FOUNDATIONONE®LIQUID CDx

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Technical Information

Table of Contents

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1 Intended Use

FoundationOne Liquid CDx is a qualitative next generation sequencing based *in vitro* diagnostic test that uses targeted high throughput hybridization-based capture technology to detect and report substitutions, insertions and deletions (indels) in 311 genes, rearrangements in four (4) genes and copy number alterations in three (3) genes. FoundationOne Liquid CDx utilizes circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood of cancer patients collected in FoundationOne Liquid CDx cfDNA blood collection tubes included in the FoundationOne Liquid CDx Blood Sample Collection Kit. The test is intended to be used as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling.

Table 1: Companion diagnostic indications

Tumor Type	Biomarker(s) Detected	Therapy
Non-small cell lung cancer (NSCLC)	*ALK* rearrangements	ALECENSA® (alectinib)
	EGFR Exon 19 deletions and *EGFR* Exon 21 L858R substitution	IRESSA® (gefitinib) TAGRISSO® (osimertinib) TARCEVA® (erlotinib)
	MET single nucleotide variants (SNVs) and indels that lead *to MET* exon 14 skipping	TABRECTA® (capmatinib)
Prostate cancer	*BRCA1, BRCA2, ATM* alterations	LYNPARZA® (olaparib)
	BRCA1, *BRCA2* alterations	RUBRACA® (rucaparib)
Ovarian cancer	*BRCA1, BRCA2* alterations	RUBRACA® (rucaparib)
Breast cancer	*PIK3CA* mutations C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R; and H1047L, H1047R, and H1047Y	PIQRAY® (alpelisib)

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 solid malignant neoplasms.

A negative result from a plasma specimen does not mean that the patient's tumor is negative for genomic findings. Patients with the tumor types above who are negative for the mutations listed in **Table 1** should be reflexed to routine biopsy and their tumor mutation status confirmed using an FDA-approved tumor tissue test, if feasible.

Genomic findings other than those listed in Table 1 are not prescriptive or conclusive for labeled use of any specific therapeutic product.

FoundationOne Liquid CDx is a single-site assay performed at Foundation Medicine, Inc. in Cambridge, MA.

2 Contraindication

There are no known contraindications.

3 Warnings and Precautions

- 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.

- The test is not intended to replace germline testing or to provide information about cancer predisposition.

- Patients for whom no companion diagnostic alterations are detected should be considered for confirmation with an FDA-approved tumor tissue test, if possible.

4 Limitations

- For in vitro diagnostic use.

- For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.

- Genomic findings other than those listed in Table 1 of the intended use are not prescriptive or conclusive for labeled use of any specific therapeutic product.

- A negative result does not rule out the presence of an alteration in the patient's tumor.

- 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.

- The test is intended to be performed on specific serial number-controlled instruments by Foundation Medicine, Inc.

- Genomic findings from circulating cell-free DNA (cfDNA) may originate from circulating tumor DNA fragments, germline alterations, or nontumor 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, the following: *ASXL1, ATM, CBL, CHEK2, DNMT3A, JAK2, KMT2D (MLL2), MPL, MYD88, SF3B1, TET2, TP53*, and *U2AF1*. The efficacy of targeting such nontumor somatic alterations (e.g., CH) is unknown.

- The false positive rate of this test was evaluated in healthy donors. The detection rate for unique short variants in apparently healthy patients is 0.82%. Across 30,622 short variants, 58 variants had a detection rate of greater than 5%.

- The analytical accuracy for the FoundationOne Liquid CDx assay has not been demonstrated in all genes.

- The analytical accuracy for the FoundationOne Liquid CDx assay for the detection of SNVs and indels that lead to *MET* exon 14 skipping has not been demonstrated for samples with variant allele frequencies (VAF) below 0.34% for base substitutions and 0.73% VAF for small insertions and small deletions.

- TABRECTA® efficacy has not been established in patients with *MET* single nucleotide variants (SNVs) <0.21% VAF and in patients with *MET* indels <0.16% VAF tested with FoundationOne Liquid CDx.

- The precision of FoundationOne Liquid CDx was only confirmed for select variants at the limit of detection.
- The FoundationOne Liquid CDx assay does not detect heterozygous deletions.
- The FoundationOne Liquid CDx assay does not detect copy number losses/homozygous deletions in *ATM*.
- A complete assessment of the impact of cfDNA blood collection tube lot-to-lot variability on the performance of the test has not been evaluated.
- The test is not intended to provide information on cancer predisposition.
- *BRCA1/BRCA2* homozygous deletions and rearrangements were not adequately represented in all analytical studies.
- Representation of *ALK* rearrangements were limited in the analytical validation studies.
- The representation of *ATM* short variants and rearrangements was limited in the analytical validation studies.
- Performance has not been validated for cfDNA input below the specified minimum input.
- Representation of SNV and indels that lead to *MET* exon 14 skipping that represent biomarker rule category 1 and 2 (refer to Section 11.6 for CDx biomarker definition), were limited in the analytical validation studies.

5 Test Principle

The FoundationOne Liquid CDx (F1LCDx) 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. All coding exons of 309 genes are targeted; select intronic or non- coding regions are targeted in fifteen of these genes (refer to **Table 2** for the complete list of genes reported by FoundationOne Liquid CDx). Hybrid-capture selected libraries are sequenced with deep coverage using the NovaSeq® 6000 platform. Sequence data are processed using a custom analysis pipeline designed to detect genomic alterations, including base substitutions and indels in 311 genes, copy number variants in three genes, and genomic rearrangements in four genes. A subset of targeted regions in 75 genes is baited for enhanced sensitivity.

