FOUNDATIONONE® LIQUID CDx

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Technical Information

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Foundation Medicine, Inc.
150 Second Street, Cambridge, MA 02141
Phone: 617.418.2200

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 12.6 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	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
	Prostate cancer Skin cancer 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

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 detection of PIK3CA mutations in breast cancer	Breast	359	N/A	1	28	4	0	0	0
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

Gene	Alteration Subtype	Number of Samples Evaluated	Median LoD*
PIK3CA	Substitutions	6	0.34% VAF
ALK	Rearrangement (*ALK-EML4*)	1	0.24% VAF
	Rearrangement (*NPM1-ALK* Rearrangement)	1	0.94% VAF
MET	Indels[1]	1	0.28% VAF
	Substitutions[1]	2	0.40% VAF

The estimated LoDs for *BRCA1* and *BRCA2* subs and indels were confirmed at values higher than the LoDs established in **Table 13** (see Precision: Reproducibility and Reproducibility section below, **Table 22** and **Table 23** for confirmed LoD values).
[1]The LoD for these alterations was determined using clinical specimens.
[2] Quantitative reporting of %VAF/%TF has not been approved by FDA.

The platform LoD for short variants, rearrangements, and copy number losses are presented in **Table 14**. A total of 864 short variants were included in the platform LoD analysis. The enhanced sensitivity region of the bait set contains 269 of the short variants analyzed and the standard sensitivity region of the bait set contains 595 of the short variants analyzed. The estimated LoD for short variants is 0.40% for the enhanced sensitivity region and 0.82% of the standard sensitivity region. The median LoD is 30.4% tumor fraction for copy number losses.

Because a major component driving the detectability of a variant is genomic context (repetitiveness of the reference genomic region), the LoD analysis for short variants was also evaluated within categories based on genomic context as summarized in **Table 15**.

Table 12: LoD estimation by variant type

Alteration Type	Number of Variants in Analysis	Bait Set Region	Median LoD[1]	Quartile 1 to Quartile 3 LoD Range
Short Variants	269	Enhanced Sensitivity	0.40% VAF	0.33% - 0.50% VAF
	595	Standard Sensitivity	0.82% VAF	0.70% - 0.98% VAF
Rearrangements	7	Enhanced Sensitivity	0.37% VAF	0.26% - 0.47% VAF
	1	Standard Sensitivity	0.90% VAF	N/A
Copy Number Amplifications	8	N/A	21.7% TF	19.8% - 25.2% TF

VAF = variant allele frequency
TF = tumor fraction
[1] Quantitative reporting of %VAF/%TF has not been approved by FDA.

Table 15: LoD by variant subtype based on genomic context

Region	Alteration Subtype	N	Minimum LoD (VAF/TF)[1, 2]	1st Quantile LoD (VAF/TF)[1]	Median LoD (VAF/TF)[1]	3rd Quantile LoD (VAF/TF)[1]
	Short Variants: Enhanced Sensitivity Region Total	269	0.20%	0.33%	0.40%	0.50%
	Insertion/Deletion in non-repetitive region or a repetitive region of <=3 base pairs	10	0.23%	0.29%	0.31%	0.36%

Region	Alteration Subtype	N	Minimum LoD (VAF/TF)[1, 2]	1st Quantile LoD (VAF/TF)[1]	Median LoD (VAF/TF)[1]	3rd Quantile LoD (VAF/TF)[1]
Enhanced Sensitivity Region	Insertion/Deletion in a repetitive region of 4 to 6 base pairs	23	0.28%	0.37%	0.48%	0.56%
	Insertion/Deletion in a repetitive region of >=7 base pairs	6	0.33%	0.48%	0.58%	0.82%
	Substitution in a non-repetitive region or a repetitive region of <=7 base pairs	229	0.20%	0.33%	0.39%	0.49%
	Substitution in a repetitive region of >7 base pairs	1	0.32%	0.32%	0.32%	0.32%
Standard Sensitivity Region	Short Variants: High Sensitivity Region Total	595	0.40%	0.70%	0.82%	0.98%
	Insertion/Deletion in non-repetitive region or a repetitive region of <=3 base pairs	18	0.46%	0.68%	0.87%	1.00%
	Insertion/Deletion in a repetitive region of 4 to 6 base pairs	32	0.61%	0.75%	0.87%	0.95%
	Insertion/Deletion in a repetitive region of >=7 base pairs	11	0.59%	1.07%	1.15%	1.20%
	Substitution in a non-repetitive region or a repetitive region of <=7 base pairs	524	0.40%	0.70%	0.81%	0.96%
	Substitution in a repetitive region of >7 base pairs	8	0.69%	0.83%	0.96%	1.28%
Enhanced Sensitivity Region	Rearrangements	7	0.20%	0.26%	0.37%	0.47%
Enhanced/ Standard Sensitivity Region	Rearrangements	1	0.28%	0.28%	0.28%	0.28%
Standard Sensitivity Region	Rearrangements	1	0.90%	0.90%	0.90%	0.90%
NA	Copy Number Amplifications	8	19.8%	19.8%	21.7%	25.2%

[1]VAF reported for short variant and rearrangement LoD, tumor fraction reported for copy number alterations LoD.

[2] Quantitative reporting of %VAF/%TF has not been approved by FDA.

The median LoD for highly-actionable, non-CDx alterations evaluated for LoD are presented in **Table 16.** The median LoD for these targeted short variants are consistent with the platform LoD presented in **Table 14.**

Table 16: LoD for non-CDx alterations

Gene	Alteration Subtype	Number of Samples Evaluated	Median LoD[2]
BRAF	Substitutions	1	0.33% VAF
KRAS	Substitutions	2	0.33% VAF
MET[3]	Indels	1	0.41% VAF
NRAS	Substitutions	2	0.42% VAF
PALB2	Indels	1	0.37% VAF
	Substitutions	1	0.51% VAF
ERBB2	Copy Number Amplification	1	19.8% TF

VAF = variant allele frequency
TF = tumor fraction
[1] LoD for these alterations was determined using clinical specimens.
[2]Quantitative reporting of %VAF/%TF has not been approved by FDA.
[3]This LoD applies to MET alterations that do not meet the CDx rules.

9.6 Limit of Blank (LoB)

Per CLSI EP17-A2, the limit of blank (LoB) was established by profiling plasma samples from 30 asymptomatic donors with no diagnosis of cancer with 4 replicates per sample. All donors were over the age of 60 with a median age of 68 and included 15 smokers and 15 non-smokers.

As would be expected in a sampling of human plasma, especially plasma from an aged population, a small number of alterations were detected. Across 30,622 short variants, which include variants classified as VUS/benign, five variants of unknown significance had a detection rate significantly exceeding 5% on an individual variant basis: *TSC1* 965T>C, *IRF4* 1ins87, *MSH3* 186_187insGCCGCAGCGCCCGCAGCG, *IGF1R* 568C>T, WHSC1 1582C>A.

All other variants were determined to have an LoB of 0, based on the detection rate not significantly exceeding 5%. Each cancer-related alteration detected in this study was detected in replicates from a single donor, indicating that these are likely true variants present in the sample. On a per variant basis (number of unique variants detected at least once across all replicates divided by the total number of unique variants included in the analysis), the overall detection rate for short variants in this study was 0.82%. On a per variant basis (number of variants detected across all replicates divided by the total number of variants included in the analysis across all replicates), the overall detection rate for short variants in this study was 0.027% (**Table 17**).

Table 17: Detection rate for each reporting category in LoB study

Category	Unique Variant Detection Rate (Unique variants detected) / (total unique variants analyzed)	Total Variant Detection Rate (Total variants detected) / (total variants analyzed[1])
Level 1	0% (0 of 292)	0% (0 of 23,068)
Level 2	0% (0 of 10)	0% (0 of 790)
Level 3	0% (0 of 18)	0% (0 of 1,422)
Level 4	0.82% (47 of 5,760)	0.024% (107 of 455,040)
VUS	0.83% (203 of 24,542)	0.029% (555 of 1,938,818)
All categories	0.82% (250 of 30,622)	0.027% (662 of 2,419,138[1])

[1] total variants analyzed = unique variants * 79 replicates

Across 264 copy number alterations and 894 rearrangements, zero variants were detected. These results demonstrate the high specificity of FoundationOne Liquid CDx.

9.7 Potentially Interfering Substances

To evaluate the robustness of the FoundationOne Liquid CDx results in the presence of potentially interfering exogenous and endogenous substances, a total of 11 potential interferents were evaluated. These potential interferents included six endogenous substances (albumin, conjugated bilirubin, unconjugated bilirubin, cholesterol, hemoglobin and triglycerides) and five exogenous substances (DNA from another source [the microorganism Staphylococcus epidermidis], excess anticoagulant, proteinase K, ethanol and molecular index barcodes).

A total of 340 samples were tested to evaluate the potential interference of albumin, conjugated bilirubin, unconjugated bilirubin, cholesterol, hemoglobin, triglycerides, DNA from another source (the microorganism Staphylococcus epidermidis), excess anticoagulant, proteinase K, ethanol, and molecular index barcodes. An assessment of the cfDNA yield obtained during the DNA isolation, purification, and quantification steps, as well as at library construction QC (LCQC) and hybrid capture QC (HCQC) was performed. The process success rates for each step are listed in **Table 18**.

Table 18 Process success rates with interfering substances

Process	# Failed	# Pass	Total	Success Rate (%)	95% CI LB (%)	95% CI UB (%)
DNA Extraction	0	180	180	100.00	97.97	100.00
LC	1	339	340	99.71	98.37	99.99
HC	3	336	339	99.12	97.44	99.82
Sequencing	0	336	336	100.00	98.91	100.00

For each potential interferent, concordance of alteration calls was calculated relative to a control sample without interferent. The pre-defined variants included 27 short variants, 17 rearrangements, and 3 copy number variants. Of the 11 potential interferents tested across 16 conditions, concordance for all variant calls was 100% for 8 conditions and ≥97% for all conditions (**Table 19**).

Table 19: Concordance per substance for variants ≥1x LoD

Substance	Concordance	95% CI LB (Exact)	95% CI UB (Exact)	N
Triglycerides, 37 mmol/L (or 33 g/L)	100.00%	91.19%	100.00%	40
Hemoglobin, 2.0 g/L	100.00%	90.97%	100.00%	39
Albumin, 60 g/L	97.56%	87.14%	99.94%	41
Bilirubin (conjugated), 0.2 g/L	100.00%	91.59%	100.00%	42
Bilirubin (unconjugated), 0.2 g/L	97.44%	86.52 %	99.94%	39
Cholesterol Level 2, 3.88 mmol (150 mg/dL)	97.56%	87.14%	99.94%	41
Cholesterol Level 1, 6.47mmol (250 mg/dL)	97.37%	86.19%	99.93%	38
Staphylococcus epidermidis, 1 x 106 CFU/mL	100.00%	90.97%	100.00%	39
Anticoagulant, 5X nominal volume	100.00%	91.40%	100.00%	41
Proteinase K, +0.6 mg/mL	98.00%	89.35%	99.95%	50
Proteinase K, +0.3 mg/mL	100.00%	92.29%	100.00%	46
Ethanol, +2.5%	97.96%	89.15%	99.95%	49
Ethanol, +5.0%	97.92%	88.93%	99.95%	48
Molecular Index barcodes, +5%	97.22%	85.47%	99.93%	36
Molecular Index barcodes, +15%	100.00%	92.60%	100.00%	48
Molecular Index barcodes, +30%	100.00%	92.75%	100.00%	49

Taken together, these data indicate that the FoundationOne Liquid CDx assay is robust to potential specimen-related endogenous substances and exogenous contaminants or interferents.

9.8 Hybrid Capture Bait Specificity

Bait specificity was addressed through an assessment of coverage of targeted regions in FoundationOne Liquid CDx using 3,546 validation study samples. Results show that targeted genomic regions have consistently high, uniform coverage. For each genomic region associated with a predefined subset of highly-actionable alterations, between 94% to 100% of samples possessed the expected level of coverage. An in-depth, platform-wide examination of the FoundationOne Liquid CDx baitset through the analysis of HapMap process control samples revealed that, on average, 98.8% and 94.1% of platform-wide baited coding and non-coding regions, respectively, met their expected coverage levels. Samples assessed in this study consistently demonstrated high quality uniform and deep coverage across the entire genomic region targeted by the assay.

9.9 Carryover/Cross-Contamination

The study demonstrated that the risk of cross contamination (intra-plate), and carry-over contamination (inter-plate) of samples during the processing of the FoundationOne Liquid CDx assay is low. A total of 376 wells were examined for intra- and inter-plate contamination by processing and sequencing of contrived samples derived from cell lines at high input concentrations with known genomic backgrounds. Unique variants of each cell line were characterized by independent control sequencing runs. The samples were arrayed in a checkerboard fashion across four 96-well PCR plates to detect cross-contamination events. A cross-contamination rate of 0.53% (2/376) was observed in this study. These data demonstrate a low probability of cross contamination during the FoundationOne Liquid CDx process.

9.10 Precision: Repeatability and Reproducibility

Precision was evaluated for alterations associated with CDx claims, as well as tumor mutation profiling variants. Repeatability including intra-run performance (run on the same plate under the same conditions) and reproducibility including inter-run performance (run on different plates under different conditions) were assessed and compared across three reagent lots, two sequencers, and two processing runs.

