

Case Number: 102240077145  
Patient Name: Dr. Dharmasiri De Silva  
Age/Sex: 68 Yrs/Male  
Patient Location: Colombo  
Hospital Name: Aegle Omics (Private) Limited  
Physician Name: Dr. Mahendra Perera  
Date & Time of Accessioning: 15/05/2024 12:25 Hrs  
Date & Time of Reporting: 08/06/2024 17:37 Hrs

### TEST INFORMATION

liquiC<sup>ORE</sup> Paradigm is a **Next Generation Sequencing (NGS)** assay that enables the detection of clinically relevant genomic alterations (**SNVs**, **CNVs**, **Indels** and **Gene fusions**) from **circulating tumor DNA (ctDNA)**, within 531 clinically relevant genes. The assay additionally provides the **tumor mutation burden (TMB)** score and **Microsatellite Instability (MSI)** score and status for optimum therapy selection, prognostication and aids in drug discovery research and clinical trial research programs.

### SPECIMEN INFORMATION

Peripheral blood in Streck tube collected on 13/05/2024.

### CLINICAL HISTORY

Diagnosed with Neuro Endocrine Carcinoma of the Prostate in February 2023. Underwent Robotic Surgery with clear outcomes, returning to normal activities. However, experienced intractable pain, difficulty passing stools, and occasional per rectal bleeding in early 2024. Local and locoregional recurrence of the tumor observed. Treatment: Initially received 3 cycles of chemotherapy (Cisplatin and Etoposide) with marginal response. Subsequently underwent radiotherapy to cover primary site and deep iliac nodes bilaterally. Following radiotherapy, received 3 more cycles of the same chemotherapy. Current Status: PET/CT revealed residual disease in left common iliac region and aorta cava region below the left renal vein, now being irradiated. Planned for further chemotherapy with FOLFOX regimen (email).

### RESULTS

#### CLINICALLY RELEVANT GENOMIC FINDINGS

S. No.	Genomic Alterations	Associated FDA Approved Therapies (in this cancer type)	Associated FDA Approved Therapies (in other cancer type; Off Label Suggestions)	Clinical Trials
1.	<b>CFTR, p.A412Tfs</b>	<b>None</b>	<b>None</b>	<b>None</b>

\*\* One Variant of unknown significance (VUS) has been detected in BRCA1 gene. Clinical correlation is recommended.

(Please refer to page no. 3 for more details)

## RESULTS

### MULTI-GENE BIOMARKER FINDINGS

Assay Biomarker	Result	Category	Interpretation
<b>Tumor Mutation Burden</b>	<b>0 Mutations/Mb</b>	Low	Unlikely to benefit from Immuno-Oncology therapy
<b>Microsatellite Instability (MSI)</b>	<b>3.45 score</b>	Stable	Stable Microsatellite Detected

# Correlation of the genetic findings with the clinical condition of the patient is required to arrive at accurate diagnosis, prognosis or therapeutic decisions.

### TUMOR MUTATION BURDEN INTERPRETATION

TMB	Category	Interpretation
1-5 Mutations /Mb	Low	Unlikely to benefit from Immuno-Oncology therapy
6-19 Mutations/Mb	Intermediate	Moderate response anticipated towards Immuno-Oncology therapy
> 20 Mutations /Mb	High	Good and significant response expected to Immuno-Oncology therapy

## CLINICALLY RELEVANT VARIANTS SUMMARY

Gene (Exon) [Transcript]/ Translocation	Variant (cDNA syntax)	Variant (Amino acid Alteration)	Variant Allele Frequency (VAF)	Variant Classification (AMP)**	Variant Classification (ACMG)#
CFTR (10) [NM_000492.4]	c.1234_1238del	p.Ala412ThrfsTer4	12.01%	Tier2	Pathogenic
BRCA1 (15) [NM_007294.4]	c.4790C>A	p.Thr1597Asn	50.28%	Tier3	VUS
CHEK2 (9) [NM_007194.4]	c.937G>A	p.Val313Met	49.24%	Tier3	VUS