Table 2: As part of its FDA-approved intended use, the FoundationOne Liquid CDx assay interrogates 324 genes, including 309 genes with complete exonic (coding) coverage and 15 genes with only select non-coding coverage (indicated with an *).

Select regions in 75 genes (indicated in bold) are captured with increased sensitivity. Genes are captured for increased sensitivity with complete exonic (coding) coverage unless otherwise noted.

ABL1 [Exons 4-9]	*ACVR1B*	***AKT1 [Exon 3]***	*AKT2*	*AKT3*	***ALK [Exons 20-29, Introns 18,19]***	*ALOX12B*	*AMER1 (FAM123B)*	***APC***	***AR***
ARAF [Exons 4, 5, 7, 11, 13, 15, 16]	*ARFRP1*	*ARID1A*	*ASXL1*	***ATM***	***ATR***	*ATRX*	*AURKA*	*AURKB*	*AXIN1*
AXL	*BAP1*	*BARD1*	*BCL2*	*BCL2L1*	*BCL2L2*	*BCL6*	*BCOR*	*BCORL1*	*BCR* [Introns 8, 13, 14]*

***BRAF [Exons 11-18**, Introns 7-10]*	***BRCA1** [Introns 2, 7, 8, 12, 16, 19, 20]*	***BRCA2** [Intron 2]*	*BRD4*	*BRIP1*	*BTG1*	*BTG2*	***BTK [Exons 2, 15]***	*C11orf30 (EMSY)*	*C17orf39 (GID4)*
CALR	*CARD11*	*CASP8*	*CBFB*	*CBL*	***CCND1***	*CCND2*	*CCND3*	*CCNE1*	*CD22*
CD70	*CD74* [Introns 6-8]*	*CD79A*	*CD79B*	***CD274 (PD-L1)***	*CDC73*	***CDH1***	***CDK12***	***CDK4***	***CDK6***
CDK8	*CDKN1A*	*CDKN1B*	***CDKN2A***	*CDKN2B*	*CDKN2C*	*CEBPA*	*CHEK1*	***CHEK2***	*CIC*
CREBBP	***CRKL***	*CSF1R*	*CSF3R*	*CTCF*	*CTNNA1*	***CTNNB1 [Exon 3]***	*CUL3*	*CUL4A*	*CXCR4*
CYP17A1	*DAXX*	*DDR1*	***DDR2 [Exons 5, 17, 18]***	*DIS3*	*DNMT3A*	*DOT1L*	*EED*	***EGFR [Introns 7,** 15, **24-27]***	*EP300*
EPHA3	*EPHB1*	*EPHB4*	***ERBB2***	***ERBB3 [Exons 3, 6, 7, 8, 10, 12, 20, 21, 23, 24, 25]***	*ERBB4*	*ERCC4*	*ERG*	***ERRFI1***	***ESR1 [Exons 4-8]***
ETV4 [Intron 8]*	*ETV5* [Introns 6, 7]*	***ETV6* [Introns 5, 6]***	*EWSR1* [Introns 7-13]*	***EZH2 [Exons 4, 16, 17, 18]***	*EZR* [Introns 9-11]*	*FAM46C*	*FANCA*	*FANCC*	*FANCG*
FANCL	*FAS*	*FBXW7*	*FGF10*	*FGF12*	*FGF14*	*FGF19*	*FGF23*	*FGF3*	*FGF4*
FGF6	***FGFR1** [Introns 1, 5, **Intron 17]***	***FGFR2** [Intron 1, **Intron 17]***	***FGFR3 [Exons 7, 9 (alternative designation exon 10), 14, 18, Intron 17]***	*FGFR4*	*FH*	*FLCN*	*FLT1*	***FLT3 [Exons 14, 15, 20]***	***FOXL2***
FUBP1	*GABRA6*	*GATA3*	*GATA4*	*GATA6*	***GNA11 [Exons 4, 5]***	*GNA13*	***GNAQ [Exons 4, 5]***	***GNAS [Exons 1, 8]***	*GRM3*
GSK3B	*H3F3A*	*HDAC1*	*HGF*	*HNF1A*	***HRAS [Exons 2, 3]***	*HSD3B1*	*ID3*	***IDH1 [Exon 4]***	***IDH2 [Exon 4]***
IGF1R	*IKBKE*	*IKZF1*	*INPP4B*	*IRF2*	*IRF4*	*IRS2*	*JAK1*	***JAK2 [Exon 14]***	***JAK3 [Exons 5, 11, 12, 13, 15, 16]***
JUN	*KDM5A*	*KDM5C*	*KDM6A*	*KDR*	*KEAP1*	*KEL*	***KIT [Exons 8,9,11,12, 13, 17**, Intron 16]*	*KLHL6*	*KMT2A (MLL) [Introns 6, 8-11, Intron 7]*
KMT2D (MLL2)	***KRAS***	*LTK*	*LYN*	*MAF*	***MAP2K1 (MEK1) [Exons 2, 3]***	***MAP2K2 (MEK2) [Exons 2-4, 6, 7]***	*MAP2K4*	*MAP3K1*	*MAP3K13*
MAPK1	*MCL1*	***MDM2***	*MDM4*	*MED12*	*MEF2B*	*MEN1*	*MERTK*	***MET***	*MITF*

