

**ABOUT THE TEST** FoundationOne®Liquid CDx is a next generation sequencing (NGS) assay that identifies clinically relevant genomic alterations in circulating cell-free DNA.

<b>PATIENT</b>	<b>DISEASE</b> Cholangiocarcinoma (NOS)	<b>PHYSICIAN</b>	<b>ORDERING PHYSICIAN</b> Yeh, Yi-Chen	<b>SPECIMEN</b>	<b>SPECIMEN ID</b> YMTH 03/15/1950
	<b>NAME</b> Tsai Huang, Yen-Mei		<b>MEDICAL FACILITY</b> Taipei Veterans General Hospital		<b>SPECIMEN TYPE</b> Blood
	<b>DATE OF BIRTH</b> 15 March 1950		<b>ADDITIONAL RECIPIENT</b> None		<b>DATE OF COLLECTION</b> 09 January 2024
	<b>SEX</b> Female		<b>MEDICAL FACILITY ID</b> 205872		<b>SPECIMEN RECEIVED</b> 12 January 2024
	<b>MEDICAL RECORD #</b> 21455903		<b>PATHOLOGIST</b> Not Provided		

## Biomarker Findings

**Blood Tumor Mutational Burden - 0 Muts/Mb**

**ctDNA Tumor Fraction - Low (< 1.0%)**

**Microsatellite status - MSI-High Not Detected**

## Genomic Findings

*For a complete list of the genes assayed, please refer to the Appendix.*

**No reportable genomic alterations were detected. See below for more information.**

**This assay tested >300 cancer-related genes, including the following 2 gene(s) routinely assessed in this tumor type: *FGFR2*, *IDH1*.**

## Report Highlights

- **Low ctDNA Tumor Fraction** was detected; in the absence of actionable driver alterations consider reflex testing to a regulated tissue test, such as FoundationOne®CDx (p. 4)

### BIOMARKER FINDINGS

**Blood Tumor Mutational Burden - 0 Muts/Mb**

**ctDNA Tumor Fraction - Low (< 1.0%)**

**Microsatellite status - MSI-High Not Detected**

### THERAPY AND CLINICAL TRIAL IMPLICATIONS

**No therapies or clinical trials. See Biomarker Findings section**

**Low ctDNA Tumor Fraction. This result does not compromise confidence in any reported alterations. See Biomarker Finding Summary.**

**MSI-High not detected. No evidence of microsatellite instability in this sample (see Appendix section).**

## No therapies or clinical trials are associated with the Genomic Findings for this sample.

*If you have questions or comments about this result, please contact your local Customer Service team*  
<https://www.rochefoundationmedicine.com/home/contact-us.html>

**NOTE** Genomic alterations detected may be associated with activity of certain approved therapies; however, the therapies listed in this report may have varied clinical evidence in the patient's tumor type. Therapies and the clinical trials listed in this report may not be complete and/or exhaustive. Neither the therapies nor the trials identified are ranked in order of potential or predicted efficacy for this patient, nor are they ranked in order of level of evidence for this patient's tumor type. This report should be regarded and used as a supplementary source of information and not as the single basis for the making of a therapy decision. All treatment decisions remain the full and final responsibility of the treating physician and physicians should refer to approved prescribing information for all therapies. Therapies contained in this report may have been approved by the US FDA or other national authorities; however, they might not have been approved in your respective country. In the appropriate clinical context, germline testing of *APC*, *ATM*, *BAP1*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *FH*, *FLCN*, *MEN1*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *NF1*, *NF2*, *PALB2*, *PMS2*, *POLE*, *PTEN*, *RAD51C*, *RAD51D*, *RB1*, *RET*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SMAD4*, *STK11*, *TGFBR2*, *TP53*, *TSC1*, *TSC2*, *VHL*, and *WT1* is recommended.

Variant Allele Frequency is not applicable for copy number alterations.

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 Lauren L. Ritterhouse Casariego, M.D., Ph.D., Laboratory Director CLIA: 22D2027531  
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**Sample Preparation:** 150 Second St., 1st Floor, Cambridge, MA 02141 • CLIA: 22D2027531  
**Sample Analysis:** 150 Second St., 1st Floor, Cambridge, MA 02141 • CLIA: 22D2027531  
**Post-Sequencing Analysis:** 150 Second St., 1st Floor, Cambridge, MA 02141 • CLIA: 22D2027531

ORDERED TEST #    **ORD-1797022-01**

HISTORIC PATIENT FINDINGS (Biomarker Findings)	ORD-1797022-01
<b>Blood Tumor Mutational Burden</b>	0 Muts/Mb
<b>Microsatellite status</b>	MSI-High Not Detected
<b>ctDNA Tumor Fraction</b>	< 1.0%
HISTORIC PATIENT FINDINGS (Genomic Findings)	NAF%

**IMPORTANT NOTE** This comparison table refers only to genes and biomarkers assayed by prior FoundationOne®Liquid CDx or FoundationOne®CDx tests. Up to five previous tests may be shown.

For some genes in FoundationOne Liquid CDx, only select exons are assayed. Therefore, an alteration found by a previous test may not have been confirmed despite overlapping gene lists. Please refer to the Appendix for the complete list of genes and exons assayed. Variants reported for prior time points reflect reporting practices at the time of the historical test(s). Changes in variant reporting nomenclature, classification, or handling may result in the appearance of discrepancies across time points. The gene and biomarker list will be updated periodically to reflect new knowledge about cancer biology.

ctDNA Tumor Fraction may include previous Tumor Fraction results which reflect reporting practices at the time of reporting. Changes in biomarker reporting may result in the appearance of discrepancies across time points.

As new scientific information becomes available, alterations that had previously been listed as Variants of Unknown Significance (VUS) may become reportable or reportable variants may become VUS.

Tissue Tumor Mutational Burden (TMB) and blood TMB (bTMB) are estimated from the number of synonymous and non-synonymous single-nucleotide variants (SNVs) and insertions and deletions (indels) per area of coding genome sampled, after the removal of known and likely oncogenic driver events and germline SNPs. Tissue TMB is calculated based on variants with an allele frequency of  $\geq 5\%$ , and bTMB is calculated based on variants with an allele frequency of  $\geq 0.5\%$ .

Not Tested = not baited, not reported on test, or test preceded addition of biomarker or gene

Not Detected = baited but not detected on test

Detected = present (NAF% is not applicable)

NAF% = variant allele frequency percentage

Cannot Be Determined = Sample is not of sufficient data quality to confidently determine biomarker status

Please note that other aspects of this table may have changed from the previous version to reflect the most up-to-date reporting information.

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BIOMARKER FINDINGS

BIOMARKER

# Blood Tumor Mutational Burden

RESULT

0 Muts/Mb

## POTENTIAL TREATMENT STRATEGIES

### — Targeted Therapies —

On the basis of clinical evidence in solid tumors, increased blood tumor mutational burden (bTMB) may be associated with greater sensitivity to immunotherapeutic agents, including anti-PD-L1<sup>1-5</sup>, anti-PD-1<sup>2,5-8</sup>, anti-PD-1/CTLA4 therapies<sup>2,6</sup>, anti-PD-L1/CTLA4 therapies<sup>1,9-13</sup>. A Phase 2 multi-solid-tumor trial showed that bTMB  $\geq 16$  Muts/Mb (as measured by this assay) was associated with improved survival from treatment with a PD-1 inhibitor alone or in combination with a CTLA-4 inhibitor<sup>6</sup>. In non-small cell lung cancer (NSCLC), multiple clinical trials have shown patients with higher bTMB derive clinical benefit from immune checkpoint inhibitors following single-agent or combination treatments with either CTLA4 inhibitors or chemotherapy, with reported high

bTMB cutpoints ranging from 6 Muts/Mb-16 Muts/Mb<sup>3,11-13</sup>. In head and neck squamous cell carcinoma (HNSCC), a Phase 3 trial showed that bTMB  $\geq 16$  Muts/Mb (approximate equivalency  $\geq 8$  Muts/Mb as measured by this assay) was associated with improved survival from treatment with a PD-L1 inhibitor alone or in combination with a CTLA-4 inhibitor<sup>1</sup>. In colorectal cancer (CRC), a Phase 2 study showed that bTMB  $\geq 28$  Muts/Mb (approximate equivalency  $\geq 14$  Muts/Mb as measured by this assay) was associated with improved OS from a PD-L1 inhibitor alone or in combination with a CTLA-4 inhibitor<sup>9-10</sup>.

## FREQUENCY & PROGNOSIS

Among cholangiocarcinoma and gallbladder cancer cases profiled by F1LCDx, 4.8% and 8.7% of cases had a blood tumor mutational burden (bTMB) of  $>10$  Muts/Mb, respectively<sup>14</sup>. Published data investigating the prognostic implications of bTMB levels in biliary tract cancer are limited (PubMed, Jul 2023). Although cases with hypermutated biliary tract cancer were enriched in a subgroup with poor prognosis in 1 study<sup>15</sup>, TMB-high ( $\geq 10$  mut/Mb) status in biliary adenocarcinoma not treated with immunotherapy was not significantly associated with OS in another study, in which

patients with TMB-high tumors experienced numerically longer OS compared with patients with TMB-low tumors (11.5 vs. 8.4 months, adjusted HR=0.65)<sup>16</sup>.

## FINDING SUMMARY

Blood tumor mutational burden (bTMB, also known as mutation load) is a measure of the number of somatic protein-coding base substitution and insertion/deletion mutations from circulating tumor DNA in blood. TMB is affected by a variety of causes, including exposure to mutagens such as ultraviolet light in melanoma<sup>17-18</sup> and cigarette smoke in lung cancer<sup>19-20</sup>, treatment with temozolomide-based chemotherapy in glioma<sup>21-22</sup>, mutations in the proofreading domains of DNA polymerases encoded by the POLE and POLD1 genes<sup>23-27</sup>, and microsatellite instability (MSI)<sup>23,26-27</sup>. High bTMB levels were not detected in this sample. It is unclear whether the bTMB levels in this sample would be predicted to be associated with sensitivity to PD-1- or PD-L1-targeting immune checkpoint inhibitors, alone or in combination with other agents<sup>3-4,8,28-29</sup>. Depending on the clinical context, TMB testing of an alternate sample or by another methodology could be considered.

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BIOMARKER FINDINGS

BIOMARKER

# ctDNA Tumor Fraction

RESULT

Low (<1.0%)

## POTENTIAL TREATMENT STRATEGIES

### — Targeted Therapies —

Specimens with low circulating-tumor DNA (ctDNA) tumor fraction have reduced positive predictive agreement and negative predictive value compared with specimens with high ctDNA tumor fraction<sup>30</sup>. In a study of advanced non-small cell lung cancer (NSCLC), additional follow-up tissue testing identified driver alterations in 51% of patients with a low ctDNA tumor fraction<sup>30</sup>. When high ctDNA tumor fraction is not detected, it does not exclude the presence of disease burden or compromise the confidence of reported alterations. A negative result does not rule out the presence of

a mutation below the limits of detection of the assay. Patients for whom no companion diagnostic alterations are detected should be considered for confirmation with an FDA-approved or appropriately validated in other countries tumor tissue test, if available. Single observations or changes over time of circulating-tumor DNA (ctDNA) quantity are not currently part of any clinical decision-making guidelines but may be a useful indicator for future cancer management<sup>31-38</sup>.

## FREQUENCY & PROGNOSIS

In a large genomic study of 25 solid tumor types, 69% of liquid biopsy samples had ctDNA levels >1% as measured by an investigational composite tumor fraction algorithm with a median tumor fraction of 2.2% across tumor types<sup>39</sup>. Median ctDNA levels were reported to be highest in small cell lung cancer, liver, colon, and bladder tumor types and lowest in glioma and appendiceal cancers<sup>39</sup>. Higher ctDNA levels were reported for patients with metastatic (Stage 4) tumors compared with patients with localized disease (Stages 1 to 3)<sup>40</sup>. Higher ctDNA levels have been reported to be

associated with worse prognosis in a variety of advanced solid tumors<sup>41</sup>, including non-small cell lung cancer (NSCLC)<sup>42</sup>, colorectal cancer (CRC)<sup>42-43</sup>, pancreatic cancer<sup>44</sup>, Ewing sarcoma and osteosarcoma<sup>45</sup>, prostate cancer<sup>36,42,46</sup>, breast cancer<sup>42,47</sup>, leiomyosarcoma<sup>48</sup>, esophageal cancer<sup>49</sup>, and gastrointestinal cancer<sup>50</sup>.

## FINDING SUMMARY

The ctDNA tumor fraction provides an estimate of the percentage of circulating tumor DNA (ctDNA) present in a cell-free DNA (cfDNA) sample. The ctDNA tumor fraction algorithm utilized for FoundationOne Liquid CDx integrates multiple distinct genomic features, including aneuploidy and the observed allele frequencies of somatic short variants and rearrangements. Low ctDNA tumor fraction (<1.0%) was detected in this sample. For patients with a negative liquid biopsy result with low ctDNA tumor fraction, reflex tissue testing should be considered to confirm tumor mutation status, if feasible<sup>30</sup>.

