.1%, 1.0%	N	-
#5	ATM	p.(Lys578=)	c.1734A>G	2	7.7%, 2.6%	N	-
#8	PTEN	p.(Ile168Ser)	c.503T>G	1	23.70%	Y	39.10%
	PTEN	-	c.493-10T>A	1	23.30%	Y	38.40%
	PTEN	-	c.802-28T>C	2	15.9%, 1.0%	N	-
#9	ERBB3	-	c.1481-58A>G	2	6.1%, 6.7%	N	-
	ALK	-	c.3515+18C>T	1	37.60%	Y	48.80%
	NF1	p.(Ser2451Gly)	c.7351A>G	2	5.3%, 3.2%	N	-

Genomic sequencing of individual CTCs and plasma-derived cfDNA provides a comprehensive understanding of the molecular abnormalities that comprise a patient's disease in real-time. Clinicians can monitor changes in the driver mutations of a patient's disease over the course of treatment and modify care as needed.

COMPREHENSIVE SAMPLE REPORT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Multi-Analyte Testing Report

Test: Multi-Analyte Immunofluorescence and Sequencing
Study: Phase 3 Clinical Trial

Sample Information

Patient ID: 1248-03
Sample ID: 03 – C2D1 — Volume: 7.5mL — Sample Received: 2024-JAN-15
Specimen: Blood — Indication: Prostate — Report Date: 2024-JAN-28

Results – High-plex CTC Enumeration and WBC Phenotyping

ANALYTE TESTED	NORMAL RANGE	SAMPLE RESULT
CTC NUMBER	0 per 7.5 mL	86
MEV NUMBER	<30 per 7.5 mL	234
CTC:WBC HYBRID NUMBER	0 per 7.5 mL	12
ARv7+ CTC EXPRESSION	0% CTC per 7.5 mL	27.9
PSMA+ CTC EXPRESSION	0% CTC per 7.5 mL	98.8

Results – Biomarker Expression

Nucleus/CK/EpCAM | Nucleus | CK/EpCAM | CD45 | ARv7 | PSMA

ANALYTE TESTED	NORMAL RANGE	SAMPLE RESULT
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ARv7+ CTC EXPRESSION	0% CTC per 7.5 mL	27.9
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Results – Sequencing

Sample ID	Gene	AA Sequence	c.DNA	# of CTCs containing	CTC variant frequency %	Found in cfDNA	cfDNA variant frequency %
1248-03-C2D1	ERBB3	-	c.1481-58A>G	2	6.1%, 6.7%	N	-
	ALK	-	c.3515+18C>T	1	37.60%	Y	48.80%
	NF1	p.(Ser2451Gly)	c.7351A>G	2	5.3%, 3.2%	N	-

Notes:

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Comprehensive sample report of findings. Results from each testing modality are synthesized into a final testing report. This provides access to relevant information about the phenotypic and genotypic characterization of a patient's disease and immune response. This information could be used to provide personalized treatment, modify treatment based on real time assessment of tumor evolution, and ultimately improve patient outcomes.

CONCLUSIONS

This novel application of a high-plex spatial biology imaging system to liquid biopsies, in conjunction with the proven capabilities of rare cell detection and genomic analysis of single cells and cfDNA, has the potential to revolutionize liquid biopsy testing. From a single liquid biopsy sample, one can ask important questions of how the immune system relates to disease state and leverage this information to improve patient outcomes.