MKNK1	*MLH1*	***MPL [Exon 10]***	*MRE11A*	*MSH2 [Intron 5]*	*MSH3*	*MSH6*	*MST1R*	*MTAP*	***MTOR [Exons 19, 30, 39 40, 43-45, 47, 48, 53, 56]***
MUTYH	*MYB* [Intron 14]*	***MYC [Intron 1]***	*MYCL (MYCL1)*	***MYCN***	***MYD88 [Exon 4]***	*NBN*	***NF1***	*NF2*	*NFE2L2*
NFKBIA	*NKX2-1 (TTF-1)*	*NOTCH1*	*NOTCH2 [Intron 26]*	*NOTCH3*	***NPM1 [Exons 4-6, 8, 10]***	***NRAS [Exons 2, 3]***	*NSD3 (WHSC1L1)*	*NT5C2*	***NTRK1 [Exons 14, 15, Introns 8-11]***
NTRK2 [Intron 12]	***NTRK3 [Exons 16, 17]***	*NUTM1* **[Intron 1]***	*P2RY8*	***PALB2***	*PARK2*	*PARP1*	*PARP2*	*PARP3*	*PAX5*
PBRM1	*PDCD1 (PD-1)*	***PDCD1LG2 (PD-L2)***	***PDGFRA [Exons 12, 18, Introns 7, 9, 11]***	***PDGFRB [Exons 12-21, 23]***	*PDK1*	*PIK3C2B*	*PIK3C2G*	***PIK3CA [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	*PTCH1*	***PTEN***	***PTPN11***	*PTPRO*	*QKI*	*RAC1*	*RAD21*	*RAD51*	*RAD51B*
RAD51C	*RAD51D*	*RAD52*	*RAD54L*	***RAF1 [Exons 3, 4, 6, 7, 10, 14, 15, 17,*** *Introns 4-8]*	*RARA [Intron 2]*	***RB1***	*RBM10*	*REL*	***RET [****Introns 7, 8,* ***Exons 11, 13-16, Introns 9-11]***
RICTOR	*RNF43*	***ROS1 [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*	***SMO***	*SNCAIP*
SOCS1	*SOX2*	*SOX9*	*SPEN*	*SPOP*	*SRC*	*STAG2*	*STAT3*	***STK11 (LKB1)***	*SUFU*
SYK	*TBX3*	*TEK*	*TERC* {ncRNA}*	***TERT* {Promoter}***	*TET2*	*TGFBR2*	*TIPARP*	*TMPRSS2* [Introns 1-3]*	*TNFAIP3*
TNFRSF14	***TP53***	*TSC1*	*TSC2*	*TYRO3*	*U2AF1*	***VEGFA***	*VHL*	*WHSC1*	*WTI*
XPO1	*XRCC2*	*ZNF217*	*ZNF703*						

The classification criteria for all CDx variants are outlined at the end of this document.

The output of the test includes:

Category 1: Companion Diagnostic (CDx) claims noted in **Table 1** of the Intended Use

Category 2: cfDNA Biomarkers with Strong Evidence of Clinical Significance in cfDNA

Category 3: Biomarkers with Evidence of Clinical Significance in tissue supported by:
3A: strong analytical validation using cfDNA
3B: analytical validation using cfDNA

Category 4: Other Biomarkers with Potential Clinical Significance

As part of its FDA-approved intended use, copy number alterations and rearrangements are reported in the genes listed in **Table 3.**

Table 3: Genes for which copy number alterations and rearrangements are reported for tumor profiling by FoundationOne Liquid CDx

Alteration Type	Genes
Copy Number Alterations	*BRCA1, BRCA2, ERBB2*
Rearrangements	*ALK, BRCA1, BRCA2*

6 FoundationOne Liquid CDx cfDNA Blood Specimen Collection Kit Contents

Test Kit Contents
The test includes a sample shipping kit, which is sent to ordering laboratories. The shipping kit contains the following components:

- Specimen preparation and shipping instructions
- Two FoundationOne Liquid CDx cfDNA blood collection tubes (8.5 mL nominal fill volume per tube)
- Return shipping label

All other reagents, materials and equipment needed to perform the assay are used exclusively in the Foundation Medicine laboratory. The FoundationOne Liquid CDx assay is intended to be performed with serial number-controlled instruments.

7 FoundationOne Liquid CDx Sample Collection and Test Ordering

To order FoundationOne Liquid CDx, the test order form in the test kit must be fully completed and signed by the ordering physician or other authorized medical professional. Please refer to Specimen Preparation Instructions and Shipping Instructions included in the test kit.

For more detailed information, including Performance Characteristics, please find the FDA Summary of Safety and Effectiveness Data at: https://www.accessdata.fda.gov/cdrh_docs/pdf19/P190032B.pdf

8 Instruments

The FoundationOne Liquid CDx device is intended to be performed with the following instruments, as identified by specific serial numbers:

Illumina NovaSeq 6000
Beckman Biomek NXP Span-8 Liquid Handler
Thermo Scientific Kingfisher Flex DW 96
Bravo Benchbot
Hamilton STARTlet-STAR Liquid Handling Workstation

9 Performance Characteristics

Performance characteristics were established using contrived and clinical circulating cfDNA derived from blood specimens extracted from a wide range of tumor types. **Table 4** below provides a summary of the number of tumor types and variants included in each study. As summarized in this table, each study included a broad range of representative alteration types (substitutions, insertion-deletions, copy number alterations, rearrangements) in various genomic contexts across a number of genes. The validation studies included >7,000 sample replicates, >31,000 unique variants [includes variants classified as variants of unknown significance (VUS) and/or benign], >30 tumor types, representing all 324 genes targeted by the assay.

Table 4 Representation of tumor types and variants across validation studies

Study Title	Cancer Types Represented	# Unique Samples	# of Sample Replicates	# of Unique Genes	# of Unique				
					Subs	Indels	Rearrang.	Copy Number Amplif.	Copy Number Losses
Contrived Sample Functional Characterization (CSFC) Study	Breast cancer Colorectal cancer Lung cancer Contrived samples	13	1843	228	563	81	11	1	1
FoundationOne Liquid CDx to Validated NGS Tumor Tissue Test Concordance: *BRCA1* and *BRCA2* Variants	Prostate cancer Ovarian cancer	279	N/A	2	100	87	9	0	2
FoundationOne Liquid CDx to Validated NGS cfDNA Assay Concordance: *PIK3CA* mutations	Breast cancer	412	N/A	1	32	5	0	0	0
Orthogonal Concordance	23 cancer types Contrived samples	278	N/A	64	541	12	11	3	0
LoD Estimation	Prostate Contrived samples	10	877	286	1490	247	32	13	3
LoB	Healthy Donors	28	79	322	26134	4482	911	222	42
Potentially Interfering Substances	Contrived samples	9	336	18	16	11	11	1	2
Hybrid Capture Bait Specificity	25 cancer types Contrived samples	3546	N/A	324	N/A	N/A	N/A	N/A	N/A
Reagent Stability	Contrived samples	8	142	279	1090	215	32	17	2
Reagent Interchangeability	Contrived samples	8	192	20	15	11	11	1	1
Platform Precision study 1	Breast cancer Colon cancer Lung cancer Ovarian cancer Prostate cancer Skin cancer	47	1121	280	900	229	63	49	5

Study Title	Cancer Types Represented	# Unique Samples	# of Sample Replicates	# of Unique Genes	# of Unique				
					Subs	Indels	Rearrang.	Copy Number Amplif.	Copy Number Losses
	Contrived samples								
Platform Precision study 2	Lung cancer Prostate cancer Stomach cancer Colorectal cancer Bile duct cancer Breast cancer	10	230	6	6	4	0	0	0
Precision of detection of SNVs and indels that lead to *MET* exon 14 skipping	Lung Cancer	5	166	1	2	3	N/A	N/A	N/A
DNA Extraction	Colorectal cancer Prostate cancer Breast cancer Lung cancer Skin cancer	6	72	161	265	53	2	0	0
Whole Blood Sample Stability	Lung cancer Colorectal cancer Prostate cancer Breast cancer	11	22	66	75	15	1	0	0
Inverted Tube Whole Blood Sample Stability	Lung cancer Colorectal cancer Breast cancer Ovarian cancer Prostate cancer	130	260	237	594	91	5	5	0
Cross Contamination	Contrived samples	5	376	39	9	5	4	21	1
Guard Banding	Contrived samples	10	375	20	17	12	12	1	1
Clinical validation for detection of *EGFR* exon 19 deletions and L858R alterations: non-inferiority study	Lung cancer	177	N/A	1	5	7	N/A	N/A	N/A
Clinical validation study for detection of deleterious alterations in *BRCA1* and *BRCA2* in prostate cancer	Prostate cancer	199	N/A	2	44	55	8	0	1
Clinical validation study for detection of deleterious alterations in *BRCA1* and *BRCA2* in ovarian cancer	Ovarian cancer	217	N/A	2	48	49	3	0	0
Clinical validation study for detection of PIK3CA mutations in breast cancer	Breast	359	N/A	1	28	4	0	0	0

Study Title	Cancer Types Represented	# Unique Samples	# of Sample Replicates	# of Unique Genes	# of Unique				
					Subs	Indels	Rearrang.	Copy Number Amplif.	Copy Number Losses
Clinical validation study for ALK rearrangements in NSCLC	Lung cancer	249	N/A	1	13	1	11	1	0
Clinical validation study for BRCA1, BRCA2, and ATM alterations in prostate cancer	Prostate cancer	333	N/A	3	48	75	10	0	1
Clinical validation study for detection of SNVs and indels that lead to *MET* exon 14 skipping	Lung Cancer	171[α]	N/A	1	10	22	N/A	N/A	N/A
Blood Collection Tube Equivalence	Ovarian cancer Breast cancer Colorectal cancer Prostate cancer Lung cancer Skin cancer Stomach cancer	60	192	116	135	39	13	5	0
Automation Line Equivalence	Contrived samples	8	187	303	1926	337	63	61	4
Variant Report Curation	Breast cancer Colorectal cancer Lung cancer Prostate cancer Skin cancer	19	57	183	300	104	15	11	2
Pan-tumor performance (includes historical analysis)	20 cancer types	19868	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Molecular Index Barcode Performance	25 cancer types Contrived samples	7637	N/A	324	N/A	N/A	N/A	N/A	N/A
FoundationOne Liquid LDT to FoundationOne Liquid CDx Concordance	25 cancer types	927	N/A	73	1815	376	109	46	N/A
FoundationOne Liquid CDx to Validated cfDNA NGS Assay Concordance: *MET* exon 14 (Primary Analysis)	Lung Cancer	172	N/A	1	11	21	N/A	N/A	N/A

* Variants detected include variants classified as VUS and benign.

[α]The number shown includes samples tested at lower cfDNA inputs of ≥ 20 ng and <30 ng cfDNA input based on pre-specified assay procedures and processed only if the samples passed pre-specified in-process quality criteria.