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**APPENDIX**
**Variants of Unknown Significance**

**NOTE** One or more variants of unknown significance (VUS) were detected in this patient's tumor. These variants may not have been adequately characterized in the scientific literature at the time this report was issued, and/or the genomic context of these alterations makes their significance unclear. We choose to include them here in the event that they become clinically meaningful in the future.

**ASXL1**

 NM\_015338.5: c.3487G>A  
 (p.D1163N)  
 chr20:31024002

**BRCA2**

 NM\_000059.3: c.7052C>G  
 (p.A2351G)  
 chr13:32929042

**CDK12**

 NM\_016507.2: c.3853G>A  
 (p.D1285N)  
 chr17:37686949

**KDR**

 NM\_002253.2: c.3721G>A  
 (p.D1241N)  
 chr4:55948744

**MET**

 NM\_000245.2: c.284G>A  
 (p.C95Y)  
 chr7:116339422

**NTRK3**

 NM\_002530.2: c.1837C>A  
 (p.L613I)  
 chr15:88476295

**RAD21**

 NM\_006265.2: c.1602A>T  
 (p.E534D)  
 chr8:117862875

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**APPENDIX**

Genes assayed in FoundationOne®Liquid CDx

FoundationOne Liquid CDx interrogates 324 genes, including 309 genes with complete exonic (coding) coverage and 15 genes with only select non-coding coverage (indicated with an \*); 75 genes (indicated in bold) are captured with increased sensitivity and have complete exonic (coding) coverage unless otherwise noted.

<b>ABL1</b> Exons 4-9	ACVR1B	<b>AKT1</b> Exon 3	AKT2	AKT3	<b>ALK</b> Exons 20-29, Introns 18, 19	ALOX12B	AMER1 (FAM123B or WTX)	<b>APC</b>
<b>AR</b>	<b>ARAF</b> Exons 4, 5, 7, 11, 13, 15, 16	ARFRP1	ARID1A	ASXL1	<b>ATM</b>	<b>ATR</b>	ATRX	AURKA
AURKB	AXIN1	AXL	BAP1	BARD1	BCL2	BCL2L1	BCL2L2	BCL6
BCOR	BCORL1	BCR* Introns 8, 13, 14	<b>BRAF</b> Exons 11-18, Introns 7-10	<b>BRCA1</b> Introns 2, 7, 8, 12, 16, 19, 20	<b>BRCA2</b> Intron 2	BRD4	BRIP1	BTG1
BTG2	<b>BTK</b> Exons 2, 15	CALR	CARD11	CASP8	CBFB	CBL	<b>CCND1</b>	CCND2
CCND3	CCNE1	CD22	CD70	CD74* Introns 6-8	CD79A	CD79B	<b>CD274</b> (PD-L1)	CDC73
<b>CDH1</b>	<b>CDK12</b>	<b>CDK4</b>	<b>CDK6</b>	CDK8	CDKN1A	CDKN1B	<b>CDKN2A</b>	CDKN2B
CDKN2C	CEBPA	CHEK1	<b>CHEK2</b>	CIC	CREBBP	<b>CRKL</b>	CSF1R	CSF3R
CTCF	CTNNA1	<b>CTNNB1</b> Exon 3	CUL3	CUL4A	CXCR4	CYP17A1	DAXX	DDR1
<b>DDR2</b> Exons 5, 17, 18	DIS3	DNMT3A	DOT1L	EED	<b>EGFR</b> Introns 7, 15, 24-27	EMSY (C11orf30)	EP300	EPHA3
EPHB1	EPHB4	<b>ERBB2</b>	<b>ERBB3</b> Exons 3, 6, 7, 8, 10, 12, 20, 21, 23, 24, 25	ERBB4	ERCC4	ERG	<b>ERRF1</b>	<b>ESR1</b> Exons 4-8
ETV4* Intron 8	ETV5* Introns 6, 7	<b>ETV6*</b> Introns 5, 6	EWSR1* Introns 7-13	<b>EZH2</b> Exons 4, 16, 17, 18	EZR* Introns 9-11	FANCA	FANCC	FANCG
FANCL	FAS	FBXW7	FGF10	FGF12	FGF14	FGF19	FGF23	FGF3
FGF4	FGF6	<b>FGFR1</b> Introns 1, 5, Intron 17	<b>FGFR2</b> Intron 1, Intron 17	<b>FGFR3</b> Exons 7, 9 (alternative designation exon 10), 14, 18, Intron 17	FGFR4	FH	FLCN	FLT1
<b>FLT3</b> Exons 14, 15, 20	<b>FOXL2</b>	FUBP1	GABRA6	GATA3	GATA4	GATA6	GID4 (C17orf39)	<b>GNA11</b> Exons 4, 5
GNA13	<b>GNAQ</b> Exons 4, 5	<b>GNAS</b> Exons 1, 8	GRM3	GSK3B	H3-3A (H3F3A)	HDAC1	HGF	HNFI1A
<b>HRAS</b> Exons 2, 3	HSD3B1	ID3	<b>IDH1</b> Exon 4	<b>IDH2</b> Exon 4	IGF1R	IKBKE	IKZF1	INPP4B
IRF2	IRF4	IRS2	JAK1	<b>JAK2</b> Exon 14	<b>JAK3</b> Exons 5, 11, 12, 13, 15, 16	JUN	KDM5A	KDM5C
KDM6A	KDR	KEAP1	KEL	<b>KIT</b> Exons 8, 9, 11, 12, 13, 17, Intron 16	KLHL6	KMT2A (MLL) Introns 6, 8-11, Intron 7	KMT2D (MLL2)	<b>KRAS</b>

