Abstract
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Comparative Performance of Targeted and Comprehensive Somatic NGS Panels in Paired Tumor and Liquid Biopsy Samples from a Prospective Solid Tumor Cohort

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INTRODUCTION

Circulating tumor DNA (ctDNA) analysis has emerged as a powerful and minimally invasive approach for detecting tumor-associated genetic alterations, monitoring disease evolution, and guiding precision oncology decisions. Despite its promise, concordance between ctDNA and traditional tissue-based next-generation sequencing (NGS) remains variable, and it is influenced by both biological and technical factors such as tumor heterogeneity, ctDNA shedding dynamics, assay design, and sequencing depth (1-3). To better understand these variables, we compared two somatic NGS strategies in matched tumor and plasma samples from patients with solid tumors. One approach utilized a targeted amplicon-based panel with distinct gene content between specimen types, while the other employed a comprehensive hybrid-capture panel with harmonized content across tissue and plasma. Our goal was to assess the degree of variant concordance, clinical relevance of detected alterations, and the relative strengths and limitations of each platform in real-world testing scenarios.

STUDY AND METHODS

STUDY

We prospectively enrolled **61 patients** diagnosed with advanced cancer (**Table 1**). Tumor tissue, plasma and buffy coat were collected from each patient. All patients consented to sample collection for this research study prior to study initiation. Criteria for inclusion and sample collection are specified in **Table 2**.

Table 1. Tumor types

Diagnosis	Cases
Lung	20
Colorectal	12
Gastric	9
Breast	8
Ovarian	3
Liver	2
Pancreas	2
Prostate	2
Bladder	1
Cervix uteri	1
Fallopian tube	1
Total	61

Table 2. Inclusion criteria

STUDY INCLUSION CRITERIA
* Consented subjects (all subjects over 18 years old)
* Confirmed diagnosis of the specified cancer types.
* Stage III and IV tumors exclusively.
* Acceptance of any tumor grade or subtype.
* Preference for resections over biopsies.
* Preferably, FFPE blocks; alternatively, freshly cut slides were accepted.
* Primary tumors and secondary (metastatic) tumors were accepted.
* Both treated and untreated subjects were accepted.
* Tumor content (cellularity) at minimum 20% for resections.
* Necrosis content below 20%.
* Tumor area above 10 mm² in FFPE slides.
* Collection of whole blood before surgery.
* Whole blood and FFPE samples were collected as close in time as possible, with a maximum gap of 3 months.
* Collection of whole blood and FFPE samples without intervening aggressive treatment.
* Acceptance of subjects under neoadjuvant treatments if required by the standard of care (S.O.C) only.
* Plasma samples should not have shown significant germline DNA contamination.
* Tissue samples were to be fixed in 10% Neutral Buffered Formalin (NBF).
* Buffy coat was collected in a separate tube.

SEQUENCING AND DATA ANALYSIS

Matched formalin-fixed paraffin-embedded (FFPE) and blood samples were prospectively collected from 61 patients with advanced solid tumors. FFPE samples were tested using a hotspot panel (Pillar Biosciences OncoReveal® Multi-Cancer Panel, 60 genes) and a large hybrid-capture panel (Illumina TSO500, 523 genes, full exonic coverage). Plasma was analyzed using a ctDNA-optimized version of the small panel (Core LBx Pillar Biosciences, 104 genes, hotspot) and the TSO500 ctDNA panel. Because the hybrid-capture panel encompassed a broader genomic territory, concordance was assessed only across genes and regions covered by both assays.

Tumor tissue samples were analyzed with the TSO500 tissue pipeline (v2.2.1) local app, which uses BWA for alignment against the GRCh37/hg19 reference genome. Annotation is performed using Nirvana v3.2.3. The ctDNA samples were analyzed with the TSO500 ctDNA pipeline (v2.1.1), executed on Illumina Connected Analytics (ICA), which uses DRAGEN v3.10.9 for alignment and variant calling, also against GRCh37/hg19, and variants were annotated using Nirvana v3.2.6. Both pipelines leverage UMI-based error correction.