9.1 Concordance – Comparison to an Orthogonal cfDNA NGS Method #1

The detection of short variants and rearrangements by the FoundationOne Liquid CDx assay was compared to that of an externally validated cfDNA NGS assay in 74 genes common to both assays across 278 samples that represented an array of tumor types (>50 unique disease ontologies across 23 cancer types). The cancer types (# samples) included lung [NSCLC (75) and other (3)]; breast (54); prostate (32); colorectal [colon (27) and rectal (6)]; liver (11); ovarian (6); pancreas (9); gastrointestinal (7); bile duct (2); esophageal (5); skin (6); cervical (1); anal (1); bladder (1); gallbladder (1); salivary gland (2); thymus (1); thyroid (3); uterine (2); fallopian tube (1); head and neck (1); soft tissue (1); and unknown primary (19). The study included samples selected from clinical FoundationOne Liquid testing (n=268) and contrived samples consisting of fragmented gDNA diluted in clinical cfDNA to represent rare alterations (n=10).

Using the externally validated NGS assay as the comparator, the analysis demonstrated a short variant PPA of 96.2% with a 95% two-sided CI of [94.8%-97.4%]. The short variant NPA was >99.9% with a 95% two-sided CI of [99.9%-100.0%]. The respective PPA of base substitutions and indels with a 95% two-sided CI was 96.1% [94.6%-97.3%] and 100.0% [85.2%-100.0%]. The respective NPA and 95% two-sided CI of base substitutions and indels was >99.9% [99.9%-100.0%] and 100.0% [99.89%-100.0%] (**Table 5).**

Table 5. Concordance of short variants called in FoundationOne Liquid CDx and the cfDNA comparator assay (n= 902 positive variants, n= 152,832 negative variants* by the comparator assay)

Variant Type	FoundationOne Liquid CDx(+) Comparator(+)	FoundationOne Liquid CDx(-) Comparator(+)	FoundationOne Liquid CDx(+) Comparator(-)	FoundationOne Liquid CDx(-) Comparator(-)	PPA [95% CI]	NPA [95% CI]	OPA [95% CI]
All Short Variants	868	34	8	152824	96.2% [94.8%-97.4%]	>99.9% [99.9%-100.0%]	>99.9% [99.9%-100.0%]
Base Substitutions	845	34	8	149511	96.1% [94.6%-97.3%]	>99.9% [99.9%-100.0%]	>99.9% [99.9%-100.0%]
Indels	23	0	0	3361	100.0% [85.2%- 100.0%]	100.0% [99.9%- 100.0%]	100.0% [99.9%- 100.0%]

* Variants detected include variants classified as VUS and benign.

For the concordance of rearrangement detection between FoundationOne Liquid CDx and the comparator assay, the observed rearrangement PPA was 100.0%, with a 95% two-sided CI of [59.0%-100.0%]. The NPA was 99.8%, with a 95% two-sided CI [99.5%-100.0%] (**Table 6**).

Table 6. Concordance of rearrangements called in FoundationOne Liquid CDx and the cfDNA comparator assay (n= 7 positive, n=1685 negative* as determined by the comparator assay)

	Comparator (+)	Comparator (-)	Total
FoundationOne Liquid CDx (+)	7	3	10
FoundationOne Liquid CDx (-)	0	1682	1682
Total	7	1685	1692
	PPA: 100.0% [59.0% - 100.0%]	**NPA: 99.8% [99.5% - 100.0%]**	**OPA: 99.8% [99.5% - 100.0%]**

* Variants detected include variants classified as VUS and benign.

Assessment of a subset of highly-actionable alterations were compared between the two assays. The analysis resulted in a PPA of 100% across all eligible highly-actionable alterations called in the comparator assay (**Table 7**).

Table 7. Concordance of CDx alterations called between FoundationOne Liquid CDx and the comparator assay (n = 74)

Targeted Alteration	*n*	PPA [95% CI]	NPA [95% CI]
BRCA1 short variants	1	100% [2.5%-100.0%]	100% [98.7%-100.0%]
BRCA2 short variants	2	100% [15.8%-100.0%]	100% [99.3%-100.0%]
EGFR exon 19 deletions	11	100% [71.5%-100.0%]	100% [99.7%-100.0%]
EGFR L858R	10	100% [69.2%-100.0%]	100% [98.7%-100.0%]
PIK3CA base substitutions	49	100% [92.7%-100.0%]	100% [99.9%-100.0%]
ALK rearrangements	1	100% [2.5%-100.0%]	99.9% [99.7%-100.0%]

These data demonstrate that the FoundationOne Liquid CDx assay and an externally-validated NGS assay are highly concordant across the 74 genes common between the two panels.

9.2 Concordance – FoundationOne Liquid CDx to validated NGS tumor tissue assay (*BRCA1* and *BRCA2* alterations)

Samples from a total of 279 prostate and ovarian cancer patients were tested and the concordance evaluated between FoundationOne Liquid CDx and the validated NGS tumor tissue assay for the detection of deleterious alterations in *BRCA1* or *BRCA2*. As summarized below, a PPA of 88.03% and an NPA of 95.68% were observed on a sample level (**Table 8**). As summarized in **Table 9**, an overall PPA of 87.28% and an NPA of 99.83% were observed at the variant level. Some discordance is expected based on biological differences and sampling times between tumor tissue and plasma samples. Considering the impact of biological differences between analytes, these data demonstrate a high concordance between FoundationOne Liquid CDx and the validated NGS tumor tissue assay for the detection of deleterious alterations in *BRCA1* or *BRCA2*.