\*\*Four Tiered Classification System based as per Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer, by Association for Molecular Pathology with liaison representation from the American College of Medical Genetics and Genomics, American Society of Clinical Oncology, and College of American Pathologists. [PMID: [27993330](#), [28157586](#)]

**Tier1:** variants with strong clinical significance for therapy, prognosis and diagnosis for the same tumor type

**Tier2:** variants with potential clinical significance for therapy, prognosis and diagnosis for the different tumor type

**Tier3:** variants of unknown clinical significance (VUS)

**Tier4:** variants deemed benign or likely benign.

#Five Tiered Classification based on ACMG guidelines [PMID: [25741868](#)]

Pathogenic

Likely Pathogenic

Variant of Unknown Significance (VUS)

Likely Benign

Benign

## TEST METHODOLOGY

### Variant Assessment:

**Massive Parallel Sequencing (Next Generation Sequencing):** ctDNA from the submitted specimen was enriched for the complete coding regions and splice site junctions of genes listed below using a custom bait- capture system. Paired End Sequencing was performed with 2x100/2x150 chemistry, on an Illumina platform. Reads were assembled and were aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. Data was filtered and analyzed to identify variants of interest and interpreted in the context of a single most damaging, clinically relevant transcript for the purpose of the report, indicated as a part of variant details. Enrichment and analysis focus on the coding sequence of the indicated transcripts, 5-10bp of flanking intronic sequence, and other specific genomic regions demonstrated to be causative of disease at the time of assay design.

### **Tools and Databases employed for analysis:**

Clinvar, OMIM, HGMD, UCSC genome browser, Uniprot, Ensembl, dbSNP, gnomAD, ExAC, Pubmed, Dgap, icgc, Kaviar, various bioinformatics analysis, predictive tools and disease specific databases used as available and appropriate. Such tools/databases would be mentioned wherever used.

## QC METRICS-DNA

MEAN_TARGET_COVERAGE	46.53
PCT_TARGET_BASES_30X	4.68 %
AFTER FILTERING Q30_RATE	93.63 %

## TEST METHODOLOGY

### Tumor Mutation Burden:

**Clinical interpretation:** Association of high TMB with improved response to immune checkpoint inhibitors such as nivolumab, ipilimumab, pembrolizumab, and atezolizumab in cancer types including non-small cell lung cancer, melanoma, and urothelial carcinoma. TMB could be a useful predictive biomarker for response to pembrolizumab therapy in patients with previously treated recurrent or metastatic advanced solid tumors (1-3).

### **Methodology:**

Tumor Mutation Burden (TMB) analysis was performed based on Next Generation Sequencing analysis from ctDNA isolated from a peripheral blood sample using the Illumina platform. TMB is calculated using nonsynonymous, in-frame indel, and frameshift indel mutations. The assay detects and annotates low frequency somatic variants (SNPs) from 531 genes related to cancer biology with a size of 1.8Mb.

### **Limitations:**

Optimal thresholds for classification of TMB values into high, intermediate, and low categories are not yet standardized across different methods and panels. A TMB cutoff is a function of the gene panel (genomic footprint and bioinformatics platform) that is used in a given study. Since data obtained from a given gene panel cannot be directly applied to another panel without a conversion algorithm, direct comparisons of results between panels can be very problematic. Certain cancer types, such as uterine, bladder and colon cancers exhibited greater variability in panel TMB values, compared with lung and head and neck cancers. The Cancer Genome Atlas compares TMB values derived using WES, which is currently considered as the gold standard for calculating TMB.

### **References:**

1. Chan TA et al. Ann Oncol. 2019
2. Goodman AM et al. Mol Cancer Ther. 2017
3. Van Allen EM et al. Science.2016
4. Denis LJ et al. Cancer Cell 2021



  
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## TEST METHODOLOGY

### Microsatellite Instability (MSI) by NGS :

#### Methodology:

NGS Illumina Sequencing platform was used to assess Microsatellite Instability analysis. Raw sequencing data is processed through bioinformatics pipelines. The data is aligned to the reference genome, and microsatellite somatic loci are identified. Total 58 somatic sites were evaluated to assess MSI.

