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TECHNICAL NOTE

Use of cfDNA as input for TruSight™ Oncology 500 v2

Enabling one solution for FFPE and cfDNA samples

One workflow

Integrate cfDNA into an existing FFPE workflow instead of adopting a separate liquid biopsy solution

Greater efficiency

Streamline laboratory operations to minimize errors and improve turnaround times

Broader scope

Expand CGP research applications with cfDNA, enabling more comprehensive and flexible study designs

Introduction

Comprehensive genomic profiling (CGP) using targeted next-generation sequencing (NGS) can be applied in oncology research laboratories to characterize DNA variants from both solid tumor tissue and liquid biopsy samples.[1-3] TruSight Oncology 500 v2 is a hybrid–capture–based assay designed for CGP of formalin-fixed, paraffin-embedded (FFPE) DNA and RNA input and enables research of key variant classes, including small DNA variants, copy number alterations, and gene fusions. The assay also enables assessment of the immuno-oncology (IO) signatures microsatellite instability (MSI) and tumor mutational burden (TMB), as well as evaluation of homologous recombination deficiency (HRD).[4] TruSight Oncology 500 ctDNA v2 is a separate workflow solution that enables CGP research from circulating tumor DNA (ctDNA), providing broad coverage of tumor-derived alterations and assessment of blood-based TMB (bTMB) and blood-based MSI (bMSI).[5]

Laboratories using TruSight Oncology 500 v2 for tissue profiling may seek to expand their research capabilities to include ctDNA analysis; however, implementing a separate assay and workflow for ctDNA can increase training requirements, limit batching flexibility, and introduce handling variability. A library preparation workflow that supports both FFPE DNA/RNA and cfDNA inputs can streamline laboratory operations, increase throughput, and provide greater flexibility in sample processing and sequencing. Although TruSight Oncology 500 v2 and TruSight Oncology 500 ctDNA v2 are distinct assays designed for different input types, their library preparation and enrichment workflows are closely aligned,[4,5] making evaluation of cell-free (cfDNA) input within the TruSight Oncology 500 v2 workflow of interest for laboratories seeking workflow consistency.

This technical note evaluates the feasibility of using cfDNA as input into the TruSight Oncology 500 v2 workflow in a limited proof-of-concept study, with performance assessed in comparison to the TruSight Oncology 500 ctDNA v2 workflow. Key metrics included coverage, analytical sensitivity, and analytical specificity across variant classes, bMSI, and bTMB, using plasma-derived cfDNA and a reference control with DRAGEN™ TruSight Oncology 500 ctDNA analysis.* Results of the study demonstrate that the TruSight Oncology 500 v2 assay can be used with cfDNA input, enabling laboratories to expand to liquid biopsy research applications while maintaining a consistent library preparation process.

* HRD analysis was not performed since genomic scar signatures are not reliably measurable in low–tumor-fraction cfDNA.[6]

Methods

Samples

Deidentified plasma samples were commercially sourced and included seven samples from normal donors (samples 1–7) and four samples from individuals with cancer (samples 8–11). Circulating cfDNA was extracted using the QIAamp Circulating Nucleic Acid Kit (QIAGEN, Catalog no. 55114) according to manufacturer instructions. In addition, one contrived sample prepared using Seraseq ctDNA Complete Mutation Mix AF0.5% reference standard (SeraCare, Catalog no. 0710-0531) was included in the study (sample 12).

Extracted cfDNA samples were quantified using a 5300 Fragment Analyzer (Agilent, Catalog no. M5311AA) with the HS Large Fragment 50 kb Kit (Agilent, Catalog no. DNF-464-0500). The Seraseq ctDNA Complete Mutation Mix reference control was quantified using the Agilent 4200 TapeStation system (Agilent, Catalog no. G2991BA) with the Cell-free DNA ScreenTape (Agilent, Catalog no. 5067-5630) and Cell-free DNA Reagents (Agilent, Catalog no. 5067-5631). Optimizing cfDNA quantification is described in detail in the Accurate quantification of cfDNA for use in TruSight Oncology 500 ctDNA technical note.

Library preparation and sequencing

To test the performance of TruSight Oncology 500 v2 assay with cfDNA, libraries were prepared in accordance with the TruSight Oncology 500 v2 Product Documentation using cfDNA directly as input. All library preparation, enrichment, and indexing steps were conducted manually, with the exception of the fragmentation step, which was omitted. Instead, cfDNA samples were diluted to 5 ng and 20 ng input levels each in a total volume of 50 µl, consistent with the TruSight Oncology 500 v2 protocol.

For comparison, libraries for the same samples were prepared using TruSight Oncology 500 ctDNA v2 with cfDNA input following the TruSight Oncology 500 ctDNA v2 Product Documentation. All library preparation, enrichment, and indexing steps were performed manually according to the protocol.

Prepared libraries were sequenced on a NovaSeq™ 6000Dx Sequencing System† using paired-end 151-bp reads with dual 10-bp indexes on an S4 flow cell.

† The NovaSeq 6000Dx Sequencing System was used in RUO mode.

Data analysis

Sequencing data were analyzed using DRAGEN TruSight Oncology 500 ctDNA Analysis Software v2.1. Quality control and performance metrics, including coverage and variant detection metrics, were evaluated using standard analysis outputs. As no TruSight Oncology 500 v2-specific resource bundle is available for the ctDNA workflow, the analysis was performed using ctDNA workflow resource files (eg, systemic noise and CNV panel of normals).

Results

QC metric performance

Quality control (QC) metrics were evaluated across all twelve samples prepared using TruSight Oncology 500 v2 to evaluate coverage depth and uniformity.

Median exon coverage across the twelve samples using 20 ng input demonstrated consistent sequencing performance, with all samples exceeding the lower specification limit (LSL) (Figure 1).

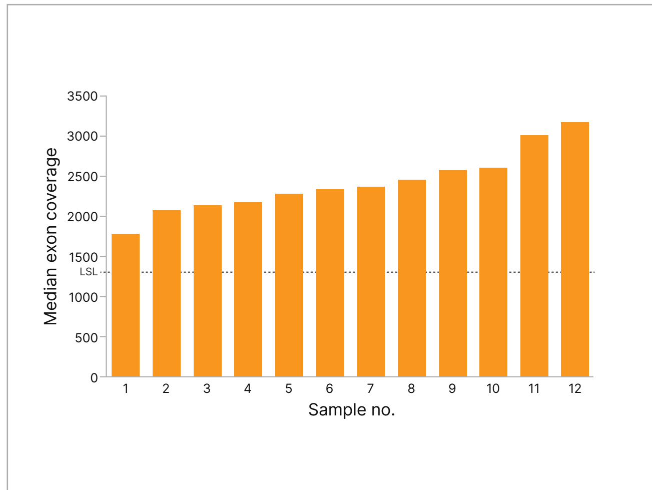

Figure 1: Median exon coverage for cfDNA samples assessed with TruSight Oncology 500 v2
Median exon coverage is shown for 12 unique samples prepared using 20 ng input and processed with TruSight Oncology 500 v2, including plasma-derived cfDNA from normal donors (sample nos. 1–7) and from individuals with cancer (sample nos. 8–11), and a contrived ctDNA reference sample (sample no. 12). All samples exceeded the assay lower specification limit (LSL), indicating consistent sequencing performance across the evaluated samples.

Coverage uniformity was assessed using the percentage of exons achieving ≥ 1000× coverage, with high exon-level coverage observed across samples, supporting adequate depth for downstream variant detection analyses (Figure 2).

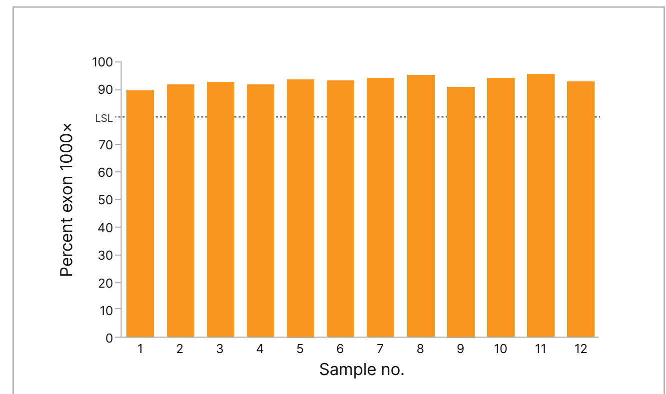

Figure 2: Exon coverage ≥ 1000× in cfDNA samples assessed with TruSight Oncology 500 v2
Coverage uniformity was evaluated as the percentage of exons achieving at least 1000× coverage across all 12 samples prepared with 20 ng input and processed with TruSight Oncology 500 v2. High exon-level coverage was observed across samples and exceeded the assay lower specification limit (LSL), supporting sufficient sequencing depth for downstream variant detection analyses.

Analytical specificity and analytical sensitivity

Analytical specificity in normal cfDNA samples

High analytical specificity was observed across variant classes, assays, and DNA input amounts. For both TruSight Oncology 500 v2 and TruSight Oncology 500 ctDNA v2, analytical specificity for small DNA variants, gene amplifications, gene deletions, gene rearrangements, and microsatellite instability status was 100% or > 99.999% at both 5 ng and 20 ng input (Table 1).

Small DNA variant analytical specificity in normal cfDNA samples remained high across individual samples, with overall analytical specificity exceeding 99.99% for all assay and input combinations (Table 2). Gene amplification analytical specificity was 100% across all evaluated samples, inputs, and assays, with no false positives observed (Table 3). Similarly, fusion detection demonstrated 100% analytical specificity across all

Table 1: Analytical specificity, analytical sensitivity, and IO gene signatures baseline performance for TruSight Oncology 500 v2 and TruSight Oncology 500 ctDNA v2 in a limited study

cfDNA input	Variant class	TruSight Oncology 500 v2		TruSight Oncology 500 ctDNA v2	
		5 ng	20 ng	5 ng	20 ng
Analytical specificity (n = 7)[a]	SNVs	> 99.999%	> 99.999%	> 99.992%	> 99.999%
	Gene amplifications	100%	100%	100%	100%
	Gene deletions	100%	100%	100%	100%
	Gene rearrangements	100%	100%	100%	100%
	bMSI status	100%	100%	100%	100%
Analytical sensitivity (n = 1)[b]	SNVs at 0.5%	89.5%	100%	57.9%	95%
	Gene amplifications	100%	66.7%	100%	66.7%
	Gene rearrangements	66.7%	100%	33%	100%
IO gene signatures baseline performance (n = 7)[c]	bMSI SumJSD average	0.003	0.012	0.002	0.019
	bTMB score average	1.79	1.91	0.68	2.12

a. Analytical specificity analysis results are from seven normal cfDNA samples.
b. Analytical sensitivity analysis results are from one contrived control sample (Seraseq cfDNA complete mutation mix AF0.5%).
c. IO gene signatures baseline performance results are from seven normal cfDNA samples.

bMSI, blood-based microsatellite instability; bTMB, blood-based tumor mutational burden; IO, immuno-oncology; SNV small nucleotide variant; VAF, variant allele frequency.

Table 2: Small DNA variant analytical specificity in normal cfDNA samples

Sample no.	TruSight Oncology 500 v2				TruSight Oncology 500 ctDNA v2			
	5 ng		20 ng		5 ng		20 ng	
	Total FPs	Analytical specificity	Total FPs	Analytical specificity	Total FPs	Analytical specificity	Total FPs	Analytical specificity
1	0	100%	4	99.9998%	2	99.998%	4	99.9998%
2	not available	not available	15	99.9992%	6	98.4887%	10	99.9992%
3	0	100%	2	99.9999%	4	99.9053%	1	99.9999%
4	9	99.9982%	12	99.9993%	8	99.9972%	5	99.9997%
5	0	100%	0	100%	1	99.505%	1	99.9999%
6	3	99.9996%	5	99.9997%	3	98.3607%	2	99.9998%
7	2	99.9997%	5	99.9997%	4	99.1323%	2	99.9998%
Overall analytical specificity	14	> 99.9996%	43	> 99.9997%	28	> 99.9928%	25	> 99.9998%

FP, false positive.

Table 3: Gene amplification analytical specificity in normal cfDNA samples

Sample no.	TruSight Oncology 500 v2				TruSight Oncology 500 ctDNA v2			
	5 ng		20 ng		5 ng		20 ng	
	Total FPs	Analytical specificity	Total FPs	Analytical specificity	Total FPs	Analytical specificity	Total FPs	Analytical specificity
1	0	100%	0	100%	0	100%	0	100%
2	not available	not available	0	100%	0	100%	0	100%
3	0	100%	0	100%	0	100%	0	100%
4	0	100%	0	100%	0	100%	0	100%
5	0	100%	0	100%	0	100%	0	100%
6	0	100%	0	100%	0	100%	0	100%
7	0	100%	0	100%	0	100%	0	100%
Overall analytical specificity	N/A	100%	N/A	100%	N/A	100%	N/A	100%

FP, false positive; N/A, not applicable.

Table 4: Fusion analytical specificity in normal cfDNA samples

Sample no.	TruSight Oncology 500 v2				TruSight Oncology 500 ctDNA v2			
	5 ng		20 ng		5 ng		20 ng	
	Total FPs	Analytical specificity	Total FPs	Analytical specificity	Total FPs	Analytical specificity	Total FPs	Analytical specificity
1	0	100%	0	100%	0	100%	0	100%
2	not available	not available	0	100%	0	100%	0	100%
3	0	100%	0	100%	0	100%	0	100%
4	0	100%	0	100%	0	100%	0	100%
5	0	100%	0	100%	0	100%	0	100%
6	0	100%	0	100%	0	100%	0	100%
7	0	100%	0	100%	0	100%	0	100%
Overall analytical specificity	N/A	100%	N/A	100%	N/A	100%	N/A	100%

FP, false positive; N/A, not applicable.

Analytical sensitivity in the contrived reference sample

Analytical sensitivity of TruSight Oncology 500 v2 and TruSight Oncology 500 ctDNA v2 assays, both using cfDNA input, was assessed in a limited study using a single replicate of the Seraseq ctDNA Complete Mutation Mix reference standard.

Analytical sensitivity for small DNA variant detection was evaluated across 19 known variants present at approximately 0.5% VAF. Analytical sensitivity increased with DNA input, reaching 100 percent for TruSight Oncology 500 v2 at 20 ng (Table 1 and Table 5).

Analytical sensitivity of gene amplification detection was assessed using a contrived reference sample containing three expected amplifications. All amplifications were detected at 5 ng input, while two of three were detected at 20 ng input for both assays (Table 6).

Fusion detection analytical sensitivity was evaluated for three expected fusions in the contrived reference sample. All fusions were detected at 20 ng input, with lower analytical sensitivity observed at 5 ng input (Table 7).

Table 5: Small DNA variant analytical sensitivity in a limited study using a reference sample (n = 1)

Gene	Variant	TruSight Oncology 500 v2 Detection Rate		TruSight Oncology 500 ctDNA v2 Detection Rate	
		5 ng	20 ng	5 ng	20 ng
AKT1	E17K	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
ALK	F1174L	100% (1/1)	100% (1/1)	0% (0/1)	100% (1/1)
ALK	G120R	100% (1/1)	100% (1/1)	0% (0/1)	100% (1/1)
BRAF	V600E	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
BRCA1	K65FS*47	0% (0/1)	100% (1/1)	0% (0/1)	0% (0/1)
BRCA2	R2645FS*3	0% (0/1)	100% (1/1)	100% (1/1)	100% (1/1)
EGFR	E746_A750del ELREA	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
EGFR	L747	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
EGFR	L858R	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
EGFR	S752_I759 del SPKANKEI	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
EGFR	T790M	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
ERBB2	A775_G776 ins YVMA	100% (1/1)	100% (1/1)	0% (0/1)	100% (1/1)
KIT	D816V	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
KRAS	G12C	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
KRAS	G12D	100% (1/1)	100% (1/1)	0% (0/1)	100% (1/1)
KRAS	Q61H	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
NRAS	Q61R	100% (1/1)	100% (1/1)	0% (0/1)	100% (1/1)
PIK3CA	H1047R	100% (1/1)	100% (1/1)	0% (0/1)	100% (1/1)
PIK3CA	N1068fs*4	100% (1/1)	100% (1/1)	0% (0/1)	100% (1/1)
Overall analytical sensitivity		89.5% (17/19)	100% (19/19)	57.9% (11/19)	95% (18/19)

Table 6: Gene amplification analytical sensitivity in a limited study using a reference sample (n = 1)

Gene	Expected fold change	TruSight Oncology 500 v2				TruSight Oncology 500 ctDNA v2			
		5 ng		20 ng		5 ng		20 ng	
		Observed fold change	Detection rate	Observed fold change	Detection rate	Observed fold change	Detection rate	Observed fold change	Detection rate
ERBB2	1.28	1.31	100% (1/1)	1.32	100% (1/1)	1.32	100% (1/1)	1.31	100% (1/1)
MET	1.20	1.38	100% (1/1)	1.44	100% (1/1)	1.40	100% (1/1)	1.42	100% (1/1)
MYC	1.18	1.19	100% (1/1)	not detected	0% (0/1)	1.21	100% (1/1)	not detected	0% (0/1)
Overall analytical sensitivity	N/A	N/A	100% (3/3)	N/A	66.7% (2/3)	N/A	100% (3/3)	N/A	66.7% (2/3)

N/A, not applicable.