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LTK	LYN	MAF	<b>MAP2K1</b> (MEK1) Exons 2, 3	<b>MAP2K2</b> (MEK2) Exons 2-4, 6, 7	MAP2K4	MAP3K1	MAP3K13	MAPK1
MCL1	<b>MDM2</b>	MDM4	MED12	MEF2B	MEN1	MERTK	<b>MET</b>	MITF
MKNK1	MLH1	<b>MPL</b> Exon 10	MRE11 (MRE11A)	MSH2 Intron 5	MSH3	MSH6	MST1R	MTAP
<b>MTOR</b> Exons 19, 30, 39, 40, 43-45, 47, 48, 53, 56	MUTYH	MYB* Intron 14	<b>MYC</b> Intron 1	MYCL (MYCL1)	<b>MYCN</b>	<b>MYD88</b> Exon 4	NBN	<b>NF1</b>
NF2	NFE2L2	NFKBIA	NKX2-1	NOTCH1	NOTCH2 Intron 26	NOTCH3	<b>NPM1</b> Exons 4-6, 8, 10	<b>NRAS</b> Exons 2, 3
NSD2 (WHSC1 or MMSET)	NSD3 (WHSC1L1)	NT5C2	<b>NTRK1</b> Exons 14, 15, Introns 8-11	NTRK2 Intron 12	<b>NTRK3</b> Exons 16, 17	NUTM1* Intron 1	P2RY8	<b>PALB2</b>
PARP1	PARP2	PARP3	PAX5	PBRM1	PDCD1 (PD-1)	<b>PDCD1LG2</b> (PD-L2)	<b>PDGFRA</b> Exons 12, 18, Introns 7, 9, 11	<b>PDGFRB</b> Exons 12-21, 23 9, 11
PDK1	PIK3C2B	PIK3C2G	<b>PIK3CA</b> Exons 2, 3, 5-8, 10, 14, 19, 21 (Coding Exons 1, 2, 4-7, 9, 13, 18, 20)	PIK3CB	PIK3R1	PIM1	PMS2	POLD1
POLE	PPARG	PPP2R1A	PPP2R2A	PRDM1	PRKAR1A	PRKCI	PRKN (PARK2)	PTCH1
<b>PTEN</b>	<b>PTPN11</b>	PTPRO	QKI	RAC1	RAD21	RAD51	RAD51B	RAD51C
RAD51D	RAD52	RAD54L	<b>RAF1</b> Exons 3, 4, 6, 7, 10, 14, 15, 17, Introns 4-8	RARA Intron 2	<b>RB1</b>	RBM10	REL	<b>RET</b> Introns 7, 8, Exons 11, 13-16, Introns 9-11
RICTOR	RNF43	<b>ROS1</b> Exons 31, 36-38, 40, Introns 31-35	RPTOR	RSPO2* Intron 1	SDC4* Intron 2	SDHA	SDHB	SDHC
SDHD	SETD2	SF3B1	SGK1	SLC34A2* Intron 4	SMAD2	SMAD4	SMARCA4	SMARCB1
<b>SMO</b>	SNCAIP	SOCS1	SOX2	SOX9	SPEN	SPOP	SRC	STAG2
STAT3	<b>STK11</b>	SUFU	SYK	TBX3	TEK	TENT5C (FAM46C)	TERC* ncRNA	<b>TERT*</b> Promoter
TET2	TGFBR2	TIPARP	TMPRSS2* Introns 1-3	TNFAIP3	TNFRSF14	<b>TP53</b>	TSC1	TSC2
TYRO3	U2AF1	<b>VEGFA</b>	VHL	WT1	XPO1	XRCC2	ZNF217	ZNF703