#### Clinical implication:

The MSS phenotype suggests the presence of normal DNA mismatch repair function within the tumor. Current data suggest that advanced stage solid tumors with defective DNA mismatch repair (MSI-H) are likely to respond to treatment with immunotherapies.

These results decrease the likelihood but do not eliminate the possibility that this individual has HNPCC/Lynch syndrome. These results do not rule out the possibility that this individual's tumor is due to an inherited defect in another gene not involved in DNA mismatch repair. A significant fraction of clinically defined HNPCC cases (30% or more) do not have defective DNA mismatch repair as the underlying genetic basis of their disease. Additionally, we cannot rule out the possibility that this tumor could represent a sporadic occurrence. If there is a strong personal or family history of HNPCC/Lynch syndrome related cancers for this patient or if this individual has multiple tumors, consider microsatellite instability (MSI) and immunohistochemical staining (IHC) on a different tumor to further evaluate the possible role of defective DNA mismatch repair for this individual or family. A genetic consult may be of benefit .

#### Limitations:

The finding of tumor microsatellite instability does not distinguish between somatic and germline alterations.

Test results should be interpreted in the context of clinical findings, family history, and other laboratory data.

Errors in our interpretation of results may occur if information given to us is inaccurate or incomplete. Of note, the literature suggests that MSI analysis on neoadjuvant chemo radiated tumor specimens may influence MSI status and lead to an erroneous interpretation of results .

## LIQUID BIOPSY CHALLENGES/LIMITATIONS

The practice of “liquid biopsy” as a diagnostic, prognostic and theranostic tool in cancer patients is an appealing approach, since it is non-invasive, cost-effective and allows easy treatment monitoring. However, the adaptability of liquid biopsy in routine clinical practice is not easy, because of several reasons:

- **False Negatives:** Inability to extract ctDNA, ctDNA’s short half-life and low signal-to-noise ratio. Low signal to noise ratio occurs as ctDNA represents a very small percentage of cfDNA. Hence, there is a need for very sensitive extraction assays, which are currently not available
- **False Positives:** A liquid biopsy test can call multiple false positives due to fragmented cfDNA and ctDNA and again can be due to low signal to noise ratio
- **Lack of standardization:** Currently vast numbers of assays are available, but consensus is not there on the ideal technical approach. The technology is still lacking on certain important decisions, like, best sample type for liquid biopsy (CTCs/ctDNA/any other liquid from the body), best transport medium to increase sample stability, best sample extraction method, best technical approach for mutation detection
- **Tumor heterogeneity:** Tumor heterogeneity refers to the coexistence of different biological, morphological, phenotypic and genotypic profiles, between tumors (inter-tumor heterogeneity) and within tumors (intra-tumor heterogeneity). It exists at multiple levels and may be present within different tumor regions or between primary cancer and metastasis (spatial heterogeneity), or during the course of disease progression (temporal heterogeneity). The tumor microenvironment (TME), defined as the complex ecosystem in which cancer cells interact with non-cancerous cells, represents an additional source of intra-tumor heterogeneity

## GENE COVERAGE

ABL1	ARID5B	BCL6	CBL	CDKN1B
ABL2	ASXL1	BCOR	CBLB	CDKN2A
ABRAXAS1	ASXL2	BCORL1	CCN6	CDKN2B
ACVR1	ATF1	BCR	CCND1	CDKN2C
ACVR1B	ATM	BIRC3	CCND2	CEBPA
AFF1	ATR	BLM	CCND3	CFTR
AGO2	ATRX	BMPR1A	CCNE1	CHD4
AKT1	AURKA	BRAF	CD22	CHEK1
AKT2	AURKB	BRCA1	CD274	CHEK2
AKT3	AXIN1	BRCA2	CD276	CIC
ALK	AXIN2	BRD3	CD70	CREBBP
ALOX12B	AXL	BRD4	CD79A	CRKL
AMER1	B2M	BRIP1	CD79B	CRLF2
ANKRD11	BAP1	BTK	CDC73	CRTC1
APC	BARD1	BUB1B	CDH1	CSF1R
AR	BCL10	CALR	CDK12	CSF3R
ARAF	BCL11B	CARD11	CDK4	CTCF
ARID1A	BCL2	CARM1	CDK6	CTLA4
ARID1B	BCL2L1	CASP8	CDK8	CTNNA1
ARID2	BCL2L11	CBFB	CDKN1A	CTNNB1