Table 7: DNA fusion analytical sensitivity in a limited study using a reference sample (n = 1)

Fusion	Expected VAF[a]	TruSight Oncology 500 v2						TruSight Oncology 500 ctDNA v2					
		5 ng			20 ng			5 ng			20 ng		
		Observed		Detection rate	Observed		Detection rate	Observed		Detection rate	Observed		Detection rate
		VAF	Depth		VAF	Depth		VAF	Depth		VAF	Depth	
ALK-EML4	0.49	0.55%	1266	100% (1/1)	0.32%	3789	100% (1/1)	not detected	not detected	0% (0/1)	0.28%	2859	100% (1/1)
RET-NCOA4	0.56	not detected	not detected	0% (0/1)	0.26%	3119	100% (1/1)	not detected	not detected	0% (0/1)	0.13%	2363	100% (1/1)
ROS1-CD74	0.53	0.23%	1281	100% (1/1)	0.30%	3617	100% (1/1)	0.45%	892	100% (1/1)	0.33%	3617	100% (1/1)
Overall analytical sensitivity	N/A	N/A	N/A	66.7% (2/3)	N/A	N/A	100% (3/3)	N/A	N/A	33.3% (1/3)	N/A	N/A	100% (3/3)

a. Expected VAF available as average AF from digital PCR reported by SeraCare.
VAF, variant allele frequency; N/A, not applicable.

IO gene signatures baseline performance

Assessment of immuno-oncology (IO) signatures using TruSight Oncology 500 ctDNA v2 includes evaluation of bMSI and bTMB. bMSI reflects defects in DNA mismatch repair pathways and is inferred from variability across multiple genomic loci, with Jensen–Shannon distance (SumJSD) used as the evaluation metric.[7] The bTMB score represents the number of somatic mutations within coding regions and provides a quantitative measure of mutational load.[8]

bMSI performance was evaluated for normal cfDNA samples at both 5 ng and 20 ng input processed with TruSight Oncology 500 v2 and TruSight Oncology 500 ctDNA v2. All samples exhibited low SumJSD values below the bMSI-high threshold, consistent with expected microsatellite stability in normal samples (Figure 3 and Table 1).

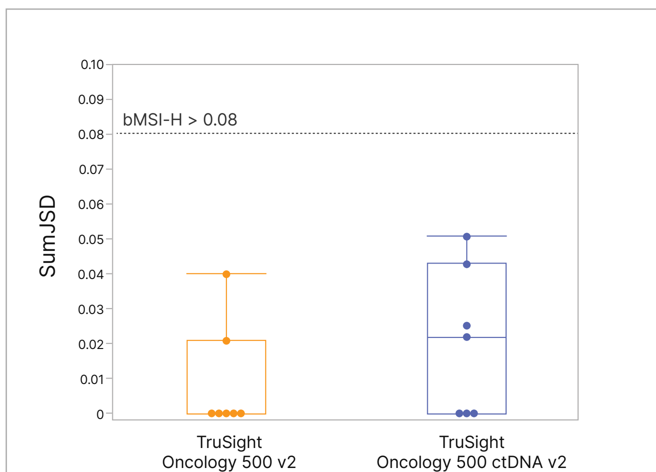

Figure 3: Comparison of bMSI assessment using SumJSD
bMSI was evaluated using the SumJSD metric for seven normal cfDNA samples processed with the TruSight Oncology 500 v2 and TruSight Oncology 500 ctDNA v2 workflows. Bars represent aggregated SumJSD values for each workflow. All samples exhibited SumJSD values below the bMSI-high threshold.

bTMB performance was assessed for samples processed with TruSight Oncology 500 v2 and TruSight Oncology 500 ctDNA v2. Low bTMB scores were observed across normal cfDNA samples at both 5 ng and 20 ng input, with consistent values across workflows and input levels (Figure 4 and Table 1). These results indicate stable estimation of bMSI and bTMB metrics when cfDNA is used as input into the TruSight Oncology 500 v2 workflow.

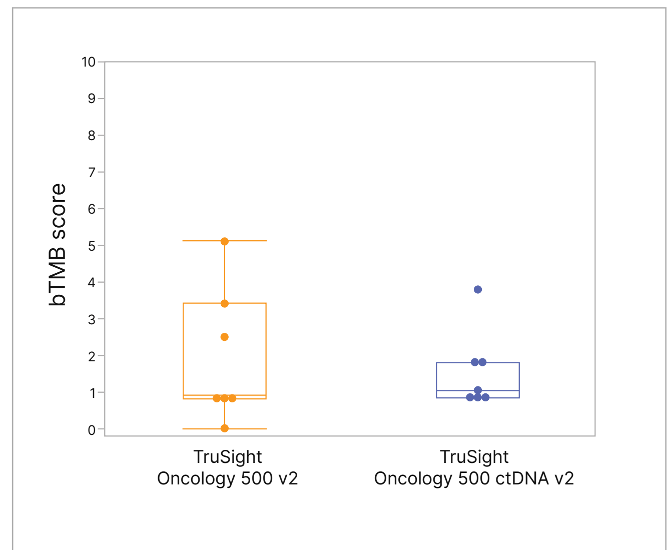

Figure 4: Comparison of bTMB assessment
bTMB was assessed using bTMB score for seven normal cfDNA samples processed with the TruSight Oncology 500 v2 and TruSight Oncology 500 ctDNA v2 workflows. Bars represent aggregated bTMB scores for each workflow. Low bTMB scores were observed across both workflows.

Summary

This limited proof-of-concept study evaluated the feasibility of using cfDNA as input into the TruSight Oncology 500 v2 workflow at 5 ng and 20 ng input amounts and assessed performance relative to TruSight Oncology 500 ctDNA v2. At 20 ng input, TruSight Oncology 500 v2 met assay quality control specifications with consistent coverage depth and uniformity across cfDNA samples. Analytical specificity in normal cfDNA exceeded 99.99% for small DNA variants. Analytical sensitivity for low-frequency variants in Seraseq ctDNA reference material varied by variant class and input amount, with higher detection generally observed at 20 ng input for both assays. Across evaluated metrics, performance of TruSight Oncology 500 v2 using cfDNA input was comparable to TruSight Oncology 500 ctDNA v2, supporting using the TruSight Oncology 500 v2 workflow for liquid biopsy research applications.

Learn more →

TruSight Oncology 500 v2

DRAGEN TruSight Oncology 500 ctDNA analysis

References

1. Wallen ZD, Nesline MK, Tierno M, et al. Genomic profiling of NSCLC tumors with the TruSight Oncology 500 assay provides broad coverage of clinically actionable genomic alterations and detection of known and novel associations between genomic alterations, TMB, and PD-L1. *Front Oncol*. 2024;14. doi:10.3389/fonc.2024.1473327
2. Pascual J, Attard G, Bidard FC, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. *Ann Oncol*. 2022;33(8):750-768. doi:10.1016/j.annonc.2022.05.520
3. Illumina. Next-generation sequencing and microarray methods for liquid biopsy in cancer research. illumina.com/content/dam/illumina-marketing/documents/gated/cancer-research-liquid-biopsy-ebook-m-gl-00678.pdf. Published 2024. Accessed December 5, 2025.
4. Illumina. TruSight Oncology 500 v2 data sheet. illumina.com/content/dam/illumina/gcs/assembled-assets/marketing-literature/trusight-oncology-500-v2-datasheet-m-gl-03460/tso500-v2-datasheet-m-gl-03460.pdf. Published 2025. Accessed February 12, 2026.
5. Illumina. TruSight Oncology 500 ctDNA v2 data sheet. illumina.com/content/dam/illumina/gcs/assembled-assets/marketing-literature/trusight-oncology-500-ctdna-v2-m-gl-02196/tso500-ctdna-v2-data-sheet-m-gl-02196.pdf. Published 2025. Accessed September 23, 2025.
6. Liu R, Roberts E, Parsons HA, et al. DirectHRD enables sensitive scar-based classification of homologous recombination deficiency. *Nucleic Acids Res*. 2025;53(8):gkaf313. doi:10.1093/nar/gkaf313
7. Kavun A, Veselovsky E, Lebedeva A, et al. Microsatellite instability: A review of molecular epidemiology and implications for immune checkpoint inhibitor therapy. *Cancers*. 2023;15(8):2288. doi:10.3390/cancers15082288
8. Sha D, Jin Z, Budczies J, Kluck K, Stenzinger A, Sinicrope FA. Tumor mutational burden as a predictive biomarker in solid tumors. *Cancer Discov*. 2020;10(12):1808-1825. doi:10.1158/2159-8290.CD-20-0522

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