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

Jon Ladd, Erin Bayer, Brock Bartels, Arista Tischner, Rachel Ponting, Arturo B. Ramirez

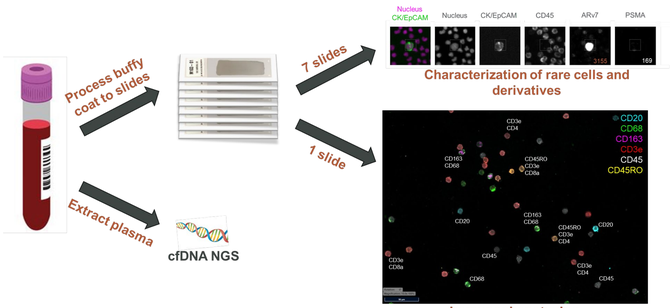
RareCyte, Inc. Seattle, WA

RARECYTE® Precision Biology for Life Sciences

BACKGROUND

There remains an untapped potential for utilizing liquid biopsy as a non-invasive, real-time window into tumor biology. In this study, we report on the use of the high-multiplex protein quantitation capability of the Orion™ spatial biology platform (RareCyte®, Inc) and cell isolation capability of the CyteFinder® rare cell platform, to perform a comprehensive liquid biopsy analysis of whole blood that provides a deeper understanding of samples. This novel technology was used to characterize circulating tumor cells (CTCs), perform immune profiling of white blood cells (WBCs), and conduct targeted mutation profiling of CTCs and cell-free DNA (cfDNA). All of these analytes can be measured from one 7.5 mL draw of blood. This array of tests was applied to cancer samples to demonstrate clinical feasibility.

METHODS



Multi-analyte workflow. Blood samples were collected into AccuCyte® blood collection tubes. Buffy coats were processed to microscope slides using AccuCyte® blood processing kits. Slides were fixed and stained with an 18-plex immuno-oncology panel and imaged using the Orion Spatial Biology platform to evaluate protein biomarker expression on CTCs and to profile WBCs. Individual CTCs and WBCs from clinical samples were isolated using the CytePicker® for downstream molecular analysis. cfDNA was extracted from plasma collected during the AccuCyte process. Isolated single CTCs, WBCs, and cfDNA were sequenced using the CleanPlex® OncoZoom® Cancer Hotspot Kit.

IMMUNE CELL PROFILING

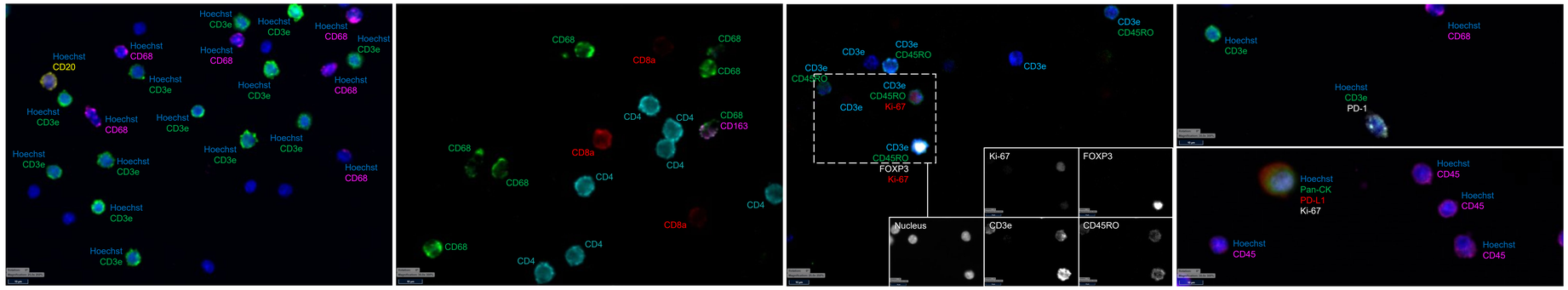
Example 18-plex Staining Panel

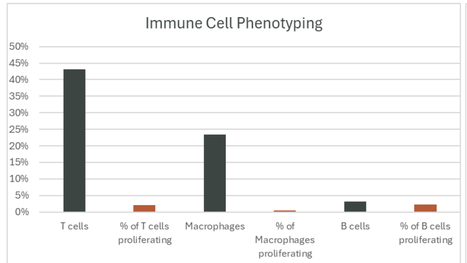
Hoechst
CD45
Ki-67
CD3e
CD20
CD4
CD163
CD8a
E-cadherin
PD-L1
PD-1
CD45RO
FOXP3
CD68
CD31
SMA
Pan-CK
Vimentin

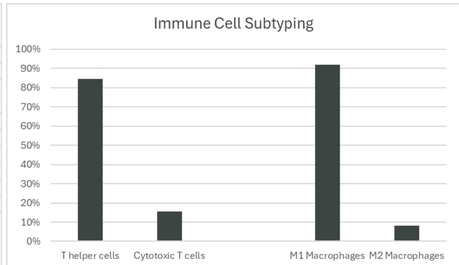
Cell Type / Cell State	Biomarkers
T cells	CD3e
T helper	CD3e, CD4
T cytotoxic	CD3e, CD8
B cells	CD20
Macrophage (CD68)	CD68
M2 Macrophage (CD163)	CD163, CD68
T reg	CD3e, CD4, FOXP3
Immune checkpoint T cell	PD-1, CD3e, CD4
Immune checkpoint macrophage	PD-L1, CD163
Immune checkpoint (tumor)	PD-L1, Pan-CK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

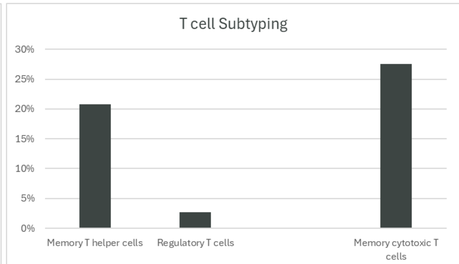
Immune cell profiling through customizable panel design. Staining panels of 17 biomarkers plus Hoechst (nuclear dye) are fully customizable to targets of interest. Panels include a broad array of markers to characterize different cell types (B cells, T cells, Macrophages, Granulocytes, Monocytes, etc.)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

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



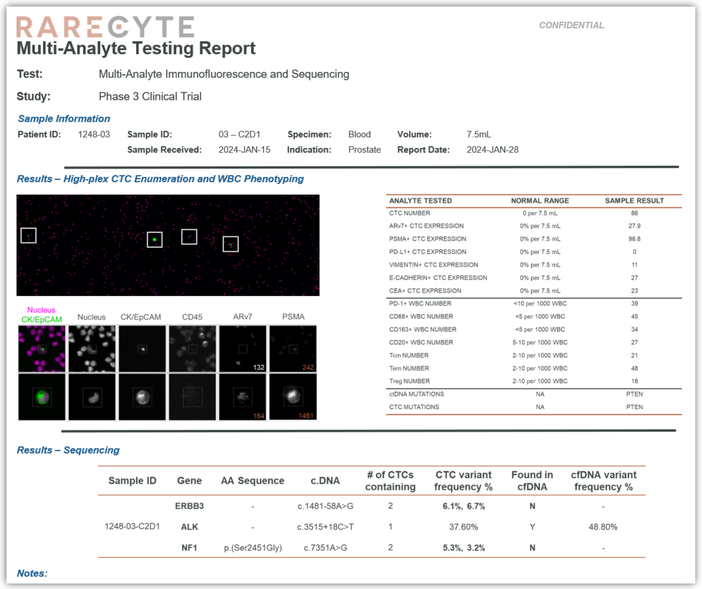






WBC profiling assesses real-time changes in immune cell numbers and phenotypes. Immunophenotyping allows clinicians to monitor a patient's immune response to treatment at regular and frequent intervals via liquid biopsy. Immunophenotyping can also be used to determine mode of action of a drug through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

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 **Specimen:** Blood **Volume:** 7.5mL
Sample Received: 2024-JAN-15 **Indication:** Prostate **Report Date:** 2024-JAN-28

Results – High-plex CTC Enumeration and WBC Phenotyping

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

ANALYTE TESTED	NORMAL RANGE	SAMPLE RESULT
CTC NUMBER	0 per 7.5 mL	86
ARv7+ CTC EXPRESSION	0% per 7.5 mL	27.9
PSMA+ CTC EXPRESSION	0% per 7.5 mL	98.8
PD-L1+ CTC EXPRESSION	0% per 7.5 mL	0
VIMENTIN+ CTC EXPRESSION	0% per 7.5 mL	11
E-CADHERIN+ CTC EXPRESSION	0% per 7.5 mL	27
CEA+ CTC EXPRESSION	0% per 7.5 mL	23
PD-1+ WBC NUMBER	<10 per 1000 WBC	39
CD68+ WBC NUMBER	<5 per 1000 WBC	45
CD163+ WBC NUMBER	<5 per 1000 WBC	34
CD20+ WBC NUMBER	5-10 per 1000 WBC	27
Tcm NUMBER	2-10 per 1000 WBC	21
Tem NUMBER	2-10 per 1000 WBC	48
Treg NUMBER	2-10 per 1000 WBC	18
cfDNA MUTATIONS	NA	PTEN
CTC MUTATIONS	NA	PTEN

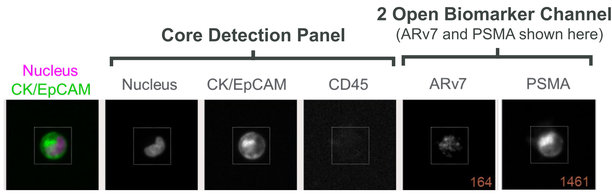
Results – Sequencing

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

Notes:

Comprehensive sample report of findings. Results from each testing modality are collated into a final report. This provides access to relevant information about the phenotypic and genotypic characterization of a patient's disease and immune response.

CHARACTERIZATION OF CIRCULATING TUMOR CELLS (CTCs)



Example CTC image. CTCs are characterized as nucleated, CK/EpCAM+, CD45-. In this example, expression of two biomarkers germane to prostate cancer, ARv7 and PSMA, are quantified in a patient sample. These biomarkers can be replaced with others pertinent to the 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.

CONCLUSIONS

This novel application of a high-plex protein-based 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. This approach allows one to ask important questions about the complex interactions between the immune system and the tumor and to follow these interactions longitudinally in real time over the course of the disease. The goal is to leverage this unprecedented depth of analysis from a single tube of blood to improve patient outcomes and better understand the biological complexity of disease. Future directions of this work are to study rare types of WBCs that indicate immune activation and response to the tumor, and the detection and longitudinal monitoring of cell therapies such as CAR T cells after infusion.