Targeted Sequencing of Circulating Tumour Cells Captured by Parsortix System Enables Low Frequency Variant Analysis with VarMap™ Pan-Cancer NGS Panel

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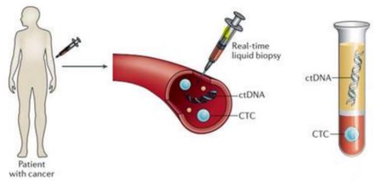
Introduction

Circulating tumour cells (CTCs) and circulating tumour DNA (ctDNA) offer a minimally invasive liquid biopsy technique to evaluate the molecular profile of cancer patients in real-time. ANGLE's Parsortix® system is a microfluidic technology able to harvest and capture viable CTCs from patient blood for downstream analysis. Analysing both CTCs and cell-free DNA (cfDNA) concurrently can provide a more comprehensive view of the genetic alterations present in a patient's cancer, as CTCs may harbour additional mutations not detected in cfDNA alone.



Klaus Pantel and Catherine Alix-Panabieres. Liquid biopsy and minimal residual disease — latest advances and implications for cure. Nat Rev Clin Oncol 16, 409–424 (2019). https://doi.org/10.1038/s41571-019-0187-3

Workflow

Blood samples were collected by venipuncture from three healthy volunteers (HVs) and thirty-seven cancer patients (13 breast, 14 lung and 10 ovarian cancer patients). The plasma samples were isolated by centrifugation and stored, while the cellular fractions were processed using Parsortix instrument to enrich CTCs. DNA samples, extracted using Allprep DNA/mRNA nano kit or QIAamp circulating nucleic acid kit, from the Parsortix-enriched CTC or plasma, respectively, were used for library preparation using VarMap™ Pan-Cancer library preparation kit (NUPROBE, USA) and targeted NGS was performed on the Illumina's NextSeq 2000 platform.

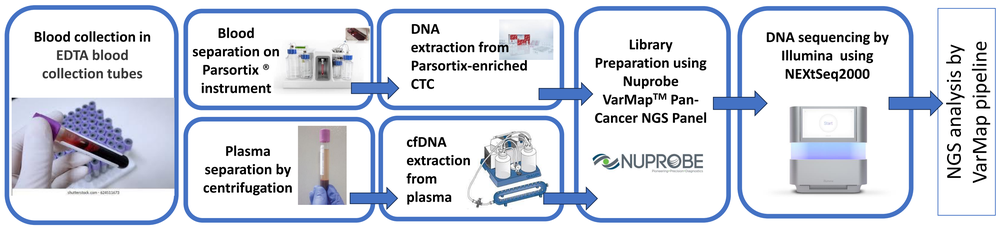


Figure 1: Workflow from liquid biopsy to CTC isolation and NGS analysis. Blood samples were collected into EDTA tubes and processed within 24 hours. Plasma was removed by centrifugation and stored. The remaining blood samples (cellular fractions) were processed using Parsortix® system to enrich for CTCs. DNA was extracted from both CTC and plasma (cfDNA) and subjected to targeted sequencing using the VarMap™ Pan-Cancer NGS Panel from NUPROBE, which interrogates 61 cancer-associated genes.

For Research Use Only. Not For Use In Diagnostic Procedures.

Analytical results

Fifteen samples, collected from 3 HVs, were spiked with different levels of cultured cell lines (0, 2, 5, 10, and 20 cells). The cell lines used in this study, SKMEL-28 and H1975, harbour known mutations; VAFs for expected mutations are tabulated in Figure 2. No mutation was detected for unspiked samples, as expected. All mutations were detected at 10 and 20 cell levels, except for EGFR L858R detected in 2/3 replicates at the 20-cell level due to technical error. When 5 cells were spiked, the detection rate was variable depending on target: 100% for TP53 R273H, 66% for BRAF V600E and EGFR T790M and 33% for PTEN T167A. The other expected mutations (EGFR L858R and PIK3CA p.G118D) were not detected.

Gene	Unspiked	Unspiked	Unspiked	2-cell	2-cell	2-cell	5-cell	5-cell	5-cell	10-cell	10-cell	10-cell	20-cell	20-cell	20-cell
BRAF p.V600E	0	0	0	1.1	0.35	0.66	0	0.62	0.78	2.1	1.7	1.3	1.7	1.2	1.3
	0%			100%			67%			100%			100%		
EGFR p.T790M	0	0	0	0	0.75	1	0	0.8	0.46	1.2	1.6	2.6	5	1.1	4.1
	0%			67%			67%			100%			100%		
EGFR p.L858R	0	0	0	0	0	0	0	0	0	0.87	0.68	1.1	0.98	0.77	0
	0%			0%			0%			100%			67%		
PIK3CA p.G118D	0	0	0	0	0	0.8	0	0	0	2.6	0.99	1.4	3.3	1.8	2.3
	0%			33%			0%			100%			100%		
PTEN p.T167A	0	0	0	0.4	0.5	0.46	0	0.59	0	1.7	1.8	2.4	5.3	0.97	2.6
	0%			100%			33%			100%			100%		
TP53 p.R273H	0	0	0	0	0.4	0.41	0.19	0.25	0.62	0.36	0.98	0.5	1.9	0.59	1.2
	0%			67%			100%			100%			100%		

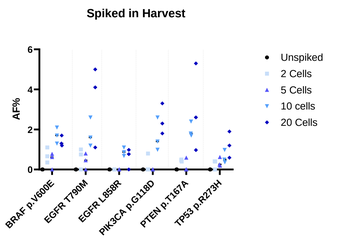
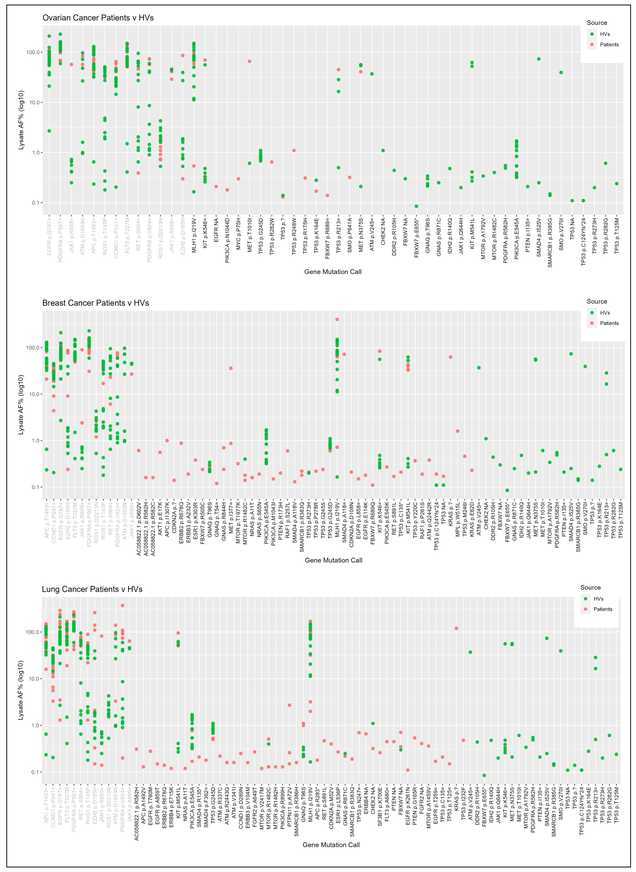


Figure 2: Positive calls and VAFs of expected mutations. The figure presents the analytical results obtained by spiking cultured SKMEL-28 (melanoma) and H1975 (non-small cell lung cancer) cell lines into blood samples from three healthy volunteers (HVs). The table displays the allele frequencies of expected somatic mutations detected in various genes, including BRAF, CDK4, EGFR, PIK3CA, PTEN, and TP53, at different spiking concentrations (2, 5, 10, and 20 cells) across the three HV donors.

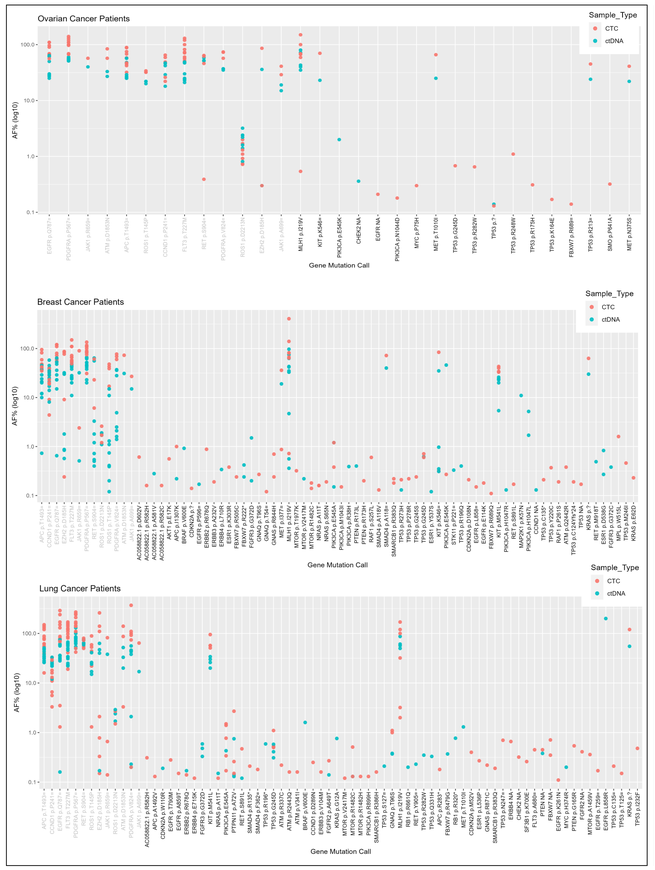
Patient's results

A total of 37 cancer patient blood samples were included in this study, with matched CTC-DNA and cfDNA analysed (13 breast, 14 lung and 10 ovarian cancer patients). Figure 3A shows the comparison of positive calls between HVs and patients by cancer type, with greyed-out mutations indicating frequently occurring variants present in the genomic DNA reference material, as stated in VarMap's Instruction For Use. Most of the mutations identified in patients are between 0.1 and 1.0% VAF. Similarly, in Figure 3B, a comparison of positive calls between CTC and cfDNA is presented. Across all 3 types of cancer, higher percentages of mutations were identified exclusively on CTCs compared to cfDNA as summarised (Figure 3C). Mutations co-detected in CTC and cfDNA fractions were at a frequency of 61%, 30% and 34% for ovarian, breast and lung cancer patients respectively, demonstrating complementary data can be obtained by investigating both sample types.

A) Mutation profile comparison of CTC DNA samples, from cancer patient and HV



B) Mutation profile comparison between CTC-DNA and cfDNA from cancer patient samples



C) CTC, cfDNA and Concordance mutation% by cancer type

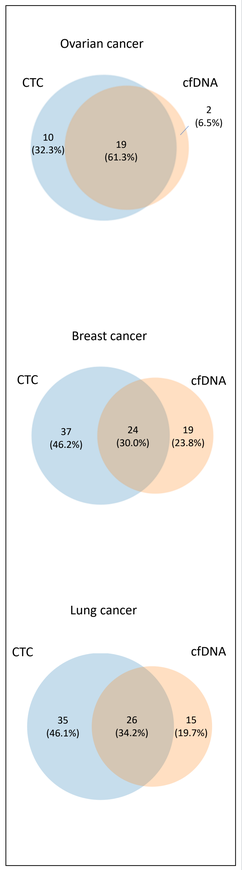


Figure 3: Positive mutation calls on CTC and cfDNA. (A) Comparison of positive mutation calls on CTC DNA originating from healthy donors and ovarian, breast and lung cancer patients respectively. VAF axis presented in log10 range. **(B)** Comparison of positive mutation calls between CTC and cfDNA originating from ovarian, breast and lung cancer respectively. VAF axis presented in log10 range. **(C)** Venn diagram summarising the percentage of mutation positive calls exclusive to each sample type and the percentage of shared variants. Greyed out mutations indicate frequently occurring mutations in genomic DNA reference material as stated in NUPROBE VarMap's pan-cancer IFU.

Conclusions

- The analytical data showcases the ability of detecting mutations in Parsortix-enriched CTC samples, using VarMap™ Pan-Cancer NGS Panel, from spiked cancer cell lines with high analytical sensitivity and analytical specificity across multiple genes.
- A number of mutations were exclusively detected in either the CTC or cfDNA samples with a higher number of exclusive mutations reported in CTC samples compared to cfDNA.
- The added value of CTC profiling using the Parsortix system is its ability to capture a comprehensive picture of the tumour's genetic heterogeneity. By interrogating both CTCs and cfDNA in parallel, this integrated approach holds promise for potentially guiding personalized cancer treatment selection, monitoring therapeutic responses, and improving overall clinical management strategies for patients.