Results for a subset of highly-actionable alterations

A set of 39 unique samples were used to evaluate the precision of FoundationOne Liquid CDx for detecting a set of highly-actionable variants, including 8 contrived samples representing various targeted alterations and 31 clinical samples. The samples representing CDx alterations are summarized in **Table 20**. Additional non-CDx variants were evaluated as summarized in **Table 21.**

Table 20: CDx sample set assessed for precision

CDx Biomarker	Targeted Alteration	Disease Ontology of Patient from which Sample was Derived
ALK rearrangements	*ALK-EML4* Rearrangement	Contrived sample
	ALK-EML4 Rearrangement	Lung adenocarcinoma
	ALK-NPM1 Rearrangement	Contrived sample
ATM alterations	*ATM* 5318delA	Contrived sample
	ATM I2012fs*4	Prostate cancer
	ATM splice site 8850+1G>A	Prostate cancer
	ATM-EXPH5 Truncation	Prostate cancer
BRCA1 and *BRCA2* alterations	*BRCA1 E23fs*17*	Ovary cancer
	BRCA1 Q780*	Ovary high grade serous carcinoma
	BRCA1 Rearrangement	Unknown primary malignant neoplasm
	*BRCA1*_2475delC	Contrived sample

CDx Biomarker	Targeted Alteration	Disease Ontology of Patient from which Sample was Derived
	*BRCA1*_2612C>TT	Contrived sample
	*BRCA2*_3599_3600delGT	Contrived sample
	*BRCA2*_4284_4285insT	Contrived sample
	*BRCA2*_5351delA	Contrived sample
	BRCA2 G267*	Ovary serous carcinoma
	BRCA2 Loss (15 of 26)	Prostate acinar adenocarcinoma
	BRCA2 Loss *(26 of 26)*	Prostate acinar adenocarcinoma
	BRCA2 S2988fs*12	Ovary cancer
	BRCA2- EDA Truncation	Prostate cancer
EGFR exon 19 deletions and *EGFR exon 21 L858R* alterations	*EGFR E746_A750del*	Non-small cell lung carcinoma
	EGFR_E746_A750del	Contrived sample
	EGFR L858R	Contrived sample
	EGFR L858R	Non-small cell lung carcinoma
MET exon 14 skipping alterations	*MET* exon 14 splice site 2888-17_2888-3del15	Non-small cell lung carcinoma
	MET exon 14 splice site 3005_3028+3>C	Non-small cell lung carcinoma
PIK3CA alterations	*PIK3CA E542K*	Contrived sample
	PIK3CA E542K, D549N	Contrived sample
	PIK3CA H1047R	Contrived sample
	PIK3CA E542K	Breast carcinoma
	PIK3CA E545K	Breast carcinoma
	PIK3CA H1047R	Breast cancer

Table 13: Non-CDx sample set assessed for precision

Non-CDx Targeted Alteration	Targeted Alteration	Disease Ontology of Patient from which Sample was Derived
BRAF alterations	*BRAF L597R*	Contrived sample
	BRAF V600E	Contrived sample
	BRAF V600E	Skin melanoma
	BRAF V600K	Skin melanoma
EGFR exon 20 T790M substitution	*EGFR* exon 20 T790M substitution	Contrived sample
KRAS alterations	*KRAS* G12D	Contrived sample
	KRAS G13D	Contrived sample
	KRAS G12L	Colon adenocarcinoma
	KRAS Q61R	Colon adenocarcinoma
MET exon 14 alterations	*MET* 3029-1G>T	Contrived sample
	MET 3933delC	Contrived sample
NRAS alterations	*NRAS* exon 2,3,4 substitutions	Contrived sample
PALB2 alterations	*PALB2* 2422G>T	Contrived sample
	PALB2 2724delA	Contrived sample
ERBB2 CNA	*ERBB2* CNA	Contrived sample
	ERBB2 CNA	Breast carcinoma

Target alterations were assessed at two target levels each (near LoD and 2-3x LoD) for the contrived samples, and at one target level (1-1.5x LoD) for clinical cfDNA samples. Each sample was divided into 24 aliquots, with 12 duplicates being processed on the same plate under the same conditions. Across 47 samples (31 clinical specimens at one dilution level and 8 contrived samples across two dilution levels), a total of 57 unique alterations were evaluated. The repeatability and reproducibility of CDx alterations tested at >1x LoD is summarized in **Table** 22.

Table 14 Repeatability and Reproducibility of CDx alterations targeted in precision study at >1x LoD[1]

Variant Type	Alteration	Repeatability [%] {95% exact CI [%])	Reproducibility [%] {95% exact CI [%])	Level Tested[2]
ALK Rearrangement	*ALK_EML4*	100 (73.54, 100)	100 (85.75, 100)	0.64% VAF
	ALK_EML4	100 (71.51, 100)	100 (85.18, 100)	0.89% VAF
	ALK_EML4	100 (73.54, 100)	100 (85.75, 100)	1.39% VAF
	ALK-NPM1	100 (73.54, 100)	100 (85.75, 100)	0.64% VAF
ATM Short variant	*ATM*_5318delA	100 (73.54, 100)	100 (85.75, 100)	0.77% VAF
	*ATM*_5318delA	100 (71.51, 100)	100 (85.18, 100)	1.04% VAF
	*ATM*_6034_6035insCAGA AGTA	100 (71.51, 100)	100 (85.18, 100)	0.86% VAF
	*ATM*_8850+1G>A	100 (73.54, 100)	100 (85.75, 100)	0.56% VAF
ATM Rearrangement	*ATM-EXPH5* Truncation	100 (73.54, 100)	100 (85.75, 100)	1.13% VAF
BRCA1 Short variant	*BRCA1*_2338C>T	100 (73.54, 100)	100 (85.75, 100)	1.11% VAF
	*BRCA1*_2475delC	100 (73.54, 100)	100 (85.75, 100)	0.61% VAF
	*BRCA1*_2475delC	100 (73.54, 100)	100 (85.75, 100)	0.93% VAF
	*BRCA1*_2612C>TT	100 (71.51, 100)	100 (85.18, 100)	0.51% VAF
	*BRCA1*_68_69delAG	100 (73.54, 100)	100 (85.75, 100)	0.66% VAF
	*BRCA1*_P871fs*32	100 (73.54, 100)	100 (85.75, 100)	1.08% VAF
BRCA1 Rearrangement	*BRCA1-BRCA1*	100 (73.54, 100)	100 (85.75, 100)	0.87% VAF
BRCA2 Short Variant	*BRCA2*_3599_3600delGT	100 (73.54, 100)	100 (85.75, 100)	0.58% VAF
	*BRCA2*_3599_3600delGT	100 (73.54, 100)	100 (85.75, 100)	0.92% VAF
	*BRCA2*_4284_4285insT	100 (73.54, 100)	100 (85.75, 100)	0.94% VAF
	*BRCA2*_4284_4285insT	100 (71.51, 100)	100 (85.18, 100)	1.26% VAF
	*BRCA2*_5351delA	100 (73.54, 100)	100 (85.75, 100)	1.22% VAF
	*BRCA2*_5351delA	100 (73.54, 100)	100 (85.75, 100)	1.85% VAF
	*BRCA2*_5351delA	100 (71.51, 100)	100 (85.18, 100)	1.07% VAF
	*BRCA2*_5351delA	100 (73.54, 100)	100 (85.75, 100)	2.24% VAF
	*BRCA2*_5465_5466insA	100 (73.54, 100)	100 (85.75, 100)	0.92% VAF
	*BRCA2*_5465_5466insA	100 (71.51, 100)	100 (85.18, 100)	1.19% VAF
	*BRCA2*_8961_8964delGA GT	100 (73.54, 100)	100 (85.75, 100)	1.07% VAF
	*BRCA2*_c.799G>T	83.33 (51.59, 97.91)	91.67 (73.0, 98.97)	0.5% VAF
	*BRCA2*_c.9097_9098insA	54.55 (23.38, 83.25)	21.74 (7.46, 43.7)	0.71% VAF
	*BRCA2*_c.9097_9098insA	83.33 (51.59, 97.91)	91.67 (73.0, 98.97)	1.03% VAF
BRCA2 Copy Number Loss	*BRCA2*_loss	91.67 (61.52, 99.79)	87.5 (67.64, 97.34)	39.43% TF
BRCA2 Rearrangement	*BRCA2-EDA*	100 (71.51, 100)	100 (85.18, 100)	0.48% VAF
EGFR Short variant	*EGFR*_2369C>T	100 (73.54, 100)	100 (85.75, 100)	0.44% VAF
	*EGFR*_2369C>T	100 (73.54, 100)	100 (85.75, 100)	0.66% VAF
	*EGFR*_2369C>T	100 (71.51, 100)	100 (85.18, 100)	0.36% VAF
	*EGFR*_2369C>T	100 (73.54, 100)	100 (85.75, 100)	0.65% VAF
	*EGFR*_2369C>T	100 (73.54, 100)	100 (85.75, 100)	1.26% VAF
	*EGFR*_2573T>G	100 (73.54, 100)	100 (85.75, 100)	0.46% VAF
	*EGFR*_2573T>G	100 (73.54, 100)	100 (85.75, 100)	0.68% VAF
	*EGFR*_2573T>G	100 (73.54, 100)	100 (85.75, 100)	0.68% VAF
	*EGFR*_2573T>G	100 (71.51, 100)	100 (85.18, 100)	0.95% VAF
	*EGFR*_2573T>G	100 (73.54, 100)	100 (85.75, 100)	0.64% VAF
	*EGFR*_2573T>G	100 (73.54, 100)	100 (85.75, 100)	1.64% VAF
	*EGFR*_E746_A750del	100 (73.54, 100)	100 (85.75, 100)	0.51% VAF

Variant Type	Alteration	Repeatability [%] {95% exact CI [%])	Reproducibility [%] {95% exact CI [%])	Level Tested[2]
	*EGFR*_E746_A750del	100 (73.54, 100)	100 (85.75, 100)	0.74% VAF
	*EGFR*_E746_A750del	100 (73.54, 100)	100 (85.75, 100)	0.93% VAF
	*EGFR*_E746_A750del	100 (71.51, 100)	100 (85.18, 100)	1.2% VAF
	*EGFR*_E746_A750del	100 (71.51, 100)	100 (85.18, 100)	0.51% VAF
	*EGFR*_E746_A750del	100 (73.54, 100)	100 (85.75, 100)	1.01% VAF
	*EGFR*_E746_A750del	100 (71.51, 100)	100 (84.56, 100)	0.34% VAF
MET Short variant	*MET* exon14 splice site 2888-17_2888-3del15	100 (73.5, 100)	100 (85.8, 100)	1.17% VAF
	MET exon 14 splice site 3005_3028+3>C	100 (73.5, 100)	100 (85.8, 100)	1.67% VAF
PIK3CA Short variant	*PIK3CA*_1624G>A	100 (73.54, 100)	100 (85.75, 100)	0.89% VAF
	*PIK3CA*_1633G>A	100 (73.54, 100)	100 (85.75, 100)	0.45% VAF
	*PIK3CA*_1633G>A	100 (73.54, 100)	100 (85.75, 100)	0.66% VAF
	*PIK3CA*_1633G>A	100 (73.54, 100)	100 (85.75, 100)	0.5% VAF
	*PIK3CA*_1634A>C	100 (73.54, 100)	100 (85.75, 100)	0.52% VAF
	*PIK3CA*_1634A>C	100 (71.51, 100)	100 (85.18, 100)	0.70% VAF
	PIK3CA _1637A>G	90.91 (58.72, 99.77)	95.65 (78.05, 9.89)	0.49% VAF
	*PIK3CA*_1637A>G	100 (73.54, 100)	100 (85.75, 100)	0.92% VAF
	*PIK3CA*_1645G>A	100 (73.54, 100)	100 (85.75, 100)	0.48% VAF
	*PIK3CA*_1645G>A	100 (73.54, 100)	100 (85.75, 100)	0.73% VAF
	*PIK3CA*_3140A>G	100 (71.51, 100)	100 (85.18, 100)	0.41% VAF
	*PIK3CA*_3140A>G	100 (73.54, 100)	100 (85.75, 100)	0.76% VAF
	*PIK3CA*_3140A>G	100 (73.54, 100)	100 (85.75, 100)	1.04% VAF

[1]Clinical samples were mostly tested at 2x – 3x LoD rather than 1x – 1.5x LoD
[2]Quantitative reporting of %VAF/%TF has not been approved by FDA.

As observed in the **Table 22** above, three BRCA2 positive samples (c.799G>T, c.9097_9098insA, and a *BRCA2* loss) demonstrated poor performance for both repeatability and reproducibility. For the *BRCA2* specimen harboring the c.799G>T, the average %VAF was determined to be 0.5%, near the LoD of 0.4% for this variant type. The *BRCA2* c.9097_9098insA variant is an insertion of an A in a highly repetitive homopolymer region of eight As, which impacts sensitivity. In the LoD study, a 93% hit rate was observed at the highest level tested, 1.16% VAF, indicating that the levels evaluated in this precision analysis were below the LoD for this variant. The replicates for the clinical sample harboring the BRCA2 loss were processed at below the minimum cfDNA input.

Of 53 targeted alterations, repeatability of 100% was observed for 43 alterations and ≥90% repeatability was observed for 53 alterations. For the targeted variants assessed, the overall repeatability was 96.39% (95% two-sided exact CIs [95.28%, 97.30%]).

Of 55 targeted alterations, reproducibility of 100% was observed for 42 alterations and ≥90% reproducibility was observed for 55 alterations. For the targeted variants assessed, the overall reproducibility was 97.33% (95% 2-sided exact CIs [96.67 %, 97.89%]).

The repeatability and reproducibility of non-CDx alterations tested at ≥1x LoD are summarized in **Table 23**.

Table 15: Repeatability and Reproducibility of non-CDx alterations targeted in precision study at ≥1x LoD

Variant Type	Alteration	Repeatability [%] {95% exact CI [%])	Reproducibility [%] {95% exact CI [%])	Level Tested[1]
BRAF Short variant	*BRAF*_1790T>G	90.91 (58.72, 99.77)	95.65 (78.88, 99.89)	0.42% VAF
	*BRAF*_1790T>G	100 (73.54, 100)	100 (85.75, 100)	0.85% VAF
	*BRAF*_1798_1799GT>AA	91.67 (61.52, 99.79)	95.83 (78.88, 99.89)	0.36% VAF
	*BRAF*_1799T>A	100 (71.51, 100)	100 (85.18, 100)	0.72% VAF

Variant Type	Alteration	Repeatability [%] {95% exact CI [%])	Reproducibility [%] {95% exact CI [%])	Level Tested[1]
	*BRAF*_1799T>A	100 (73.54, 100)	100 (85.75, 100)	1.38% VAF
	*BRAF*_1799T>A	100 (73.54, 100)	100 (85.75, 100)	0.44% VAF
KRAS Short variant	*KRAS*_182A>G	100 (73.54, 100)	100 (85.75, 100)	0.53% VAF
	*KRAS*_34_35GG>CT	100 (73.54, 100)	100 (85.75, 100)	0.49% VAF
	*KRAS*_35G>A	100 (73.54, 100)	100 (85.75, 100)	0.89% VAF
	*KRAS*_35G>A	100 (71.51, 100)	100 (85.18, 100)	1.12% VAF
	*KRAS*_38G>A	100 (73.54, 100)	100 (85.75, 100)	0.55% VAF
	*KRAS*_38G>A	100 (73.54, 100)	100 (85.75, 100)	0.82% VAF
	*KRAS*_38G>A	100 (71.51, 100)	100 (85.18, 100)	0.57% VAF
	*KRAS*_38G>A	100 (73.54, 100)	100 (85.75, 100)	0.92% VAF
MET Short variant	*MET*_3029-1G>T	81.82 (48.22, 97.72)	91.30 (71.96, 98.93)	0.30% VAF
	*MET*_3933delC	100 (73.54, 100)	100 (85.75, 100)	0.69% VAF
	*MET*_3933delC	100 (73.54, 100)	100 (85.75, 100)	0.96% VAF
NRAS Short variant	*NRAS*_34G>T	100 (73.54, 100)	100 (85.75, 100)	0.69% VAF
	*NRAS*_34G>T	100 (73.54, 100)	100 (85.75, 100)	0.96% VAF
	*NRAS*_35G>A	100 (73.54, 100)	100 (85.75, 100)	0.84% VAF
	*NRAS*_c.35G>A	63.64 (30.79, 89.07)	82.61 (61.22, 95.05)	0.48% VAF
PALB2 Short variant	*PALB2*_2422G>T	100 (71.51, 100)	100 (85.18, 100)	0.47% VAF
	*PALB2*_2422G>T	100 (73.54, 100)	100 (85.75, 100)	0.92% VAF
	*PALB2*_2724delA	100 (73.54, 100)	100 (85.75, 100)	0.52% VAF
	*PALB2*_2724delA	100 (73.54, 100)	100 (85.75, 100)	0.74% VAF
ERBB2 CN Amplification	*ERBB2* amplification	100 (73.54, 100)	100 (85.75, 100)	35.78% VAF
	ERBB2 amplification	100 (73.54, 100)	100 (85.75, 100)	39.79% VAF
	ERBB2 amplification	100 (73.54, 100)	100 (85.75, 100)	61.73% VAF

[1] Quantitative reporting of %VAF/%TF has not been approved by FDA.

Precision of Platform Variants

Across 39 unique samples, including 8 contrived samples, and 31 clinical samples, a total of 1,240 variants were evaluated with variant types including substitutions, indels, rearrangements, and copy number alterations. The number of variants in each variant bin are summarized in **Table 24.**

Table 16: Number of each variant type

Variant Category	N
Substitutions	**898**
Substitution in a non-repetitive region or a repetitive region of <=7 base pairs	882
Substitution in a repetitive region of >7 base pairs	16
Indels	**228**
Insertion/Deletion in non-repetitive region or a repetitive region of <=3 base pairs	52
Insertion/Deletion in a repetitive region of 4 to 6 base pairs	118
Insertion/Deletion in a repetitive region of >=7 base pairs	58
Rearrangements	**60**

Variant Category	N
Copy Number Alterations	**54**
Copy Number Amplification	49
Copy Number Loss	5
Total	**1240**

The overall repeatability for all variants were 99.47% with 95% 2-sided exact CIs (99.45%, 99.48%). The repeatability result for each variant type are summarized in **Table 25.**

Table 17: Assessment of repeatability of tumor mutation profiling variants per type

Variant Type	# of Concordant Pairs	# of Total Pairs	Repeatability (%)	95% two-sided exact CIs (%)
Substitution	498765	501084	99.54	(99.52, 99.56)
Indels	126475	127224	99.41	(99.37, 99.45)
Rearrangements	33105	33480	98.88	(98.76, 98.99)
Copy Number Alterations	29880	30132	99.16	(99.05, 99.26)

The overall reproducibility results were 99.59% with the 95% 2-sided exact CIs (99.58%, 99.60%). The reproducibility result for each variant type are summarized in **Table 26.**

Table 18: Assessment of reproducibility of tumor mutation profiling variants per type

Variant Type	# of Concordant Replicates	# of Total Replicates	Reproducibility (%)	95% two-sided exact CIs (%)
Substitution	1002981	1006658	99.63	(99.62, 99.65)
Indels	254509	255588	99.58	(99.55, 99.60)
Rearrangements	66723	67260	99.20	(99.13, 99.27)
Copy Number Alterations	60115	60534	99.31	(99.24, 99.7)

Confirmation of LoD and Precision in Clinical Specimens

Twenty-nine clinical cfDNA samples targeting variants at near the estimated LoD were evaluated to confirm LoD and precision in clinical specimens. The mean level tested in most cases were higher than the estimated LoD as shown in **Table 27** and **Table 28**. Twenty-six had 100% reproducibility, one had 95.8% reproducibility, and two samples had reproducibility below 90%. Of these two samples, one contained a *BRCA2* loss that had 87.5% reproducibility. This sample was processed with a cfDNA input mass below the recommended minimum and was also below LoD. The other sample harbored a *BRCA2* substitution (c.799G>T) with 91.67% reproducibility. The average VAF of this variant was 0.5% across replicates, which is near the LoD for this variant type (median LoD of 0.4% VAF). A summary of the Confirmation of LoD and Precision results for CDx variants are provided in **Table 27.** A summary of the Confirmation of LoD and Precision results for non-CDx variants are provided in **Table 28**.

Table 19: CDx variant confirmation of LoD and precision in clinical specimens

Target Alteration	LoD	Mean Level Tested[2]	Reproducibility (%)	95% Two-sided exact CIs (%)
*ATM I2012fs*4*	0.51% VAF	0.86% VAF	100	(85.18, 100)
ATM splice site 8850+1G>A	0.51% VAF	0.56% VAF	100	(85.75, 100)
*BRCA1 E23fs*17*	0.38% VAF	0.66% VAF	100	(85.75, 100)
BRCA1 Q780*	0.34% VAF	1.11%VAF	100	(85.75, 100)

Target Alteration	LoD	Mean Level Tested[2]	Reproducibility (%)	95% Two-sided exact CIs (%)
BRCA1 Rearrangement	0.87% VAF[1]	0.87% VAF	100	(85.75, 100)
BRCA2 799G>T	0.40% VAF	0.50% VAF	91.67	(73.0, 98.97)
BRCA2 Loss	48.1% TF	39.43%TF	87.50	(67.64, 97.34)
BRCA2 S2988fs*12	0.36% VAF	1.07% VAF	100	(85.75, 100)
BRCA2- EDA Truncation	0.48% VAF[1]	0.48% VAF	100	(85.18, 100)
EGFR E746_A750del	0.27% VAF	0.34% VAF	100	(84.56, 100)
EGFR L858R	0.34% VAF	1.64% VAF	100	(85.75, 100)
EGFR L858R	0.34% VAF	0.64% VAF	100	(85.75, 100)
PIK3CA E542K	0.34% VAF	0.89% VAF	100	(85.75, 100)
PIK3CA E545K	0.34% VAF	0.5% VAF	100	(85.75, 100)
PIK3CA H1047R	0.34% VAF	1.04% VAF	100	(85.75, 100)
ALK-EML4 Rearrangement	0.24% MAF	1.39 %MAF	100	(85.75, 100)

[1] LoD determined in this confirmation of LoD and precision study
[2] Quantitative reporting of %VAF/%TF has not been approved by FDA.

Table 20: Non-CDx variant confirmation of LoD and precision in clinical specimens

Target Alteration	LoD	Mean Level Tested[1]	Reproducibility (%)	95% Two-sided exact CIs (%)
BRAF V600E	0.33% VAF	0.44% VAF	100	(85.75, 100)
BRAF V600K	0.33% VAF	0.36% VAF	95.8	(78.88, 99.89)
EGFR T790M	0.34% VAF	1.26% VAF	100	(85.75, 100)
KRAS G12L	0.33% VAF	0.49% VAF	100	(85.75, 100)
KRAS Q61R	0.33% VAF	0.53% VAF	100	(85.75, 100)
ERBB2 CNA	19.8% TF	61.73% TF	100	(85.75, 100)

[1] Quantitative reporting of %VAF/%TF has not been approved by FDA.

A second study with 10 samples targeting variants at 1-1.5x LoD was performed to confirm LoD and precision in clinical specimens. Similar to above, each sample was divided into 24 aliquots, with 12 duplicates being processed on the same plate under the same conditions. Each sample was tested across 24 replicates. Six samples were included in the primary analysis for samples with ≥30 ng DNA input. Three had 100% reproducibility, one had 95.7% reproducibility, one had 91.7% reproducibility, and one had 91.3% reproducibility. The other four samples had a majority of sample replicates with DNA input <30 ng. A summary of the Confirmation of LoD and Precision results for CDx alterations are provided in **Table 29**.

Table 21: CDx variant confirmation of LoD and precision in clinical specimens

Target Alteration	LoD	Mean Level Tested[1]	Reproducibility (95% CI)	95% CIs
BRCA1 1395T>A	0.34% VAF	0.51% VAF	100%	[86.2%, 100%]
BRCA2 5351_5352insA	0.36% VAF	0.34% VAF	87.5%	[69.0%, 95.7%]
EGFR 2235_2249del	0.27% VAF	0.45% VAF	95.7%	[79.0%, 99.2%]
PIK3CA 1637A>G	0.34% VAF	0.44% VAF	91.7%	[74.2%, 97.7%]

[1] Quantitative reporting of %VAF/%TF has not been approved by FDA.

As summarized in **Table 29** above, all CDx variants with ≥30 ng DNA input had reproducibility ≥95% with the exception of one variant (BRCA2 5351_5352insA) which was tested at a variant allele fraction below the LoD.

Additionally, one of the 10 samples evaluated in this study targeted a non-CDx *BRCA2* substitution. Reproducibility of 100% was observed as summarized in **Table 30**.

Table 30: Non-CDx variant confirmation of LoD and precision in a clinical specimen

Target Alteration	LoD	Mean Level Tested*	Reproducibility (95% CI)	95% CIs
BRCA2 8524C>T	0.37% VAF	0.57% VAF	100%	[85.7%, 100%]
NRAS 34G>T	0.42% VAF	0.55% VAF	91.3%	[73.2%, 97.6%]

*Quantitative reporting of %VAF/%TF has not been approved by FDA.

A third study with 5 samples targeting variants at 1-1.5x and 2-3x LoD was performed to confirm LoD and precision in NSCLC clinical specimens with *MET* exon 14 skipping alterations. Similar to above, each sample was divided into 24 aliquots, with 12 duplicates being processed on the same plate under the same conditions. Each sample was tested across 24 replicates. **Table 31** provides the repeatability and reproducibility data for the 5 samples evaluated for precision. Two samples evaluated at 2-3x LoD had 100% reproducibility while three samples evaluated at 1-1.5x LoD had 95.8%, 95.8% and 95.7% reproducibility respectively. Two samples in **Table 31** were processed in the original precision study at 2-3x LoD and are reflected in **Table 20**. A summary of the Confirmation of LoD results for *MET* exon 14 skipping alterations in clinical specimens is provided in **Table 32**.

Table 31: Repeatability and Reproducibility of *MET* exon 14 skipping CDx alterations targeted in precision study at ≥1x LoD

Variant Type	Alteration	Targeted VAF[1,2]	Repeatability [%] (95% score CI [%])	Reproducibility [%] (95% score CI [%])	Level Calculated
MET Short Variants	*MET* exon14 splice site 2888-35_2889>A	0.50%	91.7 (64.6, 98.5)	95.8 (79.8, 99.3)	0.28% VAF
	MET exon14 splice site 3028+1G>T	0.50%	91.7 (64.6, 98.5)	95.8 (79.8, 99.3)	0.45% VAF
	MET exon14 splice site 3028+1G>T	1.00%	100 (74.1, 100)	100 (85.7, 100)	0.85% VAF
	MET exon14 splice site 3028+2T>C	0.50%	90.9 (62.3, 98.4)	95.7 (79.0, 99.2)	0.35% VAF
	MET exon14 splice site 3028+2T>C	1.00%	100 (75.8, 100)	100 (86.2, 100)	0.76% VAF
	MET exon14 splice site 2888-17_2888-3del15	1.00%	100 (73.5, 100)	100 (85,7, 100)	1.17% VAF
	MET exon14 splice site 3005_3028+3>C	1.00%	100 (73.5, 100)	100 (85.7, 100)	1.67% VAF

[1]Targeted 0.50% VAF represents 1 - 1.5x LoD and 1.00% represents 2 - 3x LoD. LoD here refers to LoD determined using the Q3 LoD from all the substitutions in a non-repetitive region or a repetitive region of <=7 base pairs in the Narrow-High region (see Table 15) in the F1 Liquid CDx platform which is extrapolated to represent *MET* exon 14 skipping base substitutions.
[2] Quantitative reporting of %VAF/%TF has not been approved by FDA.

Table 32: CDx variant confirmation of LoD and precision in NSCLC clinical specimens with *MET* exon 14 skipping alterations

Target Alteration	LoD[1]	Targeted % VAF[1,2]	Mean Level Calculated	Reproducibility	95% score CIs (%)
MET exon14 splice site 2888-35_2889>A	0.41% VAF	0.50% VAF	0.28% VAF	95.8%	[79.8%, 99.3%]

Target Alteration	LoD[1]	Targeted % VAF[1,2]	Mean Level Calculated	Reproducibility	95% score CIs (%)
MET exon14 splice site 3028+1G>T	0.49% VAF	0.50% VAF	0.45% VAF	95.8%	[79.8%, 99.3%]
MET exon14 splice site 3028+2T>C	0.49% VAF	0.50% VAF	0.35% VAF	95.7%	[79.0%, 99.2%]

[1]Targeted 0.50% VAF represents 1 - 1.5x LoD. LoD here refers to LoD determined using the Q3 LoD from all the substitutions in a non-repetitive region or a repetitive region of <=7 base pairs in the Narrow-High region (see Table 15) in the F1 Liquid CDx platform which is extrapolated to represent *MET* exon 14 skipping base substitutions.

[2] Quantitative reporting of %VAF/%TF has not been approved by FDA.

10 Reagent Lot Interchangeability

The interchangeability of critical reagent lots for library construction (LC), hybrid capture (HC) and sequencing within the FoundationOne Liquid CDx assay was evaluated by testing eight (8) contrived samples from either enzymatically fragmented cell line genomic DNA containing alterations of interest or enzymatically fragmented plasmid DNA. Each of the contrived samples was tested in triplicate using two different lots each of LC, HC, and sequencing reagents. Eight reagent pairings were assessed. A total of eight analyses for each specimen were completed. 192 tests in total were included in this study. Four Master Pool Libraries (MPLs) were evaluated on each of two flowcells on a NovaSeq 6000 sequencer, using two different Sequencing reagent lots. Of the 49 alterations assessed in the sample set, 43 had a percent agreement greater than 90% (39 alterations had percentage agreement equal to 100%, one had percent agreement equal to 95.83%, one had percent agreement equal to 95.65%, and two had percent agreement equal to 91.67%), exceeding the pre-specified acceptance criteria. For the remaining six alterations the observed detection rates for these variants were similar to the predicted detection rate based on the LoD analysis. These results demonstrate the interchangeability of critical reagent lots in the FoundationOne Liquid CDx assay.

10.1 Variant Curator Precision

This study was performed to evaluate the precision of genomic variant call curation, following analysis by the FoundationOne Liquid CDx analysis pipeline. This was established by analyzing targeted alterations, including CDx alterations, and platform-wide alterations within samples used in the FoundationOne Liquid CDx Precision and LoD and Precision Confirmation Study. The study design reflected the intermediate precision design and evaluated curator precision in reporting of targeted and platform alterations. A total of 19 samples were selected for this study. Three curators were chosen randomly amongst all qualified curators to curate variant calls in a set of randomly chosen replicates from each of the 19 samples. The variant calls were generated from each sample per curator. The overall average percent agreement for targeted alterations was 93.3% (95% CI; 83.80%, 98.15%), and for platform genomic alterations was 99.14% (95% CI; 98.47%, 99.57%).

10.2 Stability

10.2.1 Reagent Stability

The reagent stability of FoundationOne Liquid CDx was assessed by analyzing data from each of eight samples in triplicate, per each of three different lots of LC, HC, and sequencing reagents. A total of nine analyses for each specimen were completed for each of six time points assessed. A total of 72 tests were assessed per time period; a total of 432 samples and six time points (one baseline timepoint and 5 subsequent experimental timepoints) were included in this study overall. Each of the three sample Master Library Pools (MPLs), representing three LC and HC reagent lots was evaluated per time point on a NovaSeq 6000 sequencer, using three different sequencing reagent lots. The analysis of baseline timepoint zero (T0) identified the baseline variant calls for each sample.

All five experimental time points have been processed and analyzed for Lot #1, Lot #2, and Lot #3. Concordance was assessed among 127,642 data points for tumor profiling variants across the five experimental timepoints. The three reagent lots achieved ≥90% concordance with the baseline variant calls for all the experimental timepoints (including the last two timepoints T4 and T5 at 12 and 13 months respectively) except for a middle timepoint T3 (9 months) which is present in **Table 33**. The reason for the failure of T3 (9 months) was due a technical error which

resulted in lower than planned DNA being transferred for LC and therefore this was not a reagent failure. Reagent stability can be claimed as 12 months after the baseline testing date.

Table 33. Concordance for Tumor Profiling Variants at Replicate Level by Reagent Lot and by Timepoint

Reagent Lot	Timepoint[1]	# Concordant	# Total	Concordance (%)	95% 2-sided score CI (%)
LOT#1	3 months	1921	1966	97.71%	[96.95%, 98.28%]
	6 months	2082	2151	96.79%	[95.96%, 97.46%]
	9 months	1916	2151	89.07%	[87.69%, 90.32%]
	12 months	1609	1656	97.16%	[96.25%, 97.86%]
	13 months	1918	1973	97.21%	[96.39%, 97.85%]
LOT#2	3 months	2083	2148	96.97%	[96.16%, 97.62%]
	6 months	2091	2160	96.81%	[95.98%, 97.47%]
	9 months	1851	2160	85.69%	[84.15%, 87.11%]
	12 months	2087	2160	96.62%	[95.77%, 97.3%]
	13 months	2089	2160	96.71%	[95.87%, 97.39%]
LOT#3	3 months	2086	2139	97.52%	[96.77%, 98.10%]
	6 months	2098	2154	97.4%	[96.64%, 97.99%]
	9 months	1855	2154	86.12%	[84.59%, 87.51%]
	12 months	2097	2154	97.35%	[96.59%, 97.95%]
	13 months	1924	1977	97.32%	[96.51%, 97.94%]

10.2.2 Whole Blood Specimen Stability

Whole blood stability and the impact of tube inversion was evaluated in freshly collected whole blood samples from the following five cancer types: non-small cell lung cancer (NSCLC), colorectal cancer (CRC), prostate, breast, and ovarian cancer. The recommended storage temperature is 18°C - 25°C. In this study, stress conditions were simulated through extended storage at elevated (35°C ± 2°C) and reduced (4° ± 2°C) temperatures.

In this interim analysis, 22 samples (11 sample pairs) were tested, including baseline (within 24 hours of collection) and experimental time points (after 10, 14, or 15 days of storage).

Overall, 100% of samples yielded a cfDNA input ≥30ng. The success rate for DNAx yield, and LC yield were 100% and the success rate of the HC yield was 96.3%. The variant analysis was conducted for variants at ≥2x LoD. For the aggregate 11 pairs of samples processed and reported, 100% agreement was observed between the baseline and experimental timepoint for short variants and rearrangements for each experimental time point. The percent agreement per sample also resulted in 100% agreement between the baseline and experimental timepoint for short variants and rearrangements. The data is summarized in **Table 34.**

Table 22: Aggregate percent agreement per temperature and experimental timepoint

Temperature	Experimental Timepoint	N	Short Variants [95% two-sided CI]	Rearrangements
4°C	7 Days	4	100.00 [89.72, 100.00]	100.00 [39.76, 100.00]
	14 Days	3	100.00 [91.40, 100.00]	N/A
	15 Days	3	100.00 [83.89, 100.00]	N/A
35°C	14 Days	1	N/A	N/A

The impact of potential interferents originating from the FoundationOne Liquid cfDNA blood collection tube (BCT) stopper on the performance of the FoundationOne Liquid CDx assay was assessed by evaluating stability of

whole blood in tubes stored in an upright or inverted position at 4°C± 2°C , 25°C± 2°C, and 35°C± 2°C for various durations (10, 14, and 15 days).

First, the success rate of the FoundationOne Liquid CDx assay for processing samples was assessed at the DNA extraction (DNAx), Library Construction (LC), Hybrid Capture (HC) and Sequencing step, based on product in-process quality control (QC) criteria. Samples stratified by the upright and the inverted condition exhibited comparable success rates above 94% at DNAx, LC, HC and Seq (**Table 35**). Thus, the stopper of the FoundationOne Liquid cfDNA BCT does not impact FoundationOne Liquid CDx test performance when stored between 4 and 35°C for up to 15 days.

Table 23: Process success rate by tube position

Process	Tube Position	# Passing Samples	# Total Samples	Success Rate (%)	95% 2-sided CIs (%)
DNA Extraction	Upright	139	147	94.6%	[89.6%, 97.2%]
	Inverted	147	150	98%	[94.3%, 99.3%]
LC	Upright	135	136	99.3%	[96%, 99.9%]
	Inverted	146	146	100%	[97.4%, 100%]
HC	Upright	134	135	99.3%	[95.9%, 99.9%]
	Inverted	143	146	97.9%	[94.1%, 99.3%]
Sequencing	Upright	134	134	100%	[97.2%, 100%]
	Inverted	143	143	100%	[97.4%, 100%]

Stability was also evaluated by comparing concordance between baseline and experimental samples. Positive percent agreement (PPA) and negative percent agreement (NPA) for alteration calls at ≥ 2x LoD were computed along with the corresponding two-sided 95% score confidence interval (CI) across all replicates by variant category using the baseline detection as reference. Note that NPA is under-estimated as variants not detected at any of the treatment conditions were not used in the analysis set and hence counted against the NPA calculation.

Concordance between baseline and experimental results from all samples in the upright and inverted position combined demonstrated > 99% PPA and NPA for the detection of short variants and rearrangements. Copy number alterations were only detected in samples treated in the inverted tube position and therefore, not included in this analysis. Furthermore, stratification by the treatment condition (2 tube positions × 3 temperatures × 3 durations) revealed >99.0% PPA and NPA for short variants and rearrangements across the combinations of tube positions, temperatures and durations tested. The data also demonstrate that the detection of copy number alterations is not impacted by the storage of blood in the inverted position at 35°C for up to 14 days. The concordance results by variant type for each of the experimental conditions are provided in **Table 36**.

Table 24: Concordance of detected alterations between baseline sample and experimental conditions for inverted tube stability study

Variant Type	Temp.	Tube Position	Exp. Time Point	N Variants Detected at Baseline Time Point	N Variants Detected at Exp. Time Point	N Variants Agree	PPA	PPA [95% CI]	N Variants Not Detected at Baseline Time Point	N Variants Not Detected at Exp. Time Point	NPA	NPA [95% CI]
Short variants	04°C	Inverted	Day 10	50	50	49	98%	[89.5%, 99.6%]	612	612	100%	[100%, 100%]
Short variants	04°C	Upright	Day 10	50	51	50	100%	[92.9%, 100%]	613	612	100%	[100%, 100%]
Short variants	04°C	Inverted	Day 14	59	58	58	98.3%	[90.9%, 99.7%]	610	611	100%	[100%, 100%]
Short variants	04°C	Upright	Day 14	44	44	44	100%	[92.0%, 100%]	611	611	100%	[100%, 100%]

Variant Type	Temp.	Tube Position	Exp. Time Point	N Variants Detected at Baseline Time Point	N Variants Detected at Exp. Time Point	N Variants Agree	PPA	PPA [95% CI]	N Variants Not Detected at Baseline Time Point	N Variants Not Detected at Exp. Time Point	NPA	NPA [95% CI]
Short variants	04°C	Inverted	Day 15	37	37	37	100%	[90.6%, 100%]	611	611	100%	[100%, 100%]
Short variants	04°C	Upright	Day 15	52	52	52	100%	[93%, 100%]	611	611	100%	[100%, 100%]
Short variants	25°C	Inverted	Day 10	78	77	76	97.1%	[91.1%, 99.2%]	627	628	100%	[100%, 100%]
Short variants	25°C	Upright	Day 10	44	44	44	100%	[92.0%, 100%]	613	613	100%	[100%, 100%]
Short variants	25°C	Inverted	Day 14	46	48	46	100%	[92.3%, 100%]	611	609	100%	[100%, 100%]
Short variants	25°C	Upright	Day 14	42	41	41	97.6%	[87.7%, 99.6%]	610	611	100%	[100%, 100%]
Short variants	25°C	Inverted	Day 15	44	44	44	100%	[92.0%, 100%]	613	613	100%	[100%, 100%]
Short variants	25°C	Upright	Day 15	49	48	48	97.8%	[89.3%, 99.6%]	616	617	100%	[100%, 100%]
Short variants	35°C	Inverted	Day 10	15	15	15	100%	[79.6%, 100%]	609	609	100%	[100%, 100%]
Short variants	35°C	Upright	Day 10	35	35	35	100%	[90.1%, 100%]	609	609	100%	[100%, 100%]
Short variants	35°C	Inverted	Day 14	55	55	55	100%	[93.4%, 100%]	611	611	100%	[100%, 100%]
Short variants	35°C	Upright	Day 14	48	47	46	95.7%	[86.0%, 98.8%]	609	610	100%	[100%, 100%]
Short variants	35°C	Inverted	Day 15	39	39	38	97.4%	[86.8%, 99.5%]	610	610	100%	[100%, 100%]
Short variants	35°C	Upright	Day 15	28	29	28	100%	[87.9%, 100%]	613	612	100%	[100%, 100%]

These results demonstrate that blood is stable in the FoundationOne Liquid CDx cfDNA BCT when stored between 4°C and 35°C for up to 15 days, in an upright or inverted position. Additional data will be generated to further evaluate whole blood stability and potential interference of the blood collection tube cap.

10.3 DNA Extraction

DNA extraction evaluated 72 samples across five cancer types: lung cancer (including NSCLC), colorectal cancer (CRC), prostate cancer, breast cancer, and skin cancer (melanoma, sarcoma), using three reagent lots and two KingFisher Magnetic Particle processors.

Reproducibility of the FoundationOne Liquid CDx DNA extraction process across KingFisher instruments and extraction reagent lots were analyzed utilizing a factorial design (3 reagent lots × 2 KingFisher instruments × 2 replicates). The success rate of the DNAx yield for three reagent lots range from 95.8% to 100.0% and two King Fisher instruments range from 97.2% to 100.0%.

Variant calls included in the concordance analysis were identified based on the majority call across all 12 replicates for a given disease ontology. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were computed across the replicates for each somatic alteration for each sample, and aggregated by

variant type (deletion, insertion, rearrangement, and substitution) for variants at ≥1x LoD. The percent agreement results by disease ontologies are: 90.3% - 99.8 % for PPA, and 99.1% - 100.0% for NPA (**Table 37**) The percent agreement results across all variant types (deletion, insertion, rearrangement and substitution) evaluated at ≥1x LoD are: 90.6% - 96.8% for PPA and 98.9% - 100.0% for NPA (**Table 38**).