CUL3	EML4	ETV6	FGF5	FZR1
CUX1	EMSY	EWSR1	FGF6	G6PD
CXCR4	EP300	EXT1	FGF7	GAB2
CYLD	EPCAM	EXT2	FGF8	GABRA6
CYP1B1	EPHA3	EZH2	FGF9	GATA1
CYP2C8	EPHA5	FANCA	FGFR1	GATA2
CYP2D6	EPHA7	FANCC	FGFR2	GATA3
CYP3A4	EPHB1	FANCD2	FGFR3	GATA4
CYP3A5	EPHB4	FANCE	FGFR4	GATA6
CYSLTR2	ERBB2	FANCF	FH	GEN1
DAXX	ERBB3	FANCG	FLCN	GLI1
DDR1	ERBB4	FANCI	FLI1	GNA11
DDR2	ERCC1	FANCL	FLT1	GNAQ
DDX41	ERCC2	FAS	FLT3	GNAS
DEK	ERCC3	FAT1	FLT4	GPS2
DEPDC5	ERCC4	FBXW7	FN1	GRIN2A
DICER1	ERCC5	FGF1	FOXA1	GRM3
DIS3	ERF	FGF10	FOXL2	GSK3B
DNAJB1	ERG	FGF12	FOXO1	H1-2
DNMT3A	ERRFI1	FGF14	FOXO3	H2BC5
DOT1L	ESR1	FGF19	FOXP1	H3-3A
DPYD	ETS1	FGF2	FOXP4	H3-3B
EED	ETV1	FGF23	FRS2	H3-5
EGFR	ETV4	FGF3	FUBP1	H3C2
EIF1AX	ETV5	FGF4	FYN	HDAC1

# liquiC<sup>ORE</sup> Paradigm

Case Number: 102240077145

Patient Name: Dr. Dharmasiri De Silva

Ordering Physician Name: Dr. Mahendra Perera

HGF	ING4	KLF4	MAX	MUTYH
HLA-A	INHA	KLF6	MCL1	MYC
HLA-B	INHBA	KLHL6	MDC1	MYCL
HLA-C	INO80	KMT2A	MDM2	MYCN
HLF	INPP4A	KMT2B	MDM4	MYD88
HNF1A	INPP4B	KMT2C	MED12	MYH11
HRAS	INPPL1	KMT2D	MEF2B	MYOD1
HSP90AA1	INSR	KNSTRN	MEN1	NBN
HSP90AB1	IRF2	KRAS	MET	NCOA3
ICOSLG	IRF4	LAMP1	MGA	NCOR1
ID3	IRS1	LATS1	MITF	NEGR1
IDH1	IRS2	LATS2	MLH1	NF1
IDH2	JAK1	LCK	MLH3	NF2
IGF1	JAK2	LMO1	MLLT10	NFE2L2
IGF1R	JAK3	LYN	MLLT3	NFKBIA
IGF2	JUN	MAF	MPL	NKX2-1
IGF2R	KAT6A	MAFB	MRE11	NKX3-1
IKBKB	KAT6B	MAGI2	MSH2	NOTCH1
IKBKE	KDM5A	MALT1	MSH3	NOTCH2
IKZF1	KDM5C	MAP2K1	MSH6	NOTCH3
IL10	KDM6A	MAP2K2	MSI1	NOTCH4
IL2	KDR	MAP2K4	MST1R	NPM1
IL21R	KEAP1	MAP3K1	MTAP	NPRL3
IL6ST	KIF5B	MAPK1	MTOR	NRAS
IL7R	KIT	MAPK3	MUC1	NRG1



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NSD1	PDGFRA	PPM1D	RAD51D	SDHA
NSD2	PDGFRB	PPP2R1A	RAD52	SDHB
NSD3	PGR	PPP2R2A	RAD54L	SDHC
NTHL1	PHF6	PPP6C	RAF1	SDHD
NTRK1	PHOX2B	PRDM1	RARA	SETBP1
NTRK2	PIK3C2B	PREX2	RASA1	SETD2
NTRK3	PIK3C2G	PRKAR1A	RB1	SF3B1
NUMA1	PIK3C3	PRKCI	RBM10	SH2B3
NUP214	PIK3CA	PRKDC	RECQL4	SH2D1A
NUP93	PIK3CB	PRKN	REL	SHH
NUP98	PIK3CD	PRSS1	RET	SLX4
NUTM1	PIK3CG	PTCH1	RHEB	SMAD2
PAK1	PIK3R1	PTEN	RHOA	SMAD3
PALB2	PIK3R2	PTGS2	RICTOR	SMAD4
PARP1	PIK3R3	PTPN11	RIT1	SMARCA4
PARP2	PIM1	PTPRD	RNF43	SMARCB1
PARP3	PLCG2	PTPRS	ROS1	SMC1A
PAX5	PLK2	PTPRT	RPS6KA4	SMC3
PAX8	PML	QKI	RPS6KB2	SMO
PBRM1	PMS1	RAC1	RPTOR	SOCS1
PBX1	PMS2	RAD21	RRAS2	SOS1
PDCD1	PNRC1	RAD50	RUNX1	SOX17
PDCD1LG2	POLD1	RAD51	RUNX1T1	SOX2
PDE4DIP	POLE	RAD51B	RXRA	SOX9
PDGFB	PPARG	RAD51C	SAMD9	SPEN



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SPINK1	STK19	TENT5C	TOP2A	VTCN1
SPOP	SUFU	TERT	TP53	WRN
SPRED1	SUZ12	TET1	TP53BP1	WT1
SRC	SYK	TET2	TP63	XPA
SRSF2	TAF1	TFE3	TRAF2	XPC
SSX1	TAL1	TGFBR1	TRAF7	XPO1
STAG1	TAP2	TGFBR2	TSC1	XRCC2
STAG2	TBX3	TIMP3	TSC2	YAP1
STAT3	TCF3	TLR4	TSHR	YES1
STAT4	TCF7L1	TMPRSS2	U2AF1	ZFH3
STAT5A	TCF7L2	TNFAIP3	UGT1A1	ZNF217
STAT5B	TCL1A	TNFRSF14	VEGFA	ZNF703
STK11	TEK	TOP1	VHL	ZRSR2

### FUSION DRIVERS

ALK	RET	ROS1	NTRK1	MET	FGFR2	FGFR3
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## LIMITATIONS AND DISCLAIMER

Despite all precautions taken, the error (administrative and technical) associated with these types of molecular diagnostic tests can be as high as 1% to 2%. Rare polymorphisms may be present that could lead to false negative or false positive results. The quality of sequencing and coverage varies between regions; many factors such as homopolymers, GC-rich regions etc. influence the quality of sequencing and coverage. This may result in an occasional error in sequence reads or lack of detection of a particular genetic variant. Furthermore, A negative (wild type) result does not rule out the presence of a mutation or rearrangement resulting in targeted fusion, that may be present but below the limits of detection of this assay. Variants that have not been confirmed by an independent analysis could represent technical artifacts. Not all variants detected may be listed in the report. Inclusion of variants is dependent upon our assessment of their clinical significance. Additionally, the presence of a mutation may not be predictive of response to therapy in all patients. The selection of any potential treatment/course of action based on this report rests solely within the decision and judgment of the treating physician and patient. Decisions on patient care should be based on the independent medical judgment of the treating physician based upon all available clinical information, according to the applicable standard of care and should not be based solely on the tests and information contained in this report. Accurate interpretation of this report is dependent on provided detailed clinical history of the patient. In the event of unavailability of detailed clinical history, the lab cannot guarantee the accuracy of the interpretation. The results and interpretation are based on current knowledge and might change in the future. Some findings listed in this report may be based on pre-clinical studies or studies not in the given patient's tumor type.

## Question?

Contact us at **+91 124 4615 615**

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The test was processed in Lab 102.