Table 8. Concordance (by sample) of FoundationOne Liquid CDx and validated NGS tumor tissue assay in prostate and ovarian cancer patients for the detection of alterations in *BRCA1* or *BRCA2*

		NGS Tumor Tissue Assay	
		Positive	**Negative**
FoundationOne Liquid CDx	**Positive**	103	7
	Negative	14	155
		PPA: 88.03% [80.91%-92.74%]	**NPA:** 95.68% [91.35%-97.89%]

Table 9. Concordance (by variant) of FoundationOne Liquid CDx and validated NGS tumor tissue assay in prostate and ovarian cancer patients for the detection of alterations in *BRCA1* or *BRCA2*

	F1LCDx+ /Tissue+	F1LCDx- /Tissue+	F1LCDx+ /Tissue-	F1LCDx-/ Tissue-	PPA (95% CI)	NPA (95% CI)
Substitutions	77	6	29	20255	92.77% (85.11%, 96.64%)	99.86% (99.79%, 99.90%)
Indels	65	3	31	16362	95.59% (87.81%, 98.49%)	99.81% (99.73%, 99.87%)
Rearrangements	4	3	7	1939	57.14% (25.05%, 84.18%)	99.64% (99.26%, 99.83%)
Copy number loss	5	10	1	263	33.33% (15.18%, 58.29%)	99.62% (97.89%, 99.93%)
Total	151	22	68	38819	87.28% (81.50%, 91.45%)	99.83% (99.78%, 99.86%)

9.3 Concordance – Comparison to an Orthogonal cfDNA NGS Method #2

The accuracy of using FoundationOne Liquid CDx as a companion diagnostic to identify breast cancer patients harboring *PIK3CA* alterations was assessed with residual plasma samples from the SOLAR-1 clinical trial. Of the remaining plasma samples, 542 were evaluable by the externally-validated NGS method and produced valid results. 418 were evaluable by FoundationOne Liquid CDx, of which 192 positive variants were detected across 188 patients, with four patients possessing two positive variants each. The distribution of counts per positive variant is listed in **Table 10**.

Table 10. Distribution of variants detected with FoundationOne Liquid CDx evaluable samples.

Protein Effect in PIK3CA	# Variant Calls (188 Positive Samples)
C420R	3
E542K	25
E545A	1
E545G	2
E545K	50
H1047L	9
H1047R	100
H1047Y	1
Q546R	1
Total	**192**

A total of 412 valid samples generated valid results with both assays. The primary analysis using NGS Method #2 as the reference assay achieved a PPA [95% CI] of 97.06% [93.27%, 99.04%], and an NPA [95% CI] of 91.74% [87.52%, 94.88%]. The contingency table for this comparison is provided in **Table 11**, with counts representing number of samples (versus number of variant calls).

The sample counts in the core 2x2 white boxes total to 412 samples. There were seven samples evaluable with FoundationOne Liquid CDx but failed (italicized in **Table 11**), as well as three samples missing from reference assay data. There were five samples unevaluable by the reference assay; three of these aligned with the 418 evaluable FoundationOne Liquid CDx samples, while two were among the 130 samples not evaluable due to insufficient plasma.

Table 11. Contingency table comparing FoundationOne Liquid CDx with the reference assay, primary analysis with 412 cases.

		Reference Assay					
		Positive	**Negative**	**Not Evaluable**	**Missing**	**Total**	
FoundationOne Liquid CDx	**Positive**	165	20	2	1	**188**	PPA_{F1L}: 89.19% [83.80%, 93.27%]
	Negative	5	222	1	2	**230**	NPA_{F1L}: 97.80% [94.93%-99.28%
	Evaluable but Failed	0	*7*	0	0	7	
	Not Evaluable	35	93	2	0	130	
	Total	205	342	5	3	555	

	PPA_{ONC}: 97.06% [93.27%, 99.04%]	NPA_{ONC}: 91.74% [87.52%, 94.88%]				OPA: 93.93% [91.17%, 96.04%]

9.4 Concordance – FoundationeOne Liquid CDx to an externally validated cfDNA NGS assay (SNVs and indels that lead to *MET* exon 14 skipping)

An analytical accuracy study was performed to demonstrate the concordance between FoundationOne Liquid CDx (F1LCDx)and an externally validated cfDNA NGS comparator (evNGS) assay for the detection of SNVs and indels that lead to *MET* exon 14 skipping. Overall, there were 74 overlapping genes targeted by the two assays and the comparator assay bait set covered the same regions as the FoundationOne Liquid CDx bait set.

The analytical accuracy study was conducted with 45 samples from the clinical bridging study with 41 samples from patients enrolled in the GEOMETRY-mono 1 trial (refer to Section 10.7 below). An additional 100 NSCLC samples were sourced from FMI's clinical archives, 38 samples from NSCLC patients previously evaluated in the accuracy study to support the original PMA P190032 (refer to section 9.1 above) and 31 externally sourced plasma samples from NSCLC cases whose tissue specimens tested positive for *MET* exon 14 skipping alterations and were subsequently tested with F1LCDx to determine their *MET* exon 14 skipping associated alteration status prior to conducting the accuracy study statistical analysis. Samples selected from FMI's clinical archives that were positive for *MET* exon 14 skipping alterations had to have a variant allele frequency (VAF) greater than or equal 0.40%.

Of the 214 samples, 179 samples had DNA yield that allowed processing with F1L CDx at the specified LC DNA input of 30ng-80ng. Thirty-five (35) samples were tested with F1L CDx at a lower LC DNA input of out of specification of 20ng-<30ng LC DNA input. Of the 179 samples that had sufficient DNA yield for testing with F1L CDx, 3 samples had a F1L CDx sequence analysis QC failure, while 4 had an evNGS QC failure.

The primary analytical concordance analysis, using the evNGS assay results as the reference, included 172 samples that passed QC with both assays. Forty-eight (48) of the 172 samples were identified as positive for *MET* exon 14 skipping alterations by FoundationOne Liquid CDx. The statistical analysis using the evNGS assay results as the reference showed a positive percent agreement (PPA) of 94.87% with 95% CI (83.11%-98.58%), a negative percent agreement (NPA) of 91.83% with 95% CI (85.80%, 95.32%), a positive predictive value (PPV) of 77.08% with 95% CI (63.46%, 86.69%) and a negative predictive value (NPV) of 98.39% with 95% CI (94.31%, 99.56%) as shown in Table 12. Since the samples were selected from different sources based on different assays, the unadjusted PPA/NPA and unadjusted PPV/NPV in Table 12 may be subject to potential bias.

Table 12: Primary Concordance Analysis Comparing Sample-level Biomarker Detection between FoundationOne Liquid CDx and Comparator Assay

		evNGS			
		***MET* ex14 positive**	***MET* ex14 negative**	**Total**	**PPV/NPV (95% CI)**
F1L CDx	***MET* ex14 positive**	37	11	48	PPV: 77.08% (63.46%, 86.69%)
	***MET* ex14 Negative**	2	122	124	NPV: 98.39% (94.31%, 99.56%)
	Total	39	133	172	
	PPA/NPA (95% CI)	PPA: 94.87% (83.11%, 98.58%)	NPA: 91.83% (85.80%, 95.32%)		

Ten (10) of the eleven (11) samples that were F1L CDx-positive/evNGS-negative [F1L CDx(+)/evNGS(-)] were discordant due to differences in variant reporting by assays. Of the 11 samples, 10 samples harbored *MET* exon 14 deletions ≥6bp detectable by the evNGS variant caller, which calls variants in the evNGS's loci of interest (LOI) and indels ≥6bp in *MET* exon 14. Since *MET* ex14 indels ≥6bp are not part of the evNGS's LOI, this variant type is filtered out and not reported by the evNGS's analysis software in the default setting, and thus are considered negatives by the evNGS comparator assay. Further the remaining one (1) sample from the 11 samples that were F1L CDx (+)/evNGS(-), contained a *MET* exon 14 deletion <6bp which cannot be called with the evNGS variant because the variant caller can only output *MET* exon 14 deletions ≥6bp. The evNGS reporting rules only correspond to biomarker rule category 3, so all 37 samples that were F1L CDx(+)/evNGS(+) had *MET* exon 14 skipping alterations that correspond to biomarker rule category 3, i.e., these samples had base substitutions and indels affecting positions 0, +1, +2, or +3 at the splice donor site of the 3′ boundary of *MET* exon 14. The evNGS assay does not call category 1 and 2 biomarkers as they are not included in their LOI. In the two (2) discordant samples that were F1L CDx negative(-)/evNGS(+), base substitutions reported by the evNGS were not detected in the variant analysis pipeline of F1L CDx.

Four (4) of the eleven (11) discordant samples that were F1L CDx(+)/evNGS(-) were from patients evaluated in the clinical therapeutic study for whom efficacy data was available. Of these 4 patients, 3 had partial response to TABRECTA, while one had progressive disease. Although these patients had discordant results, these results appear to suggest that these patient with F1L CDx(+)/evNGS(-) were *MET* exon 14 deletion positive.

9.5 Limit of Detection (Analytical Sensitivity)

The LoD for each variant type was established by processing a total of 1,069 sample replicates across ten contrived (enzymatically fragmented cell-line gDNA) samples representing short variants, rearrangements, and copy number alterations. The LoD was determined using the conservative hit rate approach for the majority of variants. A probit model was used when appropriate (when ≥3 dilution levels with hit rates between 10% and 90% were observed). LoD by hit rate was defined as the mean VAF value (for short variants and rearrangements) or mean tumor fraction value (for copy number alterations) at the lowest dilution level tested with at least 95% detection across replicates. The hit rate was computed as the number of replicates with positive variant calls per the total number of replicates tested at each level of the targeted VAF (short variants and rearrangements) or tumor fraction (copy number alterations). Short variants with hit rates of at least 95% at all dilution levels or hit rates below 95% for all dilution levels were excluded from analysis as LoD could not be reliably estimated.

The median estimated LoD for CDx alterations are presented in **Table 13.** The median LoD for targeted short variants, rearrangements, and copy number alterations were consistent with the platform LoD (**Table 14**).

Table 13: LoD estimation for CDx alterations

Gene	Alteration Subtype	Number of Samples Evaluated	Median LoD*
ATM	Indels	1	0.51% VAF
	Rearrangement (*ATM-EXPH5* Truncation[1])	1	1.13% VAF
BRCA1	Substitutions	8	0.34% VAF
	Indels	1	0.38% VAF[2]
	Rearrangement[1]	1	0.87% VAF
BRCA2	Substitutions	17	0.37% VAF
	Indels	2	0.36% VAF
	BRCA2- EDA Truncation[1]	1	0.48% VAF
	Copy Number Loss[1]	1	48.1% TF
EGFR	Substitutions (L858R substitutions)	2	0.34% VAF
	Indels (exon 19 deletions)	2	0.27% VAF

The ORR [95% CI] in the primary efficacy population was 53.8% [33.4%-73.4%] in *BRCA* Positive patients as determined by FoundationOne Liquid CDx, which is comparable to the ORR of 54.1% [40.8%-66.9%] in patients identified by the CTA **(Table 57**).

The median DOR [95% CI] was 225 days [115, 403] in FoundationOne Liquid CDx *BRCA* Positive patients from the Primary Efficacy Population. This is similar to the median DOR of 288 days [170, 403] for the Primary Efficacy Population in *BRCA* Positive patients by the CTA.

Table 57: ORR and duration of response in the primary efficacy population by CTA and FoundationOne Liquid CDx test results

	FoundationOne Liquid CDx *BRCA* Positive n = 26	**CTA *BRCA* Positive n = 61**
Confirmed ORR (CR + PR), % (n)	53.8% (14)	54.1% (33)
95% CI	33.4%, 73.4%	40.8%, 66.9%
Duration of Response (days)		
Median	225	288
95% CI	115 – 403	170 – 403

Abbreviations: *BRCA* = breast cancer gene, includes *BRCA1* and *BRCA2*; CI = confidence interval; CTA = clinical trial assay; ORR = objective response rate; CR = complete response; PR = partial response.

The ORR [95% CI] in All Patients was evaluated for *BRCA* Positive and *BRCA* Negative patients. The ORR in *BRCA* Positive patients identified from FoundationOne Liquid CDx was 40.6% [28.5%-53.6%] compared to the ORR of 46.8% [37.8%-55.9%] in *BRCA* Positive patients based on the CTA. The ORR in *BRCA* Negative patients by FoundationOne Liquid CDx and the CTA was 5.9% [2.7%-10.9%] and 13.1% [9.8%-17.0%], respectively.

Sensitivity analysis to evaluate the robustness of the clinical efficacy estimate against the unknown FoundationOne Liquid CDx results was performed using the multiple imputation method in All Patients. After imputing the missing FoundationOne Liquid CDx results, the weighted ORR [95% CI] across the imputed datasets was 45.2% [36.3%-54.1%].

10.6 Clinical Bridging Study: Detection of *PIK3CA* Alterations to Determine Eligibility for Treatment with Alpelisib

Clinical validity of using FoundationOne Liquid CDx to identify breast cancer patients harboring *PIK3CA* alterations eligible for treatment with alpelisib was assessed through retrospective testing of plasma samples collected prior to study treatment from advanced or metastatic breast cancer patients enrolled in clinical trial CBYL719C2301 (SOLAR-1). A total of 395 patients were enrolled based on CTA1 results and 177 patients were enrolled based on CTA2 results. All 395 patients enrolled based on CTA1 results were retrospectively tested by CTA2. This clinical bridging study was performed based on CTA2 results.

Samples with ≥30 ng from 375 patients were tested by FoundationOne Liquid CDx. Excluding those with invalid results for either CTA2 or CDx (4 and 12, respectively), the primary efficacy analyses were conducted using data from the 359 subjects who were CTA2-evaluable and CDx-evaluable **Table 58.**

Table 58: Concordance between FoundationOne Liquid CDx and CTA2

	CTA2			
CDx	**Positive**	**Negative**	**Invalid**	**Total**
Positive	165	0	1	166
Negative	65	129	3	197
Invalid	7	5	0	12

***BRCA1* Alterations (Protein Change)**				***BRCA2* Alterations (Protein Change)**	
L22S	C64W	G1706E	V1838E	D23Y	T2722K
I26N	R71G	A1708E		S142N	T2722R
T37K	R71K	S1715R		S142I	D2723H
C39R	R71T	S1722F		V159M	D2723G
C39G	R71M	V1736A		V211I	G2724W
C39Y	S770L	G1738R		V211L	G2748D
C39W	R1495T	G1738E		Y600C	A2911E
H41R	R1495M	K1759N		K1530N	E3002K
C44S	R1495K	L1764P		R2336P	R3052W
C44Y	E1559K	I1766N		R2336L	D3095G
C44F	E1559Q	I1766S		R2336H	D3095E
C47S	T1685A	G1770V		T2412I	N3124I
C47Y	T1685I	M1775K		R2602T	N3187K
C47F	D1692N	M1775R		W2626C	
C61S	M1689R	C1787S		I2627F	

11.5 CDx classification criteria for PIK3CA alterations, qualifying breast cancer patients for therapy with PIQRAY® (alpelisib):

Presence of PIK3CA mutation(s): H1047R; E545K; E542K; C420R; E545A; E545D [1635G>T only]; E545G; Q546E; Q546R; H1047L; or H1047Y

11.6 CDx classification criteria for SNVs and Indels that lead to *MET* exon 14 skipping:

A SNV or indel in *MET* shall be considered to result in skipping of exon 14 if one or more of the following criteria are met:

1. Deletions greater than or equal to 5 bp that affect positions -3 to -30 in the intronic region immediately adjacent to the splice acceptor site at the 5′ boundary of *MET* exon 14.
2. Indels affecting positions -1 or -2 at the splice acceptor site of the 5′ boundary of *MET* exon 14.
3. Base substitutions and indels affecting positions 0, +1, +2, or +3 at the splice donor site of the 3′ boundary of *MET* exon 14.