Table 25: Concordance summary by disease ontology at 1x LoD for DNA extraction study

Disease Ontology	Positive Detected/ Positive Total	PPA [95% two-sided CI]	Negative Detected/ Negative Total[1]	NPA [95% two-sided CI]	Overall Detected/ Total*	OPA [95% two-sided CI]
Breast Cancer	347/348	99.7% [98.4%,100.0%]	3144/3144	100.0% [99.9%,100.0%]	3491/3492	100.0% [99.8%,100.0%]
Colorectal Cancer (CRC)	1122/1188	94.4% [93.0%,95.7%]	2284/2304	99.1% [98.7%,99.5%]	3406/3492	97.5% [97.0%,98.0%]
Lung Cancer	431/432	99.8% [98.7%,100.0%]	3053/3060	99.8% [99.5%,99.9%]	3484/3492	99.8% [99.5%,99.9%]
Non-Small Cell Lung Cancer (NSCLC)	600/612	98.0% [96.6%,99.0%]	2878/2880	99.9% [99.7%,100.0%]	3478/3492	99.6% [99.3%,99.8%]
Prostate Cancer	486/492	98.8% [97.4%,99.6%]	2987/3000	99.6% [99.3%,99.8%]	3473/3492	99.5% [99.2%,99.7%]
Skin Cancer	455/504	90.3% [87.4%,92.7%]	2987/2988	100.0% [99.8%,100.0%]	3442/3492	98.6% [98.1%,98.9%]

[1]Variants detected include variants classified as VUS and benign

Table 26: Concordance summary by variant type at 1x LoD for DNA extraction study

Variant Type	Positive Detected/ Positive Total	PPA [95% two-sided CI]	Negative Detected/ Negative Total[1]	NPA [95% two-sided CI]	Overall Detected/ Total*	OPA [95% two-sided CI]
Deletions	386/ 408	94.6% [91.9%, 96.6%]	2036/ 2040	99.8% [99.5% 99.9%]	2422/ 2448	98.9% [98.4% 99.3%]
Insertions	163/ 180	90.6% [85.3%, 94.4%]	819/ 828	98.9% [97.9% 99.5%]	982/ 1008	97.4% [96.2% 98.3%]
Rearrangements	23/ 24	95.8% [78.9%, 99.9%]	120/ 120	100.0% [97.0% 100.0%]	143/ 144	99.3% [96.2% 100.0%]
Substitutions	2869/ 2964	96.8% [96.1%, 97.4%]	14358/ 14388	99.8% [99.7% 99.9%]	17227/ 17352	99.3% [99.1% 99.4%]

[1]Variants detected include variants classified as VUS and benign

These results demonstrate robustness of the FoundationOne Liquid CDx DNA extraction process across KingFisher instruments, extraction reagent lots, and cancer types.

10.4 Guard Banding/Robustness

The purpose of this validation study was to evaluate the impact on FoundationOne Liquid CDx test performance due to potential process variation with regard to uncertainty in the measurement of DNA concentration. This guard banding evaluation assessed the DNA input into each of the main process steps of the FoundationOne Liquid CDx assay (LC, HC, and sequencing).

Guard bands were evaluated relative to calculated process variability for LC, HC, and sequencing. The assessment of multiple DNA input levels into LC demonstrated robust performance and tolerance of various DNA input levels. The observed results of HC guard banding showed that the HC process is robust within the predefined specifications 1000ng to 2000ng of DNA input into HC. For sequencing, the observed distribution of coverage indicated robust performance within the predefined specifications of 1.0nM of DNA input concentration into sequencing (as summarized in **Table 39**).

Table 27: Summary of process pass and failure rate at each guard banding DNA input level

Process	Input Level		# of Pass	Pass Rate (%)
LC	-33%	20ng	20/20	100
	-20%	24ng	20/20	100
	Recommended lower limit	30ng	20/20	100
	Low input	45ng	20/20	100
	Mid-point	55ng	20/20	100
	Upper limit	80ng	20/20	100
	+20%	96ng	19/20*	95
	+33%	106ng	20/20	100
HC	-50%	500ng	18/20	90
	-20%	800ng	20/20	100
	Lower limit	1000ng	20/20	100
	Upper limit	2000ng	20/20	100
	+20%	2400ng	20/20	100
	+50%	3000ng	18/20	90
Sequencing	-50%	0.5nM	20/20	100
	-20%	0.8nM	20/20	100
	Normal input	1.0nM	20/20	100
	+20%	1.2nM	20/20	100
	+50%	1.5nM	20/20	100

* This one failure was due to failure of HC PICO DNA yield rather than LC PICO DNA yield.

10.5 Pan-Tumor Performance

A large-scale retrospective analysis was performed to demonstrate consistent test performance of FoundationOne Liquid CDx across samples derived from patients with different tumor types. This was evaluated by comparing in-process QC metrics across tumor types using historical data from samples processed in Foundation Medicine's clinical laboratory using two prior versions of the FoundationOne Liquid CDx assay. The FoundationOne Liquid CDx assay was developed based on two versions of the FoundationOne Liquid LDT assay, each of which targeted a subset of the genomic regions targeted by FoundationOne Liquid CDx. FoundationACT (FACT) targeted 62 genes and FoundationOne Liquid targeted 70 genes. The workflow is substantially similar between the assays. In order to support the use of historical data in this study, the regions commonly baited by the two previous assay versions and by FoundationOne Liquid CDx were evaluated for comparability of test performance (Section 2.15).

The sample set for this analysis included 19,868 distinct samples from 25 tumor type categories that had previously been tested using the Foundation Medicine FoundationOne Liquid and FoundationACT assays, previous versions of FoundationOne Liquid CDx. **Table 40** below includes a summary of the tissue types included in the study. Overall, 98.1% of samples yielded ≥25ng DNA, which corresponds to a DNA input mass of 20ng for library construction (LC). A total of 89.1% of samples yielded ≥36ng of DNA which corresponds to a DNA input mass of 30ng for LC. The proportion of samples with an LC yield greater than the minimum mass of 500ng and lower than the maximum mass of 27000ng was 99.9%, with one sided 95% confidence interval of [99.8%, 99.9%]. The proportion of samples with an HC yield greater than the minimum mass of 20ng and lower than the maximum mass of 2250ng was 100%, with one sided 95% confidence interval of [99.99%, 100%]. The proportion of samples which met coverage requirements was 96.1%, with one sided 95% confidence interval of [95.9%, 96.3%]. The proportion of samples which met post-sequencing requirements was 95.6%, with one sided 95% confidence interval of [95.3%, 95.8%]. The proportion of samples that generated a passing or qualified (overall pass as results are reported) result after sequencing was 91.7%, with one sided 95% confidence interval of [91.4%, 92.1%].

Table 40. F1L/FACT samples per tumor type and pass rates

Tumor Type	Sample Size	DNA Extraction Pass Rate (≥25 ng[2])	DNA Extraction Pass Rate (≥36 ng[1])	LC Yield Pass Rate	HC Yield Pass Rate	Median Coverage Pass Rate	Post-sequencing Pass Rate	Overall Pass Rate (≥36 ng[1])	Overall Pass Rate (≥25 ng[2])
Rare Tumors	1164	97.0%	86.4%	99.9%	100.0%	93.8%	94.3%	93.4%	88.4%
Biliary Cancer	171	99.4%	95.3%	100.0%	100.0%	98.8%	97%	97.5%	95.9%
Bladder Cancer	166	97.6%	85.5%	100.0%	100.0%	93.2%	98.7%	95.8%	92%
Breast Cancer	2775	97.6%	87.7%	99.9%	100.0%	96.4%	95.5%	95.8%	91.9%
Cholangiocarcinoma	377	98.9%	96.0%	99.7%	100.0%	98.7%	97.3%	97%	95.7%
Colorectal Cancer (CRC)	1640	98.5%	92.4%	99.9%	100.0%	97.5%	96.9%	96.1%	94.3%
Endocrine-Neuro Cancer	75	100.0%	85.3%	100.0%	100.0%	100.0%	93.3%	96.9%	93.3%
Endometrial Cancer	231	98.3%	88.3%	100.0%	100.0%	96.5%	95.9%	95.1%	92.5%
Esophagus Cancer	291	99.7%	92.4%	100.0%	100.0%	97.6%	96.5%	96.3%	94.1%
Glioma Cancer	59	94.9%	72.9%	100.0%	100.0%	100.0%	76.8%	86%	76.8%
Head and Neck Cancer	154	96.1%	81.8%	100.0%	100.0%	89.2%	96.2%	95.2%	85.8%
Kidney Cancer	203	99.0%	87.7%	100.0%	100.0%	95.0%	95.3%	94.9%	90.5%
Liver Cancer	109	98.2%	95.4%	100.0%	100.0%	100.0%	95.3%	95.2%	95.3%
Lung Non-Small Cell Lung Carcinoma (NSCLC)	5919	98.2%	88.8%	99.8%	100.0%	95.5%	95.6%	94.7%	91.1%
Melanoma	257	96.5%	79.8%	100.0%	100.0%	92.7%	93.5%	93.7%	86.7%
Ovary Cancer	496	97.8%	88.5%	100.0%	100.0%	95.9%	94.6%	94.5%	90.7%
Pancreas Cancer	1359	98.8%	94.0%	99.9%	100.0%	97.8%	95.8%	95%	93.6%
Peripheral Nervous System (PNS)	44	100.0%	90.9%	100.0%	100.0%	100.0%	93.2%	95%	93.2%
Prostate Cancer	1778	97.3%	87.7%	99.9%	100.0%	96.9%	95.1%	95.8%	92.1%
Small Cell Cancer	135	98.5%	93.3%	100.0%	100.0%	99.2%	99.2%	98.4%	98.5%
Soft Tissue Sarcoma	130	97.7%	83.1%	100.0%	100.0%	95.3%	91.7%	94.4%	87.4%
Stomach Cancer	267	98.9%	89.1%	100.0%	100.0%	98.1%	93.8%	95.8%	92%

Tumor Type	Sample Size	DNA Extraction Pass Rate (≥25 ng[2])	DNA Extraction Pass Rate (≥36 ng[1])	LC Yield Pass Rate	HC Yield Pass Rate	Median Coverage Pass Rate	Post-sequencing Pass Rate	Overall Pass Rate (≥36 ng[1])	Overall Pass Rate (≥25 ng[2])
Thyroid Cancer	50	98.0%	86.0%	100.0%	100.0%	100.0%	81.6%	90.7%	81.6%
Unspecified	856	98.5%	89.1%	100.0%	100.0%	95.5%	96.6%	96.3%	92.3%
Unknown Primary Carcinoma (CUP)	1162	98.1%	89.7%	100.0%	100.0%	95.2%	95.9%	94.8%	91.3%

[1] 36 ng of extracted cfDNA allows for sufficient cfDNA to process 30 ng of cfDNA
[2] 25 ng of extracted cfDNA allows for sufficient cfDNA to process 20 ng of cfDNA

Table 41 summarizes the overall sample pass rate across tumor types as well as performance metrics from key QC points in the process. These results demonstrate comparable test performance across tumor types.

Table 28: Summary of F1L/FACT sample data

QC Metric	QC Pass Rate Across Tumor Types[1]	Tumor Types with ≥ 90% QC Pass Rate
Overall report Pass/Qualified rate	76.8%~98.5%	24/25 (96%)[2]
Library Construction	99.7%~100%	25/25 (100%)[1]
Hybridization Capture	100%	25/25 (100%)[1]
Median exon coverage	89.2%~100%	24/25 (96%)[1]
Post-sequencing	76.8%~99.2%	23/25 (92%)[1]

[1] Summarized based on 25ng of Extracted cfDNA
[2] Summarized based on 36ng of Extracted cfDNA

10.6 Concordance – FoundationOne Liquid Laboratory Developed Test to FoundationOne Liquid CDx

In order to support the use of historical data from the FoundationOne Liquid LDT to evaluate performance across cancer types, a study was performed to evaluate concordance between FoundationOne Liquid CDx and the FoundationOne Liquid LDT across the genomic regions targeted by both assays. This study evaluated the concordance of 927 unique samples processed on both the FoundationOne Liquid laboratory developed test (LDT) and FoundationOne Liquid CDx assays. A total of 3,366 alterations, consisting of only those in common between the assays were evaluated. The concordance analysis using FoundationOne Liquid LDT or FoundationOne Liquid CDx as the reference assay is summarized by variant category in **Table 42**.

Table 29. Concordance between FoundationOne Liquid LDT (F1L LDT) and FoundationOne Liquid CDx (F1LCDx)

Variant/ Mutation Type	F1L CDx+ F1L LDT+	F1L CDx- F1L LDT+	F1L CDx+ F1L LDT-	F1L CDx- F1L LDT -	PPA [95% CI]	NPA [95% CI]	OPA [95% CI]
All Short Variants	2871	123	32	1171180	95.9% [95.1%-96.6%]	>99.9% [>99.9%-100.0%]	>99.9% [>99.9%-100.0%]
Base Substitutions	2415	104	31	999032	95.9% [95.0%-96.6%]	>99.9% [>99.9%-100.0%]	>99.9% [>99.9%-100.0%]
Indels	456	19	1	172148	96.0% [93.8%-97.6%]	>99.9% [>99.9%-100.0%]	>99.9% [>99.9%-100.0%]
Rearrangements	147	20	24	59587	88.0% [82.1%-92.5%]	>99.9% [>99.9%-100.0%]	99.9% [99.9%-99.9%]
Copy Number Amplifications	173	32	0	59463	84.4% [78.7%-89.1%]	99.8% [>99.9%-100.0%]	99.8% [>99.9%-100.0%]

Variant/ Mutation Type	F1L CDx+ F1L LDT+	F1L CDx- F1L LDT+	F1L CDx+ F1L LDT-	F1L CDx- F1L LDT -	PPA [95% CI]	NPA [95% CI]	OPA [95% CI]
Total	3191	175	166	1290230	94.8% [94.0%-95.5%]	>99.9% [>99.9%-100.0%]	>99.9% [>99.9%-100.0%]

The overall PPA between FoundationOne Liquid LDT and FoundationOne Liquid CDx assays, with FoundationOne Liquid LDT as the reference assay, was 94.8% with a 95% two-sided CI of [94.0%-95.5%]. The respective short variant, rearrangement, and copy number amplification PPA values, with 95% two-sided CI, were: 95.9% [95.1%-96.6%], 88.0% [82.1%-92.5%], and 84.4% [78.7%-89.1%]. These results support the agreement between FoundationOne Liquid LDT and FoundationOne Liquid CDx and the applicability of the tumor comparability analysis performed using historical FoundationOne Liquid data.

10.7 Molecular Index Barcode Performance

To evaluate the molecular index barcode performance, a total of 7,641 sequenced samples from FoundationOne Liquid CDx validation studies were analyzed with the FoundationOne Liquid CDx assay.

The overall coefficient of variation (% CV) of sequencing coverage across all barcodes was 8.95% for the enhanced sensitivity regions and 7.64% for the standard sensitivity regions. This observed small % CV includes both sample variability and barcode variability as these two components were confounded and inseparable. Results demonstrated that all 480 barcodes analyzed are detectable with low differences in sample coverage variance between barcodes, indicating comparable performance of the barcodes.

10.8 Automation Line Equivalence

An intermediate precision study was performed to establish equivalence between the Hamilton instrumentation and the Biomek/Bravo instrumentation. The study consisted of eight contrived samples run in triplicate across four runs and both instrumentation platforms resulting in a total of 192 sample replicates included in the study overall. The analysis evaluated the negative call rate (NCR) and positive call rate (PCR) for 1,309 variants from eight contrived samples. The PCR and NCR were also evaluated by the seven variant categories.

The Mann-Whitney test was used for the comparison of PCR and NCR across liquid handling platforms for each sample, all samples in aggregate, and for each variant type. The NCR across platforms for each analysis set (per sample, all samples in aggregate, per variant type) were not statistically significant ($p > 0.05$). by sample and by variant type. The PCR across platforms were not statistically significant ($p > 0.05$) with the exception of contrived sample #3, the aggregate of all samples, and substitutions in a non-repetitive region or a repetitive region of ≤7 base pairs. The PCRs for the Hamilton liquid handling platform were slightly higher than the PCRs for the Biomek/Bravo platform (92.08% versus 90.15% for sample #3, 90.75% versus 89.67% for all samples, and 91.14 versus 90.10% for substitutions in a non-repetitive region or repetitive region of ≤7 base pairs). The statistical significance observed was due to large sample sizes allowing for the detection of slight differences that are likely not meaningful in practice; therefore, the Hamilton and Biomek/Bravo liquid handling platforms are considered to be interchangeable in the FoundationOne Liquid CDx assay.

11 Clinical Validation Studies

11.1 Clinical Bridging Study: Detection of *ALK* Rearrangements to Determine Eligibility for Treatment with Alectinib

The clinical validity of using FoundationOne Liquid CDx as a companion diagnostic to identify patients with non-small cell lung cancer (NSCLC) harboring *ALK* rearrangements for treatment with alectinib was assessed through a clinical bridging study using screening (i.e., pre-alectinib treatment) plasma samples from Cohort A of the Blood First Assay Screening Trial (BFAST, BO29554).

The BFAST trial is a Phase II/III multicenter study, in which Cohort A evaluated the safety and efficacy of alectinib as a treatment for patients with advanced or metastatic NSCLC who tested positive for an *ALK* rearrangement as determined by a blood-based NGS assay (CTA).

The concordance between FoundationOne Liquid CDx and the CTA was evaluated as summarized in **Table 43.**

Table 43: **Concordance between FoundationOne Liquid CDx and the CTA for the detection of *ALK* rearrangements**

	CTA Pos	CTA Neg	Total
FoundationOne Liquid CDx Positive[1]	63	0	63
FoundationOne Liquid CDx Negative	12	174	186
Missing	4	9	13
Total	79	183	262

[1] VAF values down to 0.06%VAF were observed for *ALK* rearrangements.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) between FoundationOne Liquid CDx and the CTA using the CTA as the reference for the primary analysis set and the corresponding 95% confidence intervals were:

- PPA [95% CI]: 84.0% [73.7%, 91.4%]
- NPA [95% CI] : 100% [97.9%, 100.0%]

After adjusting for a 5% prevalence of *ALK* rearrangements in the intended use population, the Positive Predictive Value (PPV), and Negative Predictive Value (NPV) calculated using the CTA as the reference and the corresponding 95% confidence intervals were:

- PPV [95% CI]: 100.0% [94.3%, 200.0%]
- NPV [95% CI]: 93.5% [89.0%, 96.6%]

The estimated Overall Response Rate (ORR) and the corresponding 95% confidence intervals was 88.9% [78.4%, 95.4%] for the FoundationOne Liquid CDx *ALK*-positive population which is comparable with the observed ORR and the corresponding 95% confidence intervals of 87.4% [78.5%, 93.5%] for the CTA *ALK*- positive population (BFAST Cohort A).

A sensitivity analysis was performed to estimate the clinical efficacy of treating patients with alectinib when considering missing FoundationOne Liquid CDx results. The estimated ORR and the corresponding 95% confidence intervals were 90.4% [90.1%, 90.6%] for the patient population that are both CTA *ALK*+ and FoundationOne Liquid CDx *ALK*+, demonstrating the robustness of the clinical efficacy analysis to missing FoundationOne Liquid CDx results.

11.2 FoundationOne Liquid CDx Concordance Study for *EGFR* Exon 19 deletion and *EGFR* Exon 21 L858R Alteration

Clinical validity of FoundationOne Liquid CDx assay was established as a companion diagnostic to identify patients with advanced NSCLC who may be eligible for treatment with TARCEVA® (erlotinib), IRESSA® (gefitinib), or TAGRISSO® (osimertinib). Two hundred and eighty retrospective samples from NSCLC patients were included in this study, which were tested for *EGFR* exon 19 deletion and exon 21 L858R alterations (*EGFR* alterations) by the FoundationOne Liquid CDx assay and the previously approved **cobas**® *EGFR* Mutation Test v2 (Roche Molecular Systems, referred to cobas assay). Both *EGFR* alteration-positive and *EGFR* alteration-negative samples (based on CTA results) were selected from the screen failed population of an unrelated clinical trial in NSCLC. To avoid selection bias, the samples were selected starting with a specific testing date until the predefined number of 150 *EGFR* alteration-positive and 100 *EGFR* alteration-negative samples were fulfilled. Samples were tested across two replicates by the cobas assay (denoted as CCD1 and CCD2) and one replicate by FoundationOne Liquid CDx. The tested samples, from NSCLC patients, were compared against the intended use (IU) population with respect to gender to ensure the screening population is representative of the IU

population. The variant calls were evaluated based on the agreement between both the FoundationOne Liquid CDx and the cobas assay results and between the two cobas assay replicates. For any samples in which there was insufficient plasma to process both CCD1 and CCD2, processing was not performed. In total there were 177 samples with complete test results available for analysis. The agreement analysis results between FoundationOne Liquid CDx and the cobas assay for the detection of *EGFR* exon 19 deletions and L858R alterations are presented in **Table 44**.

Table 44: Agreement analysis results for EGFR exon 19 deletion and L858R separately.

Exon 19 deletion	PPAC1F	95.5%	NPAC1F	95.6%
	PPAC1C2	97.7%	NPAC1C2	98.9%
	PPAC2F	95.5%	NPAC2F	96.0%
	PPAC2C1	96.2%	NPAC2C1	99.4%
L858R	PPAC1F	100.0%	NPAC1F	95.6%
	PPAC1C2	92.9%	NPAC1C2	98.9%
	PPAC2F	100.0%	NPAC2F	94.7%
	PPAC2C1	96.0%	NPAC2C1	98.0%

The concordance of *EGFR* mutations as detected by FoundationOne Liquid CDx and the cobas assay were assessed and the data are summarized in **Table 45**.

Table 30: Concordance among CCD1, CCD2 and FoundationOne Liquid CDx results with eligible samples (n=177)

	CCD1+			**CCD1-**		
	CCD2+	**CCD2-**	**Total**	**CCD2+**	**CCD2-**	**Total**
FoundationOne Liquid CDx+	80	4	84	1	3	4
FoundationOne Liquid CDx-	2	0	2	0	87	87
Total	82	4	86	1	90	91

The agreement analysis results between FoundationOne Liquid CDx and the cobas assay are presented in **Table 46.**

Table 31: Agreement analysis results

	PPA	**NPA**
CCD2|CCD1[1]	95.3%	98.9%
CCD1|CCD2[2]	96.1%	98.7%
FoundationOne Liquid CDx|CCD1*	97.7%	95.6%
FoundationOne Liquid CDx |CCD2**	97.7%	95.4%

[1]CCD1: the 1st replicate of cobas assay as the reference
[2]CCD2: the 2nd replicate of cobas assay as the reference

The estimates of ζPPA1, ζPPA2, ζNPA1 and ζNPA2 and the corresponding one-sided 95% upper bounds confidence limit computed using the bootstrap method are presented in **Table 47.**

Table 47: Point estimate and one-Sided 95% upper confidence limit of ζPPA1, ζNPA1, ζPPA2, and ζNPA

	Point Estimate	Mean one-sided 95% upper confidence limit
ζPPA1	-2.3%	2.3%
ζNPA1	3.3%	6.6%
ζPPA2	-1.6%	4.7%
ζNPA2	3.3%	6.6%

Based on these results, FoundationOne Liquid CDx has been demonstrated to be non-inferior to the cobas assay for the detection of *EGFR* exon 19 deletions and *EGFR* exon 21 L858R mutations. This study establishes the clinical validity of the FoundationOne Liquid CDx assay for identifying patients eligible for treatment with erlotinib, gefitinib, and osimertinib.

11.3 Clinical Bridging Study: Detection of *BRCA1/BRCA2/ATM* Alterations to Determine Eligibility for Treatment with Olaparib

The clinical validity of using FoundationOne Liquid CDx as a companion diagnostic to identify patients with metastatic castrate-resistant prostate cancer (mCRPC) harboring *BRCA1, BRCA2 or ATM* alterations for treatment with olaparib was assessed through a clinical bridging study using screening (i.e., pre-olaparib treatment) plasma samples from Cohort A of the PROfound trial.

The PROfound trial is a Phase III, open label, randomized study to assess the efficacy and safety of olaparib (Lynparza™) versus enzalutamide or abiraterone acetate in men with metastatic castration-resistant prostate cancer who have failed prior treatment with a new hormonal agent and have homologous recombination repair gene mutations. Only Cohort A patients with either *BRCA1*, *BRCA2* or *ATM* mutations were tested with the FoundationOne Liquid CDx assay.

In total, 4425 patients were screened and 387 (9.6%) were randomized into the PROfound study by the CTA. Of these 387 patients, 245 patients were randomized in cohort A. 181 out of the 245 randomized patients in cohort A both consented to the use of their sample for ctDNA CDx development and had a plasma sample available for testing. In total, 181/245 (73.9%) of the Cohort A patients were tested using the FoundationOne Liquid CDx assay. Of these, 139 (76.8%) Cohort A patients had a successful FoundationOne Liquid CDx test result and 42 Cohort A patients had a failed FoundationOne Liquid CDx test result. This represents 56.7% (139/245) of total Cohort A patients with a FoundationOne Liquid CDx result. In addition, 250 non-HRRm patient samples were randomly selected for ctDNA testing from the screen-failed population to determine the NPA/NPV of the FoundationOne Liquid CDx assay. 194/250 (77.6%) screen failed non-HRRm patients were successfully tested using the FoundationOne Liquid CDx assay.

Of the 139 successfully tested Cohort A patients, 111 patients were reported as *BRCA1/BRCA2/ATM* mutation positive and 28 randomized patients were reported as biomarker negative by FoundationOne Liquid CDx. Therefore, the FoundationOne Liquid CDx ctDNA biomarker positive subgroup comprises 111 patients with *BRCA1, BRCA2, and/or ATM* mutations.

Sample accountability for this clinical bridging study is summarized in **Table 48**.

Table 48: Sample accountability for olaparib clinical bridging study

Description	Number of patients
Patients randomized into PROfound	387
Patients with qualifying *BRCA1, BRCA2,* or *ATM* alterations (Cohort A)	245

Description	Number of patients
Cohort A patients with samples tested by FoundationOne Liquid CDx	181
FoundationOne Liquid CDx results available	139
Cohort A patients, biomarker positive by FoundationOne Liquid CDx	111

Table 49 shows the agreement analysis between CLIA CTA (tissue test) and the FoundationOne Liquid CDx results for PROfound patients, including Invalid and Not Tested results

Table 49: Summary of agreement analyses for FoundationOne Liquid CDx compared against CTA tissue test, including Invalid and Not Tested results

		CTA Results (n=495)	
		Biomarker positive	**Biomarker negative**
FoundationOne Liquid CDx assay	**Biomarker positive[1]**	111	16
	Biomarker[2] negative	28	178
	Biomarker[3] Invalid	42	56
	Not Tested	64	0
Agreement analyses (only Valid results included)	**PPA (95% CI[b])**	79.9 (72.2, 86.2) [111/139]	
	NPA (95% CI[b])	91.8 (87.0, 95.2) [178/194]	
	OPA (95% CI[b])	86.8 (82.7, 90.2) [289/333]	
	PPV (95% CI[b])	66.6 (56.0, 77.2)	
	NPV (95% CI[b])	95.7 (94.3, 97.1)	

[1] VAF values down to 0.11%VAF were observed for short variants and 0.25% VAF for rearrangements in *BRCA1, BRCA2*,or *ATM*.
[2] Biomarker refers to patients with eligible *BRCA/ATM* mutations
[3] Confidence intervals calculated using Clopper-Pearson method

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) between FoundationOne Liquid CDx and the CTA using the CTA as the reference for the primary analysis set and the corresponding 95% confidence intervals were:

- PPA [95% CI]: 79.9% [72.2%, 86.2%]
- NPA [95% CI] : 91.8% [87.0%, 95.2%]
-

After adjusting for a 17.1% prevalence of BRCA1/2 and ATM alterations in the intended use population, the Positive Predictive Value (PPV), and Negative Predictive Value (NPV) calculated using the CTA as the reference and the corresponding 95% confidence intervals were:

- PPV [95% CI]: 66.6% [56.0%, 77.2%]
- NPV [95% CI]: 95.7% [94.3%, 97.1%]

The estimated radiological progression-free survival (rPFS) hazard ratio (HR) and the corresponding 95% confidence intervals were 0.331 [0.21, 0.53] for the FoundationOne Liquid CDx biomarker positive population,

which were comparable with the observed rPFS HR and the corresponding 95% confidence intervals of 0.34 [0.25, 0.47] for the CTA biomarker positive population (PROfound Cohort A).

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 median rPFS HR and corresponding [95% CI] across the imputed datasets was 0.44 [0.32, 0.59], demonstrating robustness of the analysis to missing FoundationOne Liquid CDx results.