Amplicon sequencing data were analyzed using PiVAT v2023.1.0, Pillar Biosciences' secondary analysis platform. The OncoReveal Multi-Cancer Panel (v4) combines DNA and RNA analysis from FFPE tissue, and the OncoReveal Core LBx Panel (v1) analyzes cfDNA from plasma. DNA variants—including SNVs, indels, and CNVs—are aligned with BWA to hg19 and annotated using VEP v106.1.

The gene content for the Pillar amplicon panels (60 and 104 genes) is presented in **Tables 3** and **4**. The comprehensive hybrid-capture panel (523 genes) is not displayed, as concordance analyses were restricted to overlapping genes and regions between panels.

Table 3. oncoReveal Multi-Cancer v4 with CNV gene list.

oncoReveal Multi-Cancer v4 with CNV Panel (60 genes)

ABL1	CDKN2A	FBXW7	GNAS	KIT ▲	NPM1	PTPN11	SRC
AKT1	CSF1R	FGFR1 ▲	HNF1A	KRAS ▲	NRAS	RAC1	STK11
ALK	CTNNB1	FGFR2 ▲	HRAS	MAP2K1	NTRK1	RB1	TP53
APC	DDR2	FGFR3 ▲	IDH1	MET ▲	NTRK2	RET	VHL
ATM	EGFR ▲	FLT3 ▲	IDH2	MLH1	NTRK3	ROS1	
BRAF	ERBB2 ▲	FOXL2	JAK2	MPL	PDGFRA ▲	SMAD4	
CCND1 ▲	ERBB4	GNAS	JAK3	MYC ▲	PIK3CA ▲	SMARCB1	
CDH1	EZH2	GNAQ	KDR ▲	NOTCH1	PTEN	SMO	

CNVs detected and verified by NGS reference standard are indicated by ▲.
CNVs can also be detected in genes indicated by ▲

Table 4. oncoReveal Core LBx gene list.

oncoReveal™ Core LBx Panel (104 genes)

AKT1	AXIN2	CDKN2A	EZH2	GNAQ	KRAS	MTOR	PAK7	PTPN6	ROS1	TP53
ALK	AXL	CIC	FBXW7	GNAS	▲MAP2K1	MYC	PDCD1	PTPN6	RUNX1	TSC1
APC	B2M	CREBBP	▲FGFR1	HNF1A	MAP2K2	MYOD1	▲PDGFRA	RAF1	SF3B1	U2AF1
AR	BCOR	CTCF	▲FGFR2	HRAS	MAPK1	NCOR1	▲PIK3CA	RB1	SMAD4	VHL
ARAF	BRAF	CTNNB1	▲FGFR3	IDH1	MED12	NF1	PIK3R1	RET	SOX9	
ARID1A	CARD11	▲EGFR	FLCN	IDH2	▲MET	NFE2L2	POLE	RAC1	SPOP	
ARID2	CCND1	EP300	FOXL2	JK2P1	MLH1	NOTCH1	PPP2R1A	RHEB	STAT3B	
ASXL1	CDH1	▲ERBB2	GATA3	JAK1	MLL2	NRAS	PTCH1	RHOA	SMO	
ATM	CDK4	ERBB3	GLI1	KDM5A	MRE11A	NTRK1	PTEN	RIT1	STK11	
ATRX	CDK6	ESR1	GNA11	▲KIT	MSH6	NTRK3	PTPN3	RNF43	TCF7L2	

Copy Number Amplifications (CNAs) can also be detected in genes indicated by ▲. Genes marked in green indicate full CDS coverage.

CONCORDANCE AND CLINICAL SIGNIFICANCE

Clinically significant variants were classified per AMP/ASCO/CAP Tier I/II guidelines. For discordant variants, coverage at the corresponding loci was reviewed to confirm whether the region was captured in both assays. Variants represent single nucleotide variants (SNVs) and small insertions and deletions (InDels).

REFERENCES

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RESULTS

OVERALL CONCORDANCE

In the comprehensive hybrid capture panel comparison, 6,393 variants were concordant, 1,031 were unique to FFPE, and 3,216 were unique to ctDNA. Clinically significant variants included 55 unique to tissue and 138 unique to plasma, with 44 variants in common between FFPE and ctDNA, illustrating the broader coverage of the panel (**Figure 1A**).

In the targeted amplicon panel comparison, 1,478 variants were identified in FFPE and 1,375 in plasma, with 364 concordant. Among 51 concordant clinically relevant variants, 24 were tissue-only and 13 plasma-only (**Figure 1B**). Discordance was largely attributed to non-overlapping panel content: 61% of tissue-only and 97% of plasma-only variants fell outside the coverage of the other panel. Despite the comprehensive panel's expanded detection, the amplicon panel still captured unique actionable variants in both sample types (**Figure 1B, lower panel**).

Analysis of the proportion of clinically significant variants (Tier I/II) relative to all detected variants revealed notable differences between panels and specimen types. The TSO500 comprehensive panel detected the highest proportion of clinically significant alterations among ctDNA-only variants (58.2%), compared to FFPE-only (23.2%) and concordant (18.6%) (**Figure 2A**). In contrast, for the Pillar amplicon panels, the highest percentage of clinically relevant findings was observed among concordant variants (58.0%), followed by FFPE-only (27.3%), and ctDNA-only variants (14.8%) (**Figure 2B**).

Figure 1A – Overall concordance between FFPE and ctDNA using Illumina TSO500

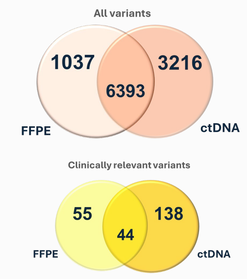


Figure 1B – Overall concordance between FFPE and ctDNA using Pillar amplicon panels

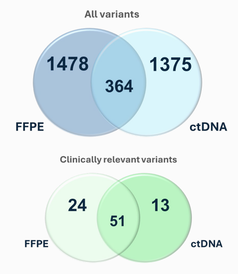


Figure 2A – Percentage of clinically relevant variants

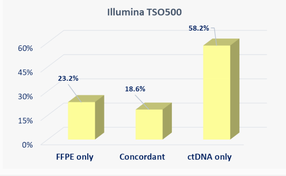
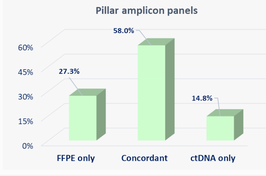


Figure 2B – Percentage of clinically relevant variants



CONCLUSIONS

For the Pillar amplicon panels, panel design differences accounted for a substantial proportion of discordant calls. Among FFPE-only variants, 66% were not covered by the Core LBx panel, while among Core LBx-only variants, 67% were not targeted by the tissue (multi-cancer) panel. This suggests a limited but asymmetric overlap in genomic content, with approximately 34% of tissue multi-cancer panel variants and 51% of Core LBx variants falling within mutually covered regions. In contrast, the large TSO500 panel, with consistent genomic coverage across sample types, demonstrated markedly higher concordance and captured more clinically significant findings, particularly in plasma.

Regarding differences in the distribution of clinically relevant variants between the two platforms, the TSO500 panel's broader genomic coverage and harmonized design may enhance detection of low-frequency or ctDNA-enriched alterations that are absent or below the limit of detection in tissue, explaining the higher proportion of ctDNA-only clinically significant calls. Conversely, the Pillar panels, with more limited and non-overlapping coverage between FFPE and ctDNA, tend to concentrate their clinical findings in concordant mutations. Together, these observations highlight that both assay types can offer valuable and complementary insights, reinforcing the importance of aligning assay selection with clinical goals, tumor type, and logistical considerations (2-4).

This study was supported by Eurofins Viracor Biopharma, Illumina and Pillar Biosciences