FoundationOne® Liquid CDx
Technical Information
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Technical Information

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ntended 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, including rearrangements in eight (8) 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 therapieslisted 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)	*LK* rearrangements	ALECENSA® (alectinib)
	EGFR exon 19 deletions and *EGFR* exon 21 L858R substitution	EGFR tyrosine kinase inhibitors approved by FDA*
	EGFR exon 20 insertions	EXKIVITY® (mobocertinib)
	MET single nucleotide variants (SNVs) and indels that lead *to MET* exon 14 skipping	TABRECTA® (capmatinib)
	ROS1 fusions**	ROZLYTREK® (entrectinib)
Prostate cancer	*BRCA1, BRCA2, TM* alterations	LYNPARZA® (olaparib)
	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)
Solid Tumors	*NTRK1/2/3* fusions**	ROZLYTREK® (entrectinib)

*For the most current information about the therapeutic products in this group, go to: https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools#Group_Labeling

Additionally, FoundationOne Liquid CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with 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** (see **Note for *NTRK1/2/3* and *ROS1* fusions) should be reflexed to routine biopsy and their tumor mutation status confirmed using an FDA-approved tumor tissue test, if feasible.

**Note: When considering eligibility for ROZLYTREK® based on the detection of *NTRK1/2/3* and *ROS1* fusions, testing using plasma specimens is only appropriate for patients for whom tumor tissue is not available for testing

Genomic findings other than those listed in **Table** 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 reportedalteration 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

1. For in vitro diagnostic use only.

2. For prescription use only. This test must be ordered by a qualified medical professional in accordance withclinical laboratory regulations.

3. Genomic findings other than those listed in **Table** of the intended use are not prescriptive or conclusive forlabeled use of any specific therapeutic product.

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

5. 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.

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

7. Genomic findings from 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: *SXL1, TM, 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.

8. The false positive rate of this test was evaluated in healthy donors. The detection rate for unique short variants in apparently healthy patients is .82%. Across 30,622 short variants, 58 variants had a detection rate of greater than 5%.

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

10. 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.

11. The analytical accuracy for the FoundationOne Liquid CDx assay for detection of *EGFR* exon 20 insertions has not been demonstrated for samples with <0.18% VAF.

12. TABRECTA® efficacy has not been established in patients with *MET* SNVs<0.21% VAF and in patients with *MET* indels <0.16% VAF tested with FoundationOne Liquid CDx.

13. ALECENSA® efficacy has not been established in patients with ALK rearrangements <0.06% VAF tested with FoundationOne Liquid CDx.

14. LYNPARZA® efficacy has not been established in prostate cancer patients with BRCA1/2 or ATM rearrangements with <0.25% VAF or with short variants in BRCA1/2 or ATM <0.11% VAF tested with FoundationOne Liquid CDx.

15. RUBRACA® efficacy has not been established in prostate cancer patients with BRCA1/2 rearrangements with <0.85% VAF or with short variants in BRCA1/2 <0.15% VAF tested with FoundationOne Liquid CDx.

16. PIQRAY® efficacy has not been established in patients with PIK3CA SNVs with <0.14% VAF tested with FoundationOne Liquid CDx.

17. EXKIVITY® efficacy has not been established in patients with *EGFR* exon 20 insertions <0.20% VAF tested with FoundationOne Liquid CDx.

18. The precision of FoundationOne Liquid CDx was only confirmed for select variants at the limit of detection (LoD).

19. The FoundationOne Liquid CDx assay does not detect heterozygous deletions.

20. The FoundationOne Liquid CDx assay does not detect copy number losses/homozygous deletions in *TM*

21. A complete assessment of the impact of cfDNA blood collection tube lot-to-lot variability on the performanceof the test has not been evaluated.

22. The test is not intended to provide information on cancer predisposition.

23. *BRCA1/BRCA2* homozygous deletions and rearrangements were not adequately represented in all analytical studies.

24. Representation of *LK* rearrangements were limited in the analytical validation studies.

25. The representation of *TM* short variants and rearrangements was limited in the analytical validation studies.

26. Performance has not been validated for cfDNA input below the specified minimum input.

27. Representation of SNV and indels that lead to *MET* exon 14 skipping that represent biomarker rule category 1and 2 (refer to Section 11.6 for CDx biomarker definition), were limited in the analytical validation studies.

28. For optimal ctDNA shed, it is recommended that blood be drawn prior to therapy or at a time of disease progression. The sensitivity of liquid biopsy is related to adequate levels of ctDNA shed. Therefore, assay performance will be dependent upon level of ctDNA shed at time of testing.

29. Due to the low prevalence of *ROS1* fusions and *NTRK1/2/3* fusions, the positive predictive value (PPV) of the test (FoundationOne Liquid CDx positive, tissue negative) may be lower than reported in test labeling.

30. FoundationOne Liquid CDx may miss a subset of patients with *NTRK1/2/3* fusion and *ROS1* fusion positive solid tumors who may derive benefit from ROZLYTREK®. In a retrospective-prospective clinical

study assessing concordance between FoundationOne Liquid CDx test results in plasma and patients whose tumor tissue tested positive and was the basis for enrollment into a clinical trial, the data demonstrated that the FoundationOne Liquid CDx test did not detect approximately 46% of potential responders with *NTRK1/2/3* fusions and 49% of responders with *ROS1* fusions.

31. ROZLYTREK® efficacy has not been established in patients with *NTRK2* fusions tested with FoundationOne Liquid CDx, given the low prevalence of the biomarker.

32. In a retrospective-prospective clinical study assessing concordance between FoundationOne Liquid CDx test results in plasma and patients whose tumor tissue tested positive and was the basis for enrollment into a clinical trial, FoundationOne Liquid CDx detected 1 of 7 different *NTRK3* fusion partners. Due to the rarity of these fusions, the accuracy of FoundationOne Liquid CDx for *NTRK3* fusions has not been adequately determined.

33. *NTRK2* fusions per the FoundationOne Liquid CDx biomarker rules for *NTRK1/2/3* fusions were not represented in analytical validation studies.

34. A study evaluating the concordance to a second method demonstrated that the agreement between FoundationOne Liquid CDx positive results and a comparator method for *NTRK1/3*, and *ROS1* was ≤ 50% (i.e., whether these are potential FoundationOne Liquid CDx false positives or false negatives by the comparator is unknown).

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. xtracted 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 eight 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 0-29, Introns 18,19]***	*ALOX12B*	*AMER1 (FAM123B)*	***APC***	***AR***
ARAF [Exons , 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 , 7, 8, 12, 16, 19, 20]*	***BRCA2** [Intron]*	*BRD4*	*BRIP1*	*BTG1*	*BTG2*	***BTK [Exons , 15]***	*C11orf30 (EMSY)*	*C17orf39 (GID4)*
CALR	*CARD11*	*CASP8*	*CBFB*	*CBL*	***CCND1***	*CCND2*	*CCND3*	*CCNE1*	*CD22*
CD70	*CD74* [Introns - 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]***	*CUL3*	*CUL4A*	*CXCR4*
CYP17A1	*DAXX*	*DDR1*	***DDR2 [Exons , 17, 18]***	*DIS3*	*DNMT3A*	*DOT1L*	*EED*	***EGFR [Introns 7,*** *15,* ***-27]***	*EP300*
EPHA3	*EPHB1*	*EPHB4*	***ERBB2***	***ERBB3 [Exons , 6, 7, 8, 10, 12, 20, 1, , 24,]***	*ERBB4*	*ERCC4*	*ERG*	***ERRFI1***	***ESR1 [Exons 4-8]***
ETV4 [Intron 8]*	*ETV5* [Introns , 7]*	***ETV6* [Introns ,]***	*EWSR1* [Introns 7-13]*	***EZH2 [Exons , 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, ,* ***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 ,]***	*GNA13*	***GNAQ [Exons ,]***	***GNAS [Exons 1, 8]***	*GRM3*
GSK3B	*H3F3A*	*HDAC1*	*HGF*	*HNF1A*	***HRAS [Exons ,]***	*HSD3B1*	*ID3*	***IDH1 [Exon 4]***	***IDH2 [Exon 4]***
IGF1R	*IKBKE*	*IKZF1*	*INPP4B*	*IRF2*	*IRF4*	*IRS2*	*JAK1*	***JAK2 [Exon 14]***	***JAK3 [Exons , 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 , 8-11, Intron 7]*
KMT2D (MLL2)	***KRAS***	*LTK*	*LYN*	*MAF*	***MAP2K1 (MEK1) [Exons ,]***	***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]*	*MSH3*	*MSH6*	*MST1R*	*MTAP*	***MTOR [Exons 19, 0, 9 0, -45, 7, 48, ,]***
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]*	*NOTCH3*	***NPM1 [Exons 4-6, 8, 10]***	***NRAS [Exons ,]***	*NSD3 (WHSC1L1)*	*NT5C2*	***NTRK1 [Exons14, 15, Introns 8-11]***
NTRK2 [Intron 12]*	***NTRK3 [Exons 16, 17]***	*NUTM1* [Intron 1]*	*2RY8*	***PALB2***	*PARK2*	*PARP1*	*PARP2*	*PARP3*	*PAX5*
PBRM1	*PDCD1(PD-1)*	***PDCD1LG2 (PD-L2)***	***PDGFRA [Exons 12, 18, Introns 7, 9, 11]***	***PDGFRB [Exons 1 - 1, 23]***	*PDK1*	*PIK3C2B*	*PIK3C2G*	***PIK3CA [Exons , , 5-8, 10, 14, 19, 21 (Coding Exons 1, , 4-7, 9, 13, 18, 20)]***	*PIK3CB*

PIK3R1	*PIM1*	*PMS2*	*POLD1*	*POLE*	*PARG*	*2R1A*	*2R2A*	*PRDM1*	*PRKAR1A*
PRKCI	*PTCH1*	***PTEN***	***PTPN11***	*PTPRO*	*QKI*	*RAC1*	*RAD21*	*RAD51*	*RAD51B*
RAD51C	*RAD51D*	*RAD52*	*RAD54L*	***RAF1 [Exons , , 6, 7, 10, 14, 15, 17,*** *Introns -8]*	*RARA [Intron]*	***RB1***	*RBM10*	*REL*	***RET [****Introns 7, 8,* ***Exons 11, 13-16, Introns 9-11]***
RICTOR	*RNF43*	***ROS1 [Exons 1, -38, 0, Introns 1-]***	*RPTOR*	*RSPO2* [Intron 1]*	*SDC4* [Intron]*	*SDHA*	*SDHB*	*SDHC*	*SDHD*
SETD2	*SF3B1*	*SGK1*	*SLC34A2* [Intron]*	*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*	*WT1*
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	*LK, BRCA1, BRCA2, NTRK1, NTRK2, NTRK3*

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 and physicians. 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 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[1] 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	3	843	228	563	8			
FoundationOne Liquid CDx to Validated NGS Tumor Tissue Test Concordance: *RCA1* and *RCA2* Variants	Prostate cancer Ovarian cancer	279	N A	2		87	9 0		2
FoundationOne Liquid CDx to Validated NGS cfDNA Assay Concordance: *PIK3CA* mutations	Breast cancer	412	N A		32	5			
Orthogonal Concordance	23 cancer types Contrived samples	278	N/A	64	541	2		3	
LoD Estimation	Prostate Contrived samples		877	286	490	247	32	3	3
LoB Study 1	Healthy Donors	28	79	322	26134	4482	911	222	42
LoB Study 2[3]	Healthy Donors	44	31	532	29507	4438	2752	222	42
Potentially Interfering Substances	Contrived samples	9	336	8	6				2

Study Title	Cancer Types Represented	# Unique Samples	# of Sample Replicates	# of Unique Genes	# of Unique				
					Subs	Indels	Rearrang.	Copy Number Amplif.	Copy Number Losses
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	42	279	90	215	32	7	2
Reagent Interchangeability	Contrived samples	8	92	20	5				
Platform Precision study 1	Breast cancer Colon cancer Lung cancer Ovarian cancer Prostate cancer Skin cancer Contrived samples	47	21	280	900	229	63	49	5
Platform Precision study 2	Lung cancer Prostate cancer Stomach cancer Colorectal cancer Bile duct cancer Breast cancer		230	6	6	4			
Precision of detection of SNVs and indels that lead to *MET* exon 14 skipping (Precision study 3)	Lung Cancer	5	66		2	3	N/A	N/A	N/A
Platform Precision study 4[3]	Ovarian cancer Prostate cancer Breast cancer Lung cancer Colon adenocarcinoma Soft tissue neuroblastoma	7	402	59	258	43	6	22	
DNA Extraction	Colorectal cancer Prostate cancer Breast cancer Lungcancer Skin cancer	6	72	61	265	53	2 0		
Whole Blood Sample Stability	Lung cancer Colorectal cancer Gastrointestinal (non-Colorectal cancer) Prostate cancer Breast cancer Ovarian cancer	74	48	206	490	76	2	4	
Inverted Tube Whole Blood Sample Stability	Lung cancer Colorectal cancer Breast cancer Ovarian cancer Prostate cancer	56	3 2	280	295	95	9	27	
Cross Contamination	Contrived samples	5	376	39	9	5	4	21	
Guard Banding	Contrived samples		375	20	7	2	2		
Guard Banding with updated LC input[3]	Contrived samples	7	5	22	6		6		

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 for detection of *R* exon 19 deletions and L858R alterations: non-inferiority study[2]	Lung cancer	77	N/A		5	7	N/A	N/A	N/A
Clinical validation study for detection of deleterious alterations in *RCA1* and *RCA2* in prostate cancer[2]	Prostate cancer	99	N/A	2	44	55	8		
Clinical validation study for detection of *PIK3CA* mutations in breast cancer[2]	Breast	359	N/A		28	4			
Clinical validation study for *ALK* rearrangements in NSCLC[2]	Lung cancer	249	N/A		3				
Clinical validation study for *BRCA1, RCA2* , and *ATM* alterations in prostate cancer[2]	Prostate cancer	333	N/A	3	48	75			
Clinical validation study for detection of SNVs and indels that lead to *MET* exon 14 skipping[2]	Lung Cancer	71 [2]	N/A			22	N/A	N/A	N/A
Clinical validation study for detection of rearrangements that lead to *NTRK* fusions[2,]	Solid Tumor	203	N A	4	N/A	N/A	2	N/A	N/A
Clinical validation study for detection of rearrangements that lead to *ROS1* fusions[2]	Lung Cancer	203	N A	8	N A	N A	7	N A	N A
Clinical validation study for detection of *R* exon 20 insertions[2]	Lung Cancer	268	N A		N A	38	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	92	6	35	39	3	5	
Automation Line Equivalence	Contrived samples	8	87	303	926	337	63	61	4
Updated LC Method Comparison Study[3]	cancer types	81	324	338	4220	364	48	6	2

Study Title	Cancer Types Represented	# Unique Samples	# of Sample Replicates	# of Unique Genes	# of Unique				
					Subs	Indels	Rearrang.	Copy Number Amplif.	Copy Number Losses
Variant Report Curation	Breast cancer Colorectal cancer Lung cancer Prostate cancer Skin cancer	9	57	83	300	4	5		2
Pan-tumor performance (includes historical analysis)	20 cancer types	9868	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	815	376	9	46	N/A
FoundationOne Liquid CDx to Validated cfDNA NGS Assay Concordance: *MET* exon 14 (Primary Analysis)	Lung Cancer	72	N/A			21	N/A	N/A	N/A
FoundationOne Liquid CDx to Validated cfDNA NGS Assay Concordance: *NTRK* fusions[4]	Solid Tumor	6	N/A	5	N/A	N/A	4	N/A	N/A
Precision and LoD Confirmation of *NTRK* Gene Fusions in a Pan-tumor Setting[4]	Solid Tumor	4	93	6	N/A	N/A	4	N/A	N/A
FoundationOne Liquid CDx to Validated cfDNA NGS Assay Concordance: *R* exon 20 insertions	Lung Cancer	51	N A		N A	25	N/A	N/A	N/A
Precision and LoD Confirmation of *R* exon 20 insertions	Lung Cancer	3	72		N/A	3	N/A	N/A	N/A

[1]Variants detected may include variants classified as VUS and benign.
[2]Clinical validation study was conducted using the original LC input range for F1LCDx (30ng-80ng, with conditional processing of samples between 20-30ng)
[3]Study was conducted to validate the new LC input range for F1LCDx (20ng 60ng).

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 next generation sequencing (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 clinicalFoundationOne 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 positive percent agreement (PPA) of 96.2% with a 95% two-sided confidence interval (CI) of [94.8%-97.4%]. The short variant negative percent agreement (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 iquid CDx(+) Comparator(+)	FoundationOne Liquid CDx(-) Comparator(+)	FoundationOne iquid CDx(+) Comparator(-)	FoundationOne iquid CDx(-) Comparator(-)	PPA [95% CI]	NPA [95% CI]	OPA [95% CI]
All Short Variants	868	34	8	52824	96.2% [94.8% 97.4%]	>99.9% [99.9% %]	>99.9% [99.9% %]
Base Substitutions	845	34	8	49511	96.1% [94.6% 97.3%]	>99.9% [99.9% %]	>99.9% [99.9% %]
Indels	23			3313	% [85.2% %]	% [99.9% %]	% [99.9% %]

* 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	1
FoundationOne Liquid CDx ( )		1682	1682
Total	7	1685	1692
	PPA: % [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 = 78)

Targeted Alteration	*n*	PPA [95% CI]	NPA [95% CI]	PPV [95% CI]	NPV [95% CI]
RCA1 short variants*	1	100% [2.5%-100%]	100% [98.7%-100%]	100% [2.5%-100%]	100% [98.7%-100%]
RCA2 short variants*	2	100% [15.8%-100%]	100% [99.3%-100%]	100% [15.8%-100%]	100% [99.3%-100%]
R exon 19 deletions*	11	100% [71.5%-100%]	100% [99.7%-100%]	100% [71.5%-100%]	100% [99.7%-100%]

Targeted Alteration	*n*	PPA [95% CI]	NPA [95% CI]	PPV [95% CI]	NPV [95% CI]
R L858R*	10	100% [69.2%-100%]	100% [98.7%-100%]	100% [69.2%-100%]	100% [98.7%-100%]
PIK3CA base substitutions*	49	100% [92.7%-100%]	100% [99.9%-100%]	100% [92.7%-100%]	100% [99.9%-100%]
ALK rearrangements*	1	100% [2.5%-100%]	99.9% [99.7%-100%]	50% [1.3%-98.7%]	100% [99.3%-100%]
NTRK1 rearrangements*	3	100% [29.2%-100%]	100% [99.8%-100%]	100% [29.2%-100%]	100% [99.3%-100%]
ROS1 rearrangements*	1	100% [20.7%-100%]	99.6% [98.0%-99.9%]	50% [9.5%-90.6%]	100% [98.6%-100%]

*The PPA and NPA for these alterations are unadjusted

These data demonstrate that the FoundationOne Liquid CDx assay and an externally-validated NGS assay are highly concordant across the 76 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	1	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 Effectin PIK3CA	# VariantCalls (188 PositiveSamples)
C420R	3
E542K	25
E545A	1
E545G	2
E545K	50
H1047L	9
H1047R	100
H1047Y	1
Q546R	1
Total	**92**

A total of 412 valid samples generated valid results with both assays. The primary analysis using NGS Method #2as 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** , 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**), 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 LiquidCDx	**Positive**	165	20	2	1	**88**	PPA 1L: 89.19% [83.80%, 93.27%]
	Negative	5	222	1	2	**230**	NPA 1L : 97.80% [94.93%-99.28%]
	Evaluable but Failed		*7*			7	
	Not Evaluable	35	93	2		130	
	Total	205	342	5	3	555	
		PPA :97.06% [93.27%, 99.04%]	NPA :91.74% [87.52%, 94.88%]				OPA: 93.93% [91.17%, 96.04%]

9.4 Concordance – FoundationOne 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 40%.

Of the 214 samples, 179 samples had DNA yield that allowed processing with F1LCDx at the specified LC DNA inputof 30ng-80ng. Thirty-five (35) samples were tested with F1LCDx 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 F1LCDx, 3 samples had a F1LCDx 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 PPA of 94.87% with 95% CI (83.11%-98.58%), a NPA of 91 83% with 95% CI (85.80%, 95.32%), a 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 2** . Since the samples were selected from different sources based on different assays, the unadjusted PPA/NPA andunadjusted PPV/NPV in **Table 2** may be subject to potential bias.

Table 2. Primary Concordance Analysis Comparing Sample-level Biomarker Detection between FoundationOne Liquid CDx and Comparator Assay

		evNGS			
		***ET* ex14 positive**	***ET* ex14 negative**	**Total**	**PPV/NPV(95% CI)**
F1LCDx	***ET* ex14 positive**	37	11	48	PPV: 77.08% (63.46%, 86.69%)
	***ET* 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 F1LCDx-positive/evNGS-negative [F1LCDx(+)/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 F1LCDx (+)/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 F1LCDx(+)/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 , +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 F1LCDx negative(-)/evNGS(+), base substitutions reported by the evNGS were not detected in the variant analysis pipeline of F1LCDx.

Four (4) of the eleven (11) discordant samples that were F1LCDx(+)/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 F1LCDx(+)/evNGS(-) were *MET* exon 14 deletion positive.

9.5 Concordance – FoundationOne Liquid CDx to an externally validated cfDNA NGS assay (NTRK1/2/3 Fusions)

An analytical accuracy study was performed to demonstrate the concordance between FoundationOne Liquid CDx and an externally validated cfDNA NGS comparator assay for the detection of *NTRK* fusions. For this study, seven (7) residual cfDNA samples were selected from patients enrolled in the STARTRK 2 trial used to support the effectiveness of the device, seven (7) residual cfDNA clinical samples were externally sourced, and 102 residual cfDNA samples were sourced from FMI's clinical archives. Overall, a total of 116 sample replicates were processed using F1LCDx in this study. Of the 116 samples, 113 were processed with the evNGS. Of the 113 samples run by both assays for this study, one (1) sample had an F1LCDx post-sequencing QC failure, while 1 had an evNGS post-sequencing QC failure.

Measures of analytical concordance for the 102 samples that passed QC with both assays were determined. Since specimens were selected based on F1LCDx and confirmed by the evNGS agreement, PPV and NPV are estimated conditional on F1LCDx. PPV was estimated as 40% (4/10) with two-sided 95% CI (16.8%, 68.7%), and NPV as 100% (92/92) with two-sided 95% CI (95.99%, 1 %) , as shown in **Table 3** , below. For informational purposes, unadjusted positive percent agreement (PPA) and negative percent agreement (NPA) are also displayed.

Table 13. Concordance Analysis Comparing Sample-level Biomarker Detection between F1LCDx and evNGS

		evNGS			
		***NTRK1/2/3* fusion positive**	***NTRK1/2/3* fusion negative**	**Total**	**PPV/NPV (95% CI)**
F1LCDx	***NTRK1/2/3* fusion positive**[1]	4	6[2]	10	PPV: 40.0% (16.8%, 68.7%)
	***NTRK1/2/3* fusion negative**		92	92	NPV: 100% (95.99%, 100%)
	Total	4	98	102	
	PPA/NPA (Unadjusted) (95% CI)	PPA: 100% (51.01%, 100%)	NPA: 93.9% (87.3%, 97.2%)		

[1]No *NTRK2* fusion positive samples were evaluated in this study
[2]These six samples were discordant due to the fusion breakpoints falling in regions that the evNGS assay does not bait for.

The six (6) samples that were *NTRK1/2/3* fusion positive by F1LCDx and *NTRK1/2/3* fusion negative by the evNGS were discordant due to the fusion breakpoints falling in regions that the evNGS assay does not bait for. Specifically, the evNGS assay did not claim to generate coverage in certain regions of interest (e.g., intron 8 of *NTRK1* and intron 5 of *ETV6*), and thus were negative by the evNGS comparator assay.

9.6 Concordance – FoundationOne Liquid CDx to an externally validated cfDNA NGS assay (*EGFR* exon 20 insertions)

An analytical accuracy study was performed to demonstrate the concordance between FoundationOne Liquid CDx and an externally validated cfDNA NGS comparator assay for the detection of *EGFR* exon 20 insertions. For this study, 101 frozen plasma samples were identified from patients enrolled in the AP32788-15-101 trial and 125 residual cfDNA samples were sourced from FMI's clinical archives. Of the 125 residual cfDNA samples, four (4) were excluded due to diluted DNA concentration being out of acceptable range or evNGS post sequencing QC failure. Of the 101 frozen plasma samples, 71 were excluded from the analysis due to insufficient cfDNA yield, diluted DNA concentration being out of acceptable range, or evNGS post-sequencing QC failure.

Overall, a total of 151 samples from NSCLC patients were processed using both F1LCDx and an externally validated cfDNA NGS assay in this study.

Analytical concordance was determined for the 151 samples that passed QC with both assays. Since specimens were selected based on F1LCDx and confirmed by the evNGS assay, positive predictive value (PPV) and negative predictive value (NPV) are estimated conditional on F1LCDx. Forty-nine (49) of the 151 samples were identified as positive for *EGFR* exon 20 insertions by both F1LCDx and evNGS. The statistical analysis showed a PPV of 100% with two-sided 95% CI [92.70%-100%] and a NPV of 99.02% with two-sided 95% CI [94.65%-99.83%], as shown in **Table 14** below.

Table 14. Concordance Analysis Comparing Sample-Level Biomarker Detection Between F1LCDx and evNGS

		evNGS			
		R **exon 20 insertion positive**	***R*** **exon 20 insertion negative**	**Total**	**PPV/NPV (95% CI[1])**
F1LCDx	***R*** **exon 20 insertion positive**	49		49	PPV: 100% (92.70%, 100%)
	R **exon 20 insertion negative**	1	101	102	NPV: 99.02% (94.65%, 99.83%)
	Total	50	1 1	151	
	PPA/NPA (Unadjusted) (95% CI[1])	PPA: 98.00% (89.50%, 99.65%)	NPA: 100% (96.34%, 100%)		

In the one (1) discordant sample that was F1LCDx-negative/evNGS positive a 3 bp *EGFR* exon 20 insertion reported by the evNGS was not detected in the variant analysis pipeline of F1LCDx.

9.7 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 9 % were observed). LoD by hit rate was defined as the mean variant allele frequency (VAF) value (for short variants and rearrangements) or mean tumor fraction (TF) 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 perthe 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.

Confirmed LoDs for CDx alterations are presented below in **Table 515** and are taken from the confirmation of LoD studies as presented in **Section 9.12**. The confirmation of LoD studies utilized clinical samples assessed near the established LoD (targeting 1x-1.5x LoD). The confirmed LoD for targeted short variants, rearrangements, and copy number alterations demonstrate at least a 95% hit rate at a level near the established LoD (**Table 16**).

Table 1515. Established and Confirmed LoD for CDx alterations

Gene	Alteration Subtype	Established LoD	Confirmed LoD (Fold LoD)
ATM	Substitutions	51% VAF	56% VAF (1.09x)
	Indels	51% VAF	.86% VAF (1.68x)
	Rearrangement	Not Determined	1.13% VAF (N/A)
RCA1	Substitutions	34% VAF	51% VAF (1.49x)
	Indels	38% VAF	.55% VAF (1.44x)

Gene	Alteration Subtype	Established LoD	Confirmed LoD (Fold LoD)
	Rearrangement	Not Determined	0.87% VAF (N/A)[1, 2]
RCA2	Substitutions	Not Determined	0.71% VAF (N/A)
	Indels	36% VAF	63% VAF (1.74x)
	Rearrangement	Not Determined	0.48% VAF (N/A)[1, 3]
	Copy Number Loss	48.1% T [4]	N A
R	Substitutions (L858R)	34% VAF	.64% VAF (1.90x)
	Indels (exon 19 deletions)	27% VAF	.45% VAF (1.65x)
	Indels (exon 20 insertions)	Not Determined	65% VAF (N/A) [1]
PIK3CA	Substitutions	.34% VAF	.39% VAF (1.14x)
ALK	Rearrangement	24% VAF	.68% VAF (2.84x)
MET	Indels (exon 14)	41% VAF	.28% VAF (0.67x)
	Substitutions (exon 14)	Not Determined	0.40% VAF (N/A)[1]
NTRK1	Rearrangement	44% VAF	75% VAF (1.70x)
NTRK3	Rearrangement	27% VAF	68% VAF (2.52x)
ROS1	Rearrangement	52% VAF	1 30% VAF (2.51x)

[1]Confirmation of LoD was performed without direct LoD establishment data. Platform LoD was used for the targeted dilution level
[2]Confirmed LoD for BRCA1 RE was using the DIBv1 primer set. LoD was also confirmed using the DIBv2 primer set at 1.27% VA
[3]Confirmed LoD for BRCA2 RE was using the DIBv1 primer set. LoD was also confirmed using the DIBv2 primer set at 1.49% VA
[4]LoD was established in a clinical sample and therefore confirmation of LoD was not applicable

The platform LoD for short variants, rearrangements, and copy number losses are presented in **Table 6.** 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 by alteration subtype was also evaluated within categories based on genomic context as summarized in **Table 16**

Table 16. LoD by variant subtype based on genomic context

Region	Alteration Subtype	LoD Unit	N	Minimum LoD	st Quantile LoD	Median LoD	3rd Quantile LoD
Enhanced Sensitivity Region	**Short Variants: Enhanced Sensitivity Region Total**	VAF	**69**	**%**	**33%**	**40%**	**50%**
	Insertion/Deletion in non-repetitive region or a repetitive region of <=3 base pairs			23%	29%	31%	36%
	Insertion/Deletion in a repetitive region of 4 to 6base pairs		23	28%	37%	48%	56%
	Insertion/Deletion in a repetitive region of >=7base pairs		6	33%	48%	58%	82%
	Substitution in a non-repetitive region or a repetitive region of <=7base pairs		229	20%	33%	39%	49%
	Substitution in a repetitiveregion of >7 base pairs			32%	32%	32%	32%
Standard SensitivityRegion	**Short Variants: High Sensitivity Region Total**	VAF	**595**	**40%**	**70%**	**82%**	**98%**

Region	Alteration Subtype	LoD Unit	N	Minimum LoD	st Quantile LoD	Median LoD	3rd Quantile LoD
	Insertion/Deletion in non-repetitive region or a repetitive region of <=3 base pairs		8	46%	68%	87%	%
	Insertion/Deletion in a repetitive region of 4 to 6base pairs		32	61%	75%	87%	95%
	Insertion/Deletion in a repetitive region of >=7base pairs			59%	7%	5%	20%
	Substitution in a non- repetitive region or a repetitive region of <=7base pairs		524	40%	70%	81%	96%
	Substitution in a repetitiveregion of >7 base pairs		8	69%	83%	96%	28%
Enhanced Sensitivity Region	**Rearrangements**	VAF	**7**	**%**	**6%**	**37%**	**47%**
Enhanced/ Standard Sensitivity Region	**Rearrangements**	VAF		**8%**	**8%**	**8%**	**8%**
Standard Sensitivity Region	**Rearrangements**	VAF		**90%**	**90%**	**90%**	**90%**
NA	**Copy Number Amplifications**	TF	**8**	**9.8%**	**9.8%**	**7%**	**5.2%**

The median LoD for highly-actionable, non-CDx alterations evaluated for LoD are presented in **Table 7** The median LoD for these targeted short variants are consistent with the platform LoD presented in **Table 15**

Table 1717. LoD for non-CDx alterations

Gene	Alteration Subtype	Number of Samples Evaluated	Median LoD[1]
RAF	Substitutions	1	33% VAF
KRAS	Substitutions	2	33% VAF
MET[2]	Indels	1	41% VAF
NRAS	Substitutions	2	42% VAF
PALB2	Indels	1	37% VAF
	Substitutions	1	51% VAF
RBB2	Copy Number Amplification	1	19.8% TF

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

9.8 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 6 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 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 unique 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 total 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 27% (**Table18**).

Table 18. 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 of 292)	% (0 of 23,068)
Level 2	% (0 of 10)	% (0 of 790)
Level 3	% (0 of 18)	% (0 of 1,422)
Level 4	.82% (47 of 5,760)	.024% (107 of 455,040)
VUS	0.83% (203 of 24,542)	.029% (555 of 1,938,818)
All categories	0.82% (250 of 30,622)	.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.

A supplemental LoB study was performed for F1LCDx to support the updated LC input range (20ng-60ng) and evaluate variants observed in gDNA. Whole blood samples from 44 healthy donors were collected to prepare two plasma cfDNA replicates per donor for a total of 88 cfDNA sample replicates. A total of 87 cfDNA replicates were run between 2 60ng DNA input, with 1 cfDNA replicate failure at the DNA extraction step. Additionally, one matched gDNA replicate per donor was isolated from buffy coat and mechanically fragmented by sonication for F1LCDx testing to obtain non-tumor variant (e.g., germline) information and support LoB analysis. A total of 44 gDNA replicates passed the QC steps.

All variants were determined to have an LoB of 0, based on the detection rate not significantly exceeding 5%. On a per unique variant basis, the overall detection rate in this study was .24%. On a total variant basis, the overall detection rate was 38%. **Table 9** provides the unique variant detection rate and overall variant LoB for variants at each variant level/category using the same definitions of unique variant detection rate and total variant detection rate as in **Table 8** . The results in **Table 9** are based on variants detected in cfDNA replicates only (variant detected in the matching gDNA replicate were subtracted) for each sample.

Table 19. 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	22% (2 of 898)	26% (2 of 78,126)
Level 2	% (0 of 1)	% (0 of 87)
Level 3a	N A (0 of 0)	N/A (0 of 0)
Level 3b	66% (2 of 302)	114% (3 of 26,274)
Level 4	25% (18 of 7,154)	35% (22 of 622,398)
VUS	23% (65 of 28,606)	38% (94 of 2,488,722)
All categories	24% (87 of 36,961)	38% (121 of 3,215,607)

[1] total variants analyzed = unique variants * 44 replicates

Across 264 copy number alterations and 2752 rearrangements, one rearrangement variant was detected. These results demonstrate the high specificity of FoundationOne Liquid CDx.

9 9 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 these substances. 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 20**

Table 20. Process success rates with interfering substances

Process	# Failed	# Pass	Total	Success Rate (%)	95% CI LB (%)	95% CI UB (%)
DNA Extraction		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		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. Ofthe 11 potential interferents tested across 16 conditions, concordance for all variant calls was 1 % for 8 conditions and ≥97% for all conditions (**Table 21**).

Table 21. Concordance per substance for variants ≥1x LoD

Substance	Detected Reps	Total Reps	Concordance	95% two-sided exact CI_lower	95% two-sided exact CI_upper
Triglycerides, 37 mmol/L (or 33 g/L)	80	8	100.00%	95.49%	100.00%
Hemoglobin, 2 g L	78	78	100.00%	95.38%	100.00%
Albumin, 60 g/L	80	82	97.56%	91.47%	99.7%
Bilirubin (conjugated), 2 g/L	84	84	100.00%	95.7%	1 %
Bilirubin (unconjugated), 2 g/L	76	78	97.44%	91.04%	99.69%
Cholesterol Level 2, 3 88 mmol (150 mg/dL)	80	82	97.56%	91.47%	99.7%
Cholesterol Level , 6 47mmol (250 mg/dL)	74	76	97.37%	90.82%	99.68%
Staphylococcus epidermidis, x 6 CFU/mL	78	78	100.00%	95.38%	100.00%
Anticoagulant, 5X nominal volume	82	82	100.00%	95.6%	1 %
Proteinase K, + 6 mg/mL	98	99	98.99%	94.50%	99.97%
Proteinase K, + 3 mg/mL	92	92	100.00%	96.07%	1 %
Ethanol, +2.5%	96	98	97.96%	92.82%	99.75%
Ethanol, +5.0%	94	95	98.95%	94.27%	99.97%
Molecular Index barcodes, +5%	70	72	97.22%	90.32%	99.66%
Molecular Index barcodes, +15%	96	96	100.00%	96.23%	100.00%
Molecular Index barcodes, +30%	98	98	100.00%	96.31%	100.00%

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

9.10 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.

9.11 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 53% (2/376) was observed in this study. These data demonstrate a low probability of cross contamination during the FoundationOne Liquid CDx process.

9.12 Precision: Reproducibility and Confirmation of LoD

Multiple Precision and Confirmation of LoD studies were performed, using both clinical and contrived samples to evaluate precision and only clinical samples for confirmation of LoD. Precision was evaluated for alterations associated with both CDx claims and tumor profiling. Target alterations were assessed at two target levels each (near LoD and 2-3x LoD) for the contrived samples, and at one level (targeting 1-1.5x LoD) for clinical cfDNA samples.

In all studies, 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. Reproducibility was assessed and compared across three lots, two sequences, and two processing runs. Samples were processed near the assay's minimum DNA input mass.

The studies evaluate the precision of FoundationOne Liquid CDx for detecting a set of highly actionable variants. **Table 22** and **Table 23** summarize the Disease Ontology (if applicable), Variant Subtype, Targeted Variant, Reproducibility, Average Measurand, and LoD for each sample with CDx variants and non-CDx variants, respectively.

Table 22. Precision and Confirmation of LoD by Targeted CDx Variant

Targeted Variant	Variant Subtype	Cancer Type	Reproducibility (%) (95% Two-sided CI)	Average Measurand	LoD
ALK_EML4_fusion	RE	Lung cancer	(86.2, 100)	68% VAF[1]	.24% VA
ALK-EML4 fusion	RE		100 (85.75, 100)	39% VAF[1]	.24% VA
ALK-EML4 fusion	RE	Contrived	(85.75, 100)	.64% VAF	24% VAF
ALK-EML4 fusion	RE		100 (85.18, 100)	89% VAF	24% VAF
ALK-NPM1 fusion	RE		78.26 (56.3, 92.54)	.4% VA	.94% VA
ALK-NPM1 fusion	RE		100 (85.75, 100)	64% VAF	94% VAF
ATM I2012fs*4	Indel	Prostate cancer	100 (85.18, 100)	86% VAF[1]	.51% VA
ATM K1773fs*3	Indel	Contrived	(85.75, 100)	.77% VAF	51% VAF
ATM K1773fs*3	Indel		(85.18, 100)	.04% VAF	51% VAF
ATM splice site 8850+1G>A	Sub	Prostate cancer	(85.75, 100)	56% VAF[1]	.51% VA
ATM-EXPH5 truncation	RE		100 (85.75, 100)	3% VAF [1]	Not Determined
BRCA N1784fs*3	Indel	Stomach cancer	87.5 (69, 95.7)	34% VAF	.36% VA
BRCA1 D825fs*21	Indel	Contrived	(85.75, 100)	61% VAF	.38% VAF

Targeted Variant	Variant Subtype	Cancer Type	Reproducibility (%) (95% Two-sided CI)	Average Measurand	LoD
BRCA1 D825fs*21	Indel		100 (85.75, 100)	93% VAF	38% VAF
BRCA1 E23fs*17	Indel	Ovary cancer	(85.75,)	66% VAF[1]	.38% VA
BRCA1 P871fs*32	Indel	Contrived	(85.18, 100)	.51% VAF	38% VAF
BRCA1 P871fs*32	Indel		100 (85.75, 100)	8% VAF	38% VAF
BRCA1 Q780*	Sub	Ovary cancer	(85.75, 100)	% VAF [1]	.34% VA
BRCA1 Y465*	Sub	Prostate cancer	(86.2, 100)	51% VAF[1]	.34% VA
BRCA1_D1840fs*32	del		95.83 (79.76, 99.26)	55% VAF [1]	.38% VA
BRCA1_N/A_truncation	RE		(86.2, 100)	27% VAF[1]	Not Determined
BRCA1_S646fs*5	del	Ovary cancer	(85.69, 100)	54% VAF[1]	.38% VA
BRCA1_Y1563*	Sub		(86.2, 100)	66% VAF[1]	.51% VA
BRCA1-BRCA1 deletion	RE		(85.75, 100)	87% VAF[1]	.28% VA [2]
BRCA2 C1200fs*1	Indel	Contrived	(85.75, 100)	.58% VAF	36% VAF
BRCA2 C1200fs*1	Indel		100 (85.75, 100)	92% VAF	36% VAF
BRCA2 G267*	Sub	Ovary cancer	9 67 (73, 98.97)	5% VAF	Not Determined
BRCA2 N1784fs*7	Indel	Contrived	(85.75, 100)	.22% VAF	36% VAF
BRCA2 N1784fs*7	Indel		100 (85.75, 100)	85% VAF	36% VAF
BRCA2 N1784fs*7	Indel		100 (85.18, 100)	7% VAF	36% VAF
BRCA2 N1784fs*7	Indel		100 (85.75, 100)	2 24% VAF	36% VAF
BRCA2 N1822fs*2	Indel		100 (85.75, 100)	92% VAF	36% VAF
BRCA2 N1822fs*2	Indel		100 (85.18, 100)	9% VAF	36% VAF
BRCA2 Q1429fs*9	Indel		(85.75, 100)	94% VAF	36% VAF
BRCA2 Q1429fs*9	Indel		(85.18, 100)	26% VAF	36% VAF
BRCA2 S2988fs*12	Indel	Ovary cancer	(85.75,)	7% VAF [1]	.36% VA
BRCA2 T3033fs*11	Indel	Contrived	21.74 (7.46, 43.7)	71% VAF	36% VAF
BRCA2 T3033fs*11	Indel		91.67 (73, 98.97)	3% VAF	36% VAF
BRCA2_CDH17_truncation	RE	Prostate cancer	(86.2, 100)	.49% VAF[1]	Not Determined
BRCA2_E2198fs*4	del	Ovarian cancer	(86.2, 100)	65% VAF[1]	.36% VA
BRCA2_G995fs*4	del	Prostate cancer	95.83 (79.76, 99.26)	63% VAF [1]	.36% VA
BRCA2_loss	CN		(86.2, 100)	53.11% TF[1]	48.1% TF
BRCA2_loss	CN		87.5 (67.64, 97.34)	39.43% TF	48.1% TF
BRCA2_N/A_truncation	RE		70.83 (50.83, 85.09)	32% VAF	48% VAF
BRCA2_N3124I	Sub	Ovarian cancer	(86.2, 100)	74% VAF[1]	.49% VA
BRCA2_Q1361*	sub	Prostate cancer	(85.69, 100)	71% VAF[1]	.49% VA
BRCA2-EDA truncation	RE		(85.18, 100)	48% VAF[1]	.47% VA [2]
EGFR E746 A750del	Indel	Lung cancer	95.7 (79, 99.2)	45% VAF[1]	.27% VA
EGFR E746_A750del	Indel		100 (84.56, 100)	34% VAF[1]	.27% VA
EGFR E746_A750del	Indel	Contrived	(85.75, 100)	.51% VAF	27% VAF
EGFR E746_A750del	Indel		(85.75,)	74% VAF	27% VAF
EGFR E746_A750del	Indel		(85.75,)	93% VAF	27% VAF
EGFR E746_A750del	Indel		100 (85.18, 100)	2% VAF	27% VAF
EGFR E746_A750del	Indel		(85.18,)	51% VAF	27% VAF
EGFR E746_A750del	Indel		(85.75,)	% VAF	27% VAF
EGFR L858R	Sub	Lung cancer	(85.75, 100)	64% VAF[1]	.34% VA
EGFR L858R	Sub		100 (85.75, 100)	64% VAF[1]	.34% VA
EGFR L858R	Sub	Contrived	100 (85.75, 100)	46% VAF	34% VAF

Targeted Variant	Variant Subtype	Cancer Type	Reproducibility (%) (95% Two-sided CI)	Average Measurand	LoD
EGFR L858R	Sub		(85.75,)	68% VAF	34% VAF
EGFR L858R	Sub		(85.75,)	68% VAF	34% VAF
EGFR L858R	Sub		(85.18,)	95% VAF	34% VAF
EGFR ex20 insertion H773_V774insH	Indel	Lung Cancer	(86.2, 100)	98% VAF[1]	Not Determined
EGFR ex20 insertion V769_D770insASV	Indel		(86.2, 100)	28% VAF[1]	Not Determined
EGFR ex20 insertion D770_N771insSVD	Indel		100 (86.2, 100)	65% VAF[1]	Not Determined
ETV6-NTRK3 fusion	RE	Thyroid cancer	(86.20, 100)	82% VAF[1]	.27% VA
ETV6-NTRK3 fusion	RE	Contrived	95.83 (78.88, 99.89)	32% VAF	474% VAF[2]
ETV6-NTRK3 fusion	RE		95.83 (78.88, 99.89)	59% VAF	474% VAF[2]
ETV6-NTRK3 fusion	RE	Lung cancer	(85.75, 100)	26.33% VAF	474% VAF[2]
ETV6-NTRK3 fusion	RE	Salivary gland cancer	(85.69, 100)	.68% VAF[1]	.27% VA
GOPC-ROS1 fusion	RE	Contrived	86.96 (66.41, 97.22)	35% VAF	474% VAF[2]
GOPC-ROS1 fusion	RE		91.67 (73, 98.97)	91% VAF	474% VAF[2]
MET exon14 splice site 2888-35_2889>A	Indel	Lung cancer	95.8 (79.8, 99.3)	28% VAF[1]	.41% VA
MET exon14 splice site 3028+1G>T	Sub		95.8 (79.8, 99.3)	45% VAF[1]	Not Determined
MET exon14 splice site 3028+2T>C	Sub		95.7 (79.0, 99.2)	35% VAF[1]	Not Determined
MET exon14splice site 3028+1G>T	Sub		(85.7, 100)	.85% VAF	Not Determined
MET exon14splice site 3028+2T>C	Sub		(85.75, 100)	.76% VAF	Not Determined
MET splice site 3029-1G>T	Sub	Contrived	62.5 (40.59, 81.2)	.21% VAF	Not Determined
MET splice site 3029-1G>T	Sub		91.3 (71.96, 98.93)	3% VAF	Not Determined
MET splice site 2888-7_2888-3del15	Indel	Lung cancer	(85.75, 100)	7% VAF [1]	.41% VA
MET splice site 3005_3028+3>C	Indel		100 (85.75, 100)	67% VAF[1]	.41% VA
MPRIP-NTRK1 fusion	RE	Contrived	69.57 (47.08, 86.79)	49% VAF	44% VAF
MPRIP-NTRK1 fusion	RE		87.5 (67.64, 97.34)	.69% VA	.44% VA
PIK3CA E542K	Sub	Breast cancer	(85.75, 100)	89% VAF[1]	.34% VA
PIK3CA E545A	Sub	Contrived	(85.75, 100)	.52% VAF	34% VAF
PIK3CA E545A	Sub		100 (85.18, 100)	7% VAF	34% VAF
PIK3CA E545K	Sub	Breast cancer	(85.75, 100)	5% VAF[1]	.34% VA
PIK3CA E545K	Sub	Contrived	(85.75, 100)	.45% VAF	34% VAF
PIK3CA E545K	Sub		(85.75,)	66% VAF	34% VAF
PIK3CA H1047R	Sub	Breast cancer	(85.75, 100)	4% VAF [1]	.34% VA
PIK3CA H1047R	Sub	Contrived	(85.18, 100)	.41% VAF	34% VAF
PIK3CA H1047R	Sub		(85.75,)	76% VAF	34% VAF
PIK3CA Q546R	Sub	Breast cancer	91.7 (74.2, 97.7)	44% VAF	.34% VAF
PIK3CA Q546R	Sub	Contrived	95.65 (78.05, 99.89)	49% VAF	34% VAF
PIK3CA Q546R	Sub		(85.75,)	92% VAF	34% VAF
PIK3CA_H1047R	Sub	Breast cancer	95.65 (79.01, 99.23)	39% VAF [1]	.34% VA
PTEN_loss	CN		100 (85.75, 100)	46.89% TF[1]	2.7% TF
ROS1-CD74 fusion	RE	Lung cancer	(85.75, 100)	32% VAF[1]	.52% VAF
ROS1-EZR fusion	RE		100 (85.75, 100)	3% VAF[1]	.52% VAF
SLC34A2-ROS1 fusion	RE	Contrived	(85.75, 100)	3% VAF	.284% VAF[2]

Targeted Variant	Variant Subtype	Cancer Type	Reproducibility (%) (95% Two-sided CI)	Average Measurand	LoD
SLC34A2-ROS1 fusion	RE		(85. 8, 100)	36% VAF	284% VAF[2]
TPM3-NTRK1 fusion	RE	Lung cancer	91.67 (73, 98.97)	8 48% VAF	44% VAF
TPM3-NTRK fusion	RE	Contrived	(85.75, 100)	.3% VAF	44% VAF
TPM3-NTRK1 fusion	RE		100 (85.75, 100)	4% VAF	44% VAF
TPM3-NTRK1 fusion	RE	Colon cancer	100 (85.69, 100)	83% VAF[1]	44% VAF
TPR-NTRK1 fusion	RE	Thyroid cancer	100 (85.69, 100)	75% VAF[1]	44% VAF[2]

[1] LoD was confirmed for these variants with hit rate (same as the reproducibility) which met the acceptance criteria defined in respective study.

[2] LoD was not determined for these specific variants; platform LoD for the variant type is listed

Table 23. Precision and Confirmation of LoD by Targeted Non-CDx Variant

Targeted Variant	Variant Subtype	Cancer Type	Reproducibility (%) (95% Two-sided CI)	Average Measurand	LoD
BRAF L597R	Sub	Contrived	95.65 (78.05, 99.89)	42% VAF	49% VAF
BRAF L597R	Sub		(85.75, 1)	85% VAF	49% VAF
BRAF V600E	Sub	Skin cancer	(85.75, 100)	44% VAF [1]	33% VAF
BRAF V600E	Sub	Contrived	(85.18, 100)	72% VAF	49% VAF
BRAF V600E	Sub		(85.75, 100)	38% VAF	49% VAF
BRAF V600K	Indel	Skin cancer	95.83 (78.88, 99.89)	36% VAF[1]	33% VAF
BRCA2 R2842C	Sub	Lung cancer	(85.7, 100)	57% VAF [1]	49% VAF
EGFR S492R	Sub	Colon cancer	71.4 (45.4, 88.3)	39% VAF	.34% VAF
EGFR T790M	Sub	Lung cancer	(85.75, 100)	26% VAF [1]	34% VAF
EGFR T790M	Sub	Contrived	(85.18, 100)	36% VAF	49% VAF
EGFR T790M	Sub		(85.75, 1)	65% VAF	49% VAF
EGFR T790M	Sub		(85.75, 1)	44% VAF	49% VAF
EGFR T790M	Sub		(85.75, 1)	66% VAF	49% VAF
ERBB2_amplification	CN	Breast cancer	(85.75, 100)	61.73% T [1]	9.8% TF
ERBB2_amplification	CN	Lung cancer	(85.69, 100)	% T [1]	9.8% TF
ERBB2_amplification	CN	Colon cancer	(86.2, 100)	31.05% T [1]	9.8% TF
ERBB2_amplification	CN	Unknown primary cancer	(85.69, 100)	33.12% T [1]	9.8% TF
ERBB2_amplification	CN	Contrived	(85.75, 100)	35.78% TF	25.2% TF
ERBB2_amplification	CN		100 (85.75, 100)	39.79% TF	25.2% TF
ERBB2_amplification	CN	Soft tissue cancer	(0, 13.8)	54.53% TF	9.8% TF
ERBB2_amplification	CN	Lung cancer	(0, 14.31)	54.8% TF	19.8% TF
KRAS G12D	Sub	Contrived	(85.75, 100)	89% VAF	49% VAF
KRAS G12D	Sub		(85.18, 1)	2% VAF	49% VAF
KRAS G12L	Sub	Colon cancer	(85.75, 100)	49% VAF [1]	33% VAF
KRAS G13D	Sub	Contrived	(85.75, 100)	55% VAF	49% VAF
KRAS G13D	Sub		(85.75, 1)	82% VAF	49% VAF
KRAS G13D	Sub		(85.18, 1)	57% VAF	49% VAF
KRAS G13D	Sub		(85.75, 1)	92% VAF	49% VAF
KRAS Q61R	Sub	Colon cancer	(85.75, 100)	53% VAF [1]	33% VAF
MET L1312fs*4	Indel	Contrived	(85.75, 100)	69% VAF	56% VAF
MET L1312fs*4	Indel		100 (85.75, 100)	96% VAF	56% VAF
NRAS G12C	Sub		(85.75, 1)	69% VAF	49% VAF
NRAS G12C	Sub		(85.75, 1)	96% VAF	49% VAF

Targeted Variant	Variant Subtype	Cancer Type	Reproducibility (%) (95% Two-sided CI)	Average Measurand	LoD
NRAS G12C	Sub	Lung cancer	91.3 (73.2, 97.6)	.55% VAF	42% VAF
NRAS G12D	Sub	Contrived	82.61 (61.22, 95.05)	48% VAF	49% VAF
NRAS G12D	Sub		(85.75, 1)	84% VAF	49% VAF
NTRK2-N/A rearrangement	RE		95.83 (78.88, 99.89)	85% VAF	897% VAF
NTRK2-N/A rearrangement	RE		95.83 (78.88, 99.89)	2 3% VAF	897% VAF
PALB2 G808*	Sub		(85.18, 100)	47% VAF	49% VAF
PALB2 G808*	Sub		(85.75, 100)	92% VAF	49% VAF
PALB2 K908fs*15	Indel		100 (85.75, 100)	52% VAF	56% VAF
PALB2 K908fs*15	Indel		100 (85.75, 100)	74% VAF	56% VAF
PALB2 N280fs*8	Indel	Colon cancer	(56.6, 100)	48% VAF [1]	37% VAF
PIK3CA D549N	Sub	Contrived	(85.75, 100)	48% VAF	49% VAF
PIK3CA D549N	Sub		(85.75, 1)	73% VAF	49% VAF
PTEN_loss	CN		75 (53.29, 90.23)	44.04% TF	2.7% TF
PTEN_loss	CN		(85.75, 100)	59.26% TF	2.7% TF
RET-CCDC6 fusion	RE		95.83 (78.88, 99.89)	22% VAF	474% VAF
RET-CCDC6 fusion	RE		100 (85.75, 00)	39% VAF	474% VAF

[1] LoD was confirmed for these variants with hit rate (same as the reproducibility) which met the acceptance criteria defined in respective study.

[2] LoD was not determined for these specific variants; platform LoD for the variant type is listed.

Assessment of Tumor Profiling Variants

Across 39 unique samples, including 8 contrived samples, and 31 clinical samples, a total of 1,240 variants were evaluated for reproducibility and repeatability of tumor profiling variants, with variant types including substitutions, indels, rearrangements, and copy number alterations. The number of variants in each variant bin are summarized in **Table 24.** The overall repeatability for all variants were 99.47% with 95% 2-sided exact CIs [99.45%, 99.48%]. The overall reproducibility results were 99.59% with the 95% 2 sided exact CIs [99.58%, 99.60%]. The repeatability and reproducibility results for each variant type are summarized in **Table 24**

Table 24. Number of each variant type

Variant Category	N	# of Pairs Agree/ # of Total Pairs	Repeatability (%) [95% Two-Sided Exact CIs (%)]	# of Replicates Agree/ # of Total Replicates	Reproducibility (%) [95% Two-Sided Exact CIs (%)]
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**	126475 / 127224	99.41 [99.37, 99.45]	254509 / 255588	99.58 [99.55, 99.60]
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				

Variant Category	N	# of Pairs Agree/ # of Total Pairs	Repeatability (%) [95% Two-Sided Exact CIs (%)]	# of Replicates Agree/ # of Total Replicates	Reproducibility (%) [95% Two-Sided Exact CIs (%)]
Rearrangements	**60**	33105 / 33480	98.88 [98.76, 98.99]	66723 / 67260	99.20 [99.13, 99.27]
Copy Number Alterations	**54**	29880 / 30132	99.16 [99.05, 99.26]	60115 / 60534	99.31 [99.24, 99.7]
Copy Number Amplification	49				
Copy Number Loss	5				
Total	**240**	**688225 / 691920**	**99.47 [99.45, 99.48]**	**384328 / 390040**	**99.59 [99.58, 99.60]**

9 3 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. ight reagent pairings were assessed. A total of eight analyses for each specimen were completed. A total of 192 tests 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

9.14 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%).

9 5 Stability

9 5 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. ach 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. Thethree 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 25**. The reason for the failure of T3 (9 months) was 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.

Table 25. Concordance for Tumor Profiling Variants at Replicate Level by Reagent Lot and by Timepoint

Reagent Lot	Timepoint[1]	# Concordant	# Total	Concordance (%)	95% 2-sided scoreCI (%)
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%]

A supplemental study is being conducted to evaluate the stability of updated LC reagents. The study will confirm that reagent stability can be claimed as 12 months for the F1LCDx assay with the changed reagents

9.15.2 Whole Blood Specimen Stability

The recommended storage temperature is 18°C 25°Cn this study stress conditionswere 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 was 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 26**

Table 26. 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), LC, 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 27**). 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 27. 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 28**

Table 28. Concordance of detected alterations between baseline sample and experimental conditions for inverted tube stability study

Variant Type	Temp.	Tube osition	xp. Time oint	Variants etected at Baseline Time oint	Variants etected at xp. Time oint	Variants Agree	A	A [95% CI]	Variants ot etected at Baseline Time oint	Variants ot etected at Exp. Time oint	A	NPA[95% CI]
Short variants	04°C	Inverted	Day 10	0	0	9	98%	[89.5%, 99.6%]	12	12	100%	[100%, 100%]
Short variants	04°C	Upright	Day 10	0	1	0	100%	[92.9%, 100%]	13	12	100%	[100%, 100%]
Short variants	04°C	Inverted	Day 14	9			98.3%	[90.9%, 99.7%]	10	11	100%	[100%, 100%]
Short variants	04°C	Upright	Day 14				100%	[92.0%, 100%]	11	11	100%	[100%, 100%]
Short variants	04°C	Inverted	Day 15	3	37	3	100%	[90.6%, 100%]	11	11	100%	[100%, 100%]
Short variants	04°C	Upright	Day 15	2	2	2	100%	[93%, 100%]	11	11	100%	[100%, 100%]
Short variants	25°C	Inverted	Day 10				97.1%	[91.1%, 99.2%]	27	28	100%	[100%, 100%]
Short variants	25°C	Upright	Day 10				100%	[92.0%, 100%]	13	13	100%	[100%, 100%]
Short variants	25°C	Inverted	Day 14				100%	[92.3%, 100%]	11	09	100%	[100%, 100%]

Variant Type	Temp.	Tube osition	xp. Time oint	Variants etected at Baseline Time oint	Variants etected at xp. Time oint	Variants Agree	A	A [95% CI]	Variants ot etected at Baseline Time oint	Variants ot etected at Exp. Time oint	A	NPA[95% CI]
Short variants	25°C	Upright	Day 14	2	1	1	97.6%	[87.7%, 99.6%]	10	11	100%	[100%, 100%]
Short variants	25°C	Inverted	Day 15				100%	[92.0%, 100%]	13	13	100%	[100%, 100%]
Short variants	25°C	Upright	Day 15	9			97.8%	[89.3%, 99.6%]	16	17	100%	[100%, 100%]
Short variants	35°C	Inverted	Day 10	1	15	1	100%	[79.6%, 100%]	09	09	100%	[100%, 100%]
Short variants	35°C	Upright	Day 10	3	35	3	100%	[90.1%, 100%]	09	09	100%	[100%, 100%]
Short variants	35°C	Inverted	Day 14				100%	[93.4%, 100%]	11	11	100%	[100%, 100%]
Short variants	35°C	Upright	Day 14				95.7%	[86.0%, 98.8%]	09	10	100%	[100%, 100%]
Short variants	35°C	Inverted	Day 15	39	39	3	97.4%	[86.8%, 99.5%]	10	10	100%	[100%, 100%]
Short variants	35°C	Upright	Day 15	28	29	28	100%	[87.9%, 100%]	13	12	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.

9.16 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 29**) 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 30**).

Table 29. Concordance summary by disease ontology at 1x LoD for DNA extraction study

Disease Ontology	Positive Detected/ Positive Total	PPA [95% two-sidedCI]	Negative Detected/ Negative Total[1]	NPA [95% two-sidedCI]	Overall Detected/ Total*	OPA [95% two-sidedCI]
Breast Cancer	347/348	99.7% [98.4%,100.0%]	3144/3144	% [99.9%,100.0%]	3491/3492	% [99.8%,100.0%]
Colorectal Cancer (CRC)	22/1188	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%]

Disease Ontology	Positive Detected/ Positive Total	PPA [95% two-sidedCI]	Negative Detected/ Negative Total[1]	NPA [95% two-sidedCI]	Overall Detected/ Total*	OPA [95% two-sidedCI]
Non-SmallCell 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%]
ProstateCancer	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	% [99.8%,100.0%]	3442/3492	98.6% [98.1%,98.9%]

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

Table 30. Concordance summary by variant type at 1x LoD for DNA extraction study

Variant Type	Positive Detected/ Positive Total	PPA [95% two-sidedCI]	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	63/ 180	90.6% [85.3%, 94.4%]	819/ 828	98.9% [97.9%, 99.5%]	982/ 8	97.4% [96.2%, 98.3%]
Rearrangements	23/ 24	95.8% [78.9%, 99.9%]	20/ 120	% [97.0%, 100.0%]	43/ 44	99.3% [96.2%, 100.0%]
Substitutions	2869/ 2964	96.8% [96.1%, 97.4%]	4358/ 4388	99.8% [99.7%, 99.9%]	7227/ 7352	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.

9.17 Guard Banding/Robustness

This validation study evaluated the impact on FoundationOne Liquid CDx test performancedue 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 31**).

Table 31. Summary of process pass and failure rate at each guard banding DNA input level

Process[1]	Input Level		# of Pass	Pass Rate (%)
HC	50%	5 ng	18/20	9
	20%	8 ng	20/20	1
	Lower limit	1000ng	20/20	1
	Upper limit	2000ng	20/20	1
	+20%	2400ng	20/20	1
	+50%	3000ng	18/20	9
Sequencing	50%	5nM	20/20	1
	20%	8nM	20/20	1

Process[1]	Input Level		# of Pass	Pass Rate (%)
	Normal input	1 nM	20/20	1
	+20%	1 2nM	20/20	1
	+50%	1 5nM	20/20	1

[1] Results for guardbanding of LC input levels can be found in **Table 32** below.

A second guard banding study was conducted to evaluate the impact of a range of cfDNA input masses (50% below the lower limit and 33% above the upper limit) for F1LCDx using an updated LC input range (20-60ng). Results from this second study are described in **Table 32** and **Table 33.** All 105 sample replicates tested in this study passed processing and post-sequencing metric specifications as shown in **Table 32** below. The results demonstrate robust performance across the intended DNA input range.

Table 32. Processing Success Rates by cfDNA Input Level for F1LCDx

Process QC	cfDNA Input Level	cfDNA Input (ng)	# Total	# Pass	# Fail	Success Rate	95% Two-sided Score CI
LC	50%		2	2		%	[84.54%, 100%]
	Lower limit	2	2	2		%	[84.54%, 100%]
	Mid-point	4	2	2		%	[84.54%, 100%]
	Upper limit	6	2	2		%	[84.54%, 100%]
	+33%	8	2	2		%	[84.54%, 100%]
HC	50%		2	2		%	[84.54%, 100%]
	Lower limit	2	2	2		%	[84.54%, 100%]
	Mid-point	4	2	2		%	[84.54%, 100%]
	Upper limit	6	2	2		%	[84.54%, 100%]
	+33%	8	2	2		%	[84.54%, 100%]
Sequencing	50%		2	2		%	[84.54%, 100%]
	Lower limit	2	2	2		%	[84.54%, 100%]
	Mid-point	4	2	2		%	[84.54%, 100%]
	Upper limit	6	2	2		%	[84.54%, 100%]
	+33%	8	2	2		%	[84.54%, 100%]
Post-sequencing QC	50%		2	2		%	[84.54%, 100%]
	Lower limit	2	2	2		%	[84.54%, 100%]
	Mid-point	4	2	2		%	[84.54%, 100%]
	Upper limit	6	2	2		%	[84.54%, 100%]
	+33%	8	2	2		%	[84.54%, 100%]

Table 33. Aggregate Percent Agreement Across All Targeted Variants per cfDNA Input Level for F1LCDx

cfDNA Input Level	cfDNA Input (ng)	Agreement (# Variants Detected / Total # Variants) [95% Two-sided Score CI]
50%	1	92.86% (117/126) [86.98%, 96.2%]
Lower limit	2	99.21% (125/126) [95.64%, 99.86%]
Mid-point	4	100% (126/126) [97.04%, 100%]
Upper limit	6	100% (126/126) [97.04%, 100%]
+33%	8	100% (126/126) [97.04%, 100%]

9.18 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 34** 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 LC. A total of 89.1% of samples yielded ≥36ng of DNA which corresponds to a DNA input mass of3 ng for LC. The proportion of samples with an LC yield greater than the minimum mass of 500ng and lower thanthe 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%, 1 0%]. 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 34. 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	64	97.0%	86.4%	99.9%	%	93.8%	94.3%	93.4%	88.4%
Biliary Cancer	71	99.4%	95.3%	100.0%	100.0%	98.8%	97%	97.5%	95.9%
Bladder Cancer	66	97.6%	85.5%	%	%	93.2%	98.7%	95.8%	92%
Breast Cancer	2775	97.6%	87.7%	99.9%	%	96.4%	95.5%	95.8%	9 9%
Cholangio-carcinoma	377	98.9%	96.0%	99.7%	%	98.7%	97.3%	97%	95.7%
Colorectal Cancer (CRC)	640	98.5%	92.4%	99.9%	%	97.5%	96.9%	96.1%	94.3%
Endocrine-Neuro Cancer	75	%	85.3%	%	%	%	93.3%	96.9%	93.3%
Endometrial Cancer	231	98.3%	88.3%	%	%	96.5%	95.9%	95.1%	92.5%
Esophagus Cancer	291	99.7%	92.4%	%	%	97.6%	96.5%	96.3%	94.1%
Glioma Cancer	59	94.9%	72.9%	%	%	%	76.8%	86%	76.8%
Head and Neck Cancer	54	96.1%	8 8%	%	%	89.2%	96.2%	95.2%	85.8%
Kidney Cancer	203	99.0%	87.7%	%	%	95.0%	95.3%	94.9%	9 5%
Liver Cancer	9	98.2%	95.4%	%	%	%	95.3%	95.2%	95.3%
Lung Non-Small Cell Lung Carcinoma (NSCLC)	5919	98.2%	88.8%	99.8%	%	95.5%	95.6%	94.7%	91.1%
Melanoma	257	96.5%	79.8%	%	%	92.7%	93.5%	93.7%	86.7%
Ovary Cancer	496	97.8%	88.5%	%	%	95.9%	94.6%	94.5%	9 .7%
Pancreas Cancer	359	98.8%	94.0%	99.9%	%	97.8%	95.8%	95%	93.6%
Peripheral Nervous System (PNS)	44	%	9 9%	%	%	%	93.2%	95%	93.2%

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])
Prostate Cancer	778	97.3%	87.7%	99.9%	%	96.9%	95.1%	95.8%	92.1%
Small Cell Cancer	35	98.5%	93.3%	%	%	99.2%	99.2%	98.4%	98.5%
Soft Tissue Sarcoma	30	97.7%	83.1%	%	%	95.3%	91.7%	94.4%	87.4%
Stomach Cancer	267	98.9%	89.1%	%	%	98.1%	93.8%	95.8%	92%
Thyroid Cancer	50	98.0%	86.0%	%	%	%	81.6%	9 7%	81.6%
Unspecified	856	98.5%	89.1%	%	%	95.5%	96.6%	96.3%	92.3%
Unknown Primary Carcinoma (CUP)	62	98.1%	89.7%	%	%	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 35 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 35. Summary of F1L/FACT sample data

QC Metric	QC Pass Rate AcrossTumor 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

9.19 Concordance – FoundationOne Liquid Laboratory Developed Test (LDT) 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 36**

Table 36. Concordance between FoundationOne Liquid LDT (F1L LDT) and FoundationOne Liquid CDx (F1LCDx)

Variant/ Mutation Type	F1LCDx+ F1L LDT+	F1LCDx- F1L LDT+	F1LCDx+ F1L LDT-	F1LCDx- F1L LDT -	PPA [95% CI]	NPA [95% CI]	OPA [95% CI]
All Short Variants	2871	23	32	71180	95.9% [95.1% 96.6%]	>99.9% [>99.9% %]	>99.9% [>99.9% %]
Base Substitutions	2415	4	31	999032	95.9% [95.0% 96.6%]	>99.9% [>99.9% %]	>99.9% [>99.9% %]
Indels	456	9		72148	96.0% [93.8% 97.6%]	>99.9% [>99.9% %]	>99.9% [>99.9% %]
Rearrangements	47	20	24	59587	88.0% [82.1% 92.5%]	>99.9% [>99.9% %]	99.9% [99.9% 99.9%]

Variant/ Mutation Type	F1LCDx+ F1L LDT+	F1LCDx- F1L LDT+	F1LCDx+ F1L LDT-	F1LCDx- F1L LDT -	PPA [95% CI]	NPA [95% CI]	OPA [95% CI]
Copy Number Amplifications	73	32		59463	84.4% [78.7% 89.1%]	99.8% [>99.9%-100.0%]	99.8% [>99.9%-100.0%]
Total	3191	75	66	290230	94.8% [94.0% 95.5%]	>99.9% [>99.9% %]	>99.9% [>99.9% %]

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.

9.20 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.

9.21 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.

9.22 Updated LC Method Comparison Study

A method comparison study was conducted to demonstrate comparable performance between F1LCDx assay using original and updated LC input ranges. ighty-one (81) clinical cfDNA samples from 10 unique disease ontologies were processed in triplicate to create 243 sample replicates. Samples were processed at the lower range for cfDNA input, 30ng for the original recommended minimum for LC input and 20ng for the updated minimum for LC input. 1815 unique targeted variants were analyzed, including CDx variants and variants from all alteration sub-types.

For each of the 81 samples, two of the three replicates were processed with F1LCDx around a 30ng input level, using the previous LC method, (referred to as CCD_1 and CCD_2) and the third replicate was processed with F1LCDx around a 20ng input level, using the updated LC method, (referred to as UCD_{ALL}). Two hundred and forty-three (243) sample replicates tested in this study passed all QC metrics. A non-inferiority analysis was performed. Aggregated PPA and NPA across all 1815 targeted variants were calculated for pairwise

comparisons between CCD_1 and CCD_2. PPAs and NPAs for all targeted variants were also calculated for either CCD_1 or CCD_2 versus UCD_{ALL}. Agreement differences were calculated with corresponding 95% upper 1 sided bounds. The upper bounds of the 1-sided 95% CIs for agreement differences ζ_{PPA1} ζ_{PPA2} ζ_{NPA1} and ζ_{NPA2} were all <1% for UCD_{ALL}. Therefore, the F1LCDx assay using the updated LC input range was demonstrated to be non-inferior to F1LCDx using the original LC input range for the detection of CDx and non CDx variants.

10 Clinical Validation Studies

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 *LK* rearrangements for treatment with alectinib was assessed through aclinical 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 *LK* rearrangement asdetermined by a blood-based NGS assay (CTA).

The concordance between FoundationOne Liquid CDx and the CTA was evaluated as summarized in **Table 37.**

Table 37. Concordance between FoundationOne Liquid CDx and the CTA for the detection of ALK rearrangements

	CTA Pos	CTA Neg	Total
FoundationOne Liquid CDxPositive[1]	63		63
FoundationOne Liquid CDxNegative	12	174	186
Missing	4	9	13
Total	79	183	262

[1] VAF values down to .06%VAF were observed for *ALK* rearrangements.

The PPA and 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 *LK* rearrangements in the intended use population, the PPV and 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 *LK* -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 *LK* 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 *LK* + and FoundationOne Liquid CDx *LK* +, demonstrating the robustness of the clinical efficacy analysis to missing FoundationOne Liquid CDx results.

.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 as 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 38**

Table 38. Agreement analysis results for *EGFR* exon 19 deletion and L858R separately.

Exon 9 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%

858R	PPAC1F	%	NPAC1F	95.6%
	PPAC1C2	92.9%	NPAC1C2	98.9%
	PPAC2F	%	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 39**

Table 39. 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		2		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 40.**

Table 40. 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%

	PPA	NPA
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 41.**

Table 41. 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.

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 TM* 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 *TM* 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. In cohort A, 181 out of the 245 randomized patients both consented to the use of their sample for ctDNA CDx development and had a plasma sample available fortesting. 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. A total of 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 TM* mutations.

Sample accountability for this clinical bridging study is summarized in **Table 42**

Table 42. 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
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 43 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 43. Summary of agreement analyses for FoundationOne Liquid CDx compared against CTA tissue test

		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	
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, RCA2*, or *ATM*
[2] Biomarker refers to patients with eligible *BRCA/ATM* mutations
[3] Confidence intervals calculated using Clopper-Pearson method

The PPA and NPA between FoundationOne LiquidCDx 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 PPV and NPV calculated using the CTA as the referenceand 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 [21, .53] for the FoundationOne Liquid CDx biomarker positive population, which were comparable with the observed rPFS HR and the corresponding 95% confidence intervals of 34 [0.25, .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 .44 [0.32, .59], demonstrating robustness of the analysis to missing FoundationOne Liquid CDx results.

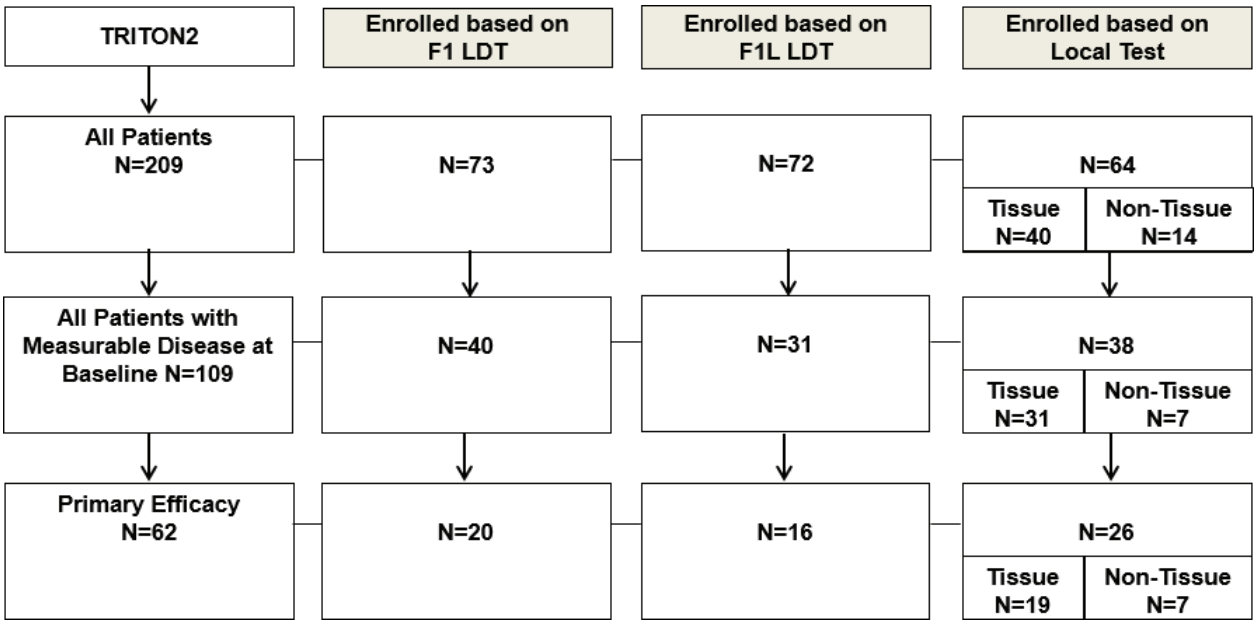
4 Clinical Bridging Study: Detection of BRCA1 and BRCA2 Alterations to Determine Eligibility of mCRPC Patients for Treatment with Rucaparib

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 44**

Table 44. Sample accountability for rucaparib prostate clinical bridging study

Description	Number
All Patients in TRITON2	209
Total samples available for retesting by oundationOne Liquid CDx	192
Patients with evaluable FoundationOne Liquid CDx data and cfDNA input ≥ 30ng (All Patients)	161

Description	Number
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 andcfDNA 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 45** and **Table 46**

Table 45. 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 .15%VAF were observed for short variants and 0.85%VAF for rearrangements in *RCA1* 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 46. 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	8	183

[1] VAF values down to .15%VAF were observed for short variants and 0.85%VAF for rearrangements in *RCA1* or *RCA2*

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 47**).

Table 47. ORR in the primary efficacy population by CTA and FoundationOne Liquid CDx test results

Primary EfficacyPopulation	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 = clinicaltrial 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.

5 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 onCTA2 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 48.**

Table 48. Concordance between FoundationOne Liquid CDx and CTA2

	CTA2			
CDx	**Positive**	**Negative**	**Invalid**	**Total**
Positive	165		1	166
Negative	65	129	3	197
Invalid	7	5		12
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 = .46, 95% CI: 30, 0.70).

As summarized in **Table 49**, the PFS hazard ratio for the 165 tissue CTA2-positive, FoundationOne Liquid CDx-positive patients was 0.46 (95% CI: 30, .70). Median PFS was 11.0 months for the alpelisib plus fulvestrant armversus 3.6 months for the placebo plus fulvestrant arm.

Table 49. 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 /Placeboqd + Fulv[1]
No of events (%)	54 (64.3)	67 (82.7)	.46 (0.30, 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 adjustedfor 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 (MICE0 method. After imputing the missing FoundationOne Liquid CDx results, the hazard ratio was estimated to be 0.63 (95% CI: 45, .87), demonstrating robustness of the clinical efficacy analysis to missing FoundationOne Liquid CDx results.

10.6 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**) was established with clinicaldata 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 cMETinhibitor, 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 skippingstatus 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. fficacywas 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 ReviewCommittee (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 betweenFoundationOne 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 positivepatients, 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 **Table 50** and **Table 5** , 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 50. 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 .16%VAF were observed for *MET* short variants.
N: The total number of patients. It is the denominator for percentage (%) calculationn: Number of patients with agreement between CTA and CDx
(1) The 95% CI calculated using Clopper-Pearson method

Table 51. 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 .16%VAF were observed for *MET* short variants.
N: The total number of patients. It is the denominator for percentage (%) calculationn: 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 improvementin ORR by BIRC assessments in patients with *MET* exon 14 deletion positive tumors in each cohort.

Table 52 and **Table 53** present the clinical efficacy of TABRECTA analyzed in CTA-positive patients who were tested asCDx-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 ≥20ng cfDNA input with valid results for analysis of ORR. In Cohort 5b there were 16 patients, all of whom met the ≥30ng cfDNA input.

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

Table 52. 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 53. 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 theORR 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” samplerequirement 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 arerobust to missing F1LCDx results.

7 Clinical Bridging Study: Detection of ROS1 Fusions to Determine Eligibility for Treatment with Entrectinib

The clinical performance of using FoundationOne Liquid CDx as a companion diagnostic to identify NSCLC patients harboring *ROS1* fusions eligible for treatment with entrectinib (**Table**) was assessed in this clinical bridging study. All available pre-entrectinib treatment plasma samples from patients enrolled in ALKA, STARTRK-1, and STARTRK-2 clinical trials were tested by FoundationOne Liquid CDx as part of this clinical bridging study. Only samples from STARTRK-2 were available for testing by F1LCDx.

A clinical bridging study was conducted to evaluate the 1) the concordance between the F1LCDx assay and the CTAs used for clinical trial enrollment for the detection of *ROS1* fusions and 2) the clinical efficacy of entrectinib treatment in patients who would be eligible for therapy based on *ROS1* fusions positive as determined by F1LCDx.

A total of 255 patients were included in the clinical bridging study. Of these 255 patients, 161 were determined as *ROS1* fusion positive based on testing by the CTAs. Initially, the clinical bridging study included 51 *ROS1*

fusion positive NSCLC patients from the new drug application (NDA) efficacy population, 41 additional *ROS1* fusion positive, *ROS1* inhibitor-naive patients with NSCLC with measurable disease who had insufficient follow up (<12 months) at the time of the NDA submission, 67 *ROS1* fusion positive patients with NSCLC who were enrolled prior to October 31, 2018, and two (2) patients with prior *ROS1* inhibitor treatment and used only for the concordance evaluation. In total, clinical outcome data from 161 *ROS1* fusion positive patients (as determined by the CTAs) enrolled before October 31, 2018 (based on the May 1, 2019 clinical data cutoff date) were planned for use in the bridging analysis. Of the 94 *ROS1* fusion negative samples (as determined by the CTAs), 73 were patients enrolled in the clinical trial by the CTAs as *NTRK1/2/3* fusion positive. The remaining 21 *ROS1* fusion negative samples were FFPE tissue-matched plasma samples procured from a commercial source, with tissue testing by one of the CTAs used for clinical trial enrollment. Only samples from STARTRK-2 were available for testing by F1LCDx and, thus, 218 of the 255 samples were evaluated by retrospective F1LCDx testing. Among them, 203 samples met the F1LCDx quality control metrics, and 175 samples met the recommended sample input of cfDNA ≥ 30ng. An additional 28 samples met the minimum F1LCDx sample input criteria of cfDNA ≥ 20ng. Sample accountability for this clinical bridging study is summarized in **Table 54**

Table 54. Sample Accountability for the *ROS1* Clinical Bridging Study

Source of samples	Total # of samples (n=255)	Sample fail/ unavailable (n=52)	F1LCDx evaluable (n=203)	DNA ≥ 30 ng (n=175)	DNA ≥ 20 ng and < 30 ng (n=28)
Procured *ROS1* Negative samples	21	2	19	17	2
ROS1* Negative by CTA test	73	14	59	51	8
***ROS1* Positive by CTA test**	161	36	125	1 7	18
Total	255	52 (20.4%)	2 3 (79.6%)	175 (68.6%)	28 (11.0%)

*The CTA *ROS1*-fusion negative samples were enrolled in the clinical trials as CTA *NTRK* fusion positive

The primary analyses were conducted for the 175 patients with evaluable FoundationOne Liquid CDx results that also had a DNA input of ≥ 30 ng. The concordance between FoundationOne Liquid CDx and the CTAs is summarized in **Table 55.** Over 20 different types of CTAs with a mix of technologies (RT-PCR, FISH, NGS) and analytes (RNA and DNA) were used to enroll the patients in the clinical trials.

Table 55. Concordance result between F1LCDx and CTA for the detection of ROS1-fusions for samples with DNA content ≥30 ng (n=175).

		CTAs		
		Detected	**Not Detected**	**Total**
F1LCDx	**Detected**	55		55
	Not Detected	52	68	120
	Unevaluable	54	26	80
	Total	161	94	255
Agreement Statistics Excluding CDx-Unevaluable Results		PPA 51.4% (55/107) 95% CI*: (42.05%, 60.66%)	NPA 100% (68/68) 95% CI*: (94.65%, 100%)	
Percent Unevaluable		33.5% (54/161) 95% CI*: (26.7%, 41.1%)	27.7% (26/94) 95% CI*: (19.6%, 37.4%)	

*Calculated with Wilson 2-sided 95% CI

The following concordance statistics were calculated for this sample set using the CTA as the reference:

- PPA [95% CI]: 51.4% [42.05%, 60.66%]
- NPA [95% CI]: 100.0% [94.65%, 100%]

After adjusting for a 1% prevalence of *ROS1* rearrangements in the intended use population PPV and NPV were calculated using the CTA as the reference:

- PPV [95% CI]: 100% [93.47%, 100%]

- NPV [95% CI]: 99.51% [99.41%, 99.61%]

The discordances between the CTAs and F1LCDx among *ROS1* fusion positive patients was evaluated by stratifying the PPA into two subgroups, DNA-based NGS CTAs and RNA-based NGS CTAs. The PPA between F1LCDx and DNA-based NGS CTAs was 55.6% (10/18) with 95% two-sided CI (33.7%, 75.4%). The PPA between F1LCDx and RNA-based NGS CTAs was 50.6% (40/79) with 95% two sided CI (39.8%, 61.4%). Of the 52 CTA positive patients who were F1LCDx negative, 92.3% (48/52) did not have detectable tumor fraction as determined by F1LCDx, suggesting that the ctDNA content in these samples was low.

The clinical efficacy of entrectinib in the clinical trials was measured in ORR with either confirmed complete response (CR) or partial response (PR) based on blinded independent centralized review (BICR). Only clinical samples with clinical outcome data were used in this part of the study analysis.

The ORR in the CTA-positive population was 67.3% (107/159) with 95% two-sided CI (59.7%, 74.1%). Fifty-four (54) patients were CTA positive and had F1LCDx *ROS1* fusion-positive results. The ORR for this population was 66.7% (36/54) with 95% two-sided CI (53.4%, 77.8%). Fifty-one (51) patients were CTA positive but had F1LCDx *ROS1* negative results. The ORR for this population was 66.7% (34/51) with 95% two-sided CI (53.0%, 78.0%).

Fifty-four (54) patients were CTA positive but were unevaluable by F1LCDx. The ORR for this population was 68.5% (37/54) with 95% two-sided CI (55.3%, 79.3%) (**Table 56**).

Table 56. ORR in CTA-positive, FoundationOne Liquid CDx-positive patients

Clinical outcome	Total CTA positive population (N=159)	CTA positive and F1LCDx positive (N=54)	CTA positive and F1LCDx negative (N=51)	CTA positive and F1LCDx unevaluable (N=54)
ORR% [95% CI**]	67.3%	66.7%	66.7%	68.5%
	[59.7%, 74.1%]	[53.4%, 77.8%]	[53.0%, 78.0%]	[55.3%, 79.3%]
Complete response	14 (8.8%)	5 (9.3%)	6 (11.8%)	3 (5.6%)
Partial response	93 (58.5%)	31 (57.4%)	28 (54.9%)	34 (63.0%)
Number of responders	**N=107**	**N=36**	**N=34**	**N=37**
Duration of response				
Median± in months (range)	9.5 (1.8, 42.3)	6 4 (1.8, 20.5)	13.4 (1.9, 27.6)	11.1 (4.6, 42.3)
% with duration ≥9 months	61.7%	38.9%	70.6%	75.7%
% with duration ≥12 months	41.1%	19.4%	55.9%	48.6%
% with duration ≥18 months	19.6%	5 6%	26.5%	27.0%

**Two-sided 95% CI for each subgroup was based on the Wilson-score method
±Arithmetic median used (not Kaplan-Meier methods) since censoring data was not available

Sensitivity analysis to evaluate the robustness of the clinical efficacy estimate against the missing FoundationOne Liquid CDx results was performed using the multiple imputation method. Based on the 100 bootstrap samples with 50 times imputation estimated ORR of the FoundationOne Liquid CDx *ROS1* positive population was 67.1% [50.7%, 78.9%].

There were 70 *ROS1* positive patients by the CTAs with partial or complete response to entrectinib, who also had an F1LCDx result. Among them, only 51.4% (36/70) were positive by F1LCDx (95% CI: 39.9, 62.8). There were 35 *ROS1*-positive patients by the CTAs who did not respond to entrectinb, who also had an F1LCDx result (54-36=18 and 51-34=17). Among them, 51.4% (18/35) were positive by F1LCDx (95% CI: 35.6, 67.0).

8 Clinical Bridging Study: Detection of NTRK 1/2/3 Fusions to Determine Eligibility for Treatment with Entrectinib

The clinical performance of using FoundationOne Liquid CDx as a companion diagnostic to identify patients with solid tumors harboring *NTRK1, NTRK2,* or *NTRK3* fusions eligible for treatment with entrectinib (**Table**) was

assessed in this clinical bridging study. All patients with available plasma samples from the NDA population from ALKA, STARTRK-1, and STARTRK-2 clinical trials were tested by FoundationOne Liquid CDx as part of this clinical bridging study. Only samples from STARTRK-2 were available for testing by F1LCDx

A clinical bridging study was conducted to evaluate the 1) the concordance between the F1LCDx assay and the CTAs used for clinical trial enrollment for the detection of *NTRK* fusions and 2) the clinical efficacy of entrectinib treatment in patients who would be eligible for therapy based on *NTRK* fusions positive as determined by F1LCDx.

A total of 256 patients were included in the clinical bridging study. Of these 256 patients, 74 were determined as *NTRK* fusion-positive based on testing by the CTAs. Initially, the clinical bridging study included 54 *NTRK* fusion positive patients from the NDA efficacy population, as well as 20 *NTRK* fusion-positive patients who were enrolled after the data cutoff. Of the 182 *NTRK* fusion-negative samples, 161 were patients enrolled in the clinical trial by the CTAs as *ROS1* fusion-positive. The remaining 21 *NTRK* fusion-negative samples were FFPE tissue-matched plasma samples procured from a commercial source, with tissue testing by one of the CTAs used for clinical trial enrollment. Only samples from STARTRK-2 were available for testing by F1LCDx and, thus, 218 of the 256 samples were included for retrospective F1LCDx testing. Among them, 203 samples met the F1LCDx quality control metrics, and 175 samples met the recommended sample input of cfDNA ≥ 30ng. An additional 28 samples met the minimum F1LCDx sample input criteria of cfDNA ≥ 20ng. Sample accountability for this clinical bridging study is summarized in **Table 57**

Table 57. Sample Accountability for the *NTRK* Clinical Bridging Study

Source of samples	Total # of samples (n=256)	Sample fail/ unavailable (n=53)	F1LCDx evaluable (n=203)	DNA ≥30 ng (n=175)	DNA ≥20 ng and <30 ng (n=28)
Procured *NTRK* Negative samples	21	2	19	17	2
NTRK* Negative by CTA test	161	36	125	107	18
***NTRK* Positive by CTA test**	74	15	59	51	8
Total	256	53 (20.7%)	203 (79.3%)	175 (68.4%)	28 (10.9%)

*The CTA *NTRK*-fusion negative samples were enrolled in the clinical trial as CTA *ROS1* fusion positive.

The primary analyses were conducted for the 175 patients with evaluable FoundationOne Liquid CDx results that also had a DNA input of ≥ 30 ng. A comparison of the clinical outcomes and baseline characteristics demonstrated that the FoundationOne Liquid CDx-evaluable population was representative of the FoundationOne Liquid CDx unevaluable population in this bridging study. The concordance between FoundationOne Liquid CDx and the CTAs is summarized in **Table 58**. Over 20 different types of CTAs with a mix of technologies (RT-PCR, FISH, NGS) and analytes (RNA and DNA) were used to enroll the patients in the clinical trials.

Table 58. Concordance between FoundationOne Liquid CDx and CTAs for the detection of *NTRK1, NTRK2*, and *NTRK3* fusions

		CTAs		
		Detected	**Not Detected**	**Total**
F1LCDx	**Detected**	25		25
	Not Detected	26	124	150
	Unevaluable	23	58	81
	Total	74	182	256
Agreement Statistics Excluding CDx-Unevaluable Results		PPA: 49.0% (25/51) 95% CI*: (35.9%, 62.3%)	NPA: 100% (124/124) 95% CI*: (97.0%, 100%)	
Percent Unevaluable		31.1% (23/74) 95% CI*: (21.7%, 42.3%)	31.9% (58/182) 95% CI*: (25.5%, 39.0%)	

*Calculated with Wilson 2-sided 95% CI

The following concordance statistics were calculated for this sample set:
- PPA [95% CI]: 49.0% [35.9%, 62.3%]
- NPA [95% CI]: 100.0% [97.0%, 100%]

After adjusting for a 0.32% prevalence of *NTRK* fusions in the intended use population PPV and NPV were calculated using the CTA as the reference:
- PPV [95% CI]: 100% [86.7%,100%]
- NPV [95% CI]: 99.8% [99.79%, 99.88%]

The discordances between the CTAs and F1LCDx among *NTRK1/2/3* fusion-positive patients was evaluated by stratifying the PPA into two subgroups, DNA-based NGS CTAs and RNA-based NGS CTAs. The PPA between F1LCDx and DNA-based NGS CTAs was 65.0% (13/20) with 95% two-sided CI (43.3%, 81.9%). The PPA between F1LCDx and RNA-based NGS CTAs was 38.7% (12/31) with 95% two-sided CI (23.7%, 56.2%).

The clinical efficacy of entrectinib in the clinical trials was measured in overall response rate (ORR) with either confirmed complete response (CR) or partial response (PR) based on blinded independent centralized review (BICR). Only clinical samples with clinical outcome data were used in this part of the study analysis.

The ORR in the CTA positive population was 63.5% (47/74) with 95% two-sided CI (52.1%, 73.6%). Twenty five (25) patients were CTA positive and had F1LCDx *NTRK* positive results. The ORR for this population was 72.0% (18/25) with 95% two-sided CI (52.4%, 85.7%). Twenty-six (26) patients were CTA positive but had F1LCDx *NTRK* negative results. The ORR for this population was 57.7% (15/26) with 95% two-sided CI (38.9%, 74.5%).

Twenty-three (23) patients were CTA positive but were F1LCDx-unevaluable. The ORR for this population was 60.9% (14/23) with 95% two-sided CI (40.8%, 77.8%) (**Table 59**).

Table 59. ORR in CTA-positive, FoundationOne Liquid CDx-positive patients

Clinical outcome	Total CTA positive population (N=74)	CTA positive and F1LCDx positive (N=25)	CTA positive and F1LCDx negative (N=26)	CTA positive and F1LCDx unevaluable (N=23)
ORR% [95% CI**]	63.5%	72.0%	57.7%	6 9%
	[52.1,73.6]	[52.4, 85.7]	[38.9, 74.5]	[40.8, 77.8]
Complete response	5 (6.8%)	(0.0%)	1 (3.8%)	4 (17.4%)
Partial response	42 (56.8%)	18 (72.0%)	14 (53.8%)	10 (43.5%)
Number of responders	**N=47**	**N=18**	**N=15**	**N=14**
Duration of response				
Median± in months (range)	7.5 (1.4, 26.0)	5 9 (1.9, 16.6)	7 9 (1.4, 26)	8.3 (2.8, 25.9)
% with duration ≥9 months	44.7%	38.9%	46.7%	5 %
% with duration ≥12 months	29.8%	22.2%	40.0%	28.6%
% with duration ≥18 months	10.6%	%	13.3%	21.4%

**Two-sided 95% CI for each subgroup was based on the Wilson-score method
±Arithmetic median used (not Kaplan-Meier methods) since censoring data was not available

Sensitivity analysis to evaluate the robustness of the clinical efficacy estimate against the missing FoundationOne Liquid CDx results was performed using the multiple imputation method. Based on the 100 bootstrap samples with 50 times imputation, the estimated ORR of the FoundationOne Liquid CDx *NTRK* positive population was 67.5% [52.4%, 87.1%].

There were 33 *NTRK1/2/3*-positive patients by the CTAs with partial or complete response to entrectinib, who also had an F1LCDx result. Among them, only 54.5% (18/33) were positive by F1LCDx (95% CI: 38.0, 70.2). There were 18 CTA-positive patients who did not respond to entrectinb, who also had an F1LCDx result (25-18=7 and 26-15=11). Among them, 38.9% (7/18) were positive by F1LCDx (95% CI: 20.3, 61.4).

There were 25 patients positive for an *NTRK3* fusion in the entrectinib clinical studies. Among them, 68.0% (17/25) were negative for *NTRK3* fusions by F1LCDx. Among the 17 patients who were negative for *NTRK3* fusions by F1LCDx, 64.7% (11/17) had response to entrectinib. Further, F1LCDx detected one (1) of seven (7) different *NTRK3* fusions that were detected by the CTAs.

9 Clinical Bridging Study: Detection of *EGFR* exon 20 Insertions to Determine Eligibility for Treatment with Mobocertinib

The clinical performance of FoundationOne Liquid CDx as a companion diagnostic to identify NSCLC patients harboring *EGFR* exon 20 insertions eligible for treatment with mobocertinib was assessed in a clinical bridging study. All available plasma samples from patients enrolled in the NDA population from the AP32788-15-101 (Study 101) clinical trial were tested by F1LCDx as part of this clinical bridging study. To further support the clinical validation of F1LCDx for the detection of *EGFR* exon 20 insertions, additional CTA-positive and CTA-negative patients from the non-NDA population (i.e., patient population that were included as part of the dose escalation cohort or did not receive prior platinum treatment) of the AP32788-15-101 trial were included in the concordance analysis. Additionally, CTA-negative tissue samples (with matched plasma for F1LCDx testing) procured from commercial sources, and residual plasma samples (not tissue-matched) from the FMI clinical archive and processed in previous studies, were also included in the clinical bridging study.

The clinical bridging study evaluated 1) the concordance between the F1LCDx assay and the CTAs used for clinical trial enrollment for the detection of *EGFR* exon 20 insertions 2) the clinical efficacy of mobocertinib treatment in patients who would be eligible for therapy based on *EGFR* exon 20 insertions-positive status as determined by F1LCDx and 3) a sensitivity analysis to assess the robustness of the concordance and efficacy results subject to the missing F1LCDx results

A total of 342 patients were identified for the clinical bridging study analysis. Among 230 *EGFR* exon 20 insertion-positive patients by CTA, 46 did not have a plasma sample available for F1LCDx testing and 25 patient samples failed the F1LCDx QC metrics, resulting in a total of 159 *EGFR* exon 20 insertion-positive samples that had F1LCDx-evaluable results. Among the 159 *EGFR* exon 20 insertion-positive evaluable samples, 132 had cfDNA ≥30 ng for input to LC and were used for the primary analysis. Twenty-seven (27) *EGFR* exon 20 insertion-positive samples had cfDNA <30 ng and ≥20ng for input to LC, and these samples were included in the exploratory analysis.

Among the 112 samples that were *EGFR* exon 20 insertion-negative by CTA, 3 samples failed F1LCDx QC metrics resulting in a total of 109 *EGFR* exon 20 insertion-negative samples that had F1LCDx-evaluable results. Among the 109 *EGFR* exon 20 insertion-negative samples evaluable by F1LCDx, 100 had cfDNA ≥30 ng for input to LC and were used for the primary analysis. The remaining 9 *EGFR* exon 20 insertion-negative samples had cfDNA <30 ng and ≥20ng for input to LC, and these samples were included in the exploratory analysis. Sample accountability for this clinical bridging study is summarized in **Table 60**

Table 60. F1LCDx Sample Accountability for *EGFR* exon 20 Insertions

CTA Status	Sample Source	Study 101 Population	# of Patients[1]	# of Failed or Unavail-able Samples	F1LCDx Evaluable		
					# of F1LCDx-Evaluable Samples	# of F1LCDx Samples ≥30 ng	# of F1LCDx Samples ≥ ng and <30 ng
POSITIVE	Study 101	NDA	4	34	80	7	9
	Study 101	Non-NDA	6	37	79	6	8
	Positive Subtotal		230	7	59	32	27
NEGATIVE	Study 101	Non-NDA	43	3	40	34	6
	Procured	N A	46		46	43	3

CTA Status	Sample Source	Study 101 Population	# of Patients[1]	# of Failed or Unavail-able Samples	F1LCDx Evaluable		
					# of F1LCDx-Evaluable Samples	# of F1LCDx Samples ≥30 ng	# of F1LCDx Samples ≥ ng and <30 ng
	Retrospective	N/A	23		23	23	
	Negative Subtotal		2	3	9		9
Total			**342 (100%)**	**74 (21.6%)**	**68 (78.4%)**	**32 (67.8%)**	**36 (10.5%)**

[1]16 additional samples (15 from Study 101 and 1 procured patient sample) failed CTA testing QC.

Results for the primary concordance analysis (total n=232) is summarized in **Table 61.**

Table 61. Contingency Table Comparing *EGFR* exon 20 Insertions Status Between the CTAs and F1LCDx

		CTAs		
		Detected	**Not Detected**	**Total[1]**
F1LCDx	**Detected**	95		95
	Not Detected	37	100	137
	Unevaluable	98	12	110
	Total	230	112	342
Agreement Statistics Excluding CDx-Unevaluable Results		PPA: 72.0% (95/132) 95% CI[2]: (63.8%, 78.9%)	NPA: 100% (100/100) 95% CI[2]: (96.3%, 100%)	
Percent Unevaluable		42.6% (98/230)	1 7% (12/112)	

[1]16 additional samples (15 from Study 101 and 1 procured patient sample) failed CTA testing QC.
[2]Calculated with Wilson 2-sided 95% CI

The following concordance statistics were calculated for this sample set using the CTA as the reference:
- PPA [95% CI]: 72.0% [63.8%, 78.9%]
- NPA [95% CI]: 100% [96.3%, 100%]

Since patients were enrolled and initially tested by local CTAs, the PPV and NPV were calculated using the PPA and NPA, after adjusting for the prevalence of *EGFR* exon 20 insertions among the intention-to-treat (ITT) population. The prevalence estimate used in the adjusted agreement was 1.8%. In this analysis, F1LCDx demonstrated an adjusted PPV of 100% with 95% two-sided CI [96.1%, 100%]) and NPV of 99.5% with 95% two-sided CI [99.3%, 99.6%].

The primary clinical efficacy of mobocertinib was estimated with NDA patients from Study 101 that had samples with DNA input ≥ 30 ng. The ORR in the CTA-positive population was 28.1% (32/114) with 95% two-sided CI [20.6%, 36.9%]. Fifty-three (53) patients were CTA-positive and had F1LCDx *EGFR* exon 20 insertion-positive results. The ORR for this population (CTA+/F1LCDx+) was 32.1% (17/53) with 95% two-sided CI [21.1%, 45.5%]. Eighteen (18) patients were CTA-positive but had F1LCDx *EGFR* exon 20 insertion-negative results. The ORR for this population (CTA+/F1LCDx-) was 16.7% (3/18) with 95% two-sided CI [5.8%, 39.2%]. Forty-three (43) patients were CTA-positive but were unevaluable by F1LCDx. The ORR for this population (CTA+/F1LCDx unevaluable) was 27.9% (12/43) with 95% two-sided CI [16.7%, 42 7%] (**Table 62**).

Table 62. Primary Efficacy Analysis Results

Clinical outcome	Total CTA+ population (N=114)	CTA+/F1LCDx+ (N=53)	CTA+/F1LCDx– (N=18)	CTA+/F1LCDx unevaluable (N=43)
ORR% [95% CI[1]]	**28.1%** [20.6%, 36.9%]	**32.1%** [21.1%, 45.5%]	**6.7%** [5.8%, 39.2%]	**27.9%** [16.7%, 42.7%]
Number of responders[2]	**32**	**7**	**3**	**2**

Clinical outcome	Total CTA+ population (N=114)	CTA+/F1LCDx+ (N=53)	CTA+/F1LCDx– (N=18)	CTA+/F1LCDx unevaluable (N=43)
Median[3] duration of responsein months [95% CI]	**7.5** [7.4, 20.3]	**7.4** [3.7, N/A[4]]	N/A[5]	**20.3** [8.3, N/A[4]]
% with duration ≥6 months	59.4%	41.2%	66.7%	83.3%

[1]CI for ORR calculated with Wilson 2-sided 95% CI except in the F1LCDx+ population which was calculated using normal approximation CI using the variance.
[2]All responses were partial response.
[3]Median was determined using Kaplan-Meier estimate.
[4]The upper bound of the 95% CI was not estimable.
[5]The median could not be calculated for the CTA+/F1LCDx- subgroup due to the small sample size (the survival probability did not reach 50%).

The median DOR in the CTA-positive population that responded to mobocertinib (N=32) was 17.5 months with 95% two-sided CI [7.4, 20.3]. Seventeen (17) patients that were CTA-positive and responded to mobocertinib also had F1LCDx *EGFR* exon 20 insertion positive results. The median DOR for this population (F1LCDx+|CTA+) was 7.4 months with 95% two-sided CI [3.7, N/A]. Twelve (12) patients that were CTA positive and responded to mobocertinib were not evaluable by F1LCDx. The median DOR for this population (F1LCDx-unevaluable|CTA+) was 20.3 months with 95% two-sided CI [8.3, N A].

A sensitivity analysis was performed to assess the robustness of the concordance and efficacy results subject to the missing F1LCDx results. F1LCDx results were predicted for the F1LCDx-unevaluable patients (patients with missing or invalid F1LCDx test results), and the PPA and PPV estimates were updated with the complete set of F1LCDx results.

In the sensitivity analysis, the average PPA was 69.7% (95% CI [59.4% 80.6%]). The prevalence-adjusted PPV was still 100%. The ORR estimated for the F1LCDx-positive population was 32.6% (95% CI [17.0% 48.2%]). The sensitivity analysis results demonstrated that the concordance between CTA and F1LCDx and drug efficacy estimated in the F1LCDx-positive population were robust as calculated with the F1LCDx-evaluable patients.

11 CDx Classification Criteria

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

- The *LK* 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 *LK* breakpoint occurs within *LK* intron 19

11.2 CDx classification criteria for *EGFR* alterations, qualifying NSCLC patients for therapy with *EGFR* Tyrosine Kinase Inhibitors (TKI) approved by FDA:

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

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

Table 63 Table 64, and **Table 65** describe the criteria for classifying *BRCA1 BRCA2, or TM* alterations known to be deleterious to protein function

Table 63. Classification Criteria for BRCA1, BRCA2, and ATM

Deleterious Variant Criteria	Sequence Classification	CDx Classifier Methodology
A gene alteration that includes any ofthe	Protein truncating mutations	Sequence analysis identifies premature stop codons or frame shift indels anywhere in the gene coding region, except: 3' of and including *RCA2* K3326*

Deleterious Variant Criteria	Sequence Classification	CDx Classifier Methodology
sequence classifications	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 65**
	Homozygous deletions	Sequence analysis identifies deletionsin both gene alleles of ≥ 1 exon in size. Only reported for BRCA1 and *RCA2* . Not reported for ATM.
	Large protein truncating rearrangements	Sequence analysis identifies proteintruncating rearrangements
	Deleterious missensemutations	Curated list (**Table 64**)

Table 64. 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
R71G	V211I	G2765S
R71K	R2336P	R2832C
R1495M	R2336H	S2855_V2856delinsRI
E1559K		R3008C
D1692N		R3008H
D1692H		
R1699W		
A1708E		
G1788V		

Table 65. Intronic Variants

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

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

Table 66 and **Table 67** describe the criteria for classifying *BRCA1* or *BRCA2* alterations known to be deleterious to BRCA protein function rendering the sample *BRCA*+.

Table 66. Classification Criteria for Deleterious Tumor BRCA Variants

Qualification Criteria	Sequence Classification	Methodology
A *RCA1/2* 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 *RCA2* K3326*
	Splice site mutations	Sequence analysis identifies variant splice sequences at intron/exon junctions + 2bp of exon starts/ends

Qualification Criteria	Sequence Classification	Methodology
	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 67**)

Table 67. 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
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	

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; 545D [1635G>T only]; 545G; Q546E; Q546R; H1047L; or H1047Y

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:

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 , +1, +2, or +3 at the splice donor site of the 3′ boundary of *MET* exon 14.

11.7 CDx classification criteria for *NTRK* fusions:

Rearrangements in *NTRK1 NTRK2* or *NTRK3* shall be considered CDx biomarker positive, that is, to lead to a *NTRK1 NTRK2* or *NTRK3* RNA fusion, if the following criterion is met:

- In-strand rearrangement events that may lead to an *NTRK1 NTRK2* or *NTRK3* RNA fusion with a previously reported or novel partner gene in which the kinase domain is not disrupted. This also includes rearrangement events that result in reciprocal fusions (*NTRK* may be on either the 5' or the 3' end of the detected fusion).

In this regard out-of-strand events are considered as non-fusion rearrangements and are classified as CDx biomarker negative. Intragenic fusions in which genomic rearrangement events are wholly internal to the *NTRK1 NTRK2*, or *NTRK3* genes (i.e., *NTRK1 NTRK1 NTRK2 NTRK2 NTRK3 NTRK3* events) are also considered biomarker negative. Unidentified partners (encoded as N/A) or LINC non-coding partners are also considered CDx biomarker negative.

11.8 CDx classification criteria for *ROS1* fusions

Rearrangements in *ROS1* shall be considered CDx biomarker positive, i.e., to lead to *ROS1* RNA fusion, if the following condition is met:

- In-strand rearrangement events that may lead to a *ROS1* RNA fusion with another protein coding gene in which the *ROS1* kinase domain is not disrupted. *ROS1* must be on the 3′ end of the detected fusion.

In this regard, out-of-strand events are considered as non-fusion rearrangements and are classified as CDx biomarker negative. Intragenic fusions in which genomic rearrangement events are wholly internal to the *ROS1* (i.e., *ROS1 ROS1* events) are also considered biomarker negative. Unidentified partners (encoded as N/A) or LINC non- coding partners are also considered CDx biomarker negative. *ROS1* fusions with novel partners are required to be in frame.

11.9 CDx classification criteria for *EGFR* exon 20 insertions

CDx positivity for *EGFR* exon 20 insertions is determined if the following criterion is met:

- Any in-frame insertions affecting amino acids 762 – 775 in exon 20

FOUNDATIONONE®LIQUID CDx

PATIENT

TUMOR TYPE
Lung non-small cell lung carcinoma (NOS)

REPORT DATE

ORDERED TEST #

PATIENT
DISEASE Lung non-small cell lung carcinoma (NOS)
NAME
DATE OF BIRTH
SEX
MEDICAL RECORD #

PHYSICIAN
ORDERING PHYSICIAN
MEDICAL FACILITY
ADDITIONAL RECIPIENT
MEDICAL FACILITY ID
PATHOLOGIST

SPECIMEN
SPECIMEN ID
SPECIMEN TYPE
DATE OF COLLECTION
SPECIMEN RECEIVED

FDA-Approved Content

Companion Diagnostic (CDx) Findings

A companion diagnostic provides essential information for the safe and effective use of a corresponding drug or biological product. This table contains therapies associated with FoundationOne®Liquid CDx's FDA-approved companion diagnostic indications.

ENOMIC FINDINGS	CDx-INDICATED THERAPIES
EGFR exon 20 insertion (V769_D770insGSV)	EXKIVITY® (mobocertinib) β

Tumor Profiling esults

FoundationOne®Liquid CDx is FDA-approved to provide tumor mutation profiling results for oncology patients with solid tumors.

SHORT VARIANTS AND SELECT REARRANGEMENTS AND COPY NUMBER ALTERATIONS IDENTIFIED

Results reported in this section that are not designated as CDx findings are not prescriptive or conclusive for labeled use of any specific therapeutic product. See *professional services* section for information on the alterations listed in this section as well as any additional detected copy number alterations, gene rearrangements, or biomarkers. Please refer to the appendix for the description of the biomarker levels.

Companion lagnostic (CDx) fin ings

EGRR exon 20 insertion (V769_D77 insGS V)

Other biomarkers with potential clinical significan e

CHEK2 splice site 909- >C #

TET2 80 #

β EXKIVITY® efficacy has not been established in patients with EGFR exon 20 insertions with <0.20% VAF tested with FoundationOne Liquid CDx.

 # Variants in this gene may be derived from a nontumor source such as clonal hematopoiesis (CH). The efficacy of targeting such nontumor somatic alterations (e.g., CH) is unknown.

Please refer to appendix for Explanation of Clinical Significance Classification and for variants of unknown significance (VUS).

ABOUT THE TEST FoundationOne®Liquid CDx is a next generation sequencing (NGS) as a y that identiÃes clinically relevant genomic alteration in circulating cell-free DNA.

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ulia Elvin, M.D., Ph.D., Laboratory Director CLIA: 22D2027531
Nimesh R. Patel, M.D., Laboratory Director CLIA: 34D2044309
oundation Medicine, Inc. | 1.888.988.3639

Sample Preparation: 50 Second St., 1st Floor, Cambridge, MA 0 4 · CLIA: 22D2 75
Sample Analysis: 50 Second St., 1st Floor, Cambridge, MA 0 4 · CLIA: 22D20 75
Post-Sequencing Analysis: 50 Second St., 1st Floor. Cambridge, MA 0 4 · CLIA: 22D20 75

INTENDED USE

FoundationOne Liquid CDx is a qualitative next generation sequencing based *in vitro* diagnostic te t that uses targeted high throughput hybridization-based capture technology to detect and report substitution , in e rtion an d deletion (indels) in 311 genes, including rearrangements in eight (8) genes, and copy number alteration 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.

Additionally, FoundationOne Liquid CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with profe ional guidelines in oncology for patients with solid malignant neopla ms .

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 mutation lis ted in Table 1 (see **Note for *NTRK1/2/3* and *ROS1* fusions) should be reflexed to routine biopsy and their tumor mutation status confirmed using an FDA-approved tumor tissue test, if feasible.

**Note: When considering eligibility for ROZLYTREK® based on the detection of *NTRK1/2/3* and *ROS1* fusions, testing using plasma pecim en is o nly appropriate for patients for whom tumor tissue is not available for testing.

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 as a y performed at Foundation Medicine, Inc. in Cambridge, MA.

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 58R subs titution	EGFR Tyrosine Kinase Inhibitors (TKI) Approved by FDA*
	EGFR Exon 20 insertions	EXKIVITY® (mobocertinib)
	MET single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping	TABRECTA® (capmatinib)
	ROS1 fusions**	ROZLYTREK® (entrectinib)
Prostate cancer	*BRCA1 BRCA2 ATM* alterations	YNPARZA® (olaparib)
	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)
Solid Tumors	*NTRK1/2/3* fusions**	ROZLYTREK® (entrectinib)

**For the most current information about the therapeutic products in this group, go to: https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools#Group_Labeling*

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Sample Preparation: 50 Second St., 1st Floor, Cambridge, MA 0 4 · CLIA: 22D2 75
Sample Analysis: 50 Second St., 1st Floor, Cambridge, MA 0 4 · CLIA: 22D20 75
Post-Sequencing Analysis: 50 Second St., 1st Floor. Cambridge, MA 0 4 · CLIA: 22D20 75

TEST PRINCIPLE

e FoundationOne Liquid CDx assay is performed exclusively as a laboratory service using circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood from patients with solid malignant neoplasms. The assay employs a single DNA extraction method to obtain cfDNA from plasma from whole blood. Extracted cfDNA undergoes whole-genome shotgun library construction and hybridization-based capture of 324 cancer-related genes. 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 eight genes. A subset of targeted regions in 75 genes is baited for enhanced sensitivity.

PERFORMANCE CHARACTERISTICS

Please refer to product label:
foundationmedicine.com/F1LCDx

WARNINGS AND PRECAUTIONS

1. 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.
2. e test is not intended to replace germline testing or to provide information about cancer predisposition.
3. Patients for whom no companion diagnostic alterations are detected should be considered for confirmation with an FDA-approved tumor tissue test, if available.

LIMITATIONS

1. For *in vitro* diagnostic use.
2. For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
3. 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.
4. A negative result does not rule out the presence of an alteration in the patient's tumor.
5. 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.
6. e test is intended to be performed on specific serial number-controlled instruments by Foundation Medicine, Inc.
7. Genomic findings from cfDNA may originate from circulating tumor DNA fragments, germline alterations, or nontumor somatic alterations, such as clonal hematopoiesis (CH). Genes with alterations that may be derived from CH 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.
8. e 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%.
9. e analytical accuracy for the FoundationOne Liquid CDx assay has not been demonstrated in all genes.
10. e 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.
11. The analytical accuracy for the FoundationOne Liquid CDx assay for detection of *EGFR* exon 20 insertions has not been demonstrated for amples with <0.18% VAF.
12. TABRECTA® efficacy has not been established in patients with *MET* SNVs <0.21% VAF and in patients with *MET* indels <0.16% VAF tested with FoundationOne Liquid CDx.
13. ALECENSA® efficacy has not been established in patients with *ALK* rearrangements <0.06% VAF tested with FoundationOne Liquid CDx.
14. LYNPARZA® efficacy has not been established in prostate cancer patients with *BRCA1/2* or *ATM* rearrangements with <0.25% VAF or with short variants in *BRCA1/2* or *ATM* <0.11% VAF tested with FoundationOne Liquid CDx.
15. RUBRACA® efficacy has not been established in prostate cancer patients with *BRCA1/2* rearrangements with <0.85% VAF or with hort variants in BRCA1/2 <0.15% VAF tested with FoundationOne Liquid CDx.
16. PIQRAY® efficacy has not been established in patients with *PIK3CA* SNVs with <0.14% VAF tested with FoundationOne Liquid CDx.
17. EXKIVITY® efficacy has not been established in patients with *EGFR* exon 20 insertions with <0.20% VAF tested with FoundationOne Liquid CDx.
18. The precision of FoundationOne Liquid CDx was only confirmed for select variants at the limit of detection (LoD).
19. The FoundationOne Liquid CDx assay does not detect heterozygous deletions.
20. The FoundationOne Liquid CDx assay does not detect copy number losses/homozygous deletions in *ATM*.
21. 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.
22. The test is not intended to provide information on cancer predisposition.
23. *BRCA1/BRCA2* homozygous deletions and rearrangements were not adequately represented in all analytical studies.
24. Representation of *ALK* rearrangements were limited in the analytical validation studies.
25. The representation of *ATM* hort variants and rearrangements was limited in the analytical validation studies.
26. Performance has not been validated for cfDNA input below the specified minimum input.
27. Representation of SNV and indels that lead to *MET* exon 14 skipping that represent biomarker rule category 1 and 2, were limited in the analytical validation studies.
28. For optimal ctDNA shed, it is recommended that blood be drawn prior to therapy or at a time of disease progression. The sensitivity of liquid biopsy is related to adequate levels of ctDNA shed. Therefore, assay performance will be dependent upon level of ctDNA shed at time of testing.
29. Due to the low prevalence of *ROS1* fusions and *NTRK1/2/3* fusions, the positive predictive value (PPV) of the test (FoundationOne Liquid CDx positive, tissue negative) may be lower than reported in test labeling.
30. FoundationOne Liquid CDx may miss a subset of patients with *NTRK1/2/3* fusion and *ROS1* fusion positive solid tumors who may derive benefit from ROZLYTREK®. In a retrospective-prospective clinical study assessing concordance between FoundationOne Liquid

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CDx test results in plasma and patients whose tumor tissue tested positive and was the basis for enrollment into a clinical trial, the data demonstrated that the FoundationOne Liquid CDx test did not detect approximately 46% of potential responders with *NTRK1/2/3* fusions and 49% of responders with *ROS1*.

31. ROZLYTREK® efficacy has not been established in patients with *NTRK2* fusions tested with FoundationOne Liquid CDx, given the low prevalence of the biomarker.
32. In a retrospective-prospective clinical study assessing concordance between FoundationOne Liquid CDx test results in plasma and patients whose tumor tissue tested positive and was the basis for enrollment into a clinical trial, FoundationOne Liquid CDx detected 1 of 7 different *NTRK3* fusion partners. Due to the rarity of these fusions, the accuracy of FoundationOne Liquid CDx for *NTRK3* fusions has not been adequately determined.
33. *NTRK2* fusions per the FoundationOne Liquid CDx biomarker rules for *NTRK1/2/3* fusions were not represented in analytical validation studies.
34. A study evaluating the concordance to a second method demonstrated that the agreement between FoundationOne Liquid CDx positive results and a comparator method for *NTRK1/3*, and *ROS1* was ≤ 50% (i.e., whether these are potential FoundationOne Liquid CDx false positives or false negatives by the comparator is unknown).

LEVEL 1: COMPANION DIAGNOSTICS (CDx)

Clinical evidence should be presented from a prospectively designed clinical trial. Results can also be presented from a retrospective clinical bridging study demonstrating that the clinical endpoints are preserved using plasma samples in trials where enrollment was based on tissue test results. For follow-on markers, a clinical concordance study demonstrating non-inferiority to the original FDA-approved cfDNA-based companion diagnostic device (refer to Li, Meijuan. Statistical Methods for Clinical Validation of Follow-On Companion Diagnostic Devices via an External Concordance Study. *Statistics in Biopharmaceutical Research*. 8: 35-363, 2016) is required. In addition to the clinical validation, analytical validation for each specific Level 1 CDx biomarker should be presented.

LEVEL 2: cfDNA BIOMARKERS WITH STRONG EVIDENCE OF CLINICAL SIGNIFICANCE IN cfDNA

For a Level 2 claim of cfDNA biomarkers with strong evidence of clinical significance, clinical validation needs to be from evidence presented with FDA-approved liquid biopsy companion diagnostic biomarkers for the specific tumor type at the biomarker or variant level. Such claims should also be supported by analytical performance for each biomarker from at least LoD, precision/reproducibility, and LoD studies.

LEVEL 3A: BIOMARKERS WITH EVIDENCE OF CLINICAL SIGNIFICANCE IN TISSUE SUPPORTED BY STRONG ANALYTICAL VALIDATION USING cfDNA AND CONCORDANCE BETWEEN cfDNA AND TISSUE

Clinical evidence can be provided from tissue-based companion diagnostics. This should also be supported by analytical validation (LoD, precision, analytical accuracy, and concordance study to a tissue-based test) for the specific tumor type at the biomarker or variant level, using a representative approach for SNVs and indels. Evidence evaluating concordance between cfDNA- and tissue-samples for FDA-approved tissue markers should be demonstrated using an FDA-approved tissue test or a validated tissue test.

LEVEL 3B: BIOMARKERS WITH EVIDENCE OF CLINICAL SIGNIFICANCE IN TISSUE SUPPORTED BY ANALYTICAL VALIDATION USING cfDNA

Clinical evidence can be provided from tissue-based companion diagnostics, with analytical validity supported by a representative approach for SNVs and indels from key analytical studies (such as LoD, accuracy, and precision).

LEVEL 4: OTHER BIOMARKERS WITH POTENTIAL CLINICAL SIGNIFICANCE

Biomarkers not categorized into Levels 1, 2, or 3 can be included under Level 4 for informational purposes or to be used to direct patients toward clinical trials for which they may be eligible. Such claims can be supported by clinical rationale for inclusion in the panel. Such rationale could also include peer-reviewed publications for genes/variants in tissue, variant information from well-curated public databases, or *in vitro* pre-clinical models. Analytical validation should be supported by a representative approach for SNVs and indels from key analytical studies (such as LoD, accuracy, and precision).

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APPENDIX Genes assayed in FoundationOne®Liquid CDx

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 noncoding 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 or WTX)	***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**	*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**	*EMSY* (C11orf30)	*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	*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*	*GID4* (C17orf39)	***GNA11*** **Exons 4, 5**
GNA13	***GNAQ*** **Exons 4, 5**	***GNAS*** **Exons 1, 8**	*GRM3*	*GSK3B*	*H3-3A* (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***

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APPENDIX Genes assayed in FoundationOne®Liquid CDx

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 noncoding 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.

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**	*MRE11* (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*	*NOTCH1*	*NOTCH2* Intron 26	*NOTCH3*	***NPM1*** **Exons 4-6, 8, 10**	***NRAS*** **Exons 2, 3**
NSD2 (WHSC1 or MMSET)	*NSD3* (WHSC1L1)	*NT5C2*	***NTRK1*** **Exons 14, 15, Introns 8-11**	*NTRK2* Intron 12	***NTRK3*** **Exons 16, 17**	*NUTM1** Intron 1	*P2RY8*	***PALB2***
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*	*PRKN* (PARK2)	*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***	*SUFU*	*SYK*	*TBX3*	*TEK*	*TENT5C* (FAM46C)	*TERC** ncRNA	***TERT**** **Promoter**
TET2	*TGFBR2*	*TIPARP*	*TMPRSS2** Introns 1-3	*TNFAIP3*	*TNFRSF14*	***TP53***	*TSC1*	*TSC2*
TYRO3	*U2AF1*	***VEGFA***	*VHL*	*WTI*	*XPO1*	*XRCC2*	*ZNF217*	*ZNF703*

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FOUNDATIONONE®LIQUID CDx

PATIENT

TUMOR TYPE
Lung adenocarcinoma

REPORT DATE

ORDERED TEST #

PATIENT
DISEASE Lung adenocarcinoma
NAME
DATE OF BIRTH
SEX
MEDICAL RECORD #

PHYSICIAN
ORDERING PHYSICIAN
MEDICAL FACILITY
ADDITIONAL RECIPIENT
MEDICAL FACILITY ID
PATHOLOGIST

SPECIMEN
SPECIMEN ID
SPECIMEN TYPE
DATE OF COLLECTION
SPECIMEN RECEIVED

FDA Approved Content

Companion Diagnostic (CDx) Findings

A companion diagnostic provides essential information for the safe and effective use of a corresponding drug or biological product. This table contains therapies associated with FoundationOne®Liquid CDx's FDA-approved companion diagnostic indications.

GENOMIC FINDINGS	CDx-INDICATED THERAPIES
o Comp anion Diagnostic (CDx) alterations for FoundationOne®Liquid CDx were detected. Please consider confirmation with tumor tissue testing, such as FoundationOne®CDx, if possible.	

Tumor Profiling Results

FoundationOne®Liquid CDx is FDA-approved to provide tumor mutation profiling results for oncology patients with solid tumors.

SHORT VARIANTS AND SELECT REARRANGEMENTS AND COPY NUMBER ALTERATIONS IDENTIFIED

Results reported in this section that are not designated as CDx findings are not prescriptive or conclusive for labeled use of any specific therapeutic product. See *professional services* section for information on the alterations listed in this section as well as any additional detected copy number alterations, gene rearrangements, or biomarkers. Please refer to the appendix for the description of the biomarker levels.

Other biomarkers with potential clinical significance

ASXL1 E635fs*15 #
ASXL1 L775fs*1 #
KEAP1 W591*
KRAS G12A
STK11 P281fs*6
TP53 L145R #

 # Variants in this gene may be derived from a nontumor source such as clonal hematopoiesis (CH). The efficacy of targeting such nontumor somatic alterations (e.g., CH) is unknown.

Please refer to appendix for Explanation of Clinical Significance Classification and for variants of unknown significance (VUS).

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

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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, including rearrangements in eight (8) 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. e 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.

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 (see **Note for *NTRK1/2/3* and *ROS1* fusions) should be reflexed to routine biopsy and their tumor mutation status confirmed using an FDA-approved tumor tissue test, if feasible.

**Note: When considering eligibility for ROZLYTREK® based on the detection of *NTRK1/2/3* and *ROS1* fusions, testing using plasma specimens is only appropriate for patients for whom tumor tissue is not available for testing.

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.

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	EGFR Tyrosine Kinase Inhibitors (TKI) Approved by FDA*
	EGFR Exon 20 insertions	EXKIVITY® (mobocertinib)
	MET single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping	TABRECTA® (capmatinib)
	ROS1 fusions**	ROZLYTREK® (entrectinib)
Prostate cancer	*BRCA1*, *BRCA2*, *ATM* alterations	LYNPARZA® (olaparib)
	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)
Solid Tumors	*NTRK1/2/3* fusions**	ROZLYTREK® (entrectinib)

**For the most current information about the therapeutic products in this group, go to: https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools#Group_Labeling*

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TEST PRINCIPLE

e FoundationOne Liquid CDx assay is performed exclusively as a laboratory service using circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood from patients with solid malignant neoplasms. The assay employs a single DNA extraction method to obtain cfDNA from plasma from whole blood. Extracted cfDNA undergoes whole-genome shotgun library construction and hybridization-based capture of 324 cancer-related genes. 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 eight genes. A subset of targeted regions in 75 genes is baited for enhanced sensitivity.

PERFORMANCE CHARACTERISTICS

Please refer to product label:
foundationmedicine.com/F1LCDx

WARNINGS AND PRECAUTIONS

1. 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.
2. e test is not intended to replace germline testing or to provide information about cancer predisposition.
3. Patients for whom no companion diagnostic alterations are detected should be considered for confirmation with an FDA-approved tumor tissue test, if available.

LIMITATIONS

1. For *in vitro* diagnostic use.
2. For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
3. 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.
4. A negative result does not rule out the presence of an alteration in the patient's tumor.
5. 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.
6. e test is intended to be performed on specific serial number-controlled instruments by Foundation Medicine, Inc.
7. Genomic findings from cfDNA may originate from circulating tumor DNA fragments, germline alterations, or nontumor somatic alterations, such as clonal hematopoiesis (CH). Genes with alterations that may be derived from CH 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.
8. e 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%.
9. e analytical accuracy for the FoundationOne Liquid CDx assay has not been demonstrated in all genes.
10. e 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.
11. The analytical accuracy for the FoundationOne Liquid CDx assay for detection of *EGFR* exon 20 insertions has not been demonstrated for amples with <0.18% VAF.
12. TABRECTA® efficacy has not been established in patients with *MET* SNVs <0.21% VAF and in patients with *MET* indels <0.16% VAF tested with FoundationOne Liquid CDx.
13. ALECENSA® efficacy has not been established in patients with *ALK* rearrangements <0.06% VAF tested with FoundationOne Liquid CDx.
14. LYNPARZA® efficacy has not been established in prostate cancer patients with *BRCA1/2* or *ATM* rearrangements with <0.25% VAF or with short variants in *BRCA1/2* or *ATM* <0.11% VAF tested with FoundationOne Liquid CDx.
15. RUBRACA® efficacy has not been established in prostate cancer patients with *BRCA1/2* rearrangements with <0.85% VAF or with hort variants in BRCA1/2 <0.15% VAF tested with FoundationOne Liquid CDx.
16. PIQRAY® efficacy has not been established in patients with *PIK3CA* SNVs with <0.14% VAF tested with FoundationOne Liquid CDx.
17. EXKIVITY® efficacy has not been established in patients with *EGFR* exon 20 insertions with <0.20% VAF tested with FoundationOne Liquid CDx.
18. The precision of FoundationOne Liquid CDx was only confirmed for select variants at the limit of detection (LoD).
19. The FoundationOne Liquid CDx assay does not detect heterozygous deletions.
20. The FoundationOne Liquid CDx assay does not detect copy number losses/homozygous deletions in *ATM*.
21. 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.
22. The test is not intended to provide information on cancer predisposition.
23. *BRCA1/BRCA2* homozygous deletions and rearrangements were not adequately represented in all analytical studies.
24. Representation of *ALK* rearrangements were limited in the analytical validation studies.
25. The representation of *ATM* short variants and rearrangements was limited in the analytical validation studies.
26. Performance has not been validated for cfDNA input below the specified minimum input.
27. Representation of SNV and indels that lead to *MET* exon 14 skipping that represent biomarker rule category 1 and 2, were limited in the analytical validation studies.
28. For optimal ctDNA shed, it is recommended that blood be drawn prior to therapy or at a time of disease progression. The sensitivity of liquid biopsy is related to adequate levels of ctDNA shed. Therefore, assay performance will be dependent upon level of ctDNA shed at time of testing.
2 . Due to the low prevalence of *ROS1* fusions and *NTRK1/2/3* fusions, the positive predictive value (PPV) of the test (FoundationOne Liquid CDx positive, tissue negative) may be lower than reported in test labeling.
30. FoundationOne Liquid CDx may miss a subset of patients with *NTRK1/2/3* fusion and *ROS1* fusion positive solid tumors who may derive benefit from ROZLYTREK®. In a retrospective-prospective clinical study assessing concordance between FoundationOne Liquid

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CDx test results in plasma and patients whose tumor tissue tested positive and was the basis for enrollment into a clinical trial, the data demonstrated that the FoundationOne Liquid CDx test did not detect approximately 46% of potential responders with *NTRK1/2/3* fusions and 49% of responders with *ROS1*.

31. ROZLYTREK® efficacy has not been established in patients with *NTRK2* fusions tested with FoundationOne Liquid CDx, given the low prevalence of the biomarker.
32. In a retrospective-prospective clinical study assessing concordance between FoundationOne Liquid CDx test results in plasma and patients whose tumor tissue tested positive and was the basis for enrollment into a clinical trial, FoundationOne Liquid CDx detected 1 of 7 different *NTRK3* fusion partners. Due to the rarity of these fusions, the accuracy of FoundationOne Liquid CDx for *NTRK3* sions has not been adequately determined.
33. *NTRK2* fusions per the FoundationOne Liquid CDx biomarker rules for *NTRK1/2/3* sions were not represented in analytical validation studies.
34. A study evaluating the concordance to a second method demonstrated that the agreement between FoundationOne Liquid CDx positive results and a comparator method for *NTRK1/3*, and *ROS1* was ≤ 50% (i.e., whether these are potential FoundationOne Liquid CDx false positives or false negatives by the comparator is unknown).

LEVEL 1: COMPANION DIAGNOSTICS (CDx)

Clinical evidence should be presented from a prospectively designed clinical trial. Results can also be presented from a retrospective clinical bridging study demonstrating that the clinical endpoints are preserved using plasma samples in trials where enrollment was based on tissue test results. For follow-on markers, a clinical concordance study demonstrating non-inferiority to the original FDA-approved cfDNA-based companion diagnostic device (refer to Li, Meijuan. Statistical Methods for Clinical Validation of Follow-On Companion Diagnostic Devices via an External Concordance Study. *Statistics in Biopharmaceutical Research*. 8: 35-363, 2016) is required. In addition to the clinical validation, analytical validation for each pecific Level 1 CDx biomarker should be presented.

LEVEL 2: cfDNA BIOMARKERS WITH STRONG EVIDENCE OF CLINICAL SIGNIFICANCE IN cfDNA

For a Level 2 claim of cfDNA biomarkers with strong evidence of clinical significance, clinical validation needs to be from evidence presented with FDA-approved liquid biopsy companion diagnostic biomarkers for the specific tumor type at the biomarker or variant level. Such claims should also be supported by analytical performance for each biomarker from at least LoD, precision/reproducibility, and LoD studies.

EVE 3A: B O AR ERS W TH EV DENCE OF C N CA S GN F CANCE N T SSUE SUPPORTED BY STRONG ANA YT CA VA DAT ON US NG cfDNA AND CONCORDANCE BETWEEN cfDNA AND T SSUE

Clinical evidence can be provided from tissue-based companion diagnostics. This should also be supported by analytical validation (LoD, precision, analytical accuracy, and concordance study to a tissue-based test) for the specific tumor type at the biomarker or variant level, using a representative approach for SNVs and indels. Evidence evaluating concordance between cfDNA- and tissue-samples for FDA-approved tissue markers should be demonstrated using an FDA-approved tissue test or a validated tissue test.

LEVEL 3B: BIOMARKERS WITH EVIDENCE OF CLINICAL SIGNIFICANCE IN TISSUE SUPPORTED BY ANALYTICAL VALIDATION USING cfDNA

Clinical evidence can be provided from tissue-based companion diagnostics, with analytical validity supported by a representative approach for SNVs and indels from key analytical studies (such as LoD, accuracy, and precision).

LEVEL 4: OTHER BIOMARKERS WITH POTENTIAL CLINICAL SIGNIFICANCE

Biomarkers not categorized into Levels 1, 2, or 3 can be included under Level 4 for informational purposes or to be used to direct patients toward clinical trials for which they may be eligible. Such claims can be supported by clinical rationale for inclusion in the panel. Such rationale could also include peer-reviewed publications for genes/variants in tissue, variant information from well-curated public databases, or *in vitro* pre-clinical models. Analytical validation should be supported by a representative approach for SNVs and indels from key analytical studies (such as LoD, accuracy, and precision).

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APPENDIX Genes assayed in FoundationOne®Liquid CDx

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 noncoding coverage (indicated with an *). Select regions in 75 genes (indicated in bold) are captured with increased sensitivi . Genes are captured for increased sensitivi 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 or WTX)	***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**	*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**	*EMSY* (C11orf30)	*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	*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*	*GID4* (C17orf39)	***GNA11*** **Exons 4, 5**
GNA13	***GNAQ*** **Exons 4, 5**	***GNAS*** **Exons 1, 8**	*GRM3*	*GSK3B*	*H3-3A* (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***

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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**	*MRE11* (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*	*NOTCH1*	*NOTCH2* Intron 26	*NOTCH3*	***NPM1*** **Exons 4-6, 8, 10**	***NRAS*** **Exons 2, 3**
NSD2 (WHSC1 or MMSET)	*NSD3* (WHSC1L1)	*NT5C2*	***NTRK1*** **Exons 14, 15, Introns 8-11**	*NTRK2* Intron 12	***NTRK3*** **Exons 16, 17**	*NUTM1** Intron 1	*P2RY8*	***PALB2***
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*	*PRKN* (PARK2)	*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***	*SUFU*	*SYK*	*TBX3*	*TEK*	*TENT5C* (FAM46C)	*TERC** ncRNA	***TERT**** **Promoter**
TET2	*TGFBR2*	*TIPARP*	*TMPRSS2** Introns 1-3	*TNFAIP3*	*TNFRSF14*	***TP53***	*TSC1*	*TSC2*
TYRO3	*U2AF1*	***VEGFA***	*VHL*	*WTI*	*XPO1*	*XRCC2*	*ZNF217*	*ZNF703*

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