**ADDITIONAL ASSAYS: FOR THE DETECTION OF SELECT CANCER BIOMARKERS**

Microsatellite (MS) status  
Blood Tumor Mutational Burden (bTMB)  
ctDNA Tumor Fraction

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**APPENDIX**
**About FoundationOne® Liquid CDx**

FoundationOne Liquid CDx fulfills the requirements of the European Directive 98/79 EC for in vitro diagnostic medical devices and is registered as a CE-IVD product by Foundation Medicine's EU Authorized Representative, Qarad b.v.b.a, Ciplastraat 3, 2440 Geel, Belgium. The CE-IVD regulatory status of FoundationOne Liquid CDx is applicable in countries that accept and/or recognize the CE mark.


**ABOUT FOUNDATIONONE LIQUID CDx**

FoundationOne Liquid CDx was developed and its performance characteristics determined by Foundation Medicine, Inc. (Foundation Medicine). FoundationOne Liquid CDx may be used for clinical purposes and should not be regarded as purely investigational or for research only. Foundation Medicine's clinical reference laboratories are qualified to perform high-complexity clinical testing.

Please refer to technical information for performance specification details.

**INTENDED USE**

FoundationOne Liquid CDx is a next generation sequencing based *in vitro* diagnostic device that analyzes 324 genes. Substitutions and insertion and deletion alterations (indels) are reported in 311 genes, copy number alterations (CNAs) are reported in 310 genes, and gene rearrangements are reported in 324 genes. The test also detects the genomic signatures blood tumor mutational burden (bTMB), microsatellite instability (MSI), and ctDNA tumor fraction. FoundationOne Liquid CDx utilizes circulating cell-free DNA (cfDNA) isolated from plasma derived from the anti-coagulated peripheral whole blood of cancer patients. The test is intended to be used as a companion diagnostic to identify patients who may benefit from treatment with targeted therapies in accordance with the approved therapeutic product labeling. Additionally, FoundationOne Liquid CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with malignant neoplasms.

**TEST PRINCIPLES**

The FoundationOne Liquid CDx assay is performed exclusively as a laboratory service using circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood from patients with solid malignant neoplasms. The assay employs a single DNA extraction method to obtain

cfDNA from plasma from whole blood. Extracted cfDNA undergoes whole-genome shotgun library construction and hybridization-based capture of 324 cancer-related genes including coding exons and select introns of 309 genes, as well as only select intronic regions or non-coding regions of 15 genes. Hybrid-capture selected libraries are sequenced with deep coverage using the NovaSeq® 6000 platform. Sequence data are processed using a customized analysis pipeline designed to accurately detect genomic alterations, including base substitutions, indels, select copy number variants, and select genomic rearrangements. Substitutions and insertion and deletion alterations (indels) are reported in 311 genes, copy number alterations (CNAs) are reported in 310 genes, and gene rearrangements are reported in 324 genes. The assay also reports ctDNA tumor fraction, and genomic signatures including MSI and bTMB. A subset of targeted regions in 75 genes is baited for increased sensitivity.

**THE REPORT**

Incorporates analyses of peer-reviewed studies and other publicly available information identified by Foundation Medicine; these analyses and information may include associations between a molecular alteration (or lack of alteration) and one or more drugs with potential clinical benefit (or potential lack of clinical benefit), including drug candidates that are being studied in clinical research. *Note:* A finding of biomarker alteration does not necessarily indicate pharmacologic effectiveness (or lack thereof) of any drug or treatment regimen; a finding of no biomarker alteration does not necessarily indicate lack of pharmacologic effectiveness (or effectiveness) of any drug or treatment regimen.

**QUALIFIED ALTERATION CALLS (EQUIVOCAL)**

All equivocal calls, regardless of alteration type, imply that there is adequate evidence to call the alteration with confidence. However, the repeatability of equivocal calls may be lower than non-equivocal calls.

**COPY NUMBER LOSS CALLS**

The FoundationOne Liquid CDx assay detects copy number loss in the following genes: *BRCA1*, *BRCA2*, and *PTEN*.

**RANKING OF THERAPIES AND CLINICAL TRIALS**
*Ranking of Therapies in Summary Table*

Therapies are ranked based on the following criteria: Therapies with clinical benefit (ranked alphabetically within each evidence category),

followed by therapies associated with resistance (when applicable).

*Ranking of Clinical Trials*

Pediatric trial qualification → Geographical proximity → Later trial phase.

**LIMITATIONS**

1. For *in vitro* diagnostic use.
2. For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
3. A negative result does not rule out the presence of a mutation below the limits of detection of the assay. Patients for whom no companion diagnostic alterations are detected should be considered for confirmation with an appropriately validated tumor tissue test, if available.
4. The FoundationOne Liquid CDx assay does not detect heterozygous deletions.
5. The test is not intended to provide information on cancer predisposition.
6. Performance has not been validated for cfDNA input below the specified minimum input.
7. Tissue TMB and blood TMB (bTMB) are estimated from the number of synonymous and nonsynonymous single-nucleotide variants (SNVs) and insertions and deletions (indels) per area of coding genome sampled, after the removal of known and likely oncogenic driver events and germline SNPs. Tissue TMB is calculated based on variants with an allele frequency of ≥5%, and bTMB is calculated based on variants with an allele frequency of ≥0.5%.
8. ctDNA tumor fraction is the percentage of circulating tumor DNA (ctDNA) present in a cell-free DNA (cfDNA) sample. The ctDNA tumor fraction estimate integrates multiple distinct genomic features, including modeled aneuploidy and the observed allele frequencies of somatic short variants and rearrangements.
9. Microsatellite instability (MSI) is a condition of genetic hypermutability that generates excessive amounts of short insertion/deletion mutations in the tumor genome; it generally occurs at microsatellite DNA sequences and is caused by a deficiency in DNA mismatch repair (MMR) in the tumor. The MSI algorithm is based on genome wide analysis of 1765 microsatellite loci and not based on the 5 or 7 MSI loci described in current clinical practice guidelines for solid tissue testing.
10. Genomic findings from circulating cell-free DNA (cfDNA) may originate from circulating tumor DNA fragments, germline alterations, or

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About FoundationOne® Liquid CDx

non-tumor somatic alterations, such as clonal hematopoiesis of indeterminate potential (CHIP). Genes with alterations that may be derived from CHIP include, but are not limited to: *ASXL1*, *ATM*, *CBL*, *CHEK2*, *DNMT3A*, *JAK2*, *KMT2D* (*MLL2*), *MPL*, *MYD88*, *SF3B1*, *TET2*, *TP53*, and *U2AF1*.

11. Alterations reported may include somatic (not inherited) or germline (inherited) alterations; however, the test does not distinguish between germline and somatic alterations. If a reported alteration is suspected to be germline, confirmatory testing should be considered in the appropriate clinical context.
12. The test is not intended to replace germline testing or to provide information about cancer predisposition.

## REPORT HIGHLIGHTS

The Report Highlights includes select genomic and therapeutic information with potential impact on patient care and treatment that is specific to the genomics and tumor type of the sample analyzed. This section may highlight information including targeted therapies with potential sensitivity or resistance; evidence-matched clinical trials; and variants with potential diagnostic, prognostic, nontargeted treatment, germline, or clonal hematopoiesis implications. Information included in the Report Highlights is expected to evolve with advances in scientific and clinical research. Findings included in the Report Highlights should be considered in the context of all other information in this report and other relevant patient information. Decisions on patient care and treatment are the responsibility of the treating physician.

## VARIANTS TO CONSIDER FOR FOLLOW-UP GERMLINE TESTING

The variants indicated for consideration of follow-up germline testing are 1) limited to reportable short variants with a protein effect listed in the ClinVar genomic database (Landrum et al., 2018; 29165669) as Pathogenic, Pathogenic/Likely Pathogenic, or Likely Pathogenic (by an expert panel or multiple submitters), 2) associated with hereditary cancer-predisposing disorder(s), 3) detected at an allele frequency of >30%, and 4) in select genes reported by the ESMO Precision Medicine Working Group (Mandelker et al., 2019; 31050713) to have a greater than 10% probability of germline origin if identified during tumor sequencing. The selected genes are *ATM*, *BAP1*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *FH*, *FLCN*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *PALB2*, *PMS2*, *POLE*, *RAD51C*, *RAD51D*, *RET*, *SDHA*, *SDHB*, *SDHC*, *SDHD*,

*TSC2*, and *VHL*, and are not inclusive of all cancer susceptibility genes. The content in this report should not substitute for genetic counseling or follow-up germline testing, which is needed to distinguish whether a finding in this patient's tumor sequencing is germline or somatic. Interpretation should be based on clinical context.

## VARIANTS THAT MAY REPRESENT CLONAL HEMATOPOIESIS

Variants that may represent clonal hematopoiesis (CH) are limited to select reportable short variants in defined genes identified in solid tumors only. Variant selection was determined based on gene tumor-suppressor or oncogene status, known role in solid tumors versus hematological malignancies, and literature prevalence. The defined genes are *ASXL1*, *ATM*, *CBL*, *CHEK2*, *DNMT3A*, *IDH2*, *JAK2*, *KMT2D* (*MLL2*), *MPL*, *MYD88*, *SF3B1*, *TET2*, and *U2AF1* and are not inclusive of all CH genes. The content in this report should not substitute for dedicated hematological workup. Comprehensive genomic profiling of solid tumors detects nontumor alterations that are due to CH. Patient-matched peripheral blood mononuclear cell sequencing is required to conclusively determine if this alteration is present in tumor or is secondary to CH. Interpretation should be based on clinical context.

## NATIONAL COMPREHENSIVE CANCER NETWORK® (NCCN®) CATEGORIZATION

Biomarker and genomic findings detected may be associated with certain entries within the NCCN Drugs & Biologics Compendium® (NCCN Compendium®) ([www.nccn.org](http://www.nccn.org)). The NCCN Categories of Evidence and Consensus indicated reflect the highest possible category for a given therapy in association with each biomarker or genomic finding. Please note, however, that the accuracy and applicability of these NCCN categories within a report may be impacted by the patient's clinical history, additional biomarker information, age, and/or co-occurring alterations. For additional information on the NCCN categories, please refer to the NCCN Compendium®. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). © National Comprehensive Cancer Network, Inc. 2023. All rights reserved. To view the most recent and complete version of the guidelines, go online to [NCCN.org](http://NCCN.org). NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way.

## LEVEL OF EVIDENCE NOT PROVIDED

Drugs with potential clinical benefit (or potential lack of clinical benefit) are not evaluated for source

or level of published evidence.

## NO GUARANTEE OF CLINICAL BENEFIT

This report makes no promises or guarantees that a particular drug will be effective in the treatment of disease in any patient. This report also makes no promises or guarantees that a drug with potential lack of clinical benefit will in fact provide no clinical benefit.

## NO GUARANTEE OF REIMBURSEMENT

Foundation Medicine makes no promises or guarantees that a healthcare provider, insurer or other third party payor, whether private or governmental, will reimburse a patient for the cost of FoundationOne Liquid CDx.

## TREATMENT DECISIONS ARE THE RESPONSIBILITY OF PHYSICIAN

Drugs referenced in this Report may not be suitable for a particular patient. The selection of any, all or none of the drugs associated with potential clinical benefit (or potential lack of clinical benefit) resides entirely within the discretion of the treating physician. Indeed, the information in this Report must be considered in conjunction with all other relevant information regarding a particular patient, before the patient's treating physician recommends a course of treatment. Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given community. A treating physician's decisions should not be based on a single test, such as this test or the information contained in this report.

Certain sample of variant characteristics may result in reduced sensitivity. These include: low sample quality, deletions and insertions >40bp, or repetitive/high homology sequences. FoundationOne Liquid CDx is performed using cell-free DNA, and as such germline events may not be reported.

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About FoundationOne®Liquid CDx

**SELECT ABBREVIATIONS**

ABBREVIATION	DEFINITION
CR	Complete response
DCR	Disease control rate
DNMT	DNA methyltransferase
HR	Hazard ratio
ITD	Internal tandem duplication
MMR	Mismatch repair
Muts/Mb	Mutations per megabase
NOS	Not otherwise specified
ORR	Objective response rate
OS	Overall survival
PD	Progressive disease
PFS	Progression-free survival
PR	Partial response
SD	Stable disease
TKI	Tyrosine kinase inhibitor

**REFERENCE SEQUENCE INFORMATION**

Sequence data is mapped to the human genome, Genome Reference Consortium Human Build 37 (GRCh37), also known as hg19.

**SOFTWARE VERSION INFORMATION**

MR Suite Version (RG) 7.15.0  
 MR Reporting Config Version Config 49  
 Analysis Pipeline Version v3.29.0  
 Computational Biology Suite Version 6.29.0

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