#3119

Comparison of Circulating Tumor Cell Derived DNA and Circulating Cell-Free DNA from Simultaneous Blood Sampling of Patients with Metastatic Breast Cancer

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Background

Blood-based candidate biomarkers of disease can be monitored by analyzing circulating tumor cells (CTCs) and/or circulating cell-free DNA (cfDNA) isolated from the peripheral blood. Our primary objective is to understand the relative contributions of these circulating factors (i.e., CTCs and cfDNA) to the overall disease profile in MBC.

Conclusions

It is feasible to isolate high quality CTCs and cfDNA from the same blood collection tube to perform targeted sequencing; this streamlines specimen processing, decreases overall costs, and minimizes required blood volumes. Importantly, there is overlap in the majority of mutations identified in CTC-DNA and cfDNA, but actionable mutations (e.g., PIK3CA, EGFR) were detected in CTC-DNA only. In addition, heterogeneous CTC populations defined by ER and HER2 are evident in the same blood sample. The clinical and theranostic relevance of these findings is unclear and warrants further investigation.

Analysis is ongoing with plans to (1) complete DNA sequencing of pooled CTCs, pooled WBCs, and plasma from all cases with detectable CTCs; (2) perform DNA sequencing of single CTCs isolated from representative cases; and (3) perform DNA sequencing of FFPE tumor samples as available.

Methods: Overview of the Clinical Study

Clinically archived FFPE tumor tissue and prospective blood samples are collected through a minimal risk protocol approved by the Mayo Clinic IRB (#16-001540) from patients with MBC and objective evidence of disease progression. Blood samples include 20 mL whole blood in Streck blood collection tubes (BCTs) for platelet poor plasma (PPP); 20 mL whole blood in AccuCyte BCTs for CTCs, WBCs, and PPP; and 10 mL whole blood in EDTA BCTs for PPP. Nucleated, EpCAM+/cytokeratin+/CD45- CTCs are identified, assessed for ER/HER2 status, and isolated using a centrifugation and direct imaging platform that allows for single cell retrieval (RareCyte). DNA is extracted from PPP, CTCs, WBCs, and FFPE tumor tissue using established methods. Targeted sequencing for SNVs/indels is performed on paired WBCs and CTC-DNA, AccuCyte-cfDNA, Streck-cfDNA, EDTA-cfDNA, and tumor tissue derived FFPE-DNA using the same NGS panel and informatics pipeline (65 genes; CleanPlex OncoZoom; Paragon Genomics).

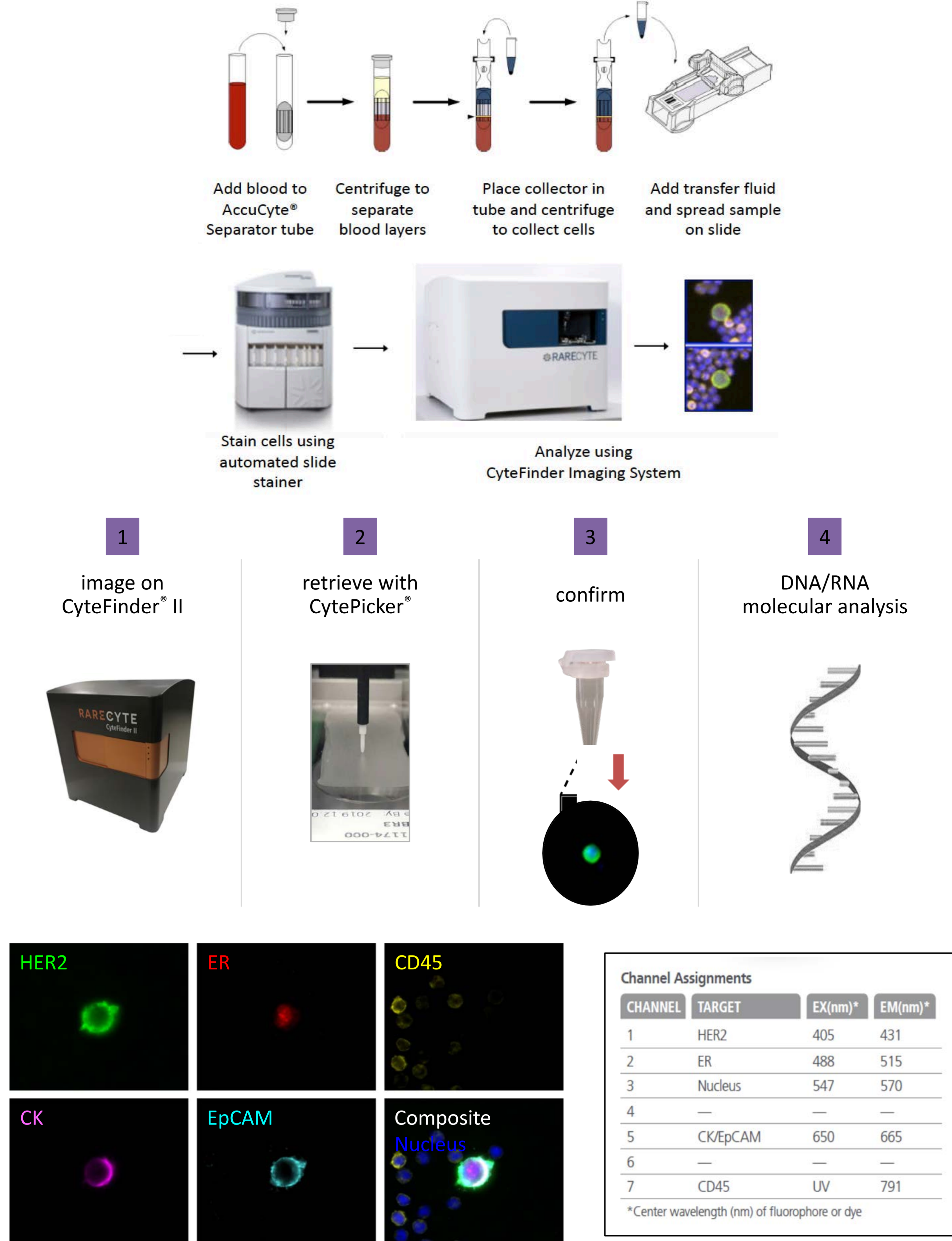
Methods: Next Generation Sequencing

Figure 1. NGS was performed using the CleanPlex OncoZoom Hotspot Panel, which targets 2900+ hotspots in 65 genes with known cancer associations. The assay uses a three-step, single tube protocol and works with intact high quality genomic DNA (e.g., CTC-DNA) as well as degraded samples (e.g., FFPE-DNA and cfDNA). Minimum input is 100 pg DNA.

CleanPlex OncoZoom Cancer Hotspot Panel Gene List

ABL1	*CTNNB1*	*FGFR3*	*JAK3*	*NF2*	*RET*
AKT1	*DDR2*	*FLT3*	*KDR*	*NOTCH1*	*SMAD4*
ALK	*DNMT3A*	*FOXL2*	*KIT*	*NPM1*	*SMARCB1*
APC	*EGFR*	*GNA11*	*KRAS*	*NRAS*	*SMO*
ATM	*ERBB2*	*GNAQ*	*MAP2K1*	*PDGFRA*	*SRC*
BRAF	*ERBB3*	*GNAS*	*MET*	*PIK3CA*	*STK11*
BRCA1	*ERBB4*	*HNF1A*	*MLH1*	*PIK3R1*	*TERT*
BRCA2	*EZH2*	*HRAS*	*MPL*	*PTCH1*	*TP53*
CDH1	*FBXW7*	*IDH1*	*MSH6*	*PTEN*	*TSC1*
CDKN2A	*FGFR1*	*IDH2*	*MTOR*	*PTPN11*	*VHL*
CSF1R	*FGFR2*	*JAK2*	*NF1*	*RB1*	

Methods: Centrifugation, Direct Imaging, Retrieval of CTCs



Channel Assignments

CHANNEL	TARGET	EX(nm)*	EM(nm)*
1	HER2	405	431
2	ER	488	515
3	Nucleus	547	570
4	—	—	—
5	CK/EpCAM	650	665
6	—	—	—
7	CD45	UV	791

*Center wavelength (nm) of fluorophore or dye

Figure 2. Peripheral blood was collected in AccuCyte® BCTs. Nucleated cells, including CTCs, were processed onto microscope slides (8 slides per 7.5 mL blood sample) using the AccuCyte® Sample Processing System. Slides were then stained with a panel of antibodies for detection of ER/HER2 on epithelial CTCs using the Ventana Discovery Ultra automated staining instrument. Slides were imaged and analyzed with the CyteFinder® Imaging System. CTC candidates were automatically identified and rank-scored using the integrated machine learning algorithm, followed by manual selection. CTCs were identified as nucleated cells with positive EpCAM and/or cytokeratin staining and negative CD45 staining. ER and HER2 status were assessed as present or absent for each CTC. Objective mean fluorescence intensity (MFI) measurements on positive and negative control cell lines were used to optimize conditions for signal to background for each marker with the following thresholds for positivity: >80 MFI for HER2 and >160 for ER.

Results: Summary of CTC Results

Subject	ER+/HER2+	ER-/HER2+	ER+/HER2-	ER-/HER2-	Total CTCs
MC_Brst_006	9	0	9	0	18
MC_Brst_007	4	0	1	0	5
MC_Brst_009	0	2	0	1	3
MC_Brst_010	56	4	44	9	113
MC_Brst_012	0	11	0	24	35
MC_Brst_014	1	0	0	4	5
MC_Brst_016	33	0	16	1	50
MC_Brst_017	2	0	31	11	44
MC_Brst_027	0	4	0	0	4
MC_Brst_030	1	0	0	0	1
MC_Brst_031	0	0	0	2	2
MC_Brst_032	0	0	0	1	1
MC_Brst_033	18	22	15	39	94
MC_Brst_034	3	0	0	2	5
MC_Brst_035	0	1	0	0	1
MC_Brst_036	1	0	0	0	1
MC_Brst_038	1	1	0	0	2
MC_Brst_041	1	0	0	0	1
MC_Brst_042	3	9	0	1	13
MC_Brst_045	3	2	0	2	7
MC_Brst_048	2	0	0	0	2
MC_Brst_049	2	8	0	4	14
MC_Brst_051	2	1	0	2	5
MC_Brst_054	1	12	0	5	18
MC_Brst_055	38	21	0	5	64

Table 1. CTC enumeration and phenotyping were completed for 36 subjects (4 of 8 slides per subject, equivalent to 3.75 mL whole blood). CTCs were detected in 25 cases with HER2 and ER results as summarized above.

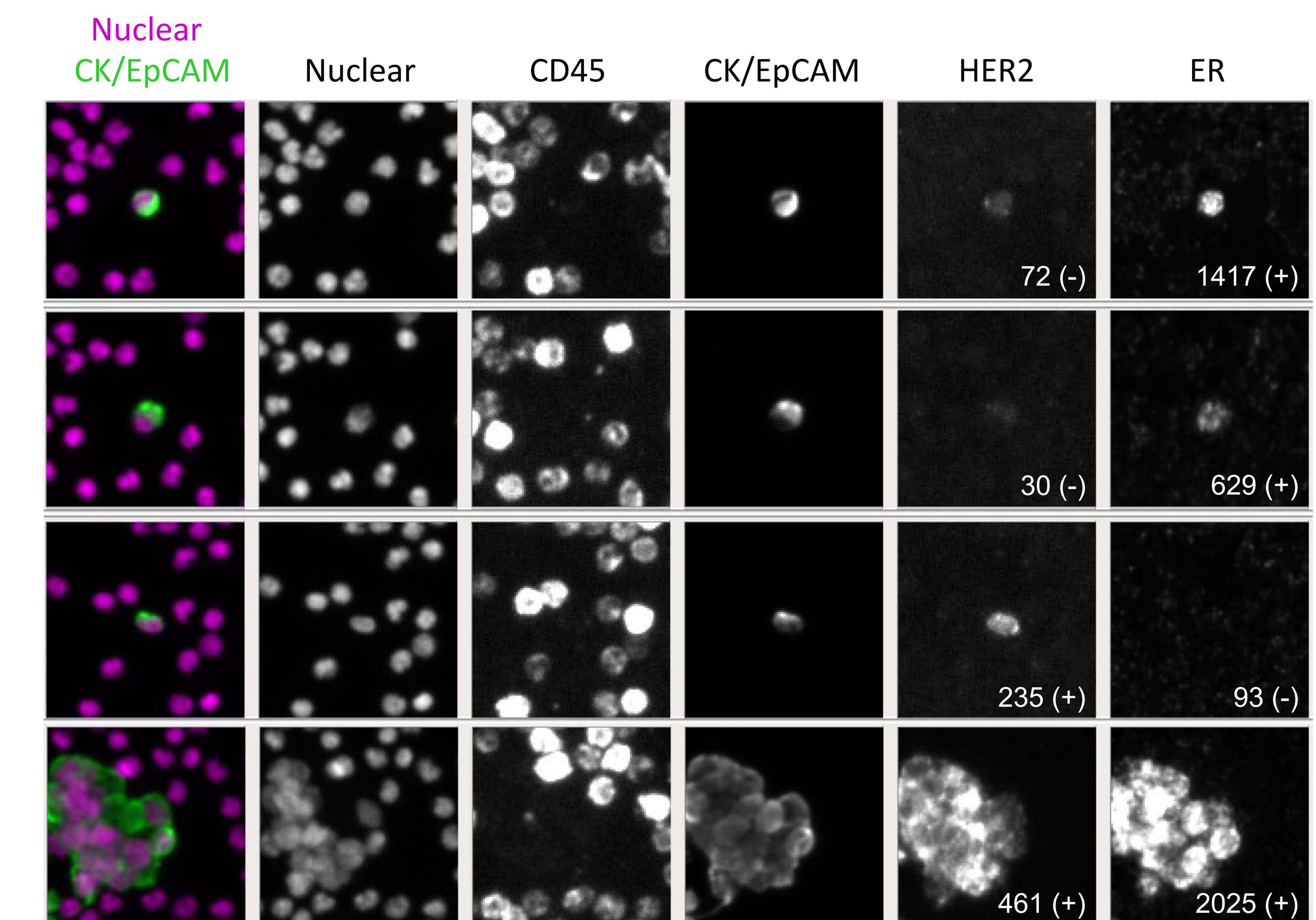


Figure 3. Heterogeneity of HER2 and ER positivity were noted among CTCs isolated from the same blood sample. An example from MC_Brst_010 is provided, where each row provides staining results for a single CTC. Four CTCs were selected for illustration, including 1 cluster (row 4). MFIs for HER2 and ER are noted. The patient was known to have ER positive (>75%) and HER2 negative (IHC 1+) disease by tissue biopsy.

Results: DNA Sequencing Summary

Subject	Primary	Metastasis	# Pooled CTCs	# Pooled WBCs	Mutations	Variant Allelic Frequency: CTCs	Variant Allelic Frequency: RC cfDNA	Variant Allelic Frequency: Streck cfDNA
MC_Brst_006	ER+/HER2-	ER+/HER2-	5	9	PIK3CA H1047R	28.5%	46.7%	45.2%
MC_Brst_007	ER+/HER2-	ER+/HER2-	3	6	PIK3CA E545K	49.5%	31.8%	Low coverage
					EGFR T790M	31.6%	0.0%	Low coverage
MC_Brst_009	ER+/HER2-	ER+/HER2-	3	3	PIK3CA H1047R	54.1%	0.0%	Low coverage
MC_Brst_010	ER+/HER2-	ER+/HER2-	5	9	PTEN R130P	11.4%	12.7%	8.9%
MC_Brst_012	ER-/HER2-	Not done	5	11	TP53 Q192*	19.2%	14.7%	14.6%
					DNMT3A W893S	0.0%	9.5%	8.0%
MC_Brst_014	ER+/HER2-	ER+/HER2-	3	6	PIK3CA E545K	20.0%	2.6%	1.4%
					EGFR R521K	10.2%	0.0%	0.0%
					BRCA2 N372H	30.8%	49.9%	48.6%
MC_Brst_016	ER+/HER2-	ER+/HER2-	5	1	PIK3CA E545K	31.7%	7.3%	Low coverage
					SMAD4 C363Y	10.2%	0.0%	Low coverage
MC_Brst_017	ER+/HER2+	ER+/HER2-	5	4	None	--	--	--
MC_Brst_027	ER-/HER2+	ER-/HER2+	3	5	RET G691S	34.5%	48.4%	47.3%
					TP53 N263D	10.2%	0.0%	0.0%
					TP53 Q136E	0.0%	9.6%	9.2%
MC_Brst_033	ER+/HER2-	ER+/HER2-	5	12	PIK3CA E542K	57.6%	34.3%	33.8%
					TP53 C135W	26.7%	14.7%	16.6%
					DNMT3A S714C	0.0%	16.0%	13.9%

Table 2. Sequencing of paired CTC-DNA, WBC-DNA, and cfDNA was performed for 10 subjects with >2 CTCs per 3.75 mL.

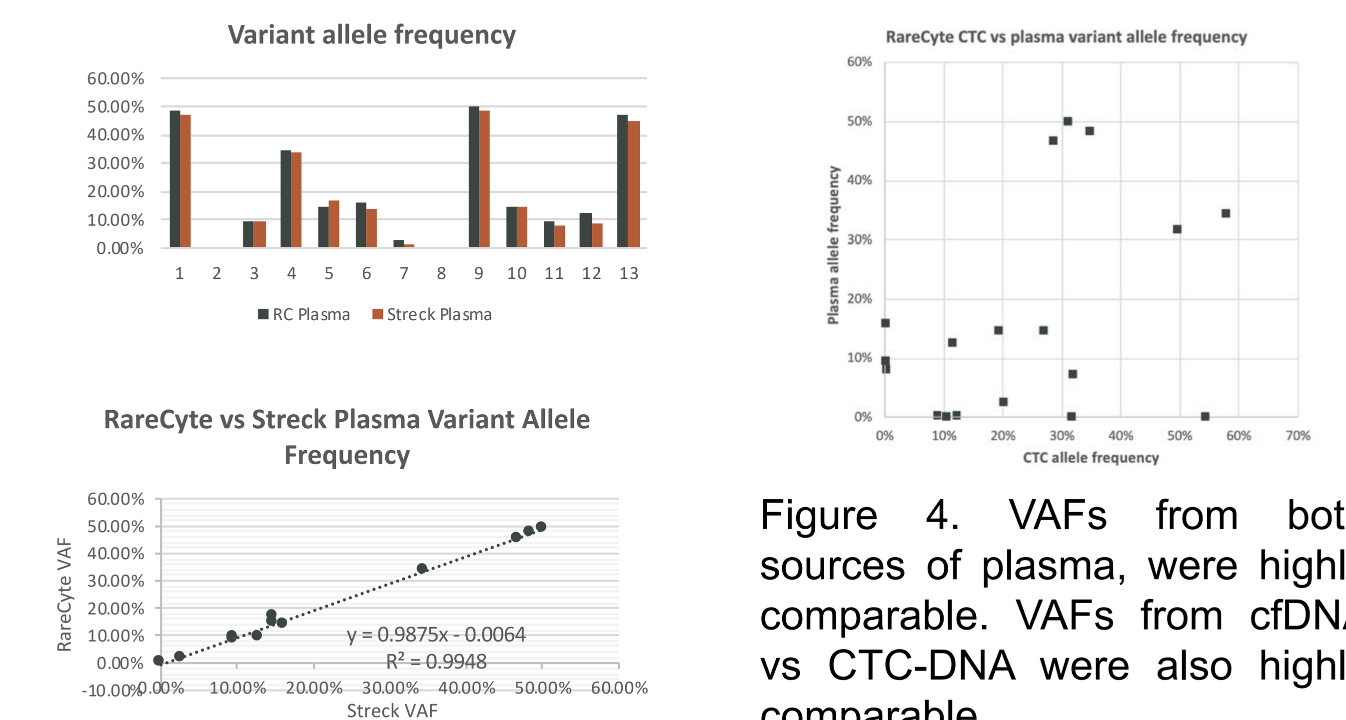


Figure 4. VAFs from both sources of plasma, were highly comparable. VAFs from cfDNA vs CTC-DNA were also highly comparable.

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Contact Information

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