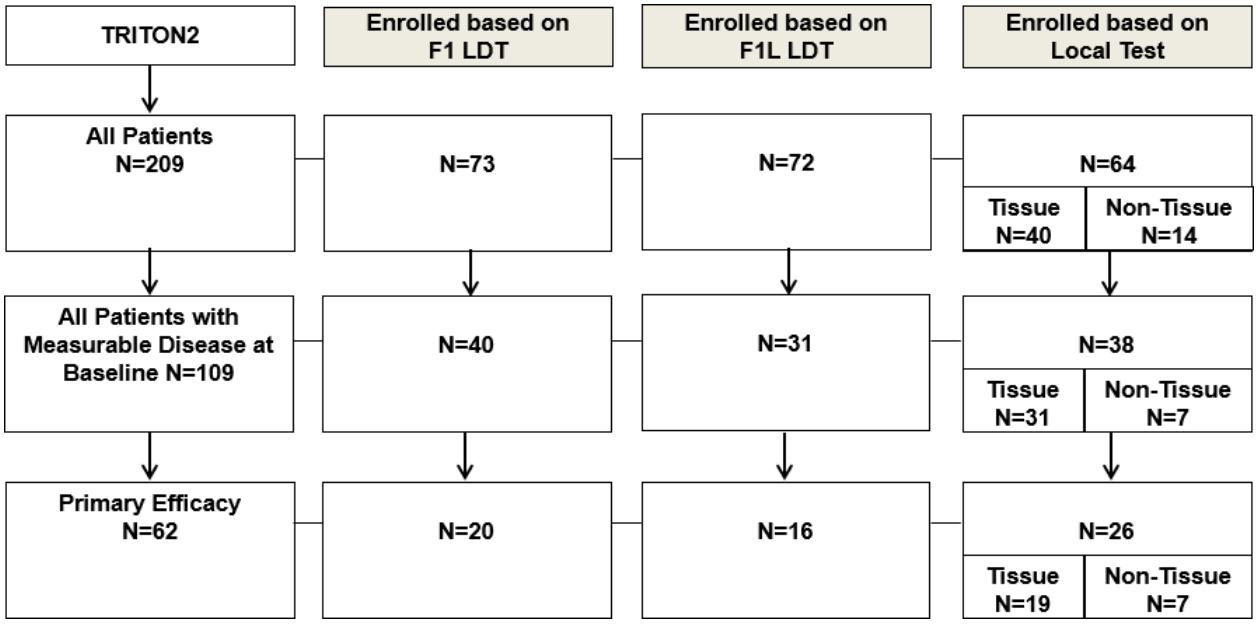
11.4 Clinical Bridging Study: Detection of *BRCA1* and *BRCA2* Alterations to Determine Eligibility of mCRPC Patients for Treatment with Rucaparib

The clinical performance of FoundationOne Liquid CDx as a companion diagnostic to identify patients with metastatic castration-resistant prostate cancer (mCRPC) harboring breast cancer gene 1 or 2 (*BRCA1* or *BRCA2*) alterations for treatment with rucaparib was demonstrated using pre-rucaparib treatment blood samples from clinical trial NCT0952534 (TRITON2). The clinical data supporting the use of rucaparib in the proposed indication was submitted as New Drug Application (NDA) 209115/S-004.

A bridging study was conducted to evaluate: 1) the concordance between BRCA1 and BRCA2 alteration status by the clinical trial assay (CTA) and FoundationOne Liquid CDx, and 2) the clinical efficacy of rucaparib treatment in patients that would be eligible for therapy based on BRCA1 and BRCA2 alteration status as determined by FoundationOne Liquid CDx.

A total of 209 patients (All Patients) from TRITON2 were included in NDA 209115/S-004. Genomic status was determined using the FoundationOne laboratory developed test [LDT] (F1 LDT), the FoundationOne Liquid LDT (F1L LDT), or a local test, as summarized in **Figure 1**.

Figure 1: TRITON2 Patient Enrollment



Pre-rucaparib treatment plasma samples were available for 92% (192/209) of the patients. FoundationOne Liquid CDx data were available for 93% (178/192) of the patients with samples tested; inadequate input material resulted in FoundationOne Liquid CDx test data being unavailable for 14 patients. In total, FoundationOne Liquid CDx data were available for 85% (178/209) of All Patients.

Of the 62 patients in the Primary Efficacy Population (those patients with measurable visceral and/or nodal disease at baseline), FoundationOne Liquid CDx test data were obtained for 84% (52/62) and used for concordance and efficacy analyses. The sample accountability for this clinical bridging study is summarized in **Table 50**.

Table 50: Sample accountability for rucaparib prostate clinical bridging study

Description	Number
All Patients in TRITON2	209
Total samples available for retesting by FoundationOne Liquid CDx	192
Patients with evaluable FoundationOne Liquid CDx data and cfDNA input ≥ 30ng (All Patients)	161
Patients with evaluable FoundationOne Liquid CDx test results and cfDNA input ≥ 20ng (All Patients)	178
Primary efficacy population in TRITON2	62
Patients with evaluable FoundationOne Liquid CDx test results and cfDNA input ≥ 30ng (Primary Efficacy Population)	48
Patients with evaluable FoundationOne Liquid CDx test results and cfDNA input ≥ 20ng (Primary Efficacy Population)	52

Concordance between FoundationOne Liquid CDx and the CTAs

The concordance of BRCA status between FoundationOne Liquid CDx and CTA test results were evaluated in all patients as summarized in **Table 51** and **Table 52**.

Table 51: Concordance between FoundationOne Liquid CDx BRCA Status and the CTA BRCA Status in All Patients with FoundationOne Liquid CDx cfDNA input ≥30ng

All Patients		CTA		
		***BRCA* Positive**	***BRCA* Negative**	**Total**
FoundationOne Liquid CDx	***BRCA* Positive**[1]	75	1	76
	***BRCA* Negative**	16	69	85
	***BRCA* Unknown**	2	1	3
	Total	93	71	164

[1] VAF values down to 0.15%VAF were observed for short variants and 0.85%VAF for rearrangements in *BRCA1* or *BRCA2*.

The PPA, NPA between FoundationOne Liquid CDx and the CTA, based on a cfDNA input ≥30ng, were determined using the CTA as the reference for all patients.

PPA (95% CI): 82.4% (73.0%, 89.6%)
NPA (95% CI): 98.6% (92.3%, 100.0%)

Table 52: Concordance between FoundationOne Liquid CDx BRCA Status and the CTA BRCA Status in All Patients with FoundationOne Liquid CDx cfDNA input ≥20ng

All Patients		CTA		
		***BRCA* Positive**	***BRCA* Negative**	**Total**
FoundationOne Liquid CDx	***BRCA* Positive**[1]	82	1	83
	***BRCA* Negative**	18	77	95
	***BRCA* Unknown**	3	2	5
	Total	103	80	183

[1] VAF values down to 0.15%VAF were observed for short variants and 0.85%VAF for rearrangements in *BRCA1* or *BRCA2*.

The PPA, NPA between FoundationOne Liquid CDx and the CTA, based on a cfDNA input ≥20ng, were determined using the CTA as the reference for all patients.

- PPA (95% CI): 82.0% (73.1%, 89.0%)
- NPA (95% CI): 98.7% (93.1%, 100%)

Efficacy Based on FoundationOne Liquid CDx Results

BRCA1 and *BRCA2* alteration status were verified retrospectively by FoundationOne Liquid CDx in 66% (41/62) of the patients in the Primary Efficacy Population. The ORR [95% CI] in the Primary Efficacy Population was 46.3% [30.7%-62.6%] in *BRCA* positive patients determined by FoundationOne Liquid CDx, which is comparable to the ORR of 43.5% [31.0%-56.7%] in patients identified by CTA (**Table 53**).

Table 53: ORR in the primary efficacy population by CTA and FoundationOne Liquid CDx test results

Primary Efficacy Population	**FoundationOne Liquid CDx**		**CTA**
	***BRCA* Positive N=38 (≥ 30 ng cfDNA input)**	***BRCA* Positive N = 41 (≥ 20 ng cfDNA input)**	***BRCA* Positive N = 62**
Confirmed ORR (CR + PR), n (%)	18 (47.4)	19 (46.3)	27 (43.5)
95% CI(%)	31.0 – 64.2	30.7 - 62.6	31.0 – 56.7

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.

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 and demonstrated that the drug efficacy in the FoundationOne Liquid CDx positive population was robust to missing FoundationOne Liquid CDx results.

11.5 Clinical Validation Study: Detection of *BRCA1* and *BRCA2* Alterations to Determine Eligibility of Ovarian Cancer Patients for Treatment with Rucaparib

The clinical performance of FoundationOne Liquid CDx as a companion diagnostic to identify patients with ovarian cancer harboring *BRCA1* or *BRCA2* alterations for treatment with rucaparib was demonstrated using pre- rucaparib treatment blood samples from the ARIEL2 study.

The bridging study was conducted to evaluate: 1) the concordance between *BRCA1* and *BRCA2* alteration status by the CTA and FoundationOne Liquid CDx, and 2) the clinical efficacy of rucaparib treatment in patients that would be eligible for therapy based on *BRCA1* and *BRCA2* alteration status as determined by FoundationOne Liquid CDx.

The ARIEL2 study is complete and enrolled 491 patients (All Patients). Pre-rucaparib treatment plasma samples were available for 55% (271/491) of patients dosed in ARIEL2. FoundationOne Liquid CDx data were available for 80% (217/271) of the patients with samples tested; 49 failures were due to insufficient remaining plasma volume or insufficient DNA extraction yield. In total, FoundationOne Liquid CDx results were available for 44% (217/491) of All Patients.

Of the 64 patients in the primary efficacy population, FoundationOne Liquid CDx results were available for 42% (27/64) and used for concordance and efficacy analyses. The sample accountability for this clinical validation study is summarized in **Table 54**.

Table 54: Sample accountability for rucaparib ovarian clinical bridging study

Description	Number
All Patients	**491**
Total samples available	**271**
Patients with FoundationOne Liquid CDx data (All Patients)	**217**
Patients with FoundationOne Liquid CDx data (Primary Efficacy Population)	**27**

The concordance between FoundationOne Liquid CDx and CTA test results was evaluated in All Patients and is summarized in **Table 55**. The Primary Efficacy Population is summarized in **Table 56.**

Table 55: Concordance between FoundationOne Liquid CDx and the CTA for the detection of *BRCA1* or *BRCA2* alterations in All Patients

	CTA Positive	CTA Negative	Total
FoundationOne Liquid CDx Positive[1]	60	4	64
FoundationOne Liquid CDx Negative	4	149	153
Missing	60	214	274
Total	124	367	491

[1] VAF values down to 0.24%VAF were observed for short variants and 5.05%VAF for rearrangements in *BRCA1* or *BRCA2*.

The PPA and NPA between FoundationOne Liquid CDx and the CTA were determined using the CTA as the reference for All Patients:

- PPA [95% CI]: 93.8% [84.8%, 98.3%]
- NPA [95% CI]: 97.4% [93.4%, 99.3%]

Table 56: Concordance between FoundationOne Liquid CDx and the CTA for the detection of *BRCA1* or *BRCA2* alterations in the primary efficacy population

	CTA Positive	CTA Negative	Total
FoundationOne Liquid CDx Positive	26	0	26
FoundationOne Liquid CDx Negative	0	1	1
Missing	35	2	37
Total	61	3	64

The PPA and NPA between FoundationOne Liquid CDx and the CTA were determined using the CTA as the reference for the Primary Efficacy Population:

- PPA [95% CI]: 100% [86.8%, 100.0%]
- NPA [95% CI]: 100% [2.5%, 100.0%]

BRCA1 and *BRCA2* alteration status was verified retrospectively by FoundationOne Liquid CDx in 41% (26/64) of the patients in the Primary Efficacy Population.

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%].

11.6 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

	CTA2			
CDx	**Positive**	**Negative**	**Invalid**	**Total**
Total	237	134	4	375

[1] VAF values down to 0.14%VAF were observed for short variants in *PIK3CA*.
Samples not tested are excluded from the analysis.
Samples tested with cfDNA input < 30 ng are excluded from the analysis.

The point estimates of PPA and NPA between FoundationOne Liquid CDx and the CTA2 assay and the corresponding 95% confidence intervals were:

- PPA [95% CI]: 71.7% [65.4%, 77.5%]
- NPA [95% CI]: 100% [97.2%, 100%]

The primary efficacy analysis in the *PIK3CA* alteration positive population identified by FoundationOne Liquid CDx was based on PFS by local investigator assessment per RECIST 1.1 criteria. Clinical efficacy of alpelisib in combination with fulvestrant for the FoundationOne Liquid CDx-positive population with cfDNA input ≥30 ng (N=165) was demonstrated with an estimated 54% risk reduction in disease progression or death in the alpelisib plus fulvestrant arm compared to the placebo plus fulvestrant arm (HR = 0.46, 95% CI: 0.30, 0.70).

As summarized in **Table 59**, the PFS hazard ratio for the 165 tissue CTA2-positive, FoundationOne Liquid CDx-positive patients was 0.46 (95% CI: 0.30, 0.70). Median PFS was 11.0 months for the alpelisib plus fulvestrant arm versus 3.6 months for the placebo plus fulvestrant arm.

Table 59: Progression-free survival in the CTA2-positive, FoundationOne Liquid CDx-positive patients (primary analysis set)

Progression free survival (months)	**Alpelisib 300mg qd + Fulvestrant N=84**	**Placebo qd + Fulvestrant N=81**	**HR (95% CI) Alpelisib 300mg qd + Fulv /Placebo qd + Fulv[1]**
No of events (%)	54 (64.3)	67 (82.7)	0.46 (0.30, 0.70)
PD (%)	52 (61.9)	61 (75.3)	
Death (%)	2 (2.4)	6 (7.4)	
No of censored (%)	30 (35.7)	14 (17.3)	
Median (95% CI)[2]	11.0 (7.3, 15.9)	3.6 (2.4, 5.8)	

[1] Hazard ratio (HR) estimated using Cox regression model stratified by the two stratification factors: presence of lung and/or liver metastases, previous treatment with any CDK4/6 inhibitor, and adjusted for clinically relevant covariates, as well as the imbalanced covariates.

[2] The 95% CI calculated from PROC LIFETEST output using the method of Brookmeyer and Crowley (1982).

CDx results from samples tested with cfDNA input < 30 ng are treated as missing.

PD = progressive disease

Sensitivity analysis to evaluate the robustness of the clinical efficacy estimate against the missing FoundationOne Liquid CDx results was performed using the multivariate imputation by chained equations (MICE) method. After imputing the missing FoundationOne Liquid CDx results, the hazard ratio was estimated to be 0.63 (95% CI: 0.45, 0.87), demonstrating robustness of the clinical efficacy analysis to missing FoundationOne Liquid CDx results.

11.7 Clinical Bridging Study: Detection of MET single nucleotide variants (SNVs) and indels that lead to MET exon 14 skipping to Determine Eligibility for Treatment with capmatinib

The clinical performance of FoundationOne Liquid CDx for detecting SNVs and indels that lead to *MET* exon 14 skipping in NSCLC patients who may benefit from treatment with capmatinib (Table 1) was established with clinical data generated from a clinical bridging study using samples from patients enrolled in the GEOMETRY mono-1 study. The study demonstrates concordance between the enrollment assay, i.e., the clinical trial assay (CTA), and the FoundationOne Liquid CDx assay and establish the effectiveness of the FoundationOne Liquid CDx assay.

GEOMETRY mono-1 was a prospectively designed, multi-center, open-label, single arm Phase II study of oral cMET inhibitor, TABRECTA(capmatinib), in adult patients with EGFR wild-type (wt), and anaplastic lymphoma kinase (ALK) negative advanced NSCLC. Patients were enrolled into multiple cohorts of the study, but the bridging study was focused on the fully-enrolled *MET* exon 14 skipping positive Cohorts 4 and 5b. Cohort 4 only enrolled pretreated (second and third line) patients with *MET* exon 14 skipping, and Cohort 5b only enrolled treatment-naïve patients with *MET* exon 14 skipping. Patients were screened for enrollment into Cohorts 4 and 5b for *MET* exon 14 skipping status using a *MET* exon 14 skipping reverse-transcriptase PCR (RT-PCR) CTA that was detected MET exon 14 skipping in a patient's tissue. Plasma samples were collected and stored prior to study treatment for retrospective testing. Patients enrolled in Cohorts 4 and 5b received 400mg of capmatinib orally twice daily in tablet form. Efficacy was evaluated every six weeks from the first day of treatment until RECIST 1.1 disease progression.

A clinical bridging study was conducted to evaluate: 1) the concordance between *MET* single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping status by the clinical trial assay (CTA) and FoundationOne Liquid CDx, and 2) the clinical efficacy of capmatinib treatment in patients that would be eligible for therapy based on *MET* biomarker positive status as determined by FoundationOne Liquid CDx.

The primary endpoint of GEOMETRY mono-1 was the overall response rate (ORR) by Blinded Independent Review Committee (BIRC) assessment by cohort to determine whether treatment with capmatinib is effective. Duration of response (DOR) as assessed by BIRC was the key secondary endpoint.

The primary concordance analysis of the status of *MET* SNVs and indels that lead to *MET* exon 14 skipping between FoundationOne Liquid CDx and the tissue CTA test results were evaluated in both analysis sets that met ≥30 ng cfDNA input and ≥20 ng cfDNA input. The analysis on the ≥30 ng cfDNA input population evaluated 150 patients (78 *MET* exon 14 skipping positive patients, and 72 *MET* exon 14 skipping negative patients), excluding invalid CDx results. The analysis on the ≥20 ng cfDNA input population evaluated 171 patients (83 *MET* exon 14 skipping positive patients, and 88 *MET* exon 14 skipping negative patients), excluding invalid CDx results.

Agreement (PPA, NPA and OPA) for combined Cohort 4 and 5b by ≥30 ng cfDNA input and ≥20 ng cfDNA input CDx are shown in **Tables 60 and 61**, below. For the 150 patients meeting the ≥30 ng cfDNA input, the PPA, NPA and OPA and respective confidence intervals were determined to be 70.5% (59.1%, 80.3%), 100% (95.0%, 100%) and 84.7% (77.9%, 90.0%). For the 171 patients meeting the ≥20 ng cfDNA input, the PPA, NPA and OPA and respective confidence intervals were determined to be 68.7% (57.6%, 78.4%), 100% (95.9, 100%) and 84.8% (78.5%, 89.8%).

Table 60: Agreement between CDx and CTA based on CTA results in combined cohorts by cfDNA input ≥ 30 ng

	Measure of agreement	Percent agreement % (n/N)	95% CI (1)
Cohort 4 and Cohort 5b (CDx sample requirement: cfDNA input ≥ 30 ng)	PPA[1]	70.5 (55/ 78)	(59.1, 80.3)
	NPA	100 (72/ 72)	(95.0, 100)
	OPA	84.7 (127/150)	(77.9, 90.0)

[1] VAF values down to 0.16%VAF were observed for *MET* short variants.
N: The total number of patients. It is the denominator for percentage (%) calculation
n: Number of patients with agreement between CTA and CDx
(1) The 95% CI calculated using Clopper-Pearson method

Table 61: Agreement between CDx and CTA based on CTA results in combined cohorts by cfDNA input ≥ 20 ng

	Measure of agreement	Percent agreement % (n/N)	95% CI (1)
Cohort 4 and Cohort 5b (CDx sample requirement: cfDNA input ≥20 ng)	PPA[1]	68.7 (57/ 83)	(57.6, 78.4)
	NPA	100 (88/ 88)	(95.9, 100)
	OPA	84.8 (145/171)	(78.5, 89.8)

[1] VAF values down to 0.16%VAF were observed for *MET* short variants.
N: The total number of patients. It is the denominator for percentage (%) calculation
n: Number of patients with agreement between CTA and CDx
(1) The 95% CI calculated using Clopper-Pearson method

Based on the PPA of 70.5% (59.1%, 80.3%) between FoundationOne Liquid CDx (F1LCDx) and the tissue CTA, reflex testing using tissue specimens to an FDA approved tissue test is recommended, if feasible, if the plasma test is negative.

Clinical effectiveness of FoundationOne Liquid CDx was evaluated by estimation of clinical efficacy in the CTA-enrolled *MET* exon 14 deletion positive patient population, as assessed by the primary objective of ORR by BIRC. The GEOMETRY mono-1 clinical trial met its primary objective demonstrating a statistically significant improvement in ORR by BIRC assessments in patients with *MET* exon 14 deletion positive tumors in each cohort.

Tables 62 and 63 present the clinical efficacy of TABRECTA analyzed in CTA-positive patients who were tested as CDx-positive ("double positive" patients) in each cohort that met the ≥30 ng cfDNA input and ≥20 ng cfDNA input CDx sample requirements, respectively. In Cohort 4 there were 39 patients with ≥30 ng cfDNA input and 41 with ≥20 ng cfDNA input with valid results for analysis of ORR. In Cohort 5b there were 16 patients, all of whom met the ≥30 ng cfDNA input.

Patients in Cohort 4 that met the ≥30 ng cfDNA input demonstrated an ORR of 51.3% (34.8%, 67.6%). Patients from Cohort 4 that met the ≥20 ng cfDNA input requirements demonstrated an ORR of 48.8% (32.9%, 64.9%). For patients in Cohort 5b, all patients met the ≥30 ng cfDNA input and demonstrated an ORR of 81.3% (54.4%, 96.0%).

Table 62: Overall response per BIRC assessment in (CTA-positive, CDx-positive) and CTA-positive patients by cohort and CDx sample requirements (Cohort 4)
(CTA+, CDx+)
CDx sample requirements

	cfDNA input ≥ 30 ng N=39		cfDNA input ≥ 20 ng N=41		CTA+ N=69	
	n (%)	95% CI (1)	n (%)	95% CI (1)	n (%)	95% CI (1)
Overall Response Rate (ORR: CR + PR)	20 (51.3)	(34.8, 67.6)	20 (48.8)	(32.9, 64.9)	28 (40.6)	(28.9, 53.1)

(1) The 95% CI calculated with the Clopper-Pearson Exact method.

Table 63. Overall response per BIRC assessment in (CTA-positive, CDx-positive) and CTA-positive patients by cohort and CDx sample requirements (Cohort 5b).
(CTA+, CDx+)
CDx sample requirements

	cfDNA input ≥ 30 ng N=16		cfDNA input ≥ 20 ng N=16		CTA+ N=28	
	n (%)	95% CI (1)	n (%)	95% CI (1)	n (%)	95% CI (1)
Overall Response Rate (ORR: CR + PR)	13 (81.3)	(54.4, 96.0)	13 (81.3)	(54.4, 96.0)	19 (67.9)	(47.6, 84.1)

(1) The 95% CI calculated with the Clopper-Pearson Exact method.

Estimated drug efficacy in FoundationOne Liquid CDx Positive (F1LCDx(+) patients

The ORR by BIRC assessment in F1LCDx(+) patients was calculated for Cohort 4 and Cohort 5b, separately. Because all CTA(-) patients are tested as negative by CDx (i.e. NPA=100%) and thus PPV is estimated as 100%, the results do not vary with Pr(CTA+) values and the ORR in F1LCDx(+) population is estimated as the same as the ORR in F1LCDx [CTA(+)/CDx(+)] population. For F1LCDx(+) patients meeting "Recommended" CDx sample requirement (cfDNA input ≥ 30 ng), the ORR (95% CI) is 51.3% (34.8%, 67.6%) in Cohort 4 and 81.3% (54.4%, 96.0%) in Cohort 5b, respectively. For CDx(+) patients meeting "Minimum" CDx sample requirement (cfDNA input ≥ 20 ng), the ORR (95% CI) is 48.8% (32.9%, 64.9%) in Cohort 4 and 81.3% (54.4%, 96.0%) in Cohort 5b, respectively.

Sensitivity analysis on missing FoundationOne Liquid CDx results

The impact of missing F1LCDx results on the concordance between CTA and F1LCDx and final drug efficacy in F1LCDx(+) patients was evaluated by imputing the missing F1LCDx results using multiple imputation method. For Cohort 4, the imputed ORR (95% CI) by BIRC were estimated to be 46.5% (32.6%, 60.9%) given "Recommended" sample requirement and 47.2% (33.3%, 61.5%) given "Minimum" sample requirement. For Cohort 5b, the imputed ORRs and two-sided 95% CIs by BIRC were estimated to be 75.3% (53.3%, 94.4%) given "Recommended" sample requirement and 78.1% (55.6%, 95.5%) given "Minimum" sample requirement. The sensitivity analysis results demonstrated that the concordance between CTA and F1LCDx and final drug efficacy in F1LCDx(+) population are robust to missing F1LCDx results.

12 CDx Classification Criteria

12.1 CDx classification criteria for *ALK* rearrangements, qualifying NSCLC patients for therapy with ALECENSA® (alectinib):

- The *ALK* rearrangement must have pathogenic driver status (FMI driver status of "known" or "likely")
- AND the disease type must be NSCLC
- AND one of the following two conditions must hold:
    1. The partner gene is *EML4*, or
    2. The *ALK* breakpoint occurs within *ALK* intron 19

12.2 CDx classification criteria for *EGFR* alterations, qualifying NSCLC patients for therapy with IRESSA® (gefitinib), TAGRISSO® (osimertinib), TARCEVA® (erlotinib):

- Base substitutions resulting in *EGFR* L858R
- In-frame deletions occurring within *EGFR* exon 19

12.3 CDx classification criteria for *BRCA1*, *BRCA2*, and *ATM* alterations, qualifying prostate cancer patients for therapy with LYNPARZA® (olaparib):

Table 64, **Table 65,** and **Table 66** describe the criteria for classifying *BRCA1*, *BRCA2, or ATM* alterations known to be deleterious to protein function

Table 64: Classification Criteria for *BRCA1, BRCA2,* and *ATM*

Deleterious Variant Criteria	Sequence Classification	CDx Classifier Methodology
A gene alteration that includes any of the sequence classifications	Protein truncating mutations	Sequence analysis identifies premature stop codons or frameshift indels anywhere in the gene coding region, except: 3' of and including *BRCA2* K3326*
	Splice site mutations	Sequence analysis identifies variant splice sequences at intron/exon junctions: within ± 2bp of exon starts/ends, or callable splice variants in Table 59
	Homozygous deletions	Sequence analysis identifies deletions in both gene alleles of ≥ 1 exon in size. Only reported for BRCA1 and *BRCA2*. Not reported for ATM.
	Large protein truncating rearrangements	Sequence analysis identifies protein truncating rearrangements
	Deleterious missense mutations	Curated list (Table 58)

Table 65: **Deleterious Missense Alterations**

BRCA1 **Protein Effect (PE)**	***BRCA2*** **Protein Effect (PE)**	***ATM*** **Protein Effect (PE)**
M1V	M1R	M1T
M1I	M1I	R2032K
C61G	V159M	R2227C
C64Y	V211L	R2547_S2549del

BRCA1 Protein Effect (PE)	*BRCA2* Protein Effect (PE)	*ATM* Protein Effect (PE)
R71G	V211I	G2765S
R71K	R2336P	R2832C
R1495M	R2336H	S2855_V2856delinsRI
E1559K		R3008C
D1692N		R3008H
D1692H		
R1699W		
A1708E		
G1788V		

Table 66: Intronic Variants

Gene	Chromosome	Position	Ref	Alt	dbSNP
ATM	chr11	108128198	T	G	rs730881346
ATM	chr11	108214102	AGTGA	A	rs730881295

12.4 CDx classification criteria for *BRCA1* and *BRCA2* alterations, qualifying prostate cancer or ovarian cancer patients for therapy with RUBRACA® (rucaparib):

Table 67 and **Table 68** describe the criteria for classifying *BRCA1* or *BRCA2* alterations known to be deleterious to BRCA protein function rendering the sample *BRCA*+.

Table 67: Classification Criteria for Deleterious Tumor *BRCA* Variants

Qualification Criteria	Sequence Classification	Methodology
A *BRCA1/2* alteration that includes any of the sequence classifications	Protein truncating mutations	Sequence analysis identifies premature stop codons anywhere in the gene coding region, except: 3' of and including *BRCA2* K3326*
	Splice site mutations	Sequence analysis identifies variant splice sequences at intron/exon junctions -/+ 2bp of exon starts/ends
	Homozygous deletions	Sequence analysis identifies deletions in both gene alleles of ≥ 1 exon in size
	Large protein truncating rearrangements	Sequence analysis identifies protein truncating rearrangements
	Deleterious missense mutations	Curated list (**Table 61**)

Table 68: **Deleterious *BRCA* Missense Alterations**

BRCA1 Alterations (Protein Change)				*BRCA2* Alterations (Protein Change)	
M1V	C61G	D1692H	G1788V	M1V	R2659T
M1T	C61Y	D1692Y	P1812A	M1T	R2659K
M1R	C64R	R1699W	A1823T	M1R	E2663V
M1I	C64G	R1699Q	V1833M	M1I	S2670L
M18T	C64Y	G1706R	W1837R	D23N	I2675V

***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	

12.5 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

12.6 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.