FOUNDATIONONE® CDx

FoundationOne®CDx
Technical Information

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Intended Use

FoundationOne®CDx (F1CDx) is a qualitative next-generation sequencing based *in vitro* diagnostic test that uses targeted high throughput hybridization-based capture technology for detection of substitutions, insertion and deletion alterations (indels), and copy number alterations (CNAs) in 324 genes and select gene rearrangements, as well as genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) using DNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor tissue specimens when using the DNAx extraction method. The test is intended for detection of substitutions and indels in 324 genes, CNAs in 15 genes and select gene rearrangements, as well as genomic signatures including MSI and TMB using DNA isolated from FFPE tumor tissue specimens when using the CoExtraction method for DNA isolation. The test is intended 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, F1CDx 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. Genomic findings other than those listed in Table 1are not prescriptive or conclusive for labeled use of any specific therapeutic product.

Table 1 Companion diagnostic indications

Tumor Type	Biomarker(s) Detected	Therapy
Non-small cell lung cancer (NSCLC)	*EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations	EGFR Tyrosine Kinase Inhibitors (TKI) approved by FDA*
	EGFR exon 20 T790M alterations	Tagrisso® (osimertinib)
	ALK rearrangements	Alecensa® (alectinib), Alunbrig® (brigatinib) Xalkori® (crizotinib), or Zykadia® (ceritinib)
	BRAF V600E	Tafinlar® (dabrafenib) in combination with Mekinist® (trametinib)
	MET single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping	Tabrecta® (capmatinib)
	ROS1 Fusions	Rozlytrek® (entrectinib)
Melanoma	*BRAF* V600E	BRAF Inhibitors approved by FDA*
	BRAF V600E and V600K	Mekinist® (trametinib) or

		BRAF/MEK Inhibitor Combinations approved by FDA*
	BRAF V600 mutation-positive	Tecentriq® (atezolizumab) in combination with Cotellic® (cobimetinib) and Zelboraf® (vemurafenib)
Breast cancer	*ERBB2* (HER2) amplification	Herceptin® (trastuzumab), Kadcyla® (ado-trastuzumab-emtansine), or Perjeta® (pertuzumab)
	PIK3CA C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y alterations	Piqray® (alpelisib)
Colorectal cancer	*KRAS* wild-type (absence of mutations in codons 12 and 13)	Erbitux® (cetuximab)
	KRAS wild-type (absence of mutations in exons 2, 3, and 4) and *NRAS* wild type (absence of mutations in exons 2, 3, and 4)	Vectibix® (panitumumab)
Ovarian cancer	*BRCA1/2* alterations	Lynparza® (olaparib)
Cholangiocarcinoma	*FGFR2* fusions and select rearrangements	Pemazyre® (pemigatinib) or Truseltiq™ (infigratinib)
Prostate cancer	Homologous Recombination Repair (HRR) gene (*BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D* and *RAD54L*) alterations	Lynparza® (olaparib)
	BRCA1, BRCA2 alterations	Akeega® (niraparib + abiraterone acetate)
Solid tumors	MSI-High	Keytruda® (pembrolizumab)
	TMB ≥ 10 mutations per megabase	Keytruda® (pembrolizumab)
	NTRK1/2/3 fusions	Rozlytrek® (entrectinib) Vitrakvi® (larotrectinib),

*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

The test is also used for detection of genomic loss of heterozygosity (LOH) from formalin-fixed, paraffin-embedded (FFPE) ovarian tumor tissue. Positive homologous recombination deficiency (HRD) status (F1CDx HRD defined as tBRCA-positive and/or LOH high) in ovarian cancer patients is associated with improved progression-free survival (PFS) from Rubraca (rucaparib) maintenance therapy in accordance with the Rubraca product label.

The F1CDx assay is performed at Foundation Medicine, Inc. sites located in Cambridge, MA and Morrisville, NC.

Contraindication
There are no known contraindications.

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. The test does not provide information about susceptibility.
- Biopsy may pose a risk to the patient when archival tissue is not available for use with the assay. The patient's physician should determine whether the patient is a candidate for biopsy.
- Reflex testing to an alternative FDA approved companion diagnostic should be performed for patients who have an *ERBB2* amplification result detected with copy number equal to 4 (baseline ploidy of tumor +2) for confirmatory testing. While this result is considered negative by F1CDx, in a clinical concordance study with an FDA approved FISH test, 70% (7 out of 10 samples) were positive, and 30% (3 out 10 samples) were negative by the FISH test with an average ratio of 2.3. The frequency of *ERBB2* copy number 4 in breast cancer is estimated to be approximately 2%[1].
  [1]HER2 overexpression occurs in 18-20% of breast cancers (Owens et al. 2004 [PMID: 15140287]; Salmon et al. 1987 [PMID:3798106]; Yaziji et al. 2004 [PMID: 15113815]). Based on the F1CDx HER2 CDx concordance study, approximately 10% of HER2 amplified samples had copy number 4. Thus, total frequency is conservatively estimated to be approximately 2%.

Limitations

- For *in vitro* diagnostic use.
- For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
- A negative result does not rule out the presence of a mutation below the limits of detection of the assay.
- Samples with <25% tumor may have decreased sensitivity for the detection of copy number alterations including *ERBB2*.
- Clinical performance of Tagrisso® (osimertinib) in patients with an *EGFR* exon 20 T790M mutation detected with an allele fraction <5% has not been established.
- Due to differences in variant calling between assays and technologies, the F1CDx assay may not identify and report approximately 16% (10/61) of copy number alterations and approximately 18% (3/17) of rearrangements.
- Confirmatory testing using a clinically validated assay should be performed for all copy number alterations and rearrangements not associated with CDx claims noted in Table 1 of the Intended Use but used for clinical decision making
- For patients with solid tumors whose samples have MSI scores >0.0041 and <0.0124, an MSI "Cannot Be Determined" result is reported. Patients with this result should be re-tested with a validated orthogonal (alternative) method as these MSI scores represent a range of scores with low reliability. The likelihood of a patient receiving this result is ~3.29% within solid tumors.
- Patients with solid tumors may also receive an MSI status reported as "Cannot Be Determined" due to a quality control (QC) failure. When all sample-level quality metrics are met, the rate of MSI "Cannot Be Determined" results due to a QC failure is 8.96%. Patients with this result should consider re-testing with FoundationOne CDx or an orthogonal (alternative) method, if clinically appropriate.
- TMB by F1CDx is determined by counting all synonymous and non-synonymous variants present at 5% allele frequency or greater (after filtering) and the total number is reported as mutations per megabase (mut/Mb) unit. Observed TMB is dependent on characteristics of the specific tumor focus tested for a patient (e.g., primary vs. metastatic, tumor content) and the testing platform used for the detection; therefore, observed TMB results may vary between different specimens for the same patient and between detection methodologies employed on the same sample. The TMB calculation may differ from TMB calculations used by other assays depending on variables such as the amount of genome interrogated, percentage of tumor, assay limit of detection (LoD), filtering of alterations included in the score, and the read depth and other bioinformatic test specifications. Refer to the SSED for a detailed description of these variables in FMI's TMB calculation https://www.accessdata.fda.gov/cdrh_docs/pdf17/P170019B.pdf. The clinical validity of TMB defined by

this panel has been established for TMB as a qualitative output for a cut-off of 10 mutations per megabase but has not been established for TMB as a quantitative score.

- Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given community.
- The test is intended to be performed on specific serial number-controlled instruments by Foundation Medicine, Inc.
- Alterations in polyT homopolymer runs may not be reliably detected in *BRCA1/2*.
- Certain large rearrangements in *BRCA1/2* including large scale genomic deletions (affecting at least one whole exon), insertions or other deleterious genomic rearrangements including inversions or transversion events, may not be detected in an estimated 5% of ovarian cancer patients with *BRCA1/2* mutations by F1CDx.
- Certain potentially deleterious missense or small in-frame deletions in *BRCA1/2* may not be reported under the "CDx associated findings" but may be reported in the "Other alterations and biomarkers identified" section in the patient report.
- Alterations at allele frequencies below the established limit of detection may not be detected consistently.
- Detection of LOH has been verified only for ovarian cancer patients
- Performance of the LOH classification has not been established for samples below 35% tumor content and with LOH scores near the cut-off of 16.
- There may be potential interference of ethanol with LOH detection. The interfering effects of xylene, hemoglobin, and triglycerides on the LOH score have not been demonstrated.
- While the overall positive percent agreement between trial enrollment assays and F1CDx was 84% (37/44), thirty percent (30%) (6/20) of patients enrolled in the VITRAKVI clinical studies using RNA-based NGS detection were negative for *NTRK* fusions by F1CDx. Four of the six patients (4/6 or 60%) that were negative for *NTRK* fusions by F1CDx had a response to larotrectinib. Therefore, F1CDx may miss a subset of patients with solid tumors with *NTRK1/2/3* fusions who may derive benefit from VITRAKVI.
- *NTRK2* fusions per the F1CDx CDx biomarker rules for *NTRK1/2/3* fusions were not well-represented in analytical validation studies.
- While the overall positive percent agreement between trial enrollment assays and F1CDx was 63.6% (21/33), 52.4% (11/21) of patients enrolled in the ROZLYTREK clinical studies using RNA-based NGS detection were negative for *NTRK* fusions by F1CDx. 36.4% (4/11) of the patients enrolled using RNA-based NGS detection that were negative for *NTRK* fusions by F1CDx had a response to ROZLYTREK.
- While the overall positive percent agreement between trial enrollment assays and F1CDx was 73.9% (34/46), 30.3% (10/33) of patients enrolled in the ROZLYTREK clinical studies using RNA-based NGS detection were negative for *ROS1* fusions by F1CDx. 50.0% (5/10) of the patients enrolled using RNA-based NGS detection that were negative for *ROS1* fusions by F1CDx had a response to ROZLYTREK
- In detecting *ALK* rearrangements, due to differences in technology, F1CDx, FISH, and IHC may identify slightly different populations. 6.5% of negatives by F1CDx may be positive by both IHC and FISH. See Section 3.4 for more information.
- Certain gene fusions, and rearrangements (RE) including but not limited to *BRCA1* RE, *BRCA2* RE, *CDK12* RE, and indels in *PALB2* were not adequately validated in the site-to-site reproducibility study and may not be detected consistently by F1CDx.

Test Principle

FoundationOne®CDx (F1CDx) is performed exclusively as a laboratory service using DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tumor samples. The assay employs two extraction methods (either DNAx or CoExtraction, an automated DNA/RNA co-extraction methodology) for DNA extraction from routine FFPE biopsy or surgical resection specimens; 50-1000 ng of DNA will undergo whole-genome shotgun library construction and hybridization-based capture of all coding exons from 309 cancer-related genes, one promoter region, one non-coding (ncRNA), and select intronic regions from 34 commonly rearranged genes, 21 of which also include the coding exons (refer to Table 2 and Table 3 for the complete list of genes included in F1CDx). In

total, the assay detects alterations in a total of 324 genes. Using the Illumina® HiSeq 4000 platform, hybrid capture–selected libraries are sequenced to high uniform depth (targeting >500X median coverage with >99% of exons at coverage >100X). Sequence data is then processed using a customized analysis pipeline designed to detect all classes of genomic alterations, including base substitutions, indels, copy number alterations (amplifications and homozygous gene deletions), and select genomic rearrangements (e.g., gene fusions). Additionally, genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB), and positive homologous recombination deficiency (HRD) status (tBRCA-positive and/or LOH high) are reported.

Table 2 Genes with full coding exonic regions included in FoundationOne®CDx for the detection of substitutions, insertions and deletions (indels), and copy number alterations (CNAs)*

ABL1	*BRAF*	*CDKN1A*	*EPHA3*	*FGFR4*	*IKZF1*	*MCL1*	*NKX2-1*	*PMS2*	*RNF43*	*TET2*
ACVR1B	**BRCA1*	*CDKN1B*	*EPHB1*	*FH*	*INPP4B*	*MDM2*	*NOTCH1*	*POLD1*	*ROS1*	*TGFBR2*
AKT1	**BRCA2*	*CDKN2A*	*EPHB4*	*FLCN*	*IRF2*	*MDM4*	*NOTCH2*	*POLE*	*RPTOR*	*TIPARP*
AKT2	*BRD4*	*CDKN2B*	**ERBB2*	*FLT1*	*IRF4*	*MED12*	*NOTCH3*	*PPARG*	*SDHA*	*TNFAIP3*
AKT3	**BRIP1*	*CDKN2C*	*ERBB3*	*FLT3*	*IRS2*	*MEF2B*	*NPM1*	*PPP2R1A*	*SDHB*	*TNFRSF14*
ALK	*BTG1*	*CEBPA*	*ERBB4*	*FOXL2*	*JAK1*	*MEN1*	*NRAS*	*PPP2R2A*	*SDHC*	*TP53*
ALOX12B	*BTG2*	**CHEK1*	*ERCC4*	*FUBP1*	*JAK2*	*MERTK*	*NT5C2*	*PRDM1*	*SDHD*	*TSC1*
AMER1	*BTK*	**CHEK2*	*ERG*	*GABRA6*	*JAK3*	*MET*	*NTRK1*	*PRKAR1A*	*SETD2*	*TSC2*
APC	*C11orf30*	*CIC*	*ERRFI1*	*GATA3*	*JUN*	*MITF*	*NTRK2*	*PRKCI*	*SF3B1*	*TYRO3*
AR	*CALR*	*CREBBP*	*ESR1*	*GATA4*	*KDM5A*	*MKNK1*	*NTRK3*	*PTCH1*	*SGK1*	*U2AF1*
ARAF	*CARD11*	*CRKL*	*EZH2*	*GATA6*	*KDM5C*	*MLH1*	*P2RY8*	*PTEN*	*SMAD2*	*VEGFA*
ARFRP1	*CASP8*	*CSF1R*	*FAM46C*	*GID4 (C17orf39)*	*KDM6A*	*MPL*	**PALB2*	*PTPN11*	*SMAD4*	*VHL*
ARID1A	*CBFB*	*CSF3R*	*FANCA*	*GNA11*	*KDR*	*MRE11A*	*PARK2*	*PTPRO*	*SMARCA4*	*WHSC1*
ASXL1	*CBL*	*CTCF*	*FANCC*	*GNA13*	*KEAP1*	*MSH2*	*PARP1*	*QKI*	*SMARCB1*	*WHSC1L1*
**ATM*	*CCND1*	*CTNNA1*	*FANCG*	*GNAQ*	*KEL*	*MSH3*	*PARP2*	*RAC1*	*SMO*	*WT1*
ATR	*CCND2*	*CTNNB1*	**FANCL*	*GNAS*	*KIT*	*MSH6*	*PARP3*	*RAD21*	*SNCAIP*	*XPO1*
ATRX	*CCND3*	*CUL3*	*FAS*	*GRM3*	*KLHL6*	*MST1R*	*PAX5*	*RAD51*	*SOCS1*	*XRCC2*
AURKA	*CCNE1*	*CUL4A*	*FBXW7*	*GSK3B*	*KMT2A (MLL)*	*MTAP*	*PBRM1*	**RAD51B*	*SOX2*	*ZNF217*
AURKB	*CD22*	*CXCR4*	*FGF10*	*H3F3A*	*KMT2D (MLL2)*	*MTOR*	*PDCD1*	**RAD51C*	*SOX9*	*ZNF703*
AXIN1	*CD274*	*CYP17A1*	*FGF12*	*HDAC1*	*KRAS*	*MUTYH*	*PDCD1LG2*	**RAD51D*	*SPEN*	
AXL	*CD70*	*DAXX*	*FGF14*	*HGF*	*LTK*	*MYC*	*PDGFRA*	*RAD52*	*SPOP*	
BAP1	*CD79A*	*DDR1*	*FGF19*	*HNF1A*	*LYN*	*MYCL*	*PDGFRB*	**RAD54L*	*SRC*	
**BARD1*	*CD79B*	*DDR2*	*FGF23*	*HRAS*	*MAF*	*MYCN*	*PDK1*	*RAF1*	*STAG2*	
BCL2	*CDC73*	*DIS3*	*FGF3*	*HSD3B1*	*MAP2K1*	*MYD88*	*PIK3C2B*	*RARA*	*STAT3*	
BCL2L1	*CDH1*	*DNMT3A*	*FGF4*	*ID3*	*MAP2K2*	*NBN*	*PIK3C2G*	*RB1*	*STK11*	
BCL2L2	**CDK12*	*DOT1L*	*FGF6*	*IDH1*	*MAP2K4*	*NF1*	*PIK3CA*	*RBM10*	*SUFU*	
BCL6	*CDK4*	*EED*	*FGFR1*	*IDH2*	*MAP3K1*	*NF2*	*PIK3CB*	*REL*	*SYK*	

BCOR	*CDK6*	*EGFR*	*FGFR2*	*IGF1R*	*MAP3K13*	*NFE2L2*	*PIK3R1*	*RET*	*TBX3*	
BCORL1	*CDK8*	*EP300*	*FGFR3*	*IKBKE*	*MAPK1*	*NFKBIA*	*PIM1*	*RICTOR*	*TEK*	
*Genes with copy number alteration reporting are limited to CDx variants when using CoExtraction method										

Table 3 Genes with select intronic regions for the detection of gene rearrangements, one with 3'UTR, one gene with a promoter region and one ncRNA gene

ALK *introns 18, 19*	*BRCA1* *introns 2, 7, 8, 12, 16, 19, 20*	*ETV4* *intron 8*	EZR *introns 9- 11*	*KIT* *intron 16*	*MYC* *intron 1*	*NUTM1* *intron 1*	*RET* *introns 7-11*	*SLC34A2* *intron 4*
BCL2 *3'UTR*	*BRCA2* *intron 2*	*ETV5* *introns 6, 7*	*FGFR1* *intron 1, 5, 17*	*KMT2A (MLL)* *introns 6-11*	*NOTCH2* *intron 26*	*PDGFRA* *introns 7, 9, 11*	*ROS1* *introns 31-35*	*TERC* *ncRNA*
BCR *introns 8, 13, 14*	*CD74* *introns 6- 8*	*ETV6** *introns 5, 6*	*FGFR2* *intron 1, 17*	*MSH2* *intron 5*	*NTRK1* *introns 8-11*	*RAF1* *introns 4-8*	*RSPO2* *intron 1*	*TERT* Promoter
BRAF *introns 7- 10*	*EGFR* *introns 7, 15, 24-27*	*EWSR1* *introns 7-13*	*FGFR3* *intron 17*	*MYB* *intron 14*	*NTRK2* *Intron 12*	*RARA* *intron 2*	*SDC4* *intron 2*	*TMPRSS2* *introns 1- 3*

**ETV6* is a common rearrangement partner for *NTRK3*

Summary and Explanation

FoundationOne®CDx (F1CDx) is a broad companion diagnostic (CDx) test for eight tumor indications. In addition to use as a companion diagnostic, F1CDx provides cancer relevant alterations that may inform patient management in accordance with professional guidelines. Information generated by this test is an aid in the identification of patients who are most likely to benefit from associated therapeutic products as noted in Table 1 of the Intended Use.

The F1CDx platform employs whole-genome shotgun library construction and hybridization-based capture of DNA extracted from FFPE tumor tissue prior to uniform and deep sequencing on the Illumina® HiSeq 4000. Following sequencing, custom software is used to determine genomic variants including substitutions, insertion and deletion variants (indels), copy number alterations (CNAs), genomic rearrangements, microsatellite instability (MSI), tumor mutational burden (TMB), and positive homologous recombination deficiency (HRD) status. The output of the test includes:

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

Category 2: Cancer Mutations with Evidence of Clinical Significance

Category 3: Cancer Mutations with Potential Clinical Significance

Sequence Analysis

Sequence data are analyzed using proprietary software developed by FMI. Sequence data are mapped to the human genome (hg19) using Burrows-Wheeler Aligner (BWA) v0.5.9.[2] PCR duplicate read removal and sequence metric collection are performed using Picard 1.47 (http://picard.sourceforge.net) and SAM tools 0.1.12a.[3] Local alignment optimization is performed using Genome Analysis Toolkit (GATK) 1.0.4705.[4] Variant calling is performed only in genomic regions targeted by the test.

Base substitution detection is performed using a Bayesian methodology, which allows for the detection of novel somatic alterations at low mutant allele frequency (MAF) and increased sensitivity for alterations at hotspot sites through the incorporation of tissue-specific prior expectations.[5] Reads with low mapping (mapping quality < 25) or base calling quality (base calls with quality ≤ 2) are discarded. Final calls are made at MAF ≥ 5% (MAF ≥ 1% at hotspots).

To detect indels, de novo local assembly in each targeted exon is performed using the de-Bruijn approach.[6] Key steps are:

- Collecting all read-pairs for which at least one read maps to the target region.
- Decomposing each read into constituent k-mers and constructing an enumerable graph representation (de-Bruijn) of all candidate non-reference haplotypes present.
- Evaluating the support of each alternate haplotype with respect to the raw read data to generate mutational candidates. All reads are compared to each of the candidate haplotypes via ungapped alignment, and a read 'vote' for each read is assigned to the candidate with best match. Ties between candidates are resolved by splitting the read vote, weighted by the number of reads already supporting each haplotype. This process is iterated until a 'winning' haplotype is selected.
- Aligning candidates against the reference genome to report alteration calls.

Filtering of indel candidates is carried out similarly to base substitutions, with an empirically increased allele frequency threshold at repeats and adjacent sequence quality metrics as implemented in GATK: % of neighboring bases mismatches < 25%, average neighboring base quality > 25, average number of supporting read mismatches ≤ 2. Final calls are made at MAF ≥ 5% (MAF ≥ 3% at hotspots).

Copy number alterations (CNAs) are detected using a comparative genomic hybridization (CGH)-like method. First, a log-ratio profile of the sample is acquired by normalizing the sequence coverage obtained at all exons and genome-wide SNPs (~3,500) against a process-matched normal control. This profile is segmented and interpreted using allele frequencies of sequenced SNPs to estimate tumor purity and copy number at each segment. Amplifications are called at segments with ≥ 6 copies (or ≥ 7 for triploid/≥ 8 for tetraploid tumors) and homozygous deletions at 0 copies, in samples with tumor purity ≥ 20%. Amplifications in ERBB2 are called positive at segments with ≥ 5 copies for diploid tumors.

Genomic rearrangements are identified by analyzing chimeric read pairs. Chimeric read pairs are defined as read pairs for which reads map to separate chromosomes, or at a distance of over 10 megabase (Mb). Pairs are clustered by genomic coordinate of the pairs, and clusters containing at least five chimeric pairs (three for known fusions) are identified as rearrangement candidates. Filtering of candidates is performed by mapping quality (average read mapping quality in the cluster must be 30 or above) and distribution of alignment positions. Rearrangements are annotated for predicted function (e.g., creation of fusion gene).

To determine a patient's MSI status, F1CDx employs a fraction based (FB) MSI algorithm to categorize a tumor specimen as MSI-High (MSI-H) or microsatellite stable (MSS). The FB-MSI algorithm calculates the fraction of microsatellite loci determined to be altered or unstable (i.e., the fraction unstable loci score) based on a genome-wide analysis across >2000 microsatellite loci. For a given microsatellite locus, non-somatic alleles are discarded, and the microsatellite is categorized as unstable if remaining alleles differ from the reference genome. The final fraction unstable loci score is calculated as the number of unstable microsatellite loci divided by the number of evaluable microsatellite loci. Two FB-MSI score thresholds are applied to classify a tumor specimen as having MSI-H or MSS status. MSI-H status is reported for patients with solid tumors whose samples have FB-MSI scores ≥ 0.0124 while MSS status is reported for patients with solid tumors whose samples have FB- MSI scores ≤ 0.0041. Per the F1CDx assay, a patient whose tumor has an MSI-H score ≥ 0.0124 is reported as eligible for treatment with KEYTRUDA. For patients with solid tumors whose samples have FB-MSI scores >0.0041 and <0.0124, an MSI "Cannot be Determined" result is reported. Patients with this result should be re- tested with a validated orthogonal (alternative) method as these MSI scores represent a range of scores with low reliability. Patients with solid tumors may also receive an MSI status reported as MSI-Cannot Be Determined due to a quality control (QC) failure. Patients with this result should consider re-testing with FoundationOneCDx or an orthogonal (alternative) method, if clinically appropriate.

Tumor mutational burden (TMB) is measured by counting all synonymous and non- synonymous substitution and indel variants present at 5% allele frequency or greater and filtering out potential germline variants according to published databases of known germline polymorphisms including Single Nucleotide Polymorphism database (dbSNP) and Exome Aggregation Consortium (ExAC). Additional germline alterations still present after database

querying are assessed for potential germline status and filtered out using a somatic-germline/zygosity (SGZ) algorithm.

Furthermore, known and likely driver mutations are filtered out to exclude bias of the data set. The resulting mutation number is then divided by the coding region corresponding to the number of total variants counted, or 793 kb. The resulting number is communicated as mutations per Mb unit (mut/Mb). Per the F1CDx assay, a patient whose tumor has a TMB ≥ 10 mut/Mb is reported as eligible for treatment with KEYTRUDA:

To compute the percentage of genomic LOH for each tumor, LOH segments are inferred across the 22 autosomal chromosomes using the genome-wide aneuploidy/copy number profile and minor allele frequencies of the more than 3500 SNPs sequenced in the Foundation Medicine's next-generation sequencing (NGS)- based platform. A comparative genomic hybridization (i.e., log-ratio profile of the sample) is obtained from the NGS sequencing data by normalizing the sequence coverage obtained at all exons and genome-wide SNPs against a process-matched normal control. This profile is segmented and interpreted using allele frequencies of sequenced SNPs to estimate copy number (Ci) and minor allele count (Mi) at each segment (i). A segment is determined to have LOH if Ci ≠ 0 and Mi = 0. Two types of LOH segments are excluded from the calculation of percent genomic LOH: (1) LOH segments spanning ≥ 90% of a whole chromosome or chromosome arm, as these LOH events usually arise through non-homologous recombination deficiency (HRD) mechanisms (e.g., mitotic nondisjunction), and (2) regions in which LOH inference is ambiguous (e.g., some small genomic regions that do not have sufficient heterozygous SNPs to support LOH calling).

After completion of the Analysis Pipeline, variant data are displayed in the FMI custom-developed CATi software applications with sequence QC metrics. As part of data analysis QC for every sample, the F1CDx assay assesses cross-contamination through the use of a SNP profile algorithm, reducing the risk of false-positive calls that could occur as a result of an unexpected contamination event. Sequence data are reviewed by trained bioinformatics personnel. Samples failing any QC metrics are automatically held and not released.

Test Kit Contents
The FoundationOne®CDx (F1CDx) test includes a sample shipping kit, which is sent to ordering laboratories. The shipping kit contains the following components:

- Specimen Preparation Instructions and Shipping Instructions
- Return Shipping Label

All other reagents, materials and equipment needed to perform the assay are used exclusively in the Foundation Medicine laboratories. The F1CDx assay is intended to be performed with serial number-controlled instruments.

Sample Collection and Test Ordering
To order FoundationOne®CDx (F1CDx), the Test Requisition Form (TRF) included 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/pdf17/P170019B.pdf

1. Instruments
The F1CDx device is intended to be performed with the following instruments, as identified by specific serial numbers:

- Agilent Technologies Benchbot Workstation with Integrated Bravo Automated Liquid Handler or Hamilton Microlab STAR/STARlet Liquid Handling Workstation
- Beckman Biomek NX^{P} Span-8 Liquid Handler or Hamilton Microlab STAR/STARlet Liquid Handling Workstation
- Hamilton AutoLys Liquid Handling Workstation
- Covaris LE220-plus Focused ultrasonicator
- Thermo Fisher Scientific KingFisher™ Flex with 96 Deep-well Head

- Illumina® cBot System
- Illumina® HiSeq 4000 System

2. Performance Characteristics

Performance characteristics were established using DNA derived from a wide range of FFPE tissue types; tissue types associated with CDx indications were included in each study. Table 4 below provides a summary of tissue types included in each study. Each study also included a broad range of representative alteration types for each class of alteration (substitution, insertion and deletion, copy number alterations, and rearrangements) in various genomic contexts across a broad selection of genes as well as analysis of genomic signatures including MSI and TMB. Table 5 provides a summary of genes and alteration types associated with the validation studies.

Table 4 Summary of tissue types included in validation studies

Tissue or Tumor Type	Limit of Detection	Precision	Pan-Tumor Analysis	NGS Concordance	Inter-Laboratory Concordance	CDx Concordance	DNA Extraction	DNA Stability (part 1)	FFPE Slide Stability	Interfering Substances	Guard Banding/Robustness	Molecular Index Barcodes	Variant Curation	Reagent Stability
Abdomen or Abdominal wall			■	■	■			■						
Adrenal Gland			■	■	■									
Anus			■	■	■									
Appendix			■	■	■									
Bladder			■	■	■		■							
Bone			■	■	■									
Brain			■	■	■		■	■						
Breast	■	■	■	■	■	■	■	■	■	■	■	■	■	
Cervix			■	■	■			■						
Chest wall			■	■	■			■						
Cholangiocarcinoma	■	■				■				**				
Colon	■	■	■	■	■	■	■	■	■	■	■	■	■	
Diaphragm			■	■	■			■						
Duodenum			*	■	■									
Ear			*	■	■									
Endometrium	■		*	■	■			■						
Esophagus			■											
Fallopian Tube			■											
Gallbladder			■	■	■			■						
Gastro-esophageal junction			■	■	■									
Head and Neck			■	■	■			■						
Kidney	■		■	■	■			■						
Larynx			*	■	■									

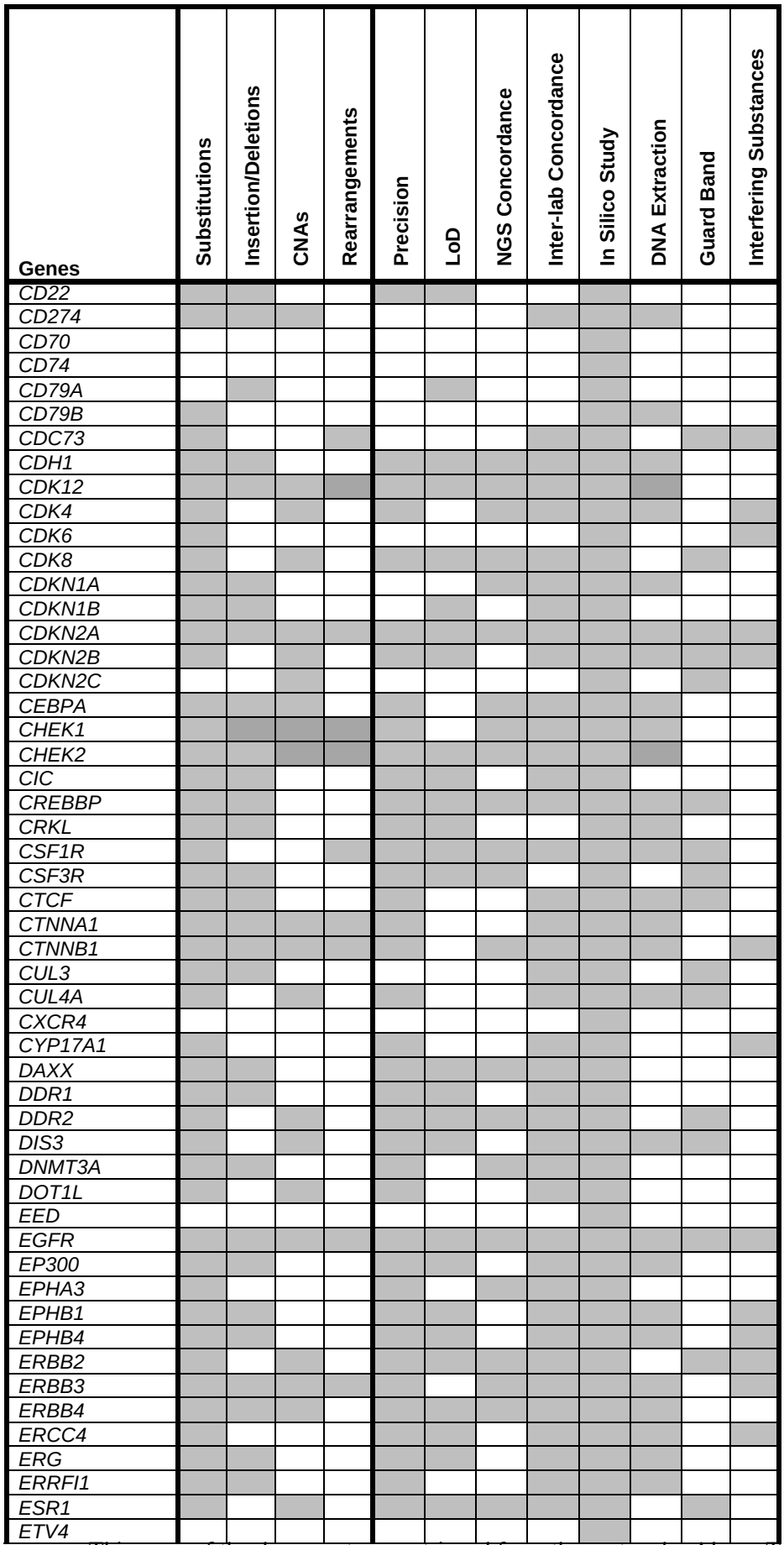
Tissue or Tumor Type	Limit of Detection	Precision	Pan-Tumor Analysis	NGS Concordance	Inter-Laboratory Concordance	CDx Concordance	DNA Extraction	DNA Stability (part 1)	FFPE Slide Stability	Interfering Substances	Guard Banding/Robustness	Molecular Index Barcodes	Variant Curation	Reagent Stability
Liver														
Lung														
Lymph Node														
Malignant effusions														
Mediastinum														
Nasal Cavity			*											
Omentum														
Ovarian														
Pancreas														
Pancreatobiliary														
Parotid Gland			*											
Pelvis														
Penis			*											
Pericardium														
Peritoneum														
Pleura			*											
Prostate														
Rare Tissues*														
Rectum			*											
Salivary Gland														
Skin (Melanoma)														
Small Intestine														
Soft Tissue														
Spleen														
Stomach														
Thyroid														
Tongue			*											
Trachea			*											
Ureter														
Uterus														
Vagina														
Vulva														
Whipple Resection														

***Included as "Rare Tissues" in Pan-Tumor Analysis**

**** Post-market study pending**

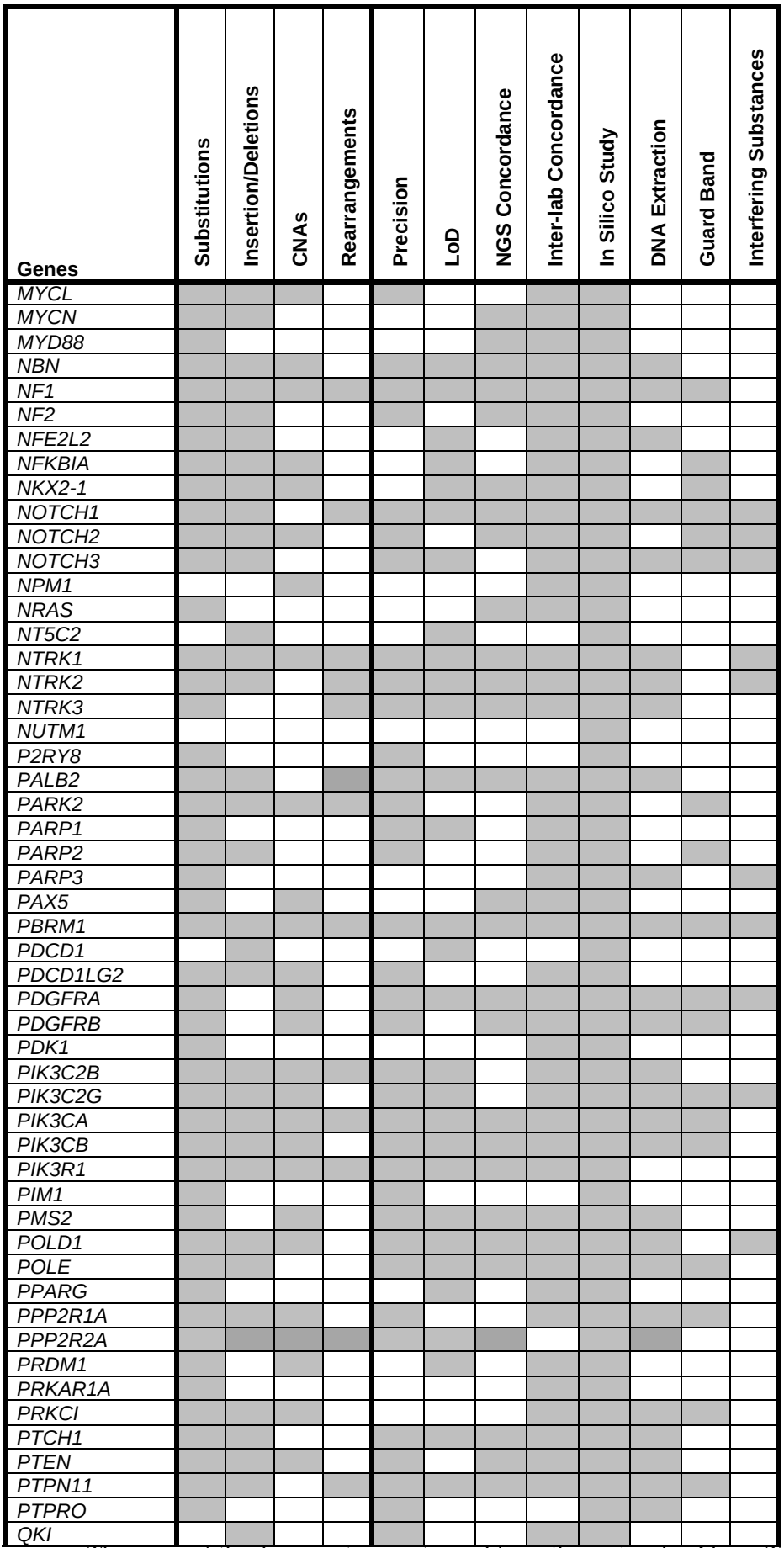
Table 5 Summary of genes and alteration types included in validation studies.

Genes	Substitutions	Insertion/Deletions	CNAs	Rearrangements	Precision	LoD	NGS Concordance	Inter-lab Concordance	In Silico Study	DNA Extraction	Guard Band	Interfering Substances
ABL1	■	■	■	■	■		■	■	■			
ACVR1B	■	■			■	■		■	■			
AKT1	■		■	■	■	■	■	■	■	■		■
AKT2	■	■	■		■		■	■	■	■		
AKT3	■	■			■	■	■	■	■			
*ALK**	■	■	■	■	■	■	■	■	■	■		
ALOX12B	■	■				■		■	■			■
AMER1 (FAM123B)	■	■			■	■		■	■			
APC	■	■	■		■	■	■	■	■	■	■	■
AR	■	■	■		■	■	■	■	■	■	■	
ARAF	■	■			■	■	■	■	■		■	■
ARFRP1	■		■		■			■	■		■	
ARID1A	■	■		■	■	■	■	■	■	■		■
ASXL1	■	■	■		■	■	■	■	■	■		
ATM	■	■	■	■	■	■	■	■	■	■		
ATR	■	■	■		■			■	■	■	■	
ATRX	■	■				■	■	■	■	■		
AURKA	■		■		■	■	■	■	■		■	
AURKB	■	■				■	■	■	■			
AXIN1	■	■			■	■		■	■			■
AXL	■		■	■	■		■	■	■	■		
BAP1	■	■	■			■	■	■	■			
BARD1	■	■		■	■	■	■	■	■			
BCL2	■	■			■		■	■	■	■		
BCL2L1			■					■	■		■	■
BCL2L2	■		■	■				■	■			
BCL6	■	■	■		■			■	■		■	
BCOR	■	■			■	■	■	■	■	■		
BCORL1	■	■			■	■	■	■	■	■	■	
BCR									■			
BRAF	■	■		■	■	■	■	■	■	■		■
BRCA1	■	■	■	■	■	■	■	■	■	■	■	■
BRCA2	■	■	■	■	■	■	■	■	■	■	■	■
BRD4	■	■			■	■		■	■	■		
BRIP1	■	■	■	■	■	■	■	■	■	■		■
BTG1	■	■			■			■	■			
BTG2									■			
BTK	■				■	■		■	■			
C11orf30 (EMSY)	■	■	■		■	■		■	■	■	■	
CALR	■						■		■			
CARD11	■	■	■		■	■		■	■			
CASP8	■	■				■		■	■		■	
CBFB									■			
CBL	■	■			■	■	■	■	■	■	■	■
CCND1	■	■	■		■	■	■	■	■	■	■	■
CCND2	■		■					■	■			
CCND3	■		■			■		■	■	■		
CCNE1	■		■			■	■	■	■	■		



Genes	Substitutions	Insertion/Deletions	CNAs	Rearrangements	Precision	LoD	NGS Concordance	Inter-lab Concordance	In Silico Study	DNA Extraction	Guard Band	Interfering Substances
ETV5									■			
ETV6				■	■	■	■		■			
EWSR1				■	■				■			
EZH2	■				■		■	■	■		■	
EZR									■			
FAM46C	■							■	■			
FANCA	■	■	■		■		■	■	■	■		
FANCC	■				■	■		■	■	■		■
FANCG	■	■	■		■	■		■	■	■		
FANCL	■	■			■	■		■	■		■	
FAS	■	■				■		■	■			
FBXW7	■	■			■	■	■	■	■	■		■
FGF10	■		■		■	■		■	■		■	
FGF12	■		■					■	■		■	
FGF14	■		■		■			■	■	■	■	■
FGF19	■	■	■		■			■	■	■	■	
FGF23	■		■			■		■	■			
FGF3	■		■	■	■	■		■	■	■	■	
FGF4	■		■		■			■	■	■	■	
FGF6	■		■					■	■	■		
FGFR1	■	■	■	■	■	■	■	■	■	■	■	■
FGFR2	■		■	■		■	■	■	■	■		
FGFR3	■				■		■	■	■	■		■
FGFR4	■	■			■	■	■	■	■		■	
FH	■	■			■			■	■			
FLCN	■	■			■	■		■	■	■		
FLT1	■	■	■	■	■	■	■	■	■	■	■	
FLT3	■		■		■		■	■	■	■	■	
FOXL2	■	■			■	■		■	■		■	
FUBP1	■	■			■			■	■	■		■
GABRA6	■	■	■		■			■	■	■		
GATA3	■	■	■		■	■	■	■	■		■	
GATA4	■	■	■			■		■	■			
GATA6	■	■	■		■	■		■	■	■		
GID4 (C17orf39)	■							■	■			
GNA11	■		■	■	■		■	■	■			
GNA13	■				■			■	■	■		
GNAQ	■					■	■	■	■			
GNAS	■		■		■	■	■	■	■		■	
GRM3	■				■	■	■	■	■	■		
GSK3B	■							■	■			
H3F3A									■			
HDAC1	■				■				■			
HGF	■				■			■	■	■		■
HNF1A	■	■			■	■	■	■	■	■		
HRAS	■	■					■	■	■			■
HSD3B1	■				■			■	■			
ID3									■			
IDH1	■	■			■	■	■	■	■	■		■
IDH2	■	■			■		■	■	■	■		
IGF1R	■	■	■		■	■	■	■	■	■		■

Genes	Substitutions	Insertion/Deletions	CNAs	Rearrangements	Precision	LoD	NGS Concordance	Inter-lab Concordance	In Silico Study	DNA Extraction	Guard Band	Interfering Substances
IKBKE	■	■	■		■	■		■	■			
IKZF1	■		■		■	■	■	■	■			
INPP4B	■				■	■		■	■	■		
IRF2	■				■			■	■			
IRF4									■			
IRS2	■	■	■		■	■		■	■	■	■	
JAK1	■	■	■	■	■	■	■	■	■	■		
JAK2	■	■	■		■		■	■	■	■		
JAK3	■	■	■			■	■	■	■	■		
JUN	■					■			■			
KDM5A	■	■	■		■	■		■	■		■	
KDM5C	■			■	■	■		■	■			
KDM6A	■	■	■		■	■	■	■	■	■		
KDR	■	■	■		■	■	■	■	■	■	■	
KEAP1	■	■			■	■		■	■	■		■
KEL	■				■			■	■	■		■
KIT	■	■	■		■	■	■	■	■	■	■	■
KLHL6	■	■	■		■			■	■	■	■	
KMT2A (MLL)	■	■	■		■	■	■	■	■	■	■	
KMT2D (MLL2)	■	■		■	■	■		■	■	■	■	
KRAS	■	■	■		■	■	■	■	■	■		
LTK	■	■			■	■		■	■	■		
LYN	■	■	■	■	■	■		■	■	■		
MAF	■				■	■			■			
MAP2K1	■						■	■	■			
MAP2K2	■			■	■	■	■	■	■			
MAP2K4	■	■	■	■			■	■	■	■		
MAP3K1	■	■	■		■	■		■	■	■	■	
MAP3K13	■		■		■	■		■	■	■	■	■
MAPK1	■	■				■	■		■			
MCL1	■		■		■		■	■	■			
MDM2	■		■		■		■		■	■		
MDM4	■		■				■	■	■	■		
MED12	■	■			■	■	■	■	■	■	■	
MEF2B	■		■		■			■	■	■	■	
MEN1	■	■			■	■	■	■	■		■	
MERTK	■							■	■			
MET	■	■			■	■	■	■	■	■		
MITF	■		■		■		■	■	■			
MKNK1	■					■		■	■	■		
MLH1	■	■	■		■	■	■	■	■			
MPL	■	■	■		■		■	■	■	■		
MRE11A	■	■	■	■	■		■	■	■			
MSH2	■	■			■	■	■	■	■		■	
MSH3	■	■			■	■			■	■		
MSH6	■	■			■	■	■	■	■	■		
MST1R	■	■			■	■		■	■	■		■
MTAP	■		■		■	■	■		■	■	■	■
MTOR	■	■			■	■	■	■	■	■		
MUTYH	■				■	■	■	■	■	■		
MYB				■				■	■			
MYC	■	■	■	■	■	■	■	■	■	■	■	



Genes	Substitutions	Insertion/Deletions	CNAs	Rearrangements	Precision	LoD	NGS Concordance	Inter-lab Concordance	In Silico Study	DNA Extraction	Guard Band	Interfering Substances
RAC1	■						■	■	■	■		
RAD21	■		■		■	■	■		■	■	■	
RAD51	■							■	■			
RAD51B (RAD51L1)	■		■	■	■	■		■	■		■	
RAD51C	■	■	■	■	■	■	■	■	■	■		
RAD51D (RAD51L3)	■	■	■	■	■	■	■		■			
RAD52	■		■		■			■	■			
RAD54L	■	■		■	■	■	■	■	■	■	■	
RAF1	■				■		■	■	■	■		■
RARA	■	■	■		■	■	■	■	■	■		
RB1	■	■	■	■	■		■	■	■	■		
RBM10	■	■				■		■	■	■		
REL	■	■						■	■			■
RET	■				■	■	■	■	■	■		
RICTOR	■		■		■	■	■	■	■	■	■	■
RNF43	■	■			■	■		■	■	■		
ROS1	■	■	■		■	■	■	■	■	■		
RPTOR	■	■		■	■	■	■	■	■	■	■	■
RSPO2									■			
SDC4									■			
SDHA	■	■	■		■	■		■	■	■	■	■
SDHB	■							■	■			
SDHC	■		■		■				■			■
SDHD	■							■	■			
SETD2	■	■			■	■		■	■	■		
SF3B1	■				■	■	■	■	■	■		
SGK1	■		■			■			■		■	
SLC34A2									■			
SMAD2	■		■		■		■	■	■			
SMAD4	■	■	■	■	■	■	■	■	■			
SMARCA4	■	■			■	■	■	■	■			
SMARCB1	■	■					■	■	■			
SMO	■	■		■	■	■	■	■	■			
SNCAIP	■	■	■		■			■	■	■	■	■
SOCS1	■							■	■			
SOX2	■		■			■		■	■		■	
SOX9	■	■			■	■		■	■	■	■	■
SPEN	■	■		■	■	■		■	■	■		■
SPOP	■		■	■	■	■	■	■	■	■		
SRC	■		■		■		■	■	■	■		
STAG2	■	■					■	■	■	■		
STAT3	■	■	■		■			■	■	■		
STK11	■	■	■	■	■		■	■	■	■		■
SUFU	■						■	■	■	■		
SYK	■				■	■		■	■	■		
TBX3	■	■	■	■	■	■		■	■	■		
TEK	■		■		■			■	■	■		
TERC			■					■	■		■	
TERT promoter	■					■		■	■	■	■	■
TET2	■	■			■	■	■	■	■	■	■	

Genes	Substitutions	Insertion/Deletions	CNAs	Rearrangements	Precision	LoD	NGS Concordance	Inter-lab Concordance	In Silico Study	DNA Extraction	Guard Band	Interfering Substances
TGFBR2	■	■			■	■	■	■	■	■		
TIPARP	■		■			■		■	■		■	■
TMPRSS2				■				■	■			
TNFAIP3	■	■	■		■	■		■	■	■		
TNFRSF14	■	■			■			■	■			
TP53	■	■	■		■	■	■	■	■	■	■	■
TSC1	■	■			■	■	■	■	■	■		
TSC2	■	■			■	■	■	■	■	■	■	■
TYRO3	■	■			■	■		■	■	■	■	
U2AF1	■						■	■	■			
VEGFA	■							■	■	■		■
VHL	■						■	■	■			
WHSC1	■	■			■	■			■	■		
WHSC1L1	■	■	■		■				■			
WT1	■	■					■	■	■	■		■
XPO1	■	■			■			■	■	■		
XRCC2				■	■				■			
ZNF217	■	■	■		■	■		■	■	■	■	
ZNF703	■	■	■		■	■		■	■	■		■

2.1 Concordance – Comparison to an Orthogonal Method

The detection of alterations by the F1CDx assay was compared to results of an externally validated NGS assay (evNGS). Overall there were 157 overlapping genes between the two assays. The comparison between short alterations, including base substitutions and short indels, detected by F1CDx and the orthogonal method included 188 samples from 46 different tumors. Additional orthogonal concordance data includes:

- 101 (53 positive, 48 negative by F1CDx) breast cancer samples were analyzed to determine concordance specific to *PIK3CA* base substitutions
- 158 (26 positive, 132 negative by F1CDx) cholangiocarcinoma samples were analyzed to determine concordance to an externally validated laboratory developed test specific to *FGFR2* fusions and select rearrangements with additional samples to be completed in the post-market setting
- 168 (50 positive, 118 negative by F1CDx) NSCLC samples were analyzed to determine concordance for detection of qualifying *MET* exon 14 base substitutions and indels
- 230 (120 positive, 110 negative by F1CDx) samples were analyzed to determine concordance specific to HRR alterations (including base substitutions, indels, rearrangements and homozygous deletions)
- 218 (76 TMB-H, 146 non-TMB-H by F1CDx) samples were analyzed to determine concordance with a CLIA validated whole exome sequencing (WES) assay for detection of TMB ≥ 10 mutations per megabase

- 626 solid tumor samples were analyzed to determine concordance of *NTRK1/2/3* fusions. These included 588 (96 positive, 492 negative by F1CDx) samples where F1CDx served as the selection assay (subset 1) and 38 (16 positive, 22 negative by F1CDx) clinical trial samples where local clinical trial assays (LCTAs) served as the selection assay (subset 2)

- 188 (84 positive, 103 negative, 1 invalid by F1CDx) samples were analyzed to determine concordance for detection of qualifying *ROS1* fusions.

- 159 unique solid tumor samples with evaluable or valid F1CDx results were analyzed to determine concordance with a PCR-based assay for detection of MSI-High (MSI-H). Additionally, 128 colorectal cancer (CRC) and 50 uterus endometrial cancer patient samples with evaluable or valid F1CDx results randomly selected from the FMI clinical commercial database were analyzed to evaluate concordance between the MSI classification (MSI-H/deficient mismatch repair (dMMR) vs non-MSI-H/proficient mismatch repair (pMMR)) determined by the F1CDx assay and mismatch repair (MMR) immunohistochemistry (IHC) assays

- 105 solid tumor samples with evaluable or valid F1CDx results were evaluated with a PCR-based assay for detection of MSI-High (MSI-H) in a supplementary study

A summary of Positive Percent Agreement (PPA), Negative Percent Agreement (NPA) and corresponding 95% two-sided exact confidence intervals (CI) is provided in Table 6 below. Differences in variants of unknown significance (VUS) alteration calls between the platforms were noted and are expected based on differences in filtering employed by F1CDx and evNGS. Negative predictive value and positive predictive value were also calculated and were found to be different than percent agreement because the two platforms filter VUS differently. Discordant alterations not related to VUS filtering were primarily caused by deletions with low allelic fraction in homopolymer regions. The F1CDx variant calling pipeline imposes a filter based on MAF of ≥0.10 for indels in homopolymer regions to reduce the likelihood of calling false positives resulting from artifacts introduced by the technology. As such, the difference observed was due to varying filter thresholds between the two platforms. Additional analytical concordance for CDx associated variants is also summarized in Table 6. For *NTRK1/2/3* fusions two analyses were conducted. A primary analysis was focused on the concordance of *NTRK1/2/3* rearrangement detection by F1CDx and evNGS assay where a sample was considered to be positive by F1CDx if any *NTRK1/2/3* rearrangements were present, otherwise it was considered as negative. This analysis was not conducted in accordance with the *NTRK1/2/3* biomarker calling rule but rather to determine the analytical accuracy of *NTRK1/2/3* rearrangement detection as *NTRK1/2/3* fusions are a subset of rearrangements, and the methodology to detect *NTRK1/2/3* rearrangement (fusions and non-fusion rearrangements) is the same. The secondary analysis focused on the concordance of *NTRK1/2/3* rearrangement detection that is predicted to result in an *NTRK1/2/3* fusion event per the F1CDx biomarker rule. In the secondary analysis, a sample was considered F1CDx positive only if it met the *NTRK1/2/3* biomarker rule, otherwise it was considered as F1CDx negative. For additional clinical concordance results for the CDx-associated variants, refer to the Summary of Clinical Studies in Section 3.

Table 6 Concordance summary for short variants inclusive of both substitutions and indels and CDx claims.

	F1CDx+ /evNGS+	F1CDx- /evNGS+	F1CDx+ /evNGS-	F1CDx- /evNGS-	PPA [95% CI][1]	NPA [95% CI][1], **
All short variants	1282	73	375	284218	94.6% [93.3%-95.8%]	99.9% [99.9%-99.9%]
Substitutions	1111	39	334	242540	96.6% [95.4%-97.6%]	99.9% [99.8%-99.9%]
Indels	171	34	41	41678	83.4% [77.6%-88.2%]	99.9% [99.9%-99.9%]

	F1CDx+ /evNGS+	F1CDx- /evNGS+	F1CDx+ /evNGS-	F1CDx- /evNGS-	PPA [95% CI][1]	NPA [95% CI][1], **
***PIK3CA* substitutions in Breast Cancer**	53	0	0	48	100.00% [93.3%-100.0%]	100.00% [92.6%-100.0%]
***FGFR2* fusions[2]**	25	2	1	130	87.08% [61.40%,98.30%]	99.59% [92.87%, 100.00%]
***MET* exon 14 SNVs and indels**	49	0	1	118	100.0% [92.8%-100.0%]	99.27% [95.4%-100.0%]
HRR gene **substitutions**	35	1	1	8243	97.22% [85.47%, 99.93%]	99.99% [99.93%, 100.00%]
HRR gene indels	75	6	2	17627	92.59% [84.57%, 97.23%]	99.99% [99.96%, 100.00%]
HRR gene **rearrangements**	10	1	5	1824	90.91% [58.72%, 99.77]	99.73% [99.36%, 99.91%]
HRR gene **copy number alterations**	20	1	3	1356	95.24% [76.18%, 99.88]	99.78% [99.36%, 99.95%]
***NTRK1, NTRK2, NTRK3* fusions**	78[3, 4]	0[3, 4]	18[3, 4]	492[3, 4]	90.00% [75.00%,100%][3, 6]	99.92% [99.92%,99.97%][3, 6]
	16[3, 5]	2[3, 5]	0[3, 5]	20[3, 5]		
	64[7, 8]	10[7, 8]	4[7, 8]	510[7, 8]	54.08% [37.94%, 71.37%][7, *]	99.98% [99.96%, 100.00%][7, *]
	15[7, 9]	3[7, 9]	0[7, 9]	20[7, 9]		
***ROS1* fusions**	79	8	2	84	90.8% [82.7%, 95.9%]	97.7% [91.9%, 99.7%]

1The PPA and NPA were calculated without adjusting for the distribution of samples enrolled using the FoundationOne Laboratory Developed Test (F1 LDT), therefore these estimates may be biased upward.
[2] PPA and NPA were adjusted using a prevalence of 9.6% to account for sampling differential.
[3]Primary analysis: a sample was considered as positive if an *NTRK1/2/3* rearrangement was detected, otherwise it was considered as negative.
[4]Subset 1: samples where F1CDx served as the selection assay. Adjusted PPA and NPA based on an estimated prevalence of 0.32% in the intended use population to account for sampling differences were 100.00% [95%CI: 95.31%, 100.00%] and 99.94% [95%CI: 99.91%, 99.96%] respectively based on the primary analysis.
[5]Subset 2: clinical trial samples where local clinical trial assays (LCTAs) served as the selection assay. PPA and NPA were 88.89% [95%CI: 67.20%, 96.90%] and 100.00% [95%CI: 83.89%, 100%] respectively based on the primary analysis.
[6] The weighted PPA and NPA are shown for the primary analysis. Bootstrap confidence intervals from 10000 simulations were used here instead of exact.
[7]Secondary analysis: a sample was considered F1CDx positive only if it met the *NTRK1/2/3* biomarker rule, otherwise it was considered as F1CDx negative.
[8] Subset 1: samples where F1CDx served as the selection assay. Adjusted PPA and NPA based on an estimated prevalence of 0.32% in the intended use population to account for sampling differences were 13.58% [95%CI: 8.66%, 25.25%] and 99.98% [95%CI: 99.96%, 100.00%] respectively based on the secondary analysis.
[9] Subset 2: clinical trial samples where local clinical trial assays (LCTAs) served as the selection assay. PPA and NPA were 83.33% [95%CI: 60.78%, 94.16%] and 100.00% [95%CI: 83.89%, 100%] respectively based on the secondary analysis.
[*] The weighted PPA and NPA are shown for the secondary analysis. Bootstrap confidence intervals from 10000 simulations were used here instead of exact.
[**] 95% 2-sided exact CIs were calculated, unless otherwise notated.

The analysis for concordance of TMB-High (≥ 10 mutations per megabase) detection was performed using a CLIA validated whole exome sequencing (WES) assay. 218 samples were evaluated, of which 89 were

not pre-screened by F1CDx (Set A) and 129 were pre-screened by F1CDx (Set B). Concordance results between F1CDx and WES for TMB calling are summarized in Table 7 below.

Table 7 Concordance summary for TMB-High.

	F1CDx+ /evWES+	F1CDx- /evWES+	F1CDx+ /evWES-	F1CDx- /evWES-	PPA [95% CI]	NPA [95% CI]
TMB ≥ 10 mutations per megabase Set A	28	7	4	50	80.00% [62.50%,90.62%]	92.59% [82.62%,98.04%]
TMB ≥ 10 mutations per megabase Set B[1]	23	1	17	88	92.31% [65.74%,100.0%]	90.84% [87.76%,93.99%]

[1]PPA and NPA were adjusted using the prevalence of TMB—High estimated at 19%.

The overall PPA and NPA were calculated based on a weighted average of the results (Set A and Set B) in the TMB concordance analysis. Overall PPA was 87.28% (95% CI [64.42%, 96.17%]) and overall NPA was 91.56% (95% CI [85.66%, 95.64%]).

To determine a patient's MSI status, F1CDx employs a fraction-based (FB) MSI algorithm to categorize a tumor specimen as MSI-High (MSI-H) or microsatellite stable (MSS). The FB-MSI algorithm calculates the fraction of microsatellite loci determined to be altered or unstable (i.e., the fraction unstable loci score) based on a genome-wide analysis across >2000 microsatellite loci. For a given microsatellite locus, non-somatic alleles are discarded, and the microsatellite is categorized as unstable if remaining alleles differ from the reference genome. The final fraction unstable loci score is calculated as the number of unstable microsatellite loci divided by the number of evaluable microsatellite loci. Two FB-MSI score thresholds are applied to classify a tumor specimen as having MSI-H or MSS status. MSI-H status is reported for patients with solid tumors whose samples have FB-MSI scores ≥ 0.0124 while MSS status is reported for patients with solid tumors whose samples have FB-MSI scores ≤ 0.0041. Patients with solid tumors whose samples have FB-MSI scores >0.0041 and <0.0124, an MSI "Cannot be Determined" result is reported.

To demonstrate the analytical accuracy of the MSI calling for the tumor profiling MSI indication, an analysis to assess concordance of MSI calling was performed using a PCR-based comparator assay. The study included 186 FFPE tumor specimens representing pan-tumor indications, of which 86 were selected with the PCR-based comparator assay, while 100 were selected with F1CDx. Of the 186 FFPE tumor specimens, 159 were evaluable with valid MSI results from both F1CDx and the comparator assay. Of the 86 samples selected with the PCR comparator assay, 24 failed to provide evaluable or valid F1CDx results due to QC failures, i.e., a 28% failure rate was observed primarily due to contamination as these samples were procured externally. Of the 100 samples selected with F1CDx, 3 failed to provide evaluable or valid F1CDx results, i.e., a 3% failure rate was observed.

The PCR-based assay-selected subset contained 62 samples and the F1CDx assay-select subset contained 97 samples. Because F1CDx employs two MSI thresholds and provides the following three outcomes based on MSI scores: MSI-H for samples with FB-MSI scores of ≥ 0.0124, MSI-Cannot be determined for samples with FB-MSI scores >0.0041 and <0.0124, and MSS for samples with FB-MSI scores of ≤ 0.0041, two sets of analyses were conducted.

- For the first concordance analysis, to represent the commercial setting, samples with F1CDx MSI scores ≥ 0.0124 were treated as MSI-H/positive in the concordance analysis. Samples with F1CDx MSI scores <0.0124 and ≤0.0041 were treated as non-MSI-H or negative results. Samples with F1CDx MSI scores ≤0.0041 were treated as non-MSI-H or negative results.

- For the second concordance analysis, different from the commercial setting, samples with F1CDx MSI scores >0.0041 (this also includes samples with MSI scores of >0.0041 and <0.0124) were treated as positive results in the concordance analysis. Samples with F1CDx MSI scores ≤0.0041 were treated as negative results.

Since the PCR comparator assay provides three outcomes, MSI-H, MSI-Low and MSS, both MSI-Low and MSS were considered negative results for the analyses, while MSI-H was considered positive.

Concordance results between F1CDx and PCR for MSI-H and MSS calling within the combined datasets. i.e., samples selected with the PCR comparator assay and F1CDx assay were calculated. PPA and NPA between F1CDx and PCR for MSI-H and MSS calling within the combined datasets, i.e., samples selected with the PCR comparator assay and F1CDx assay are calculated by determining the proportion of comparator positive and negative samples that F1CDx is able to call concordantly. PPA and NPA were calculated using the observed, unadjusted data from the sample subset enrolled with the F1CDx assay; therefore, results may be biased due to how samples were selected.

The combined PPA and NPA using the ≥ 0.0124 threshold for MSI-H calling was 98.46% (95% CI [91.79% - 99.73%]) and 96.81% (95% CI [91.03% - 98.91%]) respectively. The combined PPA and NPA using the >0.0041 threshold for MSI-H calling was 100.00% (94.42%, 100.00%) and 87.23% (79.00%, 92.64%) respectively. The results demonstrate that while the PPA for MSI-H calling ranges from 98.46% to 100.00% using both thresholds, the range of NPA results is wide, from 87.23%-96.81%. The low NPA when using the >0.0041 threshold is driven by the proportion of samples with MSI-Cannot be determined status for MSI cases with MSI >0.0041 and <0.0124 (n=10). Of the 10 samples with MSI-Cannot be Determined status due to MSI scores >0.0041 and <0.0124, 1 was MSI-High, 7 were MSS, while the remaining 2 samples had an MSI-Low status per the PCR based comparator.

While the concordance using both thresholds was high for samples from colorectal cancer (CRC) patients for the combined data sets, both PPA and NPA were 100.00%, concordance was lower for other disease ontologies (non-CRC). For non-CRC patient samples, the observed combined PPA and NPA using the ≥ 0.0124 threshold for MSI-H calling was 98.04% and 97.53% respectively, while the observed combined PPA and NPA using the >0.0041 threshold for MSI-H calling was 100.00% and 87.65% respectively. Although the number of samples from patients with uterus endometrial adenocarcinoma that had valid F1CDx results was low, 8 out 19, the observed combined PPA and NPA using the ≥ 0.0124 threshold for MSI-H calling was 100% and 66.67% respectively, while the observed combined PPA and NPA using the >0.0041 threshold for MSI-H calling was 100.00% and 33.33%.

To demonstrate the analytical accuracy of the MSI calling for the tumor profiling MSI indication, a second evaluation of the concordance between the MSI classification (MSI-High (MSI-H)/deficient mismatch repair (dMMR) vs non-MSI-H/proficient mismatch repair (pMMR)) determined by the FB-MSI caller and mismatch repair (MMR) immunohistochemistry (IHC) assays was performed through a retrospective chart review of a randomly selected set of 134 colorectal cancer (CRC) and 52 uterus endometrial cancer patients from the FMI clinical commercial database. Overall, a total of 178 samples (including 128 CRC and 50 uterus endometrial cases) passed the F1CDx QC assessment, i.e., provided evaluable or valid F1CDx results and were used as the analysis dataset.

As for the concordance study using the PCR based comparator, two sets of concordance results were conducted described above.

The IHC assays provides two outcomes, dMMR and pMMR. dMMR are considered positive results and pMMR are considered negative results for the analyses.

The PPA and NPA for the overall sample set were 85.19% and 100.00%, respectively. Point estimates and 95% two-sided CI for PPA and NPA for the overall sample set were PPA 85.19% (95%CI [67.52, 94.08%]) and NPA 100.00% (95% CI [97.52%, 100.00%]) when using the ≥ 0.0124 threshold and PPA

88.89% (95% CI [71.94%, 96.15%]) and NPA 93.38% (95% CI [88.24%, 96.36%]) when using the >0.0041 threshold. Point estimates and 95% two-sided CI for PPA and NPA for the CRC cases were PPA 78.57% (95% CI [52.41%, 92.43%]) and NPA 100.00% (95% CI [96.74%, 100.00%]) when using the ≥ 0.0124 threshold and PPA 78.57% (95% CI [52.41%, 92.43%] and NPA 95.61% (95% CI [90.14%, 98.11%]) when using the >0.0041 threshold. In totality, the evaluation passed the acceptance criteria of PPA and NPA ≥85%.

Point estimates and 95% two-sided CI for PPA and NPA for the endometrial cancer cases were 92.31% (95% CI [66.69%, 98.63%]) and 100.00% (95% CI [90.59%, 100.00%]) when using the ≥ 0.0124 threshold and PPA 100.00% (95% CI [77.19%, 100.00%] and NPA 86.49% (95% CI [72.02%, 94.08%]) when using the >0.0041 threshold.

MSI-Cannot be determined status for MSI cases with MSI >0.0041 and <0.0124 was observed in 11 samples. Of the 11 samples, 1 (9%) was dMMR while the remaining 10, (91%) had pMMR status.

An additional accuracy study was conducted to demonstrate the accuracy of MSI-H calling to support the CDx indication as an aid in identifying MSI-H status in patients with solid tumors. The additional analysis to assess concordance of MSI-H calling was performed using a PCR-based comparator assay. The study design included evaluation of a test set of 161 commercially procured samples representing different tumor types to support a solid tumor indication, including samples from 7 major organ systems, as well as 56 screen failure samples from Merck's clinical trial study KEYNOTE-158. After sample processing and screening externally at the vendor and internally at FMI, a total of 111 samples failed to yield an evaluable or valid F1CDx result, while one (1) sample that underwent F1CDx sample processing was not evaluable by the PCR assay due to poor amplification.

In total, 105 samples across multiple disease indications with valid F1CDx and PCR-based MSI statuses were evaluated within the concordance analysis. MSI assessment was dichotomized to MSI-H or non-MSI-H for both F1CDx and the PCR assay results. Samples with F1CDx MSI scores ≥ 0.0124 were treated as MSI-H/positive in the concordance analysis. Samples with F1CDx FB- MSI scores <0.0124 were treated as non-MSI-H or negative results. The PCR assay includes MSI-H, MSI-L, and MSS, which was further dichotomized into MSI-H or positive and non-MSI-H (MSI-L and MSS) or negative in the concordance analysis.

Among the 105 samples, 98 were PCR-enrolled and seven (7) were F1CDx-enrolled. Because concordance in the F1CDx-enrolled set was 100%, a prevalence adjustment does not change estimates of PPA or NPA for the dataset. As such, the analysis was simplified, and the two (2) datasets (PCR-enrolled and F1CDx-enrolled) were combined directly. Point estimates for PPA and NPA were calculated directly, along with 95% two-sided Wilson Score confidence intervals (CIs).

The combined PPA was 100.00% with 2-sided 95% CI of [87.54% - 100.00%], see Table 8. The combined NPA was 97.44% with 2-sided 95% CI of [91.12% - 99.29%], see Table 8. Two (2) samples, both from endometrial cancer patients, exhibited discordant MSI status results between the assays. Both discordant samples were PCR-enrolled and exhibited F1CDx MSI-H results (FB-MSI score of 0.0216 and 0.0192) and PCR non-MSI-H results (MSS).

Table 8 Concordance Results MSI Detection Using PCR-based Comparator Assay

	F1CDx+/ PCR+	F1CDx- /PCR +	F1CDx+ /PCR -	F1CDx- /PCR -	PPA (95% CI)	NPA (95% CI)
Combined data sets (PCR and F1CDx enrolled)	27	0	2	76	100.00% (87.54% - 100.00%)	97.44% (91.12% - 99.29%)

For CRC patients in the combined data sets, for samples that yielded evaluable results (21 out of 53, 39.6%), PPA was 100% (10/10) and NPA was 100.00% (11/11) using the ≥ 0.0124 threshold for MSI-H

calling. For non-CRC patient samples with evaluable results, the observed PPA and NPA in the combined data sets using the ≥ 0.0124 threshold for MSI-H calling were 100.00% (17/17) and 97.01% (63/65).

For the samples from patients with uterus endometrial adenocarcinoma that had evaluable F1CDx results (25 out of 58, 43.10%) the observed PPA and NPA in the combined data sets using the ≥ 0.0124 threshold for MSI-H calling were 100% (9/9) and 87.50% (14/16) respectively.

There were two (2) samples, both from patients with thyroid carcinoma, with MSI-Cannot be Determined status due to FB MSI scores >0.0041 and <0.0124; both were MSS per the PCR-based comparator.

2.2 Concordance – Comparison to FoundationOne®

To support the use of retrospective data generated using the FoundationOne® (F1 LDT), a concordance study was conducted with FoundationOne®CDx (F1CDx). This study evaluated a test set of 165 specimens. PPA and NPA between the F1CDx and F1 LDT, using the F1 LDT assay as the reference method, was calculated for all alterations, as well as for alterations binned by type: short variants, copy number alterations (CNAs) and rearrangements. A total of 2,325 variants, including 2,026 short variants, 266 CNAs and 33 rearrangements were included in the study. The study results are summarized in Table 9 below.

Table 9 Summary of inter-laboratory concordance comparing F1CDx to the F1 LDT.

	F1CDx+/F1 LDT+	F1CDx-/F1 LDT+	F1CDx+/F1 LDT-	F1CDx-/F1 LDT-	PPA	NPA
All variants	2246	33	46	322890	98.6%	99.99%
All short variants	1984	19	23	299099	99.1%	99.99%
Substitutions	1692	10	19	254854	99.4%	99.99%
Indels	292	9	4	44245	97.0%	99.99%
All CNA	230	14	22	19204	94.3%	99.9%
Amplifications	157	10	12	14671	94.0%	99.9%
Losses	73	4	10	4533	94.8%	99.8%
Rearrangements	32	0	1	4587	100.0%	99.98%

2.3 Concordance – LOH and HRD Calling Comparison to FoundationFocus™ CDx BRCA LOH

To support reporting of LOH on FoundationOne®CDx (F1CDx), a concordance study was conducted to compare results of data analyzed using the F1CDx pipeline version 3.1.3 with FoundationFocus™ CDx *BRCA* LOH (FFocus) data. This analysis included one random replicate from the FFocus LOH sPMA precision samples and one replicate from the FFocus LOH sPMA LoD study for a total of 25 samples. The study results are summarized in Table 10a below.

Table 10a Summary of LOH calling comparison agreement table

Agreement	Estimate	95% CI (exact)	Acceptance Criteria
OPA	96.0%	79.6%-99.9%	Low 95%CI >85%
PPA	94.70%	74.0%-99.9%	PPA >90%
NPA	100.00%	54.1%-100.0%	NPA>90%

Concordance for calling HRD status was evaluated by assessing data from the ARIEL3 clinical trial using the F1CDx pipeline. These data are summarized in Tables 10b and 10c below.

Table 10b. Contingency table of F1CDx v3.1.3 HRD status and FFocus HRD status in 518 samples from ARIEL3 study. Numbers in bold are the numbers of cases with determinate HRD outcome by both pipelines, and are used in agreement calculation in Table 9c.

HRD status		F1CDx v3.1.3			
		Indeterminate	Negative	Positive	Sum
FFocus	Indeterminate	22	5	0	27
	Negative	1	**156**	**8**	165
	Positive	1	**8**	**317**	326
	Sum	24	169	325	518

Table 10c. Agreement between F1CDx v3.1.3 HRD status and FFocus HRD status in 489 samples with determinate HRD outcome by both assays from ARIEL3 study.

	Percent of Agreement [95% CI]
PPA	97.5% [95.2%-98.9%]
NPA	95.1% [90.6%-97.9%]
OPA	96.7% [94.7%-98.1%]

2.4 Tissue Comparability

A large-scale retrospective analysis was conducted, using 80,715 specimens from 43 tissue types, in order to establish the comparability of assay performance across tumor tissue types. The goal of the study was to establish that assay performance after DNA extraction is independent of the tissue type from which the DNA was extracted. The retrospective analysis of data included specimens assayed using the FoundationOne (F1 LDT) assay. DNA extraction, and post-DNA extraction data were assessed for comparability of performance across tissue types. The dataset for analysis consisted of routine clinical samples analyzed using F1 LDT from March 25, 2015 to March 13, 2017.

Thirty-nine of the 43 tissue types had ≥90% of specimens passing DNA extraction QC. Specimen DNA extraction pass rates for the remaining four tissue types, lung, pancreas, pelvis and prostate, were 89.6%, 89%, 89%, and 79.7%, respectively. Each of these four tissue types have characteristically small biopsies and may also be more likely to require macro-dissection.

Of specimens entering the assay at Library Construction (LC), 39 of 43 tissue types had ≥90% of specimens resulting in a successful patient report being issued. The four tissue types below 90% include pancreatobiliary, appendix, pericardium, and prostate, and had pass rates of 83%, 88%, 79%, and 84%, respectively. For these four tissue types, the most frequent cause of failure was low tumor purity with no alterations detected. The mean LC yields across tissue types were 7,050 ng to 8,643 ng compared to the minimum required 545 ng. The percent of specimens passing the LC QC for each tissue type ranged from 98%-100%. After Hybrid Capture (HC), the mean yields across tissue types ranged from 434 ng to 576 ng, well above the minimum requirement of 140 ng. The percent of specimens passing HC across tissue types ranged from ranged from 97%-100%. The average median exon coverage assessed across tissue types ranged from 702X-793X, with percent of specimens passing QC for median coverage across tissue types ranging from 96%-100%. Uniformity of coverage was assessed by calculating the average percent of targets with >100X coverage across tissue types, and ranged from 99.0%-99.8%. The percentage of specimens passing this QC metric ranged from 98%-100%. The average sequencing error rate, assessed across tissue types, is 0.0028-0.0031, well below the required error rate (0.01) for assay acceptance. The pass rate for all tissue types was 100% for error rate. Performance data for this study is summarized in Table 11 below.

Table 11 Summary of post-DNA extraction analysis.

QC Metric Name	F1CDx QC Specification	Mean QC Performance Across Tissue Types	QC Pass Rate Across Tissue Types	Tissue types with ≥90% QC Pass Rate
Overall report Pass/Qualified rate	Pass rate: ≥90% specimens	N/A	79%-98%	39/43 (90.6%)
LC Yield	≥545 ng	7050–8643 ng	98-100%	43/43 (100%)
Library Yield after HC	≥140 ng	434-576 ng	97-100%	43/43 (100%)
Median Exon Coverage	≥250X	702-793X	96-100%	43/43 (100%)
Percent of target >100X coverage	≥95% target at ≥100X coverage	99.0%-99.8% targets	98%-100%	43/43 (100%)
Sequencing error rate	<1%	0.0028-0.0031	100%	43/43 (100%)
Noisy copy number data	N/A*	N/A	93.8-100%	43/43 (100%)

*for information only, not a specification

2.5 Analytical Specificity

2.5.1 Interfering Substances

The robustness of the FoundationOne®CDx (F1CDx) assay process was assessed while evaluating human formalin-fixed paraffin-embedded (FFPE) samples in the presence of exogenous and endogenous interfering substances. Five FFPE specimens representing five tumor types (ovary, lung, colorectal, breast and melanoma) including representative variant types (substitution, indel, amplification, homozygous deletion and rearrangement) were assessed in duplicate (Table 12). An additional 54 short alterations (substitutions and indels) were assessed. The addition of interfering substances including melanin (endogenous), ethanol (exogenous), proteinase K (exogenous), and molecular index barcodes (MIB) (exogenous) was evaluated to determine if they were impactful to F1CDx, and the results were compared to the control (no interferents) condition.

Table 12 Summary of tumor types and variant types included in study.

Tumor Type	Gene (and variant as relevant)	Variant type
CRC	*FGFR1*	Rearrangement
	BCL2L1	Amplification
	AXIN1 c.1058G>A (R353H)	Substitution
	SOX9 c.768_769insGG (R257fs*23)	Insertion
Breast cancer	*ERBB2*	Amplification
	AKT1	Amplification
	CCND1	Amplification
Lung cancer	*CDKN2A*	Homozygous Deletion
	CDKN2B	Homozygous Deletion
	EGFR	Amplification
Ovarian cancer	*BRCA1* c.5263_5264insC (Q1756fs*74)	Insertion
	ERCC4 c.2395C>T	Substitution
	TP53 c.779_779delC (S261fs*84)	Deletion
Melanoma	*BRAF* c.1799T>A (V600E)	Substitution
	TP53 c. 856G>A (E286K)	Substitution
	IGF1R	Amplification

Interfering substances included melanin, ethanol, proteinase K, and molecular index barcodes, as noted in Table 13 below. Each of the five FFPE specimens were tested in either two or four replicates each, resulting in a total of 170 data points across the five specimens (10 without interferent, 80 for evaluation of melanin, ethanol and proteinase K and 80 for molecular index barcodes) assessed in this study.

Table 13 Interfering substance evaluated.

Substances	Level	# Samples	# Replicates/Sample
No Interferent	–	5	2

Substances	Level	# Samples	# Replicates/Sample
Melanin	0.025 µg/mL	5	2
Melanin	0.05 µg/mL	5	2
Melanin	0.1 µg/mL	5	2
Melanin	0.2 µg/mL	5	2
Proteinase K	0.04 mg/mL	5	2
Proteinase K	0.08 mg/mL	5	2
Ethanol	5%	5	2
Ethanol	2.5%	5	2
MIB	0	5	4
MIB	5%	5	4
MIB	15%	5	4
MIB	30%	5	4

Substances were considered as non-interfering if, when compared to no interferent, the DNA yield is sufficient to meet the standard processing requirements of DNA isolation (≥55 ng), if the quality was sufficient to create products per the specification of library construction (≥545 ng) and hybrid capture (≥140 ng), and the sample success rate (fraction of samples that met all process requirements and specifications), across all replicates in aggregate, is ≥90%. Sequence analysis was assessed as percent agreement for each sample and calculated as the number of replicates with the correct alteration call reported per the total number of replicates processed. Percent agreement (fraction of correct calls) was computed across all replicates. The acceptance for concordance required a minimum of 90% of correct calls within each treatment category.

All samples tested at all interfering substance levels met all process requirements and specifications; achieving the acceptance criterion of ≥90%, indicating that the sample quality was not impacted by the interfering substances at the levels evaluated. The concordance of variants for the melanin, proteinase K and MIB evaluations was 100%, and was 95.3% for the ethanol evaluation, each meeting the acceptance criterion of ≥90%, indicating that the performance was not affected by the tested interferents. In addition to the variants selected to represent specific alteration types summarized in Table 11, samples included in the study harbored 54 additional short alterations (substitutions and indels) and were 100% concordant across all replicates for each variant.

See Summary of Safety and Effectiveness Data for P160018 for additional interference studies, wherein the interference of necrotic tissue, triglycerides, hemoglobin, and xylene, in addition to ethanol, proteinase K, and MIBs, was evaluated in ovarian tissue and assessed *BRCA1/2* alterations.

An additional study was performed to assess the impact of endogenous and exogenous contaminants including melanin, ethanol, proteinase K, and MIB, on TMB-H (≥ 10 mutations per megabase) and MSI calling as a qualitative biomarker. The analysis included 19 retrospective samples from 3 previous studies and all acceptance criteria were met.

To supplement the previous additional study performed to assess the impact of interfering substances, an exploratory analysis was conducted to further evaluate the effect of endogenous (melanin, hemoglobin, and triglycerides) and exogenous (proteinase K, ethanol, MIB and xylene) interfering substances on MSI calling. All samples spiked with each interfering substance had their MSI calling performance compared to control replicates which had no interfering substances. The results demonstrated 100% agreement of MSI calling between the test and control replicates. Because agreement was 100%, there is no evidence to suggest that interfering substances tested in the exploratory analysis had an adverse effect on MSI calling.

2.5.1.1 Necrosis

An evaluation of necrosis and F1CDx MSI calling performance was conducted to determine whether necrotic tissue in solid tumors interfered with F1CDx MSI calling.

Evaluation of 39 samples with a valid MSI status (sample set included FB-MSI scores ranging from 0-0.0776 and % necrosis ranging from 0-70%), as determined by both F1CDx and Promega MSI Analysis System v1.2 assays, demonstrated that the levels of necrosis present in these samples did not interfere with MSI calling. The positive percent agreement (PPA) with 95% 2- sided score CI was calculated to be 100.00% (79.61%, 100.00%) and the negative percent agreement (NPA) was calculated to 95.83% with 95% 2-sided score CI (79.76%, 99.26%). MSI concordance was evaluated for 25 samples with necrotic tissue and 14 samples without necrotic tissue. Within the necrosis-absent samples, the PPA was 100% (note: CI was not calculated due to small sample size) and the NPA was 91.67% (64.61%, 98.51%). Within the necrosis-present samples, the PPA was 100.00% (77.19%, 100.00%) and the NPA was 100.00% (75.75%, 100.00%). Comparable concordance was observed between the two subgroups in terms of PPA and NPA. In conclusion, necrosis was not found to interfere with the performance of the MSI calling of the F1CDx assay in this study when % necrosis is ≤ 70%. However, as samples above 70% necrosis were not evaluated as part of MSI calling performance, it is possible that there may be different performance in MSI biomarker QC failure when high % necrosis (i.e., above 70%) is present in tumor tissue.

2.5.2 *In silico* Analysis – Hybrid Capture Bait Specificity

Bait specificity was addressed through an assessment of coverage at the base level for targeted regions included in F1CDx. Lack of bait specificity and/or insufficient bait inclusion would result in regions of diminished high quality mapped reads due to the capture of off-target content. This analysis showed that all regions that may harbor alterations associated with companion diagnostic claims consistently have high quality (MQS ≥ 30), deep coverage ≥ 250X. When assessing the entire gene set, 99.45% of individual bases in targeted coding regions +/-2 bp of flanking intronic splice site are covered with ≥100X coverage, and 91.45% of individual bases within targeted introns platform-wide had ≥100X coverage**.**

2.5.3 Carryover/Cross-contamination

No carryover or cross-contamination was observed when alternating positive and negative samples for *BRCA1* and *BRCA2* variants, assessed in a checker-board pattern (see Summary of Safety and Effectiveness Data for P160018). In addition, data from plates with high-level confirmed *ERBB2* amplifications, *EGFR* T790M alterations or *ALK* fusions were examined for cross-contamination in adjacent wells containing confirmed negative samples. No contamination was observed.

2.6 Precision: Repeatability and Reproducibility

Repeatability and reproducibility of alterations associated with CDx claims and platform-wide alterations, including agreement for MSI, TMB, and MAF of short variants, were evaluated. Repeatability between intra-run aliquots (run on the same plate under the same conditions) and reproducibility of inter-run aliquots (run on different plates under different conditions) were assessed and compared across three different sequencers and three different reagent lots, across multiple days of performance by multiple operators.

A total of 163 samples had alterations representative of CDx associated alterations as well as exemplar alterations in a variety of genomic contexts, as shown in Tables 14 and 15 below. Each sample also included additional alterations that were included in the assessment. The maximum insertion length in this study was 30 bp and the longest deletion was 263 bp.

Table 14-1 Repeatability and Reproducibility of CDx Alterations Targeted in Precision

Gene or Biomarker	Number of Unique Samples	Alteration	Tumor Type
EGFR	3	Exon 19 Deletion	NSCLC
	2	Exon 21 L858R	
	2	Exon 20 T790M	
KRAS	3	Codons 12/13 substitution	CRC
ALK	3	Fusion	NSCLC
BRAF	3	V600E/V600K	Melanoma
ERBB2	3	Amplification	Breast cancer
PIK3CA	3[1]	E545K/H1047R/H1047L	Breast cancer
FGFR2	5[2]	FGFR2 Fusions and rearrangement[3]	Cholangiocarcinoma (CCA)

Gene or Biomarker	Number of Unique Samples	Alteration	Tumor Type
MET	8[4]	SNVs and indels that lead to Exon 14 skipping	NSCLC
ROS1	2	Fusions	NSCLC
HRR Genes	47	Base Substitutions, Indels, Rearrangements, Homozygous Deletions	Prostate
TMB	46[4]	TMB ≥ 10 mutations per megabase	Solid tumors
NTRK1 ***NTRK2*** ***NTRK3***	7[4]	Fusions	Solid tumors[5]
MSI	66[6] 46[7]	MSI-High	Solid Tumors

[1]Two samples are from the 47 samples originally included in the PMA precision study. An additional sample was analyzed in a subsequent precision study.
[2]Included 3 samples that included 24 replicates (2 runs x 2 replicates x 2 reagent lots x 3 sequencers), and two samples that included 36 replicates (2 runs x 3 replicates x 2 reagent lots x 3 sequencers)
[3]The precision study included *FGFR2-BICC1*, *FGFR2-CCDC6* fusion; *FGFR2-TFCP2* fusion, and an intron 17 rearrangement (no partner)
[4]24 replicates performed (2 runs x 2 replicates x 2 reagent lots x 3 sequencers)
[5]The precision study included 7 samples with CDx *NTRK1/2/3* fusion positive status: Four (4)) *NTRK3-EVT6* fusions, one (1) *NTRK1-TPM3* fusion, one (1) *NTRK1-LMNA* fusion, and one (1) *NTRK2-DSTYK* fusion.
[6]The precision of MSI calling was evaluated through a re-analysis of 66 samples previously evaluated for precision. These samples consisted of a set of 7 MSI-H samples, and 59 non-MSI-H samples from eight tumor types.
[7]The precision of MSI calling was evaluated through a re-analysis of 46 samples (44 MSI-H, and 2 non-MSI-H) previously evaluated for precision.

Table 14-2. Reproducibility and Repeatability for CDx Alterations with One Variant Type

Gene	Tumor Type	Number of Unique Samples	CDx Alteration	Alteration of the Sample	Fold LoD[1]	Reproducibility (95% CIs) (%)[5]	Repeatability (95% CIs) (%)[5]
EGFR	NSCLC	3	Exon 19 Deletion	*EGFR* 2253_2276delATCTCCGAAAGCCAACAAGGAAAT S752_I759del	4.84	100.0 (90.3, 100.0)	100.0
				EGFR 2235_2249delGGAATTAAGAGAAGC E746_A750del	7.51	100.0 (90.0, 100.0)	100.0
				EGFR 2236_2246delGAATTAAGAGA E746fs*13	3.76	100.0 (90.0, 100.0)	100.0
		2	Exon 21 L858R	*EGFR* 2573T>G L858R	8.71	100.0 (90.0, 100.0)	100.0
				EGFR 2573T>G L858R	11.83	100.0 (90.0, 100.0)	100.0
		2	Exon20 T790M	*EGFR* 2369C>T T790M	2.88	100.0 (90.3, 100.0)	100.0
				EGFR 2369C>T T790M	7.72	100.0 (90.3, 100.0)	100.0
KRAS	CRC	3	Codons 12/13 substitution	*KRAS* 38G>A G13D	6.83	100.0 (90.3, 100.0)	100.0
				KRAS 35G>A G12D	14.83	100.0 (90.3, 100.0)	100.0

Gene	Tumor Type	Number of Unique Samples	CDx Alteration	Alteration of the Sample	Fold LoD[1]	Reproducibility (95% CIs) (%)[5]	Repeatability (95% CIs) (%)[5]
				KRAS 34G>T G12C	10.52	100.0 (90.3, 100.0)	100.0
ALK	NSCLC	3	Rearrangement	*ALK-EML4*	8.19 (10.35)[2]	100.0 (90.3, 100.0)	100.0
				ALK-EML4	19.62 (12.58)[2]	100.0 (90.3, 100.0)	100.0
				ALK-EML4	5.46 (25.04)[2]	100.0 (90.3, 100.0)	100.0
BRAF	Melanoma	2	V600E/V600K	*BRAF* 1799T>A V600E	19.10	100.0 (90.3, 100.0)	100.0
				BRAF 1798GT>AA V600K	30.35	100.0 (90.0, 100.0)	100.0
ERBB2	Breast cancer	3	Amplification	HER2 amplification	2.60	100.0 (90.0, 100.0)	100.0
				HER2 amplification	2.04	100.0 (90.0, 100.0)	100.0
				HER2 amplification	1.92	100.0 (89.7, 100.0)	100.0
PIK3CA	Breast cancer	3	H1047R/H1047L/ E545K	*PIK3CA* 3140A>G H1047R	0.15	52.8[4] (37.0, 68.0)	27.8 (12.5, 50.9)
				PIK3CA 1633G>A E545K	0.98	100.0 (90.4, 100.0)	100.0 (82.4, 100.0)
				PIK3CA 3140A>T H1047L	0.86	100.0 (85.2, 100.0)	100.0 (71.5, 100.0)
FGFR2	Cholangiocarcinoma (CCA)	5	Fusions and rearrangement	*FGFR2-CCDC6*	4.16[3]	100.0 (90.0, 100.0)	100.0 (73.5, 100.0)
				FGFR2-BICC1	2.52[3]	100.0 (89.4, 100.0)	100.0 (71.5, 100.0)
				FGFR2-BICC1	3.37[3]	100.0 (85.8, 100.0)	100.0 (73.5, 100.0)
				FGFR2-TFCP2	3.47[3]	95.5 (77.2, 99.9)	90.0 (55.5, 99.8)
				FGFR2-N/A	2.37[3]	100.0 (85.8, 100.0)	100.0 (73.5, 100.0)
MET	NSCLC	8	SNVs and indels that lead to Exon 14 skipping	splice site 2888-10_2911del34	1.34	100.0 (85.8, 100.0)	100.0 (73.5, 100.0)
				splice site 2888-37_2888-30delCGTCTTTA	0.76	95.8 (78.9, 99.9)	91.7 (61.5, 99.8)
				splice site 2888-18_2888-5del14	4.09	100.0 (85.2, 100.0)	100.0 (71.5, 100.0)

Gene	Tumor Type	Number of Unique Samples	CDx Alteration	Alteration of the Sample	Fold LoD[1]	Reproducibility (95% CIs) (%)[5]	Repeatability (95% CIs) (%)[5]
				D1010N	0.87	95.8 (78.9, 99.9)	91.7 (61.5, 99.8)
				splice site 3028+2T>C	1.22	100.0 (85.8, 100.0)	100.0 (73.5, 100.0)
				splice site 2999_3028+4del34	0.87	100.0 (85.8, 100.0)	100.0 (73.5, 100.0)
				splice site 3028+1G>A	2.79	100.0 (85.8, 100.0)	100.0 (73.5, 100.0)
				splice site 3028_3028+2delGGT	1.61	100.0 (85.2, 100.0)	100.0 (71.5, 100.0)
NTRK1 *NTRK2* *NTRK3*	Solid Tumors	7	Fusions	*NTRK1*-TPM3	0.93 (1.42)[2]	100.0 (85.8, 100.0)	100.0 (73.5, 100.0)
				NTRK1-LMNA	1.69 (1.50)[2]	100.0 (85.2, 100.0)	100.0 (71.5, 100.0)
				NTRK2-DSTYK	1.45 (3.06)[2]	100.0 (85.2, 100.0)	100.0 (71.5, 100.0)
				NTRK3-ETV6	2.68 (6.57)[2]	100.0 (90.3, 100.0)	100.0 (73.5, 100.0)
				NTRK3-ETV6	2.42 (3.28)[2]	100.0 (90.3, 100.0)	100.0 (73.5, 100.0)
				NTRK3-ETV6	10.86 (5.64)[2]	100.0 (85.2, 100.0)	100.0 (73.5, 100.0)
				NTRK3-ETV6	0.58 (3.28)[2]	95.8 (78.9, 99.9)	91.7 (61.5, 99.8)
ROS1	NSCLC	2	Fusions	*ROS1-CD74*	2.64 (2.33)[1, 6]	100.0 (90.3, 100.0)	100.0 (73.5, 100.0)
				ROS1-CD74	3.57 (4.13)[1, 6]	100.0 (90.3, 100.0)	100.0 (73.5, 100.0)

[1] LoD was determined based on hit rate approach, which is a conservative approach that overestimates LoD. The column represents the level evaluated for the sample in relationship to the LoD for the variant.
[2] LoD for *ALK* rearrangement and *NTRK* fusions was determined by both the hit rate approach and the probit method based on fusion reads and by tumor purity. Values here are relative to the calculated LoD based on the hit rate approach for fusion reads and (tumor purity).
[3] LoD for *FGFR2* fusions and rearrangements was determined by both the hit rate approach and the probit method based on tumor purity. Values here are relative to the calculated LoD based on the hit rate approach for tumor purity.
[4] The breast sample with H1047R alteration has a MAF of 1%, which is below the LoD of 4.9% MAF.
[5] 95% 2-sided CIs for *PIK3CA* H1047R and *PIK3CA* E545K were calculated based on Wilson score method. 95% 2-sided CIs for other genes and alterations were calculated based on exact method.
[6] Values here are relative to the calculated LoD based on the hit rate approach for fusion reads and (tumor purity).

Table 14-3. Reproducibility and Repeatability for TMB

Gene or Biomarker	Tumor Type	Number of Unique Samples	Alteration	Fold LoD	Reproducibility (95% CIs) (%)[2]	Repeatability (95% CIs) (%)[2]
TMB	Solid tumors	46[1]	TMB ≥ 10 mutations per megabase	0.39-3.46	99.72% (99.18%, 99.94%)	99.54% (98.39%, 99.89%)

[1] Based on the cut-off of 10 mut/Mb for TMB-H, there were 44 TMB-H and 2 non-TMB-H samples in this precision analysis. There were 6 samples with TMB scores near the TMB-H cut-off of 10 mut/Mb. 21samples were TMB-H and near the LoD for computational purity (< 1.5x LoD).
[2] 95% 2-sided exact CIs were calculated.

Table 14-4. Reproducibility and Repeatability for HRR Genes

Gene	Variant Type	Average MAF%/TP %/Reads*	Repeatability		Reproducibility	
			SD	CV	SD	CV
Base substitutions (SUB) and Indels (ID) component						
PALB2	SUB	35.1	0.03	0.08	0.02	0.08
CHEK2	ID	45.9	0.03	0.06	0.02	0.06
PALB2	SUB	6.9	0.01	0.18	0.01	0.24
CDK12	SUB	17.2	0.01	0.07	0.02	0.12
ATM	ID	13.5	0.01	0.09	0.01	0.11
CDK12	ID	38.8	0.02	0.06	0.02	0.07
BRCA2	ID	6.0	0.00	0.11	0.00	0.11
ATM	ID	6.9	0.03	0.04	0.03	0.04
ATM	ID	15.1	0.01	0.08	0.01	0.10
CDK12	ID	5.8	0.00	0.09	0.01	0.11
BARD1	ID	12.9	0.0 1	0.07	0.01	0.09
BRCA2	ID	10.8	0.01	0.09	0.01	0.11
CHEK1	SUB	31.6	0.02	0.07	0.02	0.07
BARD1	SUB	6.5	0.08	0.11	0.01	0.19
FANCL	ID	43.6	0.03	0.07	0.03	0.08
RAD51C	1D	57.3	0.01	0.03	0.01	0.03
BRCA1	1D	9.9	0.01	0.11	0.02	0.21
BRCA2	ID	59.9	0.02	0.04	0.02	0.04
ATM	ID	49.9	0.02	0.05	0.02	0.05
BRCA2	SUB	36.3	0.03	0.07	0.03	0.09
BRCA2	SUB	52.7	0.03	0.05	0.03	0.07
BRCA2	SUB	66.3	0.03	0.04	0.03	0.05
FANCL	ID	51.3	0.0 1	0.03	0.02	0.04
BRIP1	ID	16.1	0.0 2	0.09	0.01	0.10
CDK12	ID	15.4	0.01	0.07	0.01	0.12
CHEK2	ID	44.3	0.03	0.07	0.04	0.08
BRCA2	ID	48.3	0.02	0.05	0.03	0.05
BRCA2	ID	20.4	0.01	0.09	0.02	0.12
CDK12	ID	46.5	0.02	0.04	0.02	0.04
CDK12	ID	45.7	0.02	0.05	0.02	0.05
BRIP1	ID	46.0	0.01	0.03	0.04	0.09
RAD51D	SUB	6.3	0.01	0.15	0.0 1	0.25
FANCL	ID	42.9	0.02	0.05	0.02	0.06
BRIP1	SUB	19.1	0.01	0.07	0.01	0.08

Gene	Variant Type	Average MAF%/TP %/Reads*	Repeatability		Reproducibility	
			SD	CV	SD	CV
Amplification (Amp) and HD variant component						
CHEK1	Amp	79.9	0.02	0.02	0.03	0.03
ATM	loss	82.2	0.06	0.07	0.07	0.08
BRCA1	loss	40.6	0.01	0.02	0.01	0.02
CHEK2	loss	55.9	0.03	0.06	0.05	0.09
RAD51C	loss	55.0	0.01	0.02	0.01	0.02
ATM	loss	65.4	0.16	0.25	0.16	0.25
ATM	loss	49.9	0.09	0.18	0.09	0.18
ATM	loss	43.6	0.09	0.20	0.09	0.22
Rearrangement (RE) component						
ATM	RE	153.1	17.26	0.11	27.64	0.18
BRCA1	RE	73.6	9.64	0.13	9.88	0.13
BRCA1	RE	12.7	2.90	0.22	2.92	0.22
BRCA2	RE	23.8	4.89	0.20	5.82	0.24
BRCA2	RE	14.7	2.58	0.17	3.44	0.23
BRCA2	RE	17.1	5.03	0.29	5.19	0.30
BRIP1	RE	169.7	16.54	0.09	20.89	0.12
PALB2	RE	38.6	6.60	0.17	7.82	0.20
RAD51B	RE	236.9	20.84	0.08	32.68	0.13
ATM	RE	75.8	13.27	0.17	14.71	0.19
RAD51B	RE	12.5	3.06	0.24	3.06	0.24
CHEK1	RE	65.5	8.85	0.13	9.37	0.14
BRIP1	RE	68.2	10.3	0.15	14.70	0.21

*Average MAF% for SUB/ID, Average TP% for Amp/Loss, and Average Reads for RE

Table 15 Sample set selection for platform validation

Alteration Type	Number of Unique Samples	Alteration Size	Genomic Context
Substitution	3	-	-
Short Insertion	2	1-2bp	Homopolymer Repeats
Short Insertion	2	1-2bp	Dinucleotide Repeats
Short Insertion	2	3-5bp	-
Short Insertion	2	>5bp	-
Short Deletion	2	1-2bp	Homopolymer Repeats
Short Deletion	2	1-2bp	Dinucleotide Repeats
Short Deletion	2	3-5bp	-
Short Deletion	2	>5bp	-
Amplification	3	-	-
Homozygous Deletion	3	-	-
Rearrangement	3	-	-

Note: Two samples with *PIK3CA* alterations (E545K and H1047R) were represented in both the CDx and platform validation.

The results demonstrated that the F1CDx is robust regarding the repeatability and reproducibility of calling genomic alterations. Across all samples, the pre-sequencing process failure is 1.5%, and the no call rate is 0.18% for MSI, 6.38% for TMB (all) and 0.22% for TMB (≥10 mut/Mb). Within the assessment of repeatability and reproducibility for CDx variants, all variants from all samples were 100% concordant. Percent of negative calls at each CDx variant location for wild-type samples was 100%.

Similarly, the platform-level repeatability and reproducibility showed high overall agreement across alteration bins, and high sample-level positive and negative call rates as summarized in Tables 16 and 17

below. The platform-level study included a total of 443 substitutions, 188 indels, 55 copy number amplifications, 13 copy number loss, and 18 rearrangements in the variant set across the samples.

Table 16 Reproducibility across variant bins (copy number, rearrangement, substitution, indels

Variant Bin	# of Variants	# of valid Comparisons	# of Agreements	Positive Percent Agreement	95% CI Lower Limit[1]	95% CI Upper Limit[1]
CNAs	68	67,524	67,300	99.67%	99.62%	99.71%
Rearrangements	18	17,874	17,851	99.87%	99.81%	99.92%
Substitutions	443	439,899	439,649	99.94%	99.94%	99.95%
Indels	188	186,684	186,319	99.80%	99.78%	99.82%
All Variants	717	711,981	711,119	99.88%	99.87%	99.89%

[1] 95% 2-sided exact CIs were calculated.

Table 17 Positive and negative call rates per sample for platform variants (N=717).

Alteration Type(s) Assessed		exact 95% CI			exact 95% CI[1]	
	PC Rate*	Lower	Upper	NC Rate**	Lower	Upper
CNA/RE/SUB	100.00%	99.40%	100.00%	99.98%	99.95%	99.99%
CNA/ SUB/Indel	99.37%	98.38%	99.83%	99.96%	99.92%	99.98%
SUB/Indel	100.00%	99.10%	100.00%	99.97%	99.95%	99.99%
CNA/ SUB/Indel	97.84%	96.89%	98.56%	99.84%	99.78%	99.89%
SUB/Indel	99.81%	98.94%	100.00%	99.98%	99.95%	99.99%
SUB/Indel	99.60%	97.81%	99.99%	99.94%	99.90%	99.97%
CNA/ SUB/Indel	98.33%	97.11%	99.14%	99.98%	99.96%	100.00%
SUB/Indel	100.00%	99.83%	100.00%	99.97%	99.94%	99.99%
CNA/ SUB/Indel	100.00%	99.32%	100.00%	99.98%	99.96%	100.00%
RE/ SUB/Indel	96.46%	94.14%	98.05%	99.96%	99.92%	99.98%
CNA/ SUB	98.67%	97.27%	99.46%	99.98%	99.96%	100.00%
CNA/RE/SUB/Indel	96.27%	95.39%	97.02%	99.87%	99.82%	99.91%
RE/SUB/Indel	98.23%	97.48%	98.80%	99.66%	99.58%	99.73%
CNA/ SUB/Indel	98.32%	97.57%	98.89%	99.92%	99.88%	99.95%
SUB/Indel	99.30%	98.90%	99.58%	99.90%	99.86%	99.94%
CNA/RE/SUB/Indel	85.42%	82.27%	88.20%	99.89%	99.84%	99.93%
RE/SUB/Indel	97.75%	96.42%	98.68%	99.98%	99.95%	99.99%
RE/SUB/Indel	95.30%	92.97%	97.03%	99.96%	99.93%	99.98%
CNA/RE/SUB/Indel	100.00%	98.31%	100.00%	99.89%	99.84%	99.93%
CNA/RE/SUB/Indel	100.00%	99.25%	100.00%	99.96%	99.93%	99.98%
CNA /SUB	96.83%	94.90%	98.17%	99.94%	99.90%	99.97%
CNA/RE/SUB/Indel	95.97%	94.06%	97.40%	99.98%	99.96%	100.00%
CNA/ SUB/Indel	100.00%	99.42%	100.00%	99.93%	99.89%	99.96%
CNA/RE/SUB/Indel	100.00%	99.30%	100.00%	99.95%	99.91%	99.97%
RE/SUB	100.00%	99.05%	100.00%	100.00%	99.98%	100.00%
CNA /SUB	96.99%	95.39%	98.15%	99.84%	99.79%	99.89%
CNA/RE/SUB/Indel	100.00%	98.95%	100.00%	99.93%	99.89%	99.96%
CNA/RE/SUB/Indel	99.80%	99.29%	99.98%	99.98%	99.96%	100.00%

*Abbreviations: SUB=substitution, Indel=Insertion or Deletion, CNA=Copy Number Alteration, RE=Rearrangement, PC=Positive Call, NC=Negative Call

[1] 95% 2-sided exact CIs were calculated.

For TMB determination, 13 samples met the inclusion criteria (TMB ≥ 10) for assessment of repeatability and reproducibility. Twelve of 13 samples (92.3%) met the ≤20% Coefficient of Variation (CV) requirements; one sample fell just outside this requirement with a repeatability CV of 21% and reproducibility CV of 23%. The putative source of variability was determined to be low depth of coverage for this sample.

To evaluate the performance of MSI detection using the FB-MSI caller, a prospectively designed retrospective analysis was performed using a set of 7 MSI-H samples and 59 non-MSI-H samples from eight tumor types. FFPE-derived DNA samples were selected from banked Foundation Medicine DNA samples. Each sample was tested in two or three replicates, in two runs, with three sequencers, and two or three reagent lots in a factorial design. A total of 36 replicates per sample was processed. Data analysis was performed for each sample separately. According to their MSI scores, samples were classified as MSI-H (≥ 0.0124) or non-MSI-H (<0.0124). An evaluation of within-laboratory (intermediate) precision for MSI status was performed by evaluation of reproducibility and repeatability estimated as the percent agreement for each sample. Samples or replicates with missing MSI results due to QC failure were excluded from the analysis. In total, there were 65 evaluable samples for this study, 7 MSI-H samples and 58 non-MSI samples. The 7 MSI-H samples had MSI scores ranging from 0.0226 to 0.0682 and 58 non-MSI-H samples had MSI scores ranging from 0.0043 to 0.0001. The agreement for reproducibility and repeatability was 100% for the 65 evaluable samples. For 64 of 65 samples the lower bound of the two-sided 95% score CI ranged from 89.57% to 90.36% for reproducibility and from 74.12% to 82.41% for repeatability. One sample with an average MSI score of 0.0009 had 7 evaluable replicates and agreement for reproducibility (7/7) was 100%; the lower bound of the two-sided 95% score CI was 64.57%. For the same sample the repeatability was 100%; the lower bound of the two-sided 95% score CI was 34.24%.

The same set of sample results were also analyzed using the 0.0041 threshold; samples were classified as MSS (≥0.0041) or non-MSS (>0.0041). An evaluation of intermediate precision, reproducibility, and repeatability for calling MSS vs non-MSS was performed. In total precision of MSI calling was evaluable in 56 MSS samples and 9 non-MSS samples. The agreement for reproducibility and repeatability ranged from 94.44% to 100% for the 64 of 65 evaluable samples. For 63 of 65 samples with reproducibility and repeatability ranging from 94.44% to 100% the lower bound of the two-sided 95% score CI ranged from 81.86% to 90.11% for reproducibility and from 67.20% to 82.41% for repeatability. One sample had 29 of 36 replicates failing to meet F1CDx QC metrics for MSI. For this sample, with an average MSI score of 0.000914, the agreement for reproducibility (7/7) was 100%, the lower bound of the two-sided 95% score CI was 64.57%. For the sample the repeatability was 100%; the lower bound of the two-sided 95% score CI was 34.24%. Finally, one sample had an average MSI score of 0.0043 and was close to the threshold of 0.0041. The agreement for reproducibility for this sample was 54.29% (19/35) and the lower bound of the two-sided 95% score CI was 38.19%. The agreement for repeatability for this sample was 0%.

A second precision study was conducted to evaluate intra-run repeatability and reproducibility for MSI calling by the F1CDx assay in tumors derived from 7 major organ systems (i.e., gastrointestinal, hepato-pancreatobiliary, urinary, endocrine, skin, thoracic, and reproductive) to support pan-tumor testing. This study was comprised of 44 MSI-H and 2 non-MSI samples. FFPE-derived DNA samples were selected from banked Foundation Medicine DNA samples. The study examined reagent lots and instruments as factors in inter-run reproducibility by assessing samples with two replicates in each of two separate runs, using two reagent lots, and three sequencers. According to their MSI scores, samples were classified as MSI-H (≥ 0.0124) or non-MSI-H (<0.0124). Of the 46 samples, 42 samples had agreement for reproducibility ranging from 90.48% to 100.00%, with the lower bound the two-sided 95% score CI ranging from 71.09% to 86.20%. For four (4) samples with MSI scores close to the 0.0124 cut-off, ranging from 0.0116 to 0.0133, agreement for reproducibility ranged from 52.56% to 82.61%, with the lower bound of the two-sided 95% score CI ranging from 36.81% to 62.86%. Of the 46 samples, 39 samples had agreement for repeatability ranging from 91.67% to 100.00%, with the lower bound of the two-sided 95% score CI ranging from 64.61% to 74.74%. For six (6) samples agreement for repeatability ranged from 54.55% to 83.83%, with the lower bound the two-sided 95% score CI ranging from 28.01% to 55.20%. The observed MSI scores of these six samples were close to the cut-off of 0.0124, ranged from 0.0116 to 0.0154, thus explaining the disagreement of MSI status results near the threshold. The repeatability value of the 7th sample could not be calculated because 21 of 24 replicates were removed from the analysis due to failures in F1CDx laboratory process QC or MSI QC rules.

In order to provide for a more robust assessment of the impact of sequencing reagent lots on the within-laboratory (intermediate) precision of MSI calling by F1CDx, a supplemental evaluation was conducted

using the 44 MSI-H samples and 2 non-MSI-H sample described in the second intermediate precision study above representing 7 major organ systems with a total of three (3) sequencing reagent lots. MSI within-laboratory precision and repeatability were assessed by testing each of the 46 samples with two (2) replicates, per each of two (2) separate runs (plates), using three (3) sequencing reagent lots, and three (3) HiSeq 4000 sequencers using a factorial design with a total of 36 sample replicates across the paired reagent lot/sequencer combination.

Since data from the previous study were used to support the precision assessment by comparing three (3) lots of reagents, processing failures noted in previous study were integrated into this validation study. Samples with less than 36 replicates could be evaluated, however, any sample that resulted in sequencing data for ≤12 evaluable replicates (e.g., wherein ≥24 replicates failed during processing) was not evaluated for repeatability or reproducibility. In total 1656 sample replicates were evaluated in the study. Of the 1656 sample replicates, 1518 replicates were successfully sequenced, and provided F1CDx evaluable results. Among the 46 samples, one lung adenocarcinoma sample was excluded from the analysis as all sample replicates failed LC or HC QC leaving no evaluable replicates for evaluation. Therefore, 45 of 46 samples provided F1CDx evaluable replicates that were considered in the data analysis.

According to their MSI scores, samples were classified as MSI-H (≥ 0.0124) or non-MSI-H (< 0.0124). Of the 45 samples, 41 samples had agreement for within-laboratory precision ranging from 91.18% to 100.00%, with the lower bound of the two-sided 95% score CI ranging from 77.04% to 90.36%. For 4 samples with MSI scores close to the 0.0124 cut-off, ranging from 0.0115 to 0.0138, agreement for within-laboratory precision ranged from 61.29% to 88.89%, with the lower bound of the two-sided 95% score CI ranging from 43.82% to 74.69%. Of the 45 samples, 39 samples had agreement for repeatability ranging from 92.86% to 100%, with the lower bound of the two-sided 95% score CI ranging from 68.53% to 80.64%. For 6 samples, agreement for repeatability ranged from 61.54% to 81.25%, with the lower bound the two-sided 95% score CI ranging from 35.52% to 56.99%. The observed MSI scores of these six samples were close to the cut-off of 0.0124, ranging from 0.0115 to 0.0157, thus explaining the disagreement of MSI status results near the threshold.

Refer to the Summary of Safety and Effectiveness for P170019/S029 for summary tables.

Together the results of these studies demonstrate that while the within-laboratory precision is high for samples well above and well below the two MSI score thresholds that are applied to classify a tumor specimen as having MSI-H or MSS status, there may be imprecision as shown by reproducibility and repeatability results for samples near the two thresholds for MSI calling.

2.6.1 Reagent Lot-to-Lot Reproducibility

Three lots of critical reagents were assessed for four replicates per sample in a full factorial design. Reagents were evaluated as internally prepared kits for each process step (LC, HC, sequencing). The use of three different lots of reagents did not impact performance. Twenty-seven of 28 samples (96.4%) had pairwise agreement estimates (APA and ANA) above 95%; one sample had APA estimates below 90% (85.9% to 88.7%). ANA estimates were greater than 99%. The putative source of variability was determined to be non-focal copy number amplifications with low copy number close to the calling threshold observed in one sample; no specific reagent lot performed differently among three lots for this sample.

2.6.2 Instrument-to-Instrument Reproducibility

Four replicates per sample were sequenced on each of three Illumina HiSeq4000 sequencers, serial numbers K00255, K00256, and K00257 in a full factorial design. The use of three different sequencers did not impact performance. Twenty-seven of 28 samples (96.4%) had pairwise agreement estimates (APA and ANA) at least 97%; one sample had APA estimates below 90% (86.6% to 89.2%). ANA estimates were greater than 99%. The putative source of variability was determined to be non-focal copy number amplifications with low copy number close to the calling threshold observed in one sample; no specific sequencer performed differently among three sequencers for this sample.

2.6.3 Site-to-Site Reproducibility

FMI performed a site-to-site precision study with the objective of evaluating repeatability and reproducibility of the F1CDx assay with challenging samples near the LoD across many tumor types. This study assessed the repeatability and reproducibility of the detection of alterations associated with CDx claims and other tumor profiling alterations. In addition, the study evaluated agreement for MSI, LOH and TMB calling. Repeatability between intra-run replicates (run on the same plate under the same conditions) and reproducibility of inter-run replicates (run on different plates under different conditions) were assessed and compared between the two FMI sites (Cambridge, MA and Morrisville, NC), two reagent lots, and three non-consecutive days. The study demonstrated repeatable and reproducible results across the CDx variants including:

NSCLC:

- *EGFR* exon 19 deletions, exon 21 L858R, exon 20 T790M
- *ALK* rearrangement
- *BRAF* V600E

Melanoma:

- *BRAF* V600E and V600K

Breast Cancer:

- *ERBB2* (HER2) amplification
- *PIK3CA* mutations

Colorectal Cancer:

- *KRAS* wild-type
- *NRAS* wild-type

Ovarian Cancer:

- *BRCA1/2*
- LOH

Solid Tumors:

- TMB-H (≥ 10 mutations per megabase)
- MSI-H

In the assessment of other tumor profiling alterations, the study demonstrated repeatable and reproducible results with a multivariant analysis for all alteration types, as well as MSI. The totality of the results demonstrate that the F1CDx assay has robust performance with respect to repeatability and reproducibility in calling genomic alterations across two sites (i.e., Cambridge, MA and Morrisville, NC). In summary, comparable results for FMI Cambridge and FMI Morrisville were observed when detecting CDx variants (including LOH for ovarian cancer and TMB as a qualitative biomarker [≥ 10 mutations per megabase]), tumor profiling alterations, as well as genomic signatures (e.g., MSI).

2.7 Analytical Sensitivity: Limit of Detection (LoD) and Limit of Blank (LoB)

The Limit of Detection (LoD) of alterations assessed by FoundationOne®CDx (F1CDx) was evaluated. The LoDs of seventeen (17) CDx biomarkers are summarized in Tables 18-1, 18-2 and 18-3 below. An additional twelve (12) categories of alteration types were evaluated for the F1CDx assay platform validation. FFPE tumor samples were selected for each of the variant categories. For each sample, six levels of MAF, with 13 replicates per level, were evaluated for a total of 78 replicates per sample. LoD for short variants, including substitutions and indels, is based on allele fraction. LoD for structural variants (fusions, amplifications, homozygous deletions, rearrangements) and TMB is based on computational tumor purity. Computational tumor purity is calculated by fitting the observed log-ratio and minor allele

frequency data with statistical models that predict a genome-wide copy number profile, tumor ploidy, and tumor purity (i.e., computational tumor purity). The log-ratio profile is obtained by normalizing aligned tumor sequence reads by dividing read depth by that of a process-matched normal control, followed by a GC-content bias correction using Loess regression. The minor allele frequency profile is obtained from the heterozygous genome-wide SNPs. For platform-wide LoD assessment, the indels were grouped together (other than homopolymer repeat context) as they are similar in LoD characteristics. The indels ranged from 1 bp up to 42 bp insertions and deletions up to 276 bp. Indels at homopolymer repeat context had higher LoD, with a dependency on the length of the repeat context. The LoD for representative alterations detected by the F1CDx platform is summarized in Tables 19-1 and 19-2.

Table 18-1. Summary of LoD for alterations associated with CDx claims (short variants). LoD is based on Allele Fraction.

Alteration	LoD[1] Allele Fraction (%) (95% Hit Rate)	LoD[2] Allele Fraction (%) (Probit)
EGFR L858R	2.4%	< 2.4% (all detected)
EGFR Exon 19 deletion	5.1%	3.4%
EGFR T790M	2.5%	1.8%
KRAS G12/G13	2.3%	< 2.3% (all detected)
BRAF V600E/K	2.0%	< 2.0% (all detected)
MET Exon 14 SNVs[3]	N/A	< 2.9% (all detected)
MET Exon 14 insertion and deletion[3]	N/A	5.7%
PIK3CA E542K	4.9%	Not Calculated
BRCA1/2[4] Alteration in non-repetitive or homopolymer <4 bp Deletion in 8 bp homopolymer	 N/A N/A	 5.9% 15.3%
HRR gene base substitutions	5.44% - 6.33%[5]	Not calculated
HRR gene indels	5.22% - 12.74% [5]	Not calculated

[1] LoD calculations for the CDx variants were based on the hit rate approach, as there were less than three levels with hit rate between 10% and 90% for all CDx variants (not including *BRCA*1/2 variants). LoD from the hit rate approach is defined as the lowest level with 95% hit rate (worst scenario).
[2]LoD calculations for the CDx variants based on the probit approach with 95% probability of detection.
[3] For each sample, five levels of MAF, with 10 replicates per level, were evaluated for a total of 50 replicates per sample.
[4]See Summary of Safety and Effectiveness Data for P160018.
[5] LoD defined as the lowest level with 95% hit rate or greater.

Table 18-2. Summary of LoD (C95) based on tumor purity for biomarkers associated with CDx claims.

Alteration	Tumor Purity (%) (95% Hit Rate)[1]	Tumor Purity (%) (Probit)[2]
ALK fusion	2.6%[3]	1.8%
ERBB2 amplification	25.3%[4]	19.7%
BRCA2 homozygous deletion (HD)	8.8%[5]	Not Calculated
LOH[6]	35%	30%
FGFR2 fusions	5.31%[7]	5.38%
HRR gene rearrangements[8]	20.1%[7]	Not Calculated
HRR gene homozygous deletions[8]	23.9%[7]	Not Calculated
TMB ≥ 10 mutations per megabase[8]	28.16%[7]	Not Calculated
NTRK1 fusions[9,10]	12.10%	N/A
NTRK2 fusions[9,11]	11.5%	N/A
NTRK3 fusions[9,12]	6.1%	N/A

ROS1 fusions[4]	5.8%	Not calculated
MSI-High[13]	9.76% (8.25%-15.67%)[7,14]	N/A

[1]Sensitivity calculations for the CDx variants were based on the hit rate approach, as there were less than three levels with hit rate between 10% and 90%. LoD from the hit rate approach is defined as the lowest level with 95% hit rate (worst scenario).
[2]Sensitivity calculations for the CDx variants based on the probit approach with 95% probability of detection.
[3]The number of chimeric reads for the sample evaluated is 16 at the indicated tumor fraction.
[4]The number of copy number amplifications for the sample evaluated is 6 at the indicated tumor fraction.
[5]The LoD calculation for the *BRCA2* HD was based on the hit rate approach, as there was a hit at every dilution level tested, making the probit regression not applicable.
[6]See Summary of Safety and Effectiveness Data for P160018/S001.
[7]Calculated using the 95% hit rate.
[8]For each sample, five levels of tumor purity, with 20 replicates per level except for the highest level at which 14 replicates were tested, were evaluated for a total of 94 replicates per sample.
[9]For each sample, a total of 94 tumor dilution replicates were assessed, including twenty (20) replicates for each level of tumor purity, excluding the highest level, for which only fourteen (14) replicates were performed.
[10] The LoD study included 2 samples with CDx *NTRK1* fusion positive status: one (1) *NTRK1-LMNA* fusion, and one (1) *NTRK1-TRP* fusion.
[11] The LoD study included 2 samples with CDx *NTRK2* fusion positive status: one (1) *NTRK2-BCR* fusion, and one (1) *NTRK2-GARNL3* fusion.
[12] The LoD study included 3 samples with CDx *NTRK3* fusion positive status: three (3) *NTRK3-EVT6* fusions.
N/A=not applicable.
[13] The LoD study included eight samples, four (4) from patients with CRC, one (1) from a patient with uterus endometrial carcinoma, one (1) from a patient with liver cholangiocarcinoma, one (1) from a patient with lung squamous cell carcinoma, and one (1) from a patient with kidney urothelial carcinoma.
[14] Median and range LoD displayed.

Table 18-3. Summary of analytical sensitivity based on reads for biomarkers associated with CDx claims

Alteration	Reads (95% Hit Rate)[1]
ALK rearrangements	15.54[2]
NTRK1 fusions	24.55
NTRK2 fusions	24.16
NTRK3 fusions	14.65
***ROS1* fusions**	11.85

[1]Sensitivity calculations for the CDx variants were based on the hit rate approach, as there were less than three levels with hit rate between 10% and 90%. LoD from the hit rate approach is defined as the lowest level with 95% hit rate.
[2]The LoD of *ALK* rearrangements was originally determined using tumor purity in P170019. However, data was later provided to support the LoD of *ALK* rearrangements in terms of reads.

Table 19-1. Summary of representative LoD for F1CDx platform (short variants).

Variant Category	Subcategory	N	Range LoD[1] Allele Fraction (%)
Base Substitutions	known[3]	21[2]	1.8-7.9[2]
	other[4]	166	5.9-11.8
Indels at non-homopolymer context, including insertions up to 42bp and deletions up to 276bp	Known	3	4.5-6.5
	Other	17	6.0-10.2
Indels at homopolymer context	5bp repeat	8	10.0-12.2
	6bp repeat	2	13.6-13.7
	7bp repeat	4	16.3-20.4
	8bp repeat	3	17.0-20.0

[1]LoD calculations for the platform variants were based on the hit rate approach for variants with less than three levels with hit rate between 10% and 90% and probit approach for variants with at least three levels with hit rate between 10% and 90%. LoD from the hit rate approach is defined as the lowest level with 100% hit rate (worst scenario).

[2]Data includes an alteration in the *TERT* promoter, 124C>T (LoD of 7.9%). *TERT* is the only promoter region interrogated and is highly enriched for repetitive context of poly-Gs, not present in coding regions.
[3]Alterations classified as" known" are defined as those that are listed in COSMIC
[4]Alterations classified as "other" include truncating events in tumor suppressor genes (splice, frameshift and nonsense) as well as variants that appear in hotspot locations but do not have a specific COSMIC association, or are considered variants of unknown significance (VUS) due to lack of reported evidence and conclusive change in function.

Table 19-2. Summary of representative analytical sensitivity for tumor purity for F1CDx platform alterations (copy number variants and rearrangements).

Variant Category	N	Range Tumor Purity (%)[1]
Copy Number Amplifications (CN>10)	8	9.6%-18.5%
Copy Number Amplifications (6≤CN≤10)	7	19.5%-58.3%[2]
Copy Number: Homozygous Deletions	3	33.4%-33.4%
Genomic Rearrangements	3	9.2%-14.9%

[1]Sensitivity calculations for the platform variants were based on the hit rate approach for variants with less than three levels with hit rate between 10% and 90% and probit approach for variants with at least three levels with hit rate between 10% and 90%.
[2]Max represents VUS alteration at calling threshold.

The LoB of zero was confirmed through the assessment of alterations within the LoB samples, with a percentage of false-positive results less than 5% (type I error risk α=0.05). Seventy-five (75) samples were used for the assessment of LoB. For all the alterations evaluated for LoD, the LoB of zero was confirmed. A similar study was conducted for *BRCA1/2* alterations (PMA P160018) with no false-positive *BRCA* calls observed, thus confirming the LoB of zero for *BRCA*. An additional study was conducted for TMB in twenty-one (21) samples with no false positive TMB-H calls (≥ 10 mutations per megabase) observed, thus confirming the LoB of zero for TMB. To assess LoB for MSI, a supplementary statistical analysis was performed using 111 test replicates from 10 individual tumor FFPE-derived DNA samples as well as a pool of biomarker-negative DNA derived from 30 unique FFPE tumor DNA samples. With 0/111 MSI-H results, the study met the acceptance criterion that no more than 5% of the 111 blank replicates are determined to be MSI-H and confirm LoB for MSI-H is 0. The average, median, minimum and maximum MSI scores observed were 0.0006, 0.0004, 0 and 0.0032, respectively.

2.8 Stability

2.8.1 Reagent Stability

Identical reagents with the same specifications are used following the same protocols for both the FoundationFocus CDx$_{BRCA}$ Assay and FoundationOne®CDx (F1CDx). For reagent stability performance data, see the Summary of Safety and Effectiveness Data for P160018. The claimed reagent stability is 4 months for the library construction (LC) and hybrid capture (HC) kits, and 3 months for the sequencing kits.

2.8.2 DNA Stability

Stability of DNA was evaluated through a retrospective review of data generated using the FoundationOne LDT assay. Samples from 47 unique clinical specimens from 21 different tissues of origin were evaluated. The sample set covered 200 alterations inclusive of nucleotide changes, indels, copy number amplifications, copy number losses and rearrangements. Duration of DNA storage at time of testing ranged from 48 to 464 days, with a median of 184 days and a mean of 199 days. A total of 199 of 200 alteration calls were concordant. A 242-day old sample with a single alteration call that met inclusion criteria was discordant; however, this sample was classified as not meeting all QC criteria due to other data quality issues. DNA age for the sample with discordance was 242 days. Sixteen other samples had concordant calls with DNA age >242 days. Based on these data, DNA stored in accordance with internal procedures can be stored at 4°C for up to 6 weeks and -20°C for 5 months. Further supporting this

retrospective data is a prospective study conducted using ovarian cancer samples, see the Summary of Safety and Effectiveness Data for P160018. An additional prospective DNA stability study is underway.

2.8.3 FFPE Sample Stability

The FFPE Slide Stability Study is an ongoing study with data summarized for T_0, T_1 (30 days), and T_2 (6 months). This study evaluated the stability of FFPE tumor tissue prepared as slides prior to DNA extraction for use within the FoundationOne®CDx (F1CDx) assay. Five tumor samples including ovarian, lung, colorectal cancer, melanoma and breast cancer that contained a variety of DNA alterations, as described in Table 20 below. The five samples were selected to include specific alteration types that were reflective of the CDx alterations, but were found to contain additional alterations as well (13 CNAs, one rearrangement, 53 base substitutions and five indels; refer to Table 21). To assess stability of pre-cut FFPE tissue for genomic alterations, the agreement between results from the defined time points for each sample were calculated by comparing the alteration call reported at each follow-up time point to the alteration call at baseline (T_0). Alterations at the 30-day time point and the 6-month time point are in 100% agreement with the day 0 baseline results (T_0). The FFPE slides are considered stable for at least 6 months. Further assessment at months 12 and 15 will evaluate stability of FFPE slides beyond 6 months.

Table 20. Stability results at baseline, 30 days and 6 months.

Tissue	Baseline Call (T_0)		Percent Agreement to T_0	Percent Agreement to T_0
	Gene	Variant Effect	30 days (T_1)	6 months (T_2)
Ovarian	*BRCA1*	c.1340_1341insG, p.H448fs*8	100% (2/2)	100% (2/2)
Lung	*KRAS*	c.34G>T, p.G12C	100% (2/2)	100% (2/2)
CRC	*PIK3CA*	c.3139C>T, p.H1047Y	100% (2/2)	100% (2/2)
CRC	*PIK3CA*	c.1258T>C, p.C420R	100% (2/2)	100% (2/2)
Melanoma	*CDKN2A*	Homozygous Deletion	100% (2/2)	100% (2/2)
Melanoma	*CDKN2B*	Homozygous Deletion	100% (2/2)	100% (2/2)
Breast	*ERBB2*	Amplification	100% (1/1)	100% (2/2)

Table 21. Percent agreement for each variant type.

Variant type	Number of variants	30 days (T_1) Percent Agreement (# agreement/total)	95% 2-sided CI LB, UB*	6 months (T_2) Percent Agreement (# agreement/total)	95% 2-sided CI LB, UB*
Copy Number	13	100% (23/23)	85.2%, 100.0%	100% (26/26)	86.8%, 100.0%
Rearrangement	1	100% (2/2)	15.8%, 100.0%	100% (2/2)	15.8%, 100.0%
Substitution	53	100% (98/98)	96.3%, 100.0%	100% (106/106)	96.6%, 100.0%
Insertion/Deletion	5	100% (7/7)	59.0%, 100.0%	100% (10/10)	69.2%, 100.0%

*LB: lower bound; UB: upper bound

2.9 Reagent Lot Interchangeability

Identical reagents with the same specifications are used following the same protocols for both the FoundationFocus CDx$_{BRCA}$ assay and FoundationOne®CDx. For reagent lot interchangeability performance data, see the Summary of Safety and Effectiveness Data for P160018.

2.10 General Lab Equipment and Reagent Evaluation

2.10.1 DNA Amplification

Identical reagents and equipment with the same specifications are used following the same protocols for both the FoundationFocus CDx$_{BRCA}$ Assay and FoundationOne®CDx. For DNA amplification performance data, see the Summary of Safety and Effectiveness Data for P160018.

2.10.2 DNA Extraction

The performance of DNA extraction from FFPE tumor specimens was evaluated. The DNA extraction procedure for the FoundationOne®CDx (F1CDx) assay was assessed by testing FFPE specimens including two samples per tissue type for ten different tumor tissue types including lung, breast, ovarian, melanoma, colorectal, brain, hepatic, pancreatic, thyroid, and bladder with different representative types of alterations. Samples were run in duplicate for a total of 240 extractions, employing two different KingFisher Flex Magnetic Particle Processors (120 extractions per processor) and comparing across three extraction

reagent lots (80 extractions per reagent lot). Average DNA yield was calculated across twelve (12) replicates for each sample. All average DNA yields were significantly above the minimum requirement of 55 ng, with the minimum being 758.3 ng. Only one sample aliquot of the 240 replicates failed the DNA yield specification, and the success rates based on the reagent lot and the equipment were 98.8% (79/80) and 99.2% (119/120), respectively, passing the acceptance criteria (≥90%). Concordance of all genomic alterations detected was also analyzed for all variants across 12 replicates for each sample. Table 22 provides a summary of concordance across replicates. A study with an additional ten samples will be completed post-market.

Table 22-1. Summary of concordance across replicates of DNA extraction study.

Group	$N_{concordance}$	N_{total}	Concordance	95% CI
Substitutions (All MAF)	2700	2969	90.9%	[89.9% 91.9%]
Substitutions (MAF > 10%)	1631	1637	99.6%	[99.2% 99.9%]
Substitutions (All MAF, excluding hypermutated sample)*	1663	1685	98.7%	[98% 99.1%]
Indel (All)	465	476	97.7%	[95.9% 98.8%]
Copy Number: Amplification	307	314	97.8%	[95.4% 99%]
Copy Number: Loss	132	144	91.7%	[85.9% 95.3%]
Rearrangement	84	90	93.3%	[85.9% 97.2%]

*One sample included in the study was hypermutated, harboring many alterations near LoD and exhibited evidence of external contamination. Concordance of substitutions was 80.8% for this sample.

A DNA extraction study was performed to evaluate the FoundationOne®CDx (F1CDx) assay DNA extraction procedure with respect to TMB-H (≥ 10 mutations per megabase) calling. The analysis included 35 retrospective samples and all acceptance criteria were met.

2.10.2.1 CoExtraction Validation

A concordance study was performed to evaluate the variant calling concordance between samples with the existing F1CDx DNA extraction method (DNAx, baseline, 1 replicate) and the new extraction method (CoEx, test, 1 replicate). A total of 127 FFPE tumor specimens from 12 different tissue types were included in the study. Table 22-2 below provides a summary of the concordance study results. The tumor profiling variants analyzed in this study included all reportable, non-CDx variants detected in this study.

Table 22-2. Concordance of Targeted Variants and Biomarkers – CoExtraction

	Variant/Biomarker Type	PPA (95%CI)	NPA (95% CI)
Targeted CDx Variants and Biomarkers	Indels (short variants)	88.24 (15/17) (65.66, 96.71)	100 (196/196) (98.08, 100)
	Substitutions (short variants)	100 (46/46) (92.29, 100)	98.98 (487/492) (97.64, 99.57)
	Rearrangements	100 (8/8)	100 (24/24) (86.2, 100)
	Amplifications (copy number alterations)	100 (6/6)	100 (8/8)
	Homozygous deletions (copy number alterations)	100 (1/1)	100 (13/13) (77.19, 100)
	TMB High	92.86 (26/28) (77.35, 98.02)	100 (71/71) (94.87, 100)
	MSI High	100 (11/11) (74.12, 100)	100 (89/89) (95.86, 100)

	Variant/Biomarker Type	PPA (95%CI)	NPA (95% CI)
	gLOH High (non-CDx*)	93.33 (14/15) (70.18, 98.81)	100 (2/2)
	All	**96.21 (127/132) (91.44, 98.37)**	**99.44 (890/895) (98.7, 99.76)**
Tumor Profiling Variants	Indels (short variants)	94.69 (357/377) (91.95, 96.54)	99.9957 (422896/422914) (99.9933, 99.9973)
	Substitutions (short variants)	96.07 (1685/1754) (95.05, 96.88)	99.9962 (2314383/2314472) (99.9953, 99.9969)
	Rearrangements	90.91 (40/44) (78.84, 96.41)	99.9955 (66628/66631) (99.9868, 99.9985)
	All	**95.72 (2082/2175) (94.79, 96.50)**	**99.9961 (2803907/2804017) (99.9953, 99.9967)**
*The gLOH biomarker has a complementary diagnostic claim for ovarian cancer patients and rucaparib			

A precision study was performed to assess the reproducibility and repeatability of the CoExtraction method in the F1CDx assay. The study included 47 unique FFPE tumor samples. First, six (6) curls were cut from each source block. The curls were processed with the CoExtraction method, using a combination of two unique CoExtraction reagent lots and two unique CoExtraction instrument lines. Second, the extracted DNA from each curl was subdivided into four (4) DNA sub-aliquots, for a total of 24 total replicates per source block entering precision testing (6 curl extractions x 4 extracted DNA subaliquots each = 24 total).

72 total targeted variants and biomarkers were analyzed, including 36 short variants, 8 rearrangements, 7 copy number alterations, 9 TMB, 5 MSI, and 7 gLOH (in ovarian samples only), and data analysis was performed for each variant and biomarker separately. An evaluation of within-laboratory precision was performed by evaluation of reproducibility and repeatability estimated as the percent agreement for each variant and biomarker.

The agreement for reproducibility and repeatability of short variants was 100% for the 36 variants; the lower bound of the two-sided 95% CI ranged from 84.54% to 86.20% for reproducibility and from 70.90% to 75.75% for repeatability. The agreement for reproducibility and repeatability of rearrangements was 100% for the 8 variants; the lower bound of the two-sided 95% CI ranged from 85.69% to 86.2% for reproducibility and from 74.12% to 75.75% for repeatability. The agreement for reproducibility and repeatability of copy number alterations was 100% for the 7 variants; the lower bound of the two-sided 95% CI ranged from 85.69% to 86.2% for reproducibility and from 74.12% to 75.75% for repeatability.

The agreement for reproducibility and repeatability of TMB was 100% for 7 of 9 samples; the lower bound of the two-sided 95% CI for these 7 samples ranged from 83.18% to 86.2% for reproducibility and from 67.56% to 75.75% for repeatability. For 1 TMB sample, the agreement for reproducibility and repeatability was 95.5% and 90.0% with the lower bound of the two-sided 95% CI being 78.2% and 59.58%, respectively. There was 1 TMB sample that did not meet acceptance criteria and had an agreement for reproducibility and repeatability of 16.7% and 66.7% with the lower bound of the two-sided 95% CI being 6.68% and 39.06%, respectively. A discordance investigation revealed that this was a borderline case near the calling threshold where reproducibility is expected to be most challenging.

The agreement for reproducibility and repeatability of MSI was 100% for 4 of the 5 samples; the lower bound of the two-sided 95% CI ranged from 84.54% to 86.2% for reproducibility and from 72.25% to 75.75% for repeatability. One MSI sample had an agreement for reproducibility and repeatability of 95.7% and 90.9% with the lower bound of the two-sided 95% CI being 79.01% and 62.26%, respectively.

The agreement for reproducibility and repeatability of gLOH was 100% for the 6 of 7 samples; the lower bound of the two-sided 95% CI ranged from 85.69% to 86.2% for reproducibility and from 74.12% to 75.75% for repeatability. There was 1 gLOH sample that did not meet acceptance criteria and had an agreement for reproducibility and repeatability of 47.4% and 33.3% with the lower bound of the two-sided 95% CI being 27.33% and 12.06%, respectively. A discordance investigation revealed that this was a borderline case near the calling threshold where reproducibility is expected to be most challenging.

2.11 Guard banding/Robustness

Guard banding studies were performed to evaluate the impact of process variation with regard to the measurement of DNA concentration at various stages of the process. Guard bands were evaluated relative to observed and measured process variability for Library Construction (LC), Hybrid Capture (HC), and Sequencing. Each of the three guard banding experiments demonstrated reliable and robust performance at all DNA input levels evaluated.

A total of 255 samples were processed; ninety (90) to assess DNA input into LC, ninety (90) to assess DNA input into HC, and seventy-five (75) to assess DNA input into sequencing. For LC input, five samples were run in triplicate over six different DNA input levels representing -20% and -50% from the lower limit (50 ng) to +20% and +50% from the upper limit (1000 ng) needed for LC (n=90). Five samples were run in triplicate over six DNA input levels representing -25% and -50% from the lower limit (0.5 µg) to +25% and +50% from the upper limit (2.0 µg) for HC input. The third component of the guard banding study evaluated the captured DNA input into the sequencing reaction. Five samples were run in triplicate over five different DNA input levels representing ±10% and ±20% from the required amount needed for sequencing (1.75 nM; n=75). Concordance of detected alterations was calculated for each condition across successful replicates. Results from this study support the robustness of the FoundationOne®CDx (F1CDx) process. The study design and results are shown below in Tables 23-1 through 23-4.

Table 23-1. Summary of the success rate per process and per input level, and concordance of substitutions (SUB) among successful replicates.

Process	Input Level	# of Sample Failures	Variant Type	# of Concordant Successes	# of Variant Comparisons	Success Rate (95% CI) (Number of Concordant comparisons)
LC	25 ng	1/15	SUB	184	184	100.0% (98.0%, 100.0%)
LC	40 ng	0/15	SUB	192	192	100.0% (98.1%, 100.0%)
LC	50 ng	0/15	SUB	191	192	99.5% (97.1%, 100%)
LC	1000ng	0/15	SUB	192	192	100.0% (98.1%, 100.0%)
LC	1200 ng	0/15	SUB	191	192	99.5% (97.1%, 100%)
LC	1500 ng	0/15	SUB	190	192	99.0% (96.3%, 99.9%)
HC	0.25 µg	15/15	SUB	0	0	NA* (no samples sequenced)
HC	0.375 µg	12/15	SUB	30	30	100.0% (88.4%, 100.0%)
HC	0.5 µg	1/15	SUB	166	166	100.0% (97.8%, 100.0%)
HC	2.0 µg	0/15	SUB	192	192	100.0% (98.1%, 100.0%)
HC	2.5 µg	0/15	SUB	192	192	100.0% (98.1%, 100.0%)
HC	3.0 µg	0/15	SUB	192	192	100.0% (98.1%, 100.0%)
Seq	1.4 nM	0/15	SUB	192	192	100.0% (98.1%, 100.0%)
Seq	1.575 nM	1/15	SUB	180	180	100.0% (98.0%, 100.0%)
Seq	1.75 nM	1/15	SUB	184	184	100.0% (98.0%, 100.0%)
Seq	1.925 nM	0/15	SUB	192	192	100.0% (98.1%, 100.0%)
Seq	2.1 nM	0/15	SUB	192	192	100.0% (98.1%, 100.0%)

* All samples failed at the input level of 0.25 µg and as a result, there is no data available to present for that level.

Table 23-2. Summary of the success rate per process and per input level, and concordance of insertions and deletions (INDEL) among successful replicates.

Process	Input Level	# of sample failures	Variant Type	# of concordant successes	# of variant comparisons	Success Rate (95% CI) (Number of Concordant comparisons)
LC	25 ng	1/15	INDEL	17	17	100.0% (80.5%, 100.0%)
LC	40 ng	0/15	INDEL	18	18	100.0% (81.5%, 100.0%)
LC	50 ng	0/15	INDEL	18	18	100.0% (81.5%, 100.0%)
LC	1000ng	0/15	INDEL	18	18	100.0% (81.5%, 100.0%)
LC	1200 ng	0/15	INDEL	18	18	100.0% (81.5%, 100.0%)
LC	1500 ng	0/15	INDEL	18	18	100.0% (81.5%, 100.0%)
HC	0.25 µg	15/15	INDEL	0	0	NA* (no samples sequenced)
HC	0.375 µg	12/15	INDEL	4	4	100.0% (39.8%, 100.0%)
HC	0.5 µg	1/15	INDEL	18	18	100.0% (81.5%, 100.0%)
HC	2.0 µg	0/15	INDEL	18	18	100.0% (81.5%, 100.0%)
HC	2.5 µg	0/15	INDEL	18	18	100.0% (81.5%, 100.0%)
HC	3.0 µg	0/15	INDEL	18	18	100.0% (81.5%, 100.0%)
Seq	1.4 nM	0/15	INDEL	18	18	100.0% (81. 5%, 100.0%)
Seq	1.575 nM	1/15	INDEL	16	16	100.0% (79.4%, 100.0%)
Seq	1.75 nM	1/15	INDEL	17	17	100.0% (80.5%, 100.0%)
Seq	1.925 nM	0/15	INDEL	18	18	100.0% (81.5%, 100.0%)
Seq	2.1 nM	0/15	INDEL	18	18	100.0% (81.5%, 100.0%)

* All samples failed at the input level of 0.25 µg and as a result, there is no data available to present for that level.

Table 23-3. Summary of the success rate per process and per input level, and concordance of rearrangements (RE) among successful replicates.

Process	Input Level	# of sample failures	Variant Type	# of concordant successes	# of variant comparisons	Success Rate (95% CI) (Number of Concordant comparisons)
LC	25 ng	1/15	RE	6	6	100.0% (54.1%, 100.0%)
LC	40 ng	0/15	RE	6	6	100.0% (54.1%, 100.0%)
LC	50 ng	0/15	RE	6	6	100.0% (54.1%, 100.0%)
LC	1000ng	0/15	RE	6	6	100.0% (54.1%, 100.0%)
LC	1200 ng	0/15	RE	6	6	100.0% (54.1%, 100.0%)
LC	1500 ng	0/15	RE	6	6	100.0% (54.1%, 100.0%)
HC	0.25 µg	15/15	RE	0	0	NA* (no samples sequenced)
HC	0.375 µg	12/15	RE	2	2	100.0% (15.8%, 100.0%)
HC	0.5 µg	1/15	RE	6	6	100.0% (54.1%, 100.0%)
HC	2.0 µg	0/15	RE	6	6	100.0% (54.1%, 100.0%)
HC	2.5 µg	0/15	RE	6	6	100.0% (54.1%, 100.0%)
HC	3.0 µg	0/15	RE	6	6	100.0% (54.1%, 100.0%)
Seq	1.4 nM	0/15	RE	8	9	88.9% (51.8%, 99.7%)
Seq	1.575 nM	1/15	RE	9	9	100.0% (66.4%, 100.0%)
Seq	1.75 nM	1/15	RE	8	8	100.0% (63.1%, 100.0%)

Process	Input Level	# of sample failures	Variant Type	# of concordant successes	# of variant comparisons	Success Rate (95% CI) (Number of Concordant comparisons)
Seq	1.925 nM	0/15	RE	8	9	88.9% (51.8%, 99.7%)
Seq	2.1 nM	0/15	RE	7	9	77.8% (40.0%, 97.2%)

* All samples failed at the input level of 0.25 µg and as a result, there is no data available to present for that level.

Table 23-4. Summary of the success rate per process and per input level, and concordance of copy number alterations (CN) among successful replicates.

Process	Input Level	# of sample failures	Variant Type	# of concordant successes	# of variant comparisons	Success Rate (95% CI) (Number of Concordant comparisons)
LC	25 ng	1/15	CN	128	128	100.0% (97.2%, 100.0%)
LC	40 ng	0/15	CN	132	132	100.0% (97.2%, 100.0%)
LC	50 ng	0/15	CN	132	132	100.0% (97.2%, 100.0%)
LC	1000ng	0/15	CN	132	132	100.0% (97.2%, 100.0%)
LC	1200 ng	0/15	CN	132	132	100.0% (97.2%, 100.0%)
LC	1500 ng	0/15	CN	132	132	100.0% (97.2%, 100.0%)
HC	0.25 µg	15/15	CN	0	0	NA* (no samples sequenced)
HC	0.375 µg	12/15	CN	13	14	92.9% (66.1%, 99.8%)
HC	0.5 µg	1/15	CN	107	108	99.0% (95.0 %, 100.0%)
HC	2.0 µg	0/15	CN	129	132	97.7% (93.5%, 99.5%)
HC	2.5 µg	0/15	CN	129	132	97.7% (93.5%, 99.5%)
HC	3.0 µg	0/15	CN	130	132	98.5% (94.6%, 99.8%)
Seq	1.4 nM	0/15	CN	131	132	99.2% (95.9%, 100.0%)
Seq	1.575 nM	1/15	CN	122	128	95.3% (90.1%, 98.3%)
Seq	1.75 nM	1/15	CN	128	128	100.0% (97.2%, 100.0%)
Seq	1.925 nM	0/15	CN	130	132	98.5% (94.6%, 99.8%)
Seq	2.1 nM	0/15	CN	131	132	99.2% (95.9%, 100.0%)

* All samples failed at the input level of 0.25 µg and as a result, there is no data available to present for that level.

3. Clinical Studies

Several CDx claims described in sections 3.1-3.7 and summarized in Section 3.8 were based on a non-inferiority (NI) statistical testing approach using the enrichment design presented in the paper by Li (2016)[1], when the concordance study sample is not a random sample from the companion diagnostic FoundationOne®CDx (F1CDx) intended use population and a reference standard is not available.

To assess clinical concordance, F1CDx was compared to an FDA-approved CDx (CCD). All studies based on NI passed the acceptance criteria specified in each study protocol. Clinical concordance studies, with the exception of *ALK* and *EGFR* T790M, used randomly selected samples from FMI's clinical archives (initially tested with FoundationOne) and were subject to pre-screening bias. Therefore, the concordance results may be over- or underestimated and the failure rate may be underestimated.

Additional CDx claims are described in sections 3.9-3.21 including:

- A concordance study between F1CDx and FoundationFocus CDx$_{BRCA\ LOH}$ was conducted for the reporting of *BRCA1*, *BRCA2* and loss of heterozygosity (LOH) in ovarian cancer patients.
- For the CDx indication to identify *PIK3CA* alterations in breast cancer patients intended to be treated with alpelisib, the effectiveness of the F1CDx assay was demonstrated through the clinical bridging

study using specimens from the patients screened for enrollment into the study CBYL719C2301 (SOLAR-1).

- For the CDx indication to identify *BRCA1* and *BRCA2* in ovarian patients intended to be treated with olaparib, the effectiveness was demonstrated using specimens from the patients screened for enrollment into study D0818C00001 (SOLO1).
- For the CDx indication to identify *FGFR2* fusions and select rearrangements in cholangiocarcinoma (CCA) patients to determine eligibility for treatment with pemigatinib, the effectiveness of F1CDx was demonstrated through a clinical bridging study using specimens from the patients screened for enrollment into the INCB 54828-202 (FIGHT-202) trial.
- For the indication to identify SNVs and indels that lead to *MET* exon 14 skipping in NSCLC patients to determine eligibility for treatment with capmatinib, the effectiveness of the F1CDx was demonstrated through a clinical bridging study using specimens from the patients screened for enrollment into the CINC280A2201 (GEOMETRY-mono 1) trial.
- For the CDx indication to identify mutations in homologous recombination repair (HRR) genes in metastatic castration-resistant prostate cancer (mCRPC) patients to determine eligibility for treatment with olaparib, the effectiveness of the F1CDx assay was demonstrated based on the results from the PROfound trial.
- For the CDx indication to identify solid cancer patients with TMB-H (defined as $\geq$ 10 mutations per megabase) tumors to determine eligibility for treatment with pembrolizumab, the effectiveness of the F1CDx assay was demonstrated through a prospectively-planned retrospective analysis of clinical specimens from the patients enrolled in the KEYNOTE-158 clinical trial.
- For the CDx indication to identify *NTRK1, NTRK2,* or *NTRK3* fusions in patients with solid tumors that are intended to be treated with larotrectinib, the effectiveness of the F1CDx assay was demonstrated through the clinical bridging study using specimens from patients enrolled in the LOXO-TRK-14001 (Bayer 20288, NCT02122913), -15002 (Bayer 20289, NAVIGATE, NCT02576431), and -15003 (Bayer 20290, SCOUT, NCT02637687) clinical trials.
- For the CDx indication to identify *FGFR2* fusions and select rearrangements in patients with cholangiocarcinoma (CCA) to determine eligibility for treatment with infigratinib, the effectiveness of F1CDx was demonstrated through a clinical bridging study using specimens from the patients screened for enrollment into the CBGJ398X2204 trial.
- For the CDx indication to identify *NTRK1, NTRK2,* or *NTRK3* fusions in patients with solid tumors that are intended to be treated with entrectinib, the effectiveness of the F1CDx assay was demonstrated through the clinical bridging study using specimens from patients enrolled in the ALKA-372-001 (ALKA), RXDX-101-01 (STARTRK-1), and RXDX-101-02 (STARTRK-2) clinical trials.
- For the CDx indication to identify *ROS1* fusions in patients with NSCLC that are intended to be treated with entrectinib, the effectiveness of the F1CDx assay was demonstrated through the clinical bridging study using specimens from patients enrolled in the ALKA-372-001 (ALKA), RXDX-101-01 (STARTRK-1), and RXDX-101-02 (STARTRK-2) clinical trials.

3.1 FoundationOne®CDx Concordance Study for *EGFR* Exon19del/L858R

Clinical validity of FoundationOne®CDx (F1CDx) as a companion diagnostic used for identifying patients with advanced NSCLC who may be eligible for treatment with Gilotrif® (afatinib), Iressa® (gefitinib), or Tarceva® (erlotinib) was established by retrospectively testing 282 samples from NSCLC patients. The *EGFR* diagnostic results from the F1CDx assay were compared against those obtained from the approved **cobas**® *EGFR* Mutation Test v2 (Roche Molecular Systems, referred to as **cobas**® EGFR v2 below). Samples were tested using **cobas**® EGFR v2 (CCD1) with an approximately equal number of mutation positive and negative samples, followed by testing with F1CDx and a second, replicate testing of **cobas**® EGFR v2 (CCD2). NSCLC tumor samples used for this study were not obtained from a clinical trial and had limited demographic data available. For this study age and gender data were available and were found to be similar to the pivotal study EURTAC.

Two separate concordance analyses were performed: one with samples with complete records only (N = 267), and the other with all the 282 samples, where missing data were handled by multiple imputation. Data from concordance testing are summarized in Table 24 below.

Table 24. Concordance Table with CCD1, CCD2 and F1CDx results with eligible samples.

	CCD1+				CCD1-			
	CCD2+	CCD2-	CCD2 missing	Total	CCD2+	CCD2-	CCD2 missing	Total
F1CDx+	106	0	0	106	1	1*	0	2
F1CDx-	2**	1	0	3	3	153	0	156
F1CDx Missing	3	0	0	3	1	9	2	12
Total	111	1	0	112	5	163	2	170

***** QRF006212 was the only sample where both replicates of the **cobas**® EGFR v2 assay reported negative results but F1CDx reported positive for L858R with AF 33%. Upon further review, F1CDx identified a second somatic mutation in-cis (on same allele) as that of L858R with identical AF only 17bp downstream: *EGFR* A864P. Therefore, it is suspected that this second mutation interfered with the allele-specific PCR primers of **cobas**® EGFR v2, and thus L858R went undetected.
****** QRF005867 was reported as positive for both replicates of **cobas**® EGFR v2 for exon19 deletion, but negative by F1CDx. F1CDx detected the exon19 deletion, but incorrectly annotated the variant as 2 frameshift mutations. This would have been corrected by manual curation review, which was not part of this concordance study. QRF005883 was also reported as positive for both replicates of **cobas**® EGFR v2 for exon19 deletion, but negative by F1CDx. F1CDx identified an 18bp exon 19 insertion event, with protein effect K745_E746insIPVAIK. As **cobas**® EGFR v2 is not designed to detect insertion events at exon 19, it is likely an error by **cobas**® EGFR v2.

Fifteen (15) samples were assigned as missing data for F1CDx, two of which also had missing results for CCD2. Missing data was caused by process failures or samples not meeting assay specifications.

By defining the reference standard as the consensus calls between CCD1 and CCD2, F1CDx achieved a PPA of 98.1% (106/108) (95% CI [93.5%, 99.8%]) and NPA of 99.4% (153/154) (95% CI [96.4%, 100.0%]). These data are summarized in Table 25.

Table 25. Summary of concordance data using agreement between CCD1 and CCD2 as the reference.

	CCD1+/CCD2+	CCD1-/CCD2-
F1CDX+	106	1
F1CDX-	2	153

The mutations detected by the **cobas**® EGFR v2 include all the mutations detected by *therascreen*® EGFR RGQ PCR Kit (QIAGEN), as well as a few additional exon19 deletions/L858R variants. Several concordance studies comparing the **cobas**® EGFR v2 and *therascreen*® EGFR RGQ PCR Kit have been reported in the literature[7,8,9], supporting that these two assays are concordant.

Additionally, a post-market concordance study will be completed comparing F1CDx to the *therascreen*® EGFR RGQ PCR Kit.

In addition, based on results of the FLAURA (NCT02296125) study, an additional therapeutic product, osimertinib, was approved on April 18, 2018, for the first-line treatment of patients with metastatic NSCLC whose tumors have EGFR exon 19 deletions or exon 21 L858R mutations, as detected by an FDA approved test. The companion diagnostic for this indication included the **cobas**® EGFR Mutation Test v2 (Roche Molecular Systems) whose claims were expanded, to include Tagrisso® (osimertinib) for the same *EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations as approved in the F1CDx PMA (P170019) on November 30, 2017. Consequently, Tagrisso® (osimertinib) was added to the F1CDx label for *EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations in NSCLC patients.

3.2 FoundationOne®CDx Concordance Study for *EGFR* T790M

The study established the clinical validity of the FoundationOne®CDx (F1CDx) as a companion diagnostic device used for identifying NSCLC patients harboring *EGFR* T790M that may be eligible for treatment with Tagrisso® (osimertinib). The patient samples and corresponding demographic information were obtained from AstraZeneca in connection with the clinical studies entitled AURA (NCT01802632), AURA2 (NCT02094261) and AURA3 (NCT02151981). The *EGFR* T790M diagnostic results from the F1CDx assay were compared against the consensus calls between the original T790M testing used in the AURA, AURA2 and AURA3 studies and a separate run of the FDA approved **cobas**® EGFR v2 (Roche Molecular Systems; designated as comparator companion diagnostic, CCD), using an NI approach.

Two separate concordance analyses were performed: one included samples with complete records only (N = 227), and the second analysis was with all the 312 samples, where missing data was handled by multiple imputation. A summary of concordance is presented in Table 26.

Table 26. Concordance table with CCD1, CCD2 and F1CDX results with eligible samples.

	CCD1+				CCD1-			
	CCD2+	**CCD2-**	**CCD2 missing**	**Total**	**CCD2+**	**CCD2-**	**CCD2 missing**	**Total**
F1CDx+	87	19	1	107	8	15	0	23
F1CDx-	1	4	0	5	0	93	2	95
F1CDx Missing	21	4	8	33	1	37	11	49
Total	109	27	9	145	9	145	13	167

Eighty-two samples were assigned as missing data for F1CDx, which consisted of 78 samples with no sequencing results from F1CDx and four samples with QC status as “Fail” after curation. CCD2 had 22 samples with missing data in total, in which 19 samples also had missing values in F1CDx.

The concordance analysis above shows that for the results of PPA, F1CDx is more concordant with both CCD1 and CCD2 than CCD1 is with CCD2; the opposite is true for NPA results. See the Venn Diagram below for the T790M-positive calls (Figure 2).

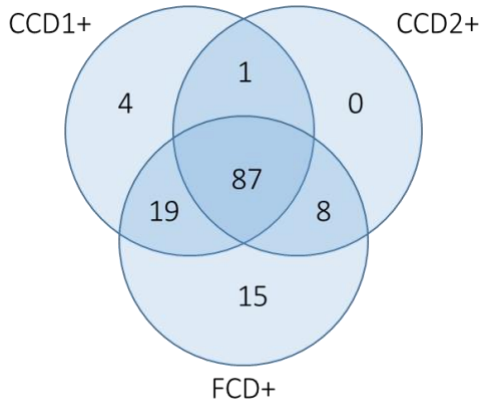


Figure 2. Venn Diagram for *EGFR* T790M-positive samples.

A difference in detection sensitivity between CCD1 and CCD2 was observed, with CCD1 appearing to be more sensitive than CCD2. This could be attributed to the fact that CCD1 was run 2-3 years ago using freshly biopsied tissue, while CCD2 testing was recently performed using DNA extracted from archival FFPE sections. Figure 3 below illustrates the relationship between allele frequency and detection by F1CDx, CCD1 and CCD2. The results demonstrated that F1CDx detects mutations at allele frequency lower than 5% which are not detected by the **cobas**® v2 assay. The clinical performance in this subset of the patient population (patients with an *EGFR* T790M mutation detected with an allele fraction <5%) and has not been established.

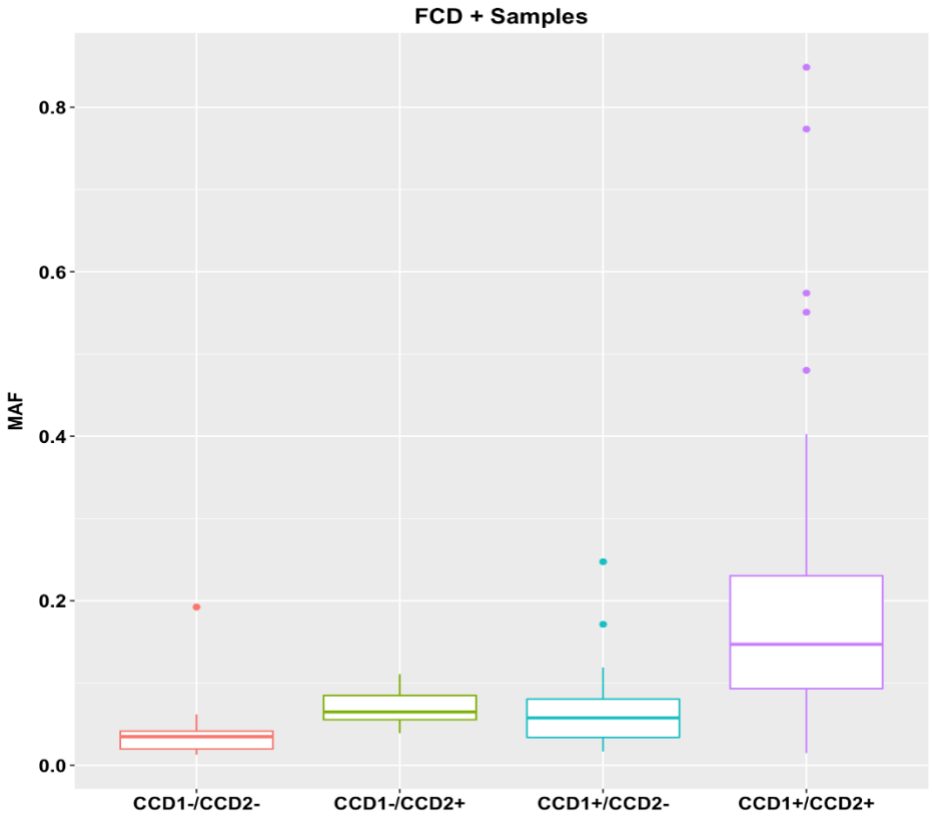


Figure 3. Distribution of MAF in F1CDx+ (FCD) samples.

By defining the reference standard as the consensus calls between CCD1 and CCD2, F1CDx achieved a PPA of 98.9% (87/88) (95% CI [93.8%, 100.0%]) and NPA of 86.1% (93/108) (95% CI [78.1%, 92.0%]) as summarized in Table 27 below.

Table 27. Summary of concordance data using agreement between CCD1 and CCD2 as the reference.

	CCD1+/CCD2+	CCD1-/CCD2-
F1CDx+	87	15
F1CDx-	1	93

3.3 FoundationOne®CDx Concordance Study for *ERBB2* (HER2)

Clinical validity of FoundationOne®CDx (F1CDx) as a companion diagnostic device used to identify patients eligible for treatment with approved HER2-directed therapies including Herceptin® (trastuzumab), Kadcyla® (ado-trastuzumab-emtansine), and Perjeta® (pertuzumab) was established. A study was performed using 317 pre-screened retrospective samples obtained from patients with advanced breast cancer. The failure rate for pre-screening is not known; however, the sample set is enriched for samples with HER2+ samples with ratio between 2 and 3 representing 27% of samples compared to the expected range of 8-10% reported in literature[10,11]. The *ERBB2* amplification positive results from the F1CDx assay were compared against those obtained from the approved HER2 FISH PharmDx® Kit (Dako Denmark A/S). The samples used for this study were not obtained from a clinical trial and had limited demographic data available. For this study age and ethnicity data were available. Age data were compared to the Danish Study for the Danish Breast Cancer Group clinical trial 89-D in 1990 and was found to have a similar distribution, though the mean age was higher for the concordance samples.

Concordance data are summarized in Table 28 below.

Table 28. Concordance Table with CCD1, CCD2 and F1CDx results with eligible samples.

	CCD1+			CCD1-		
	CCD2+	CCD2-	Total	CCD2+	CCD2-	Total
F1CDx+	101	2	103	3	3	6
F1CDx-	12	10	22	6	180	186
Total	113	12	125	9	183	192

The prevalence of the *ERBB2*/HER2 amplification mutation in the intended use (IU) population is based on the ASCO guideline and is estimated to be 17.5%. To assess the impact of prevalence for the main results of this study, a sensitivity analysis was performed using the lower and upper bound of the prevalence guideline of 15% and 20%. The sensitivity analysis also showed that there was no impact on the study conclusion. The distribution of age is similar to the IU population for all samples tested. However, there was missing demographic data from the sample population. For missing data analysis using multiple imputation, the results show that based on the missing at random (MAR) assumption, the invalid test results did not affect the conclusion of this study.

The Venn diagrams for samples tested positive or negative for *ERBB2*/HER2-amplification mutation in all three assays (F1CDx, CCD1 and CCD2) are presented in Figure 4.

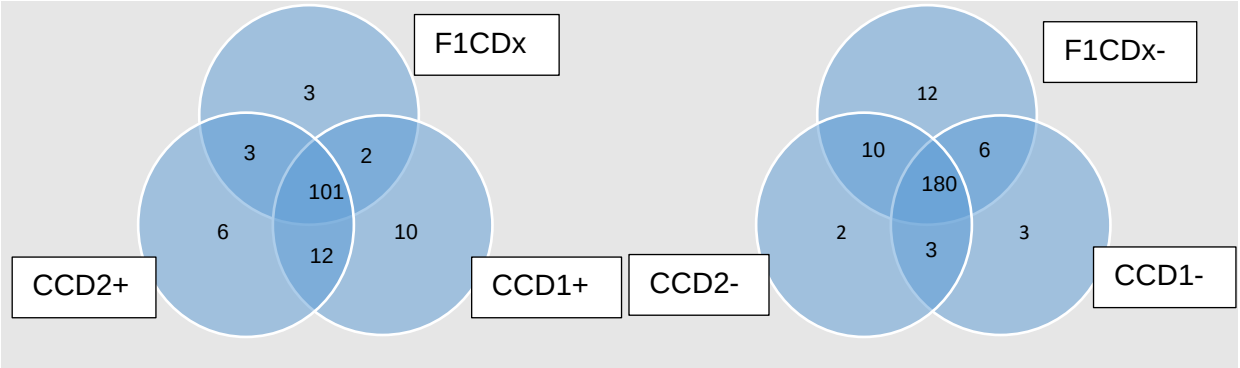


Figure 4. Venn Diagrams for *ERBB2*-amplification positive (left panel) and negative (right panel) samples.

These two Venn diagrams illustrate concordance among F1CDx, CCD1 and CCD2. For the F1CDx+ samples, concordance of F1CDx with CCD1 or CCD2 was better than concordance between the same platform tests CCD1 and CCD2; for the F1CDx- samples, F1CDx was more consistent in calling negative alterations than either CCD1 or CCD2.

Using the consensus calls between CCD1 and CCD2 as the reference standard, i.e., limiting analysis to only the samples in which CCD1 and CCD2 are in agreement, the results are shown below:

Table 29. Summary of concordance data using agreement between CCD1 and CCD2 as the reference.

	CCD1+/CCD2+	CCD1-/CCD2-
F1CDx+	101	3
F1CDx-	12	180

Based on these results, PPA is 89.4% (101/113) (95% CI [82.2%, 94.4%]) and NPA is 98.4% (180/183) (95% CI [95.3%, 99.7%]).

3.4 FoundationOne®CDx Concordance Study for *ALK*

Clinical validity of FoundationOne®CDx (F1CDx) as a companion diagnostic device used to identify non-small cell lung cancer (NSCLC) patients eligible for treatment with approved *ALK*-directed therapies including Alecensa® (alectinib), X*ALK*ori® (crizotinib), or Zykadia® (ceritinib) was established. The study was performed using 175 tumor samples from patients with histologically-confirmed NSCLC including enrolled patients as well as screen failures from the clinical trial NCT02075840, Roche study number BO28984 (also known as the ALEX study), which is a randomized, active controlled, multicenter Phase III open-label study designed to evaluate the efficacy and safety of alectinib compared with crizotinib treatment in participants with treatment-naïve *ALK* rearrangement positive advanced NSCLC. The *ALK* diagnostic results from the F1CDx panel were compared against those obtained from the FDA approved Ventana *ALK* (D5F3) CDx Assay ("Ventana IHC", Ventana Medical Systems, Inc.) and Vysis *ALK* Break-Apart FISH Probe Kit ("Vysis FISH", Abbott Molecular). The Vysis FISH assay results used were obtained from the ALEX study. In this concordance study, the majority of the samples were from the IU population of the clinical trial NCT02075840. The concordance results are summarized in Table 30 below.

Table 30. Concordance table with CCD1, CCD2 and F1CDx results with eligible samples.

	CCD1 +			CCD1 -		
	CCD2 +	**CCD2 -**	**Total**	**CCD2 +**	**CCD2 -**	**Total**
F1CDx +	78	1	79	3	0	3
F1CDx -	6*	7	13	5	75	80
Total	84	8	92	8	75	83

*Two samples harbored *ALK* rearrangements that were detected by F1CDx but were classified as negative based on the study protocol.

The Venn diagrams for samples tested positive or negative for *ALK*-rearrangement mutation in all three assays (F1CDx, CCD1 and CCD2) are shown in Figure 5.

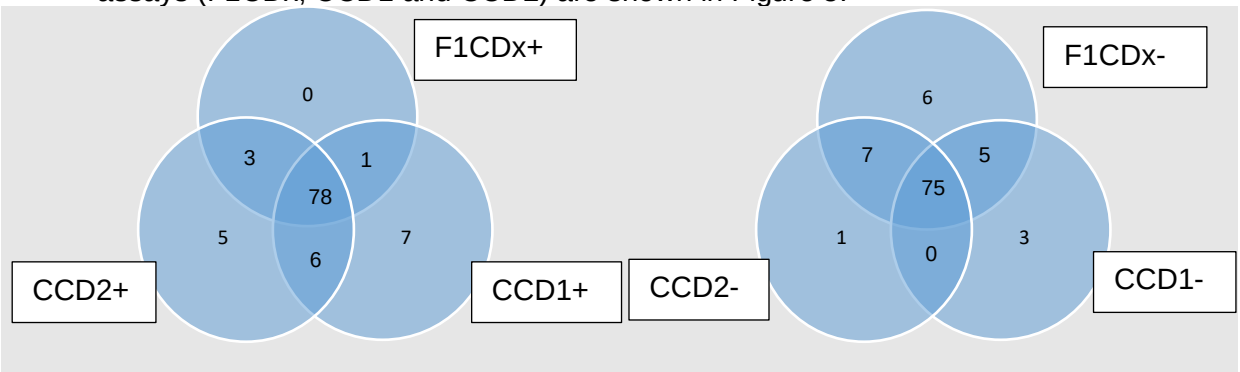


Figure 5. Venn Diagrams for *ALK*-rearrangement positive (left panel) and negative (right panel) samples.

These two Venn diagrams illustrate concordance among F1CDx, CCD1 and CCD2. A number of samples with discordant results between CCD1 and CCD2 were observed. This is expected because Vysis FISH Assay (CCD2) is a technology that probes at the DNA level while Ventana *ALK* IHC assay examines protein expression. When samples that were discordant between CCD1 and CCD2 were excluded, the concordance between F1CDx+ with CCD1+ and CCD2+ samples was superior to concordance between CCD1+ and CCD2+ samples. For the F1CDx- samples, F1CDx was more consistent in calling negative alterations than either CCD1 or CCD2.

Using the consensus calls between CCD1 and CCD2 as the reference standard, i.e. limiting analysis to only the samples in which CCD1 and CCD2 are in agreement, the results are shown below:

Table 31. Summary of concordance data using agreement between CCD1 and CCD2 as the reference.

	CCD1+/CCD2+	**CCD1-/CCD2-**

F1CDx+	78	0
F1CDx-	6*	75

*Two samples harbored *ALK* rearrangements that were detected by F1CDx but were classified as negative based on the study protocol.

Based on these results, PPA is 92.9% (78/84) (95% CI [85.1%, 97.3%]) and NPA is 100% (75/75) (95% CI [95.2%, 100.0%]).

In addition, based on results of the ALTA-1L (NCT02737501) study, an additional therapeutic product, Alunbrig (brigatinib), was approved on May 22, 2020 for the treatment of patients with metastatic NSCLC whose tumors harbor *ALK* rearrangements, as detected by an FDA approved test. The companion diagnostic for this indication included Abbott Molecular's Vysis ALK Break Apart FISH Probe Kit whose claims were expanded to include Alunbrig (brigatinib) for the same *ALK* rearrangements, as approved in the F1CDx PMA (P170019) on November 30, 2017. Subsequently, Alunbrig (brigatinib) was added on the F1CDx label for *ALK* rearrangements in NSCLC patients.

3.5 FoundationOne®CDx Concordance Study for *KRAS*

Clinical validity of FoundationOne®CDx (F1CDx) as a companion diagnostic device used to identify colorectal cancer patients that may not benefit from certain *EGFR* inhibitor treatments, including Erbitux® (cetuximab) or Vectibix® (panitumumab), due to alterations in *KRAS* was established. The study was performed using 342 retrospective samples obtained from patients with advanced front-line or later-line colorectal cancer (CRC). Samples used in this study underwent pre-screening using the FoundationOne laboratory developed test (F1 LDT) or prescreening by an external vendor to enrich for positive samples. The prescreen failure rate using the F1 LDT was 3.7% and is unknown for the external vendor. The *KRAS* diagnostic results from the F1CDx assay were compared against those obtained from the approved *therascreen*® KRAS RGQ PCR Kit (QIAGEN). The samples used for this study were not obtained from a clinical trial and had limited demographic data available. For this study age, gender and ethnicity data were available. Age and gender characteristics were found to be similar between the F1CDx concordance study and the pivotal studies, with the percentage of male samples in the concordance study being slightly lower compared to the pivotal studies (CRYSTAL and PRIME). Concordance data are summarized in Table 32 below.

Table 32. Concordance Table with CCD1, CCD2 and F1CDx results with eligible samples.

	CCD1+				**CCD1-**			
	CCD2+	**CCD2-**	**CCD2 missing**	**Total**	**CCD2+**	**CCD2-**	**CCD2 missing**	**Total**
F1CDx+	173	0	2	175	0	0	0	0
F1CDx-	0	2	0	2	1	154	7	162
F1CDx Missing	0	0	0	0	0	3	0	3
Total	173	2	2	177	1	157	7	165

Twelve (12) samples are assigned as missing data, including 3 samples with missing data in F1CDx and 9 samples with missing data in CCD2.

The prevalence of the *KRAS* mutation in the IU population is based on the CRYSTAL study for cetuximab (35.6%) and PRIME study for panitumumab (40%). The key statistics of PPA and NPA between F1CDx and the two replicates of the *therascreen*® KRAS assay (CCD1 and CCD2) were estimated based on the result in Table 33. Multiple imputation was used to impute the missing data and showed that missing data did not impact study conclusions. The summary statistics of age and sex were highly similar to the estimates from the pivotal trial CRYSTAL (for cetuximab) and PRIME (for panitumumab) studies.

By defining the reference standard as the consensus calls between CCD1 and CCD2, F1CDx achieved a PPA of 100% (173/173) (95% CI [97.9%, 100.0%]) and NPA of 100% (154/154) (95% CI [97.6%, 100.0%]).

Table 33. Summary of concordance data using agreement between CCD1 and CCD2 as the reference.

	CCD1+/CCD2+	CCD1-/CCD2-
F1CDx+	173	0
F1CDx-	0	154

3.6 FoundationOne®CDx Concordance Study for *BRAF*

Clinical validity of the FoundationOne®CDx (F1CDx) as a companion diagnostic device used to identify melanoma patients that may be eligible for treatment with approved *BRAF*-directed therapies was established. The study was performed using 305 retrospective samples obtained from patients with advanced melanoma. 157 samples used in this study underwent pre-screening using the FoundationOne laboratory developed test (F1 LDT) and 27 were prescreened by an external vendor to enrich for positive samples. The prescreen failure rate using the F1 LDT was 3.7% and is unknown for the external vendor. The *BRAF* diagnostic results from the F1CDx assay were compared against those obtained from the approved **cobas**® 4800 BRAF V600 Mutation Test (Roche Molecular Systems, Inc; referred to as the **cobas**® BRAF assay below). These samples were not obtained from a clinical trial and had demographic data limited to age and gender. The distributions of age and gender to the intended use population (BRIM-3 trial) was found to be comparable.

Concordance analysis showed that the upper bounds of 95% one-sided Confidence Interval (CI) were below 20% for all four NI hypothesis tests. Thus, it can be concluded with 95% confidence that the differences of results between F1CDx and **cobas**® BRAF assays are less than 20%, the non-inferiority (NI) margin. Concordance results are summarized in Table 34 below.

Table 34. Concordance Table with CCD1, CCD2 and F1CDx results with eligible samples.

	CCD1+			CCD1-		
	CCD2+	**CCD2-**	**Total**	**CCD2+**	**CCD2-**	**Total**
F1CDx+	166	0	166	3	14	17
F1CDx-	1	0	1	0	121	121
Total	167	0	167	3	135	138

Because the **cobas**® BRAF assay has lower sensitivity for detection of dinucleotide mutations, a separate analysis was conducted that included only eligible samples without dinucleotide mutations. A total of 273 (=305-32) samples were available for this analysis. The concordance results are summarized in Table 35.

Table 35. Concordance Table with CCD1, CCD2 and F1CDx results with eligible samples excluding samples with dinucleotide mutations detected by F1CDx.

	CCD1+			CCD1-		
	CCD2+	**CCD2-**	**Total**	**CCD2+**	**CCD2-**	**Total**
F1CDx+	149	0	149	1	1*	2
F1CDx-	1**	0	1	0	121	121
Total	150	0	150	1	122	123

*QRF006472 was the only sample where both replicates of the **cobas**® BRAF assay reported negative results but F1CDx reported positive. The Allele Frequency of this sample was 3.45% with the computational tumor purity of 10%. According to Table 4 of the **cobas**® BRAF assay insert, the **cobas**® BRAF assay can correctly detect all *BRAF* V600E mutant specimens that have a minimum % mutant DNA above 5% and when the minimum tumor content is at least 15%. Thus, the discordance can be explained by F1CDx's high sensitivity in the lower % mutant DNA and low tumor purity condition.

QRF006374 was the only sample where both replicates of the **cobas® BRAF assay reported positive results but F1CDx reported negative. A mutation was recorded in the line data (Appendix 7) having protein effect V600_K601>E,

which is a non-frameshift deletion of 3 nucleotides with CDS effect 1799_1801delTGA. This more complex mutation does result in V600E, but because of annotation differences to the canonical V600E, it was called negative by F1CDx.

PPA and NPA were calculated by defining the reference standard as the consensus calls between CCD1 and CCD2. The observed performance of **cobas**® BRAF assay has lower sensitivity for detection of dinucleotide V600 alterations (including V600K) than the single nucleotide V600E 1799T>A alteration, particularly at allele frequency below 40% detected by F1CDx, therefore, the data presented will include PPA/NPA results both with both alterations as the study was designed, as well as for V600E only in Table 36. A study using the THxID™ *BRAF* kit (bioMérieux) was conducted using 29 samples with *BRAF* V600 dinucleotide mutation detected by F1CDx and 29 negative samples to provide a better evaluation of V600 dinucleotide concordance. Out of the 51 samples with valid results from the THxID™ *BRAF* kit (Table 36), there was only one discordant result (F1CDx-/THxID+), achieving a PPA of 96.3% (26/27) (95% CI [81.0%, 99.9%]) and NPA of 100% (24/24) (95% CI [85.8%, 100.0%]).

Table 36. PPA and NPA for *BRAF* V600 detection with cobas® BRAF.

	PPA	NPA
All V600 alterations	99.4% (166/167)	89.6% (121/135)
Single nucleotide V600E (1799T>A)	99.3% (149/150)	99.2% (121/122)

Table 37. Concordance of *BRAF* dinucleotide samples with THxID™ *BRAF* kit.

Dinucleotide Samples	THxID+	THxID-	Total
F1CDx+	26	0	26
F1CDx-	1	24	25
Total	27	24	51

To support the expansion of F1CDx to identify patients with rare *BRAF* V600 mutation-positive unresectable or metastatic melanoma, who may benefit from treatment with atezolizumab (Tecentriq®) in combination with cobimetinib and vemurafenib, the accuracy of detection of the rare V600 mutations in the clinical trial was evaluated. A total of 7 *BRAF* V600D or V600R subjects were identified in the IMspire150 clinical trial through central confirmatory testing at FMI, including 5 specimens with V600R and 2 with V600D mutations. Limited data was available to confirm the V600D results. Additional real-world data was provided from melanoma samples with rare variants. While the ability to distinguish between rare V600 mutations could not be completely resolved given the lack of available clinical trial specimens, the data support the detection capability of rare *BRAF* V600 alterations by F1CDx.

3.7 FoundationOne®CDx Concordance Study for *KRAS/NRAS*

A post-market commitment for F1CDx was performed to provide additional evidence to support the clinical validity of F1CDx in identifying colorectal cancer (CRC) patients eligible for treatment with Vectibix® (panitumumab) as follow-on companion diagnostic (FCD) device, by demonstrating concordance between F1CDx and the Praxis Extended RAS Panel (Praxis test) in detecting *KRAS* and *NRAS* mutations. The study was conducted via retrospective analysis of clinical samples obtained from CRC patients. This study included 99 KRAS/NRAS mutation-positive and 96 KRAS/NRAS with valid test results mutation-negative (WT) CRC samples identified by the Praxis test. The samples used for testing in this study were external to the samples used in the original pivotal study for the FDA approval of the Praxis test (as shown in Table 39).

Patient samples were intended to reflect the intended use (IU) population of CRC patients eligible for treatment with panitumumab. Gender information was used for demographic analysis. The distribution of

the gender characteristics was similar to the panitumumab pivotal trial population. The similarity of the gender distribution demonstrated that the selected test samples were representative of the IU population. In addition, comparisons of patient and disease-related characteristics were made between the samples used in the study and the IU population used for the Praxis test approval in the *RAS* mutation-negative (wild-type) CRC patients, to ensure the screened population was representative of the IU population.

CRC samples were screened using the Praxis test (CCD1) with an approximately equal number of *KRAS/NRAS* mutation positive and negative (wild-type (WT)) samples, followed by testing with F1CDx (FCD) and a second, replicate testing of the Praxis test (CCD2). After testing was complete (CCD1, CCD2, and FCD), the variant calls were evaluated based on the agreement between both the F1CDx and the Praxis test results and the agreement between the two replicates of the Praxis test results. Concordance analysis results are summarized in Table 38 below.

Table 38. Concordance Table with CCD1, CCD2 and F1CDx results with eligible samples.

	CCD1+				CCD1-			
	CCD2+	CCD2-	Invalid CCD2	Total	CCD2+	CCD2-	Invalid CCD2	Total
FCD+	99	0	3	102	0	1	0	1
FCD-	0	0	0	0	0	95	1	96
Invalid FCD	2	0	0	2	0	21	0	21
Total	101	0	3	104	0	117	1	118

Thirty-five (35) samples are assigned as invalid data, including 23 samples with missing data in FCD, 8 samples with missing data in CCD1 and 4 samples with missing data in CCD2.

The key statistics of PPA and NPA between F1CDx and the two replicates of the Praxis test (CCD1 and CCD2) were estimated based on the result in Table 39. Multiple imputation was used to impute the missing data and showed that missing data did not impact study conclusions. F1CDx achieved a PPA of 100% (99/99) (95% CI [96.26%, 100%]) and NPA of 98.96% (95/96) (95% CI [96.88%, 100%]).

Table 39. Summary of concordance data using agreement between CCD1 and CCD2 as the reference.

	CCD1+/CCD2+	CCD1-/CCD2-
F1CDx+	99	1
F1CDx-	0	95

The data demonstrated that the concordance between F1CDx (as the FCD) and Praxis (as CCD1/CCD2) was non-inferior to the concordance between Praxis replicates CCD1 and CCD2. The upper bounds of the corresponding one-sided 95% CI met the acceptance criteria with a NI margin less than 10%.

3.8 Summary of Clinical Concordance Studies

A summary of clinical concordance study results is included in Table 40 below. The reference standard used to calculate positive percent agreement (PPA) and negative percent agreement (NPA) below is

defined as the consensus calls between the two comparator methods or comparator runs. Agreement calculations solely using consensus calls may overestimate the performance of FoundationOne®CDx (F1CDx).

Table 40. Summary of PPA and NPA for CDx concordance studies.

Biomarker	PPA	NPA	Comparator Method
EGFR exon 19 deletions and L858R	98.1% (106/108)	99.4% (153/154)	**cobas**® EGFR Mutation Test v2
EGFR T790M	98.9% (87/88)	86.1% (93/108)	**cobas**® EGFR Mutation Test v1 **cobas**® EGFR Mutation Test v2
ALK rearrangements	92.9% (78/84)	100% (75/75)	Ventana *ALK* (D5F3) CDx Assay Vysis *ALK* Break-Apart FISH Probe Kit
KRAS	100% (173/173)	100% (154/154)	*therascreen*® KRAS RGQ PCR Kit
KRAS and NRAS	100% (99/99)	98.96% (95/96)	Praxis Extended RAS Panel
ERBB2(HER2) Amplifications	89.4% (101/113)	98.4% (180/183)	Dako HER2 FISH PharmDx® Kit
BRAF V600 *BRAF* V600E *BRAF* V600 dinucleotide[2]	99.4% (166/167) 99.3% (149/150) 96.3% (26/27)	89.6% (121/135)[1] 99.2% (121/122) 100% (24/24)	**cobas**® 4800 BRAF V600 Mutation Test **cobas**® 4800 BRAF V600 Mutation Test THxID™ *BRAF* kit

[1] Sensitivity of dinucleotide detection of *BRAF* V600K and V600E was found to be significantly reduced in **cobas**® BRAF test, in particular for samples in which F1CDx detected the dinucleotides to be of lower than 40% MAF, leading to low NPA values.

[2] A study using the THxIDTM BRAF kit (bioMérieux) was conducted with samples with BRAF V600 dinucleotide mutation detected by F1CDx and BRAF V600 negative samples to provide a better evaluation of V600 dinucleotide concordance.

3.9 FoundationOne®CDx Concordance with FoundationFocus CDx$_{BRCA\ LOH}$ for *BRCA1*, *BRCA2*, and LOH calling

FoundationOne®CDx (F1CDx) and FoundationFocus CDx$_{BRCA\ LOH}$ assays are equivalent with the exception of an updated analysis pipeline in use for F1CDx and reporting software that allow for comprehensive reporting of all relevant alterations detected by the F1CDx platform. Comprehensive validation of the analysis pipeline which included robust regression testing and reanalysis of FoundationFocus CDx $_{BRCA\ LOH}$ clinical bridging sample data was performed. The assays were determined to be concordant for determining HRD status. Reanalysis of the clinical efficacy data demonstrated that F1CDx and FFocus have similar performance in identifying HRD+ patients who may benefit from rucaparib treatment. Details for the clinical studies can be found in the Summary of Safety and Effectiveness Data for PMA P160018 and P160018/S001. A summary of progression-free survival assessed by the investigator using F1CDx is provided in Table 41 below.

Table 41. Progression-free survival assessed by the investigator (invPFS) using F1CDx.

Cohort	Hazard Ratio Rucaparib vs Placebo	Number of Patients	Median invPFS (months)		95% CI
ITT	0.365	375	10.8	Rucaparib	8.3, 11.4
	P value: <.0001				
	95% CI: 0.295, 0.451	189	5.4	Placebo	5.3, 5.5
All populations assessable by FMI assays	0.377	345	10.4	Rucaparib	8.3, 11.1
	P value: <.0001				
	95% CI: 0.302, 0.469	173	5.4	Placebo	5.3, 5.5
HRD+	0.302	215	13.6	Rucaparib	10.9, 17.1
	P value: <.0001				
	95% CI: 0.224, 0.406	110	5.4	Placebo	5.1, 5.6
tBRCA+	0.240	124	16.6	Rucaparib	11.1, 22.9
	P value: <.0001				
	95% CI: 0.159, 0.364	63	5.4	Placebo	4.9, 7.1
tBRCA- LOH+	0.354	91	9.7	Rucaparib	8.2, 13.8
	P value: <.0001				
	95% CI: 0.226, 0.554	47	5.4	Placebo	2.9, 5.6

Cohort	Hazard Ratio Rucaparib vs Placebo	Number of Patients	Median invPFS (months)		95% CI
tBRCA-LOH unknown	0.176	16	8.3	Rucaparib	5.3, 24.7
	P value=0.0069				
	95% CI: 0.044, 0.711	8	4.1	Placebo	2.3, 8.2
tBRCA-LOH-	0.620	114	6.3	Rucaparib	5.4, 8.3
	P value=0.0086				
	95% CI: 0.429, 0.895	55	5.4	Placebo	4.1, 5.6

3.10 Clinical evaluation of *BRCA1/2* classification for treating ovarian cancer patients with olaparib

3.10.1 Summary of the Clinical Study – Olaparib D0818C00001 (SOLO1)

The clinical performance of F1CDx for *BRCA1/2* classification was established based on available tumor analysis using the F1CDx in the clinical study D0818C00001 (SOLO1). SOLO1 was a Phase III, randomized, double-blind, placebo-controlled, multicenter trial, that compared the efficacy of Lynparza® (olaparib) with placebo in patients with advanced ovarian, fallopian tube, or primary peritoneal cancer with *BRCA* mutation (documented mutation in *BRCA1* or *BRCA2*) following first-line platinum-based chemotherapy. A total of 391 patients were randomized (2:1) to receive Lynparza tablets 300 mg orally twice daily (n=260) or placebo (n=131). Patients were required to have a documented mutation in *BRCA1* or *BRCA2* that were known or predicted to be a loss of function mutation.

Treatment was continued for up to 2 years or until disease progression or unacceptable toxicity; however, patients with evidence of disease at 2 years, who in the opinion of the treating healthcare provider could derive further benefit from continuous treatment, could be treated beyond 2 years. Randomization was stratified by response to first-line platinum-based chemotherapy (complete or partial response). The major efficacy outcome was investigator-assessed progression-free survival (PFS) evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1.

The study was designed to recruit *BRCAm* patients, i.e., germline or somatic *BRCAm (gBRCAm* or *sBRCAm)*. At the time of study initiation, a health authority approved tumor diagnostic test was not available. Patients known to have *BRCA* mutation/s (*gBRCA,* i.e., blood or *tBRCA,* i.e., tumor) prior to randomization could enter the study based on this result provided that all such testing had been undertaken in appropriately accredited laboratories (i.e., testing done for research use only [RUO] was not acceptable). In addition, the patients must have consented to provide blood samples for a confirmatory *gBRCA* test post randomization using a blood-based germline *BRCA* test. However, patients could enter the study if they were known to have a tumor *BRCAm* (*tBRCAm*) based on a local, clinically validated test. Tumor tissue was requested for all randomized patients and where possible, retrospectively tested prior to database lock with the F1CDx assay. Since few patients underwent tumor testing during the SOLO1 recruitment period, the patients recruited were predominantly *gBRCAm* as determined by local results or a *gBRCA* clinical trial assay (CTA); however, there were 2 patients with *sBRCAm* tumors. Based on strong biological rationale, it is predicted that patients with a *BRCA* mutation that is somatic in origin will derive a similar clinical efficacy benefit to those with a mutation that is germline in origin.

3.10.2 Accountability of the PMA Cohort

Out of the 391 patients randomized in SOLO1, 368 (94.1%) had an available tumor sample for testing. Of these, 335 (85.6%) patients had a valid tumor tissue F1CDx result. Out of the 335 with a valid tumor tissue F1CDx result, 313 patients were confirmed to carry a deleterious mutation in either *BRCA1* or *BRCA2* by F1CDx. The PMA cohort represented 80.1% of the full analysis set (FAS) in SOLO1. Of the 22 patients that were not confirmed to carry a deleterious mutation by F1CDx, 12 were not confirmed to have a deleterious mutation by F1CDx in their tumor tissue due to differences in the variant classification criteria used by F1CDx compared to the *gBRCA* CTA. The remaining 10 patients that were not confirmed to carry deleterious *BRCA1/2* mutations in their tumor tissue had genomic rearrangements that consisted of large-scale genomic deletions (affecting at least one whole exon), or large-scale genomic insertions including exon duplications. These patients represented 10 out of a total of 20 randomized patients in SOLO1 that had genomic rearrangements in *BRCA1/2* detected by the *gBRCA* CTA.

3.10.3 Efficacy Evaluation

The primary efficacy endpoint was investigator assessed PFS evaluated according to RECIST, version 1.1. SOLO1 met the primary endpoint demonstrating a statistically significant improvement in investigator-assessed PFS for olaparib compared to placebo. Results from a blinded independent review were consistent.

The effectiveness of the F1CDx test was based on a subset of 313 ovarian cancer patients whose tumor tissue was confirmed to carry deleterious *tBRCAm* status. Table 42 presents a summary of key efficacy outcome variables for patients whose tissue was confirmed to have *tBRCAm* status by F1CDx. PFS in the confirmed F1CDx *tBRCAm* patients was consistent with the results of the FAS, namely that SOLO1 met the primary endpoint, demonstrating a substantial improvement in PFS for olaparib compared with placebo. The sensitivity analysis of PFS to assess possible ascertainment bias using blinded independent centralized review (BICR) in the F1CDx confirmed *tBRCAm* patient subset was consistent with the BICR-assessed PFS analysis in the FAS and confirmed its robustness. Overall, the primary efficacy outcome in the F1CDx *tBRCAm* subset were consistent with the FAS.

Table 42. Summary of key efficacy outcome variables in the FAS and in the F1CDx *tBRCAm* subset.

	FAS n=391		**F1CDx *tBRCAm* n=313**	
	Olaparib (n=260)	**Placebo (n=131)**	**Olaparib (n=206)**	**Placebo (n=107)**
PFS by Investigator Assessment				
Number of events/total number of patients (%)	102/260 (39)	96/131 (73)	80/206 (39)	81/107 (76)
Median PFS (months)[a]	Not reached	13.8	Not reached	11.9
HR (95% CI)[b]	0.30 (0.23-0.41)		0.28 (0.20-0.38)	
p-value (2-sided)[c]	p<0.0001		p<0.0001	

[a] PFS is defined as the time from randomization until data of RECIST progression or death.

[b] Hazard ratio from a Cox proportional hazards model including response to previous platinum chemotherapy (complete response versus partial response) as a covariate.

[c] The p-value is derived from a stratified log-rank test.

3.11 FoundationOne®CDx Clinical Bridging Study for *PIK3CA*

The safety and effectiveness of FoundationOne®CDx (F1CDx) for detecting *PIK3CA* alterations in breast cancer patients who may benefit from treatment with alpelisib was demonstrated in a retrospective analysis of specimens from patients enrolled in SOLAR-1. SOLAR-1 is the pivotal Phase III, randomized, double-blind, placebo-controlled study of alpelisib in combination with fulvestrant in men and postmenopausal women with hormone receptor positive (HR+), human epidermal growth factor receptor 2 negative (HER2-) locally advanced breast cancer whose disease had progressed or recurred on or after an aromatase inhibitor based treatment (with or without CDK4/6 combination) (SOLAR-1, NCT2437318).

A bridging study was conducted to assess the clinical efficacy of F1CDx in identifying *PIK3CA* alteration positive patients for treatment with alpelisib in combination with fulvestrant and the concordance between *PIK3CA* status (mutant or non-mutant) tested with the clinical trial enrollment assays (referred to as clinical trial assay [CTA1] and [CTA2]) and the F1CDx in the intent-to-test population. F1CDx was used to

retrospectively test the stored patient samples from SOLAR-1 with sufficient residual tumor material (N = 415 of the total 572 enrolled patients). Samples from 296 patients enrolled with the CTA1 (119 *PIK3CA* alteration positive patients and 177 *PIK3CA* alteration negative patients), and 119 patients enrolled with the CTA2 (115 *PIK3CA* alteration positive patients and 4 *PIK3CA* alteration negative patients), were retrospectively tested with F1CDx.

3.11.1 Safety Analysis

The F1CDx assay is not expected to directly cause actual or potential adverse effects, but test results may directly impact patient treatment risks.

3.11.2 Effectiveness Results

Concordance Analysis

The concordance between F1CDx and the two enrollment assays (CTA1 and CTA2) was assessed. The point estimates of PPA, NPA and OPA for F1CDx compared to the CTAs are provided in Table 43 and Table 44 below.

Table 43. Agreement between CDx and CTA1 based on the CTA1 results (Primary analysis set, CTA1-enrolled).

	Without invalid CDx results		With invalid CDx results	
Measure of agreement	**Percent agreement (N)**	**95% CI (1)**	**Percent agreement (N)**	**95% CI (1)**
PPA	93.8% (106/113)	(87.7%, 97.5%)	93.0% (106/114)	(86.6%, 96.9%)
NPA	98.8% (159/161)	(95.6%, 99.8%)	95.8% (159/166)	(91.5%, 98.3%)
OPA	96.7% (265/274)	(93.9%, 98.5%)	94.6% (265/280)	(91.3%, 97.0%)

(1) The 95% CI calculated using the Clopper-Pearson Exact method.
- Samples not tested are excluded from the analysis.
- Samples tested on deviation are excluded from the analysis.

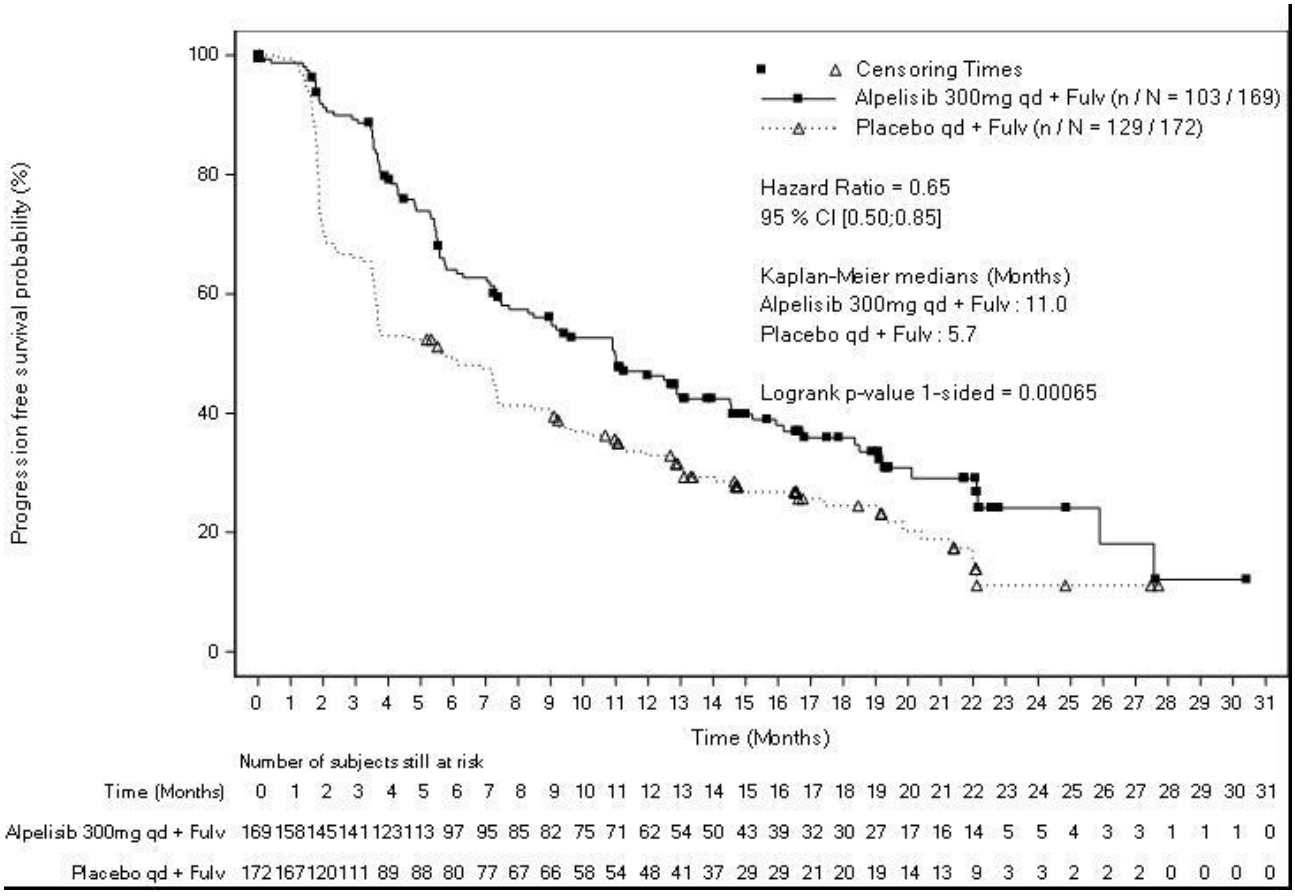
Table 44. Agreement between CDx and CTA2 based on the CTA2 results (Concordance analysis set for CTA2).

	Without invalid CDx results		With invalid CDx results	
Measure of Agreement	**Percent Agreement (N)**	**95% CI (1)**	**Percent Agreement (N)**	**95% CI (1)**
PPA	91.6% (197/215)	(87.1%, 95.0%)	90.4% (197/218)	(85.7%, 93.9%)
NPA	98.8% (162/164)	(95.7%, 99.9%)	97.0% (162/167)	(93.2%, 99.0%)
OPA	94.7% (359/379)	(92.0%, 96.7%)	93.2% (359/385)	(90.3%, 95.5%)

(1) The 95% CI calculated using the Clopper-Pearson Exact method.
- Samples not tested are excluded from the analysis.
- Samples tested on deviation are excluded from the analysis.

Clinical Efficacy Results in the SOLAR-1 Mutant Cohort

The SOLAR-1 clinical trial met its primary objective demonstrating a statistically significant improvement in PFS by investigator assessment in patients with *PIK3CA* alteration positive tumors. Supportive analysis included PFS based on blinded independent review committee (BIRC). Alpelisib in combination with fulvestrant demonstrated an estimated 35% risk reduction of disease progression or death compared to the placebo plus fulvestrant arm (HR = 0.65; 95% CI: 0.50, 0.85; p = 0.00065) in the *PIK3CA* alteration cohort. The median PFS was prolonged by a clinically relevant 5.3 months, from 5.7 months in the placebo plus fulvestrant arm to 11.0 months in the alpelisib plus fulvestrant arm (Figure 6).



Stratified Logrank test and stratified Cox model using strata defined by (i) prior CDK 4/6 inhibitor use. (ii) presence of liver and/or lung metastases.

Figure 6. Kaplan-Meier plot of progression free survival by treatment in the mutant patients randomized in the original SOLAR-1 trial (Primary analysis set).

Clinical Efficacy Results in the CDx-Positive Population

Efficacy analyses were performed for patients determined to be CDx-positive (*PIK3CA* alteration detected by F1CDx) and compared to the efficacy results in the SOLAR-1 *PIK3CA* mutant cohort. The clinical efficacy in the CDx-positive population was estimated by pooling the hazard ratios calculated for 1) the CTA1-enrolled patients that were CDx-positive and 2) the CTA2-enrolled patients that were CDx-positive.

Table 45 and Table 46 show the efficacy results in the CTA1-enrolled CDx-positive patients (HR = 0.52, 95% CI: 0.29, 0.93) and the results in the CTA2-enrolled (CTA2+, CDx+) patients (HR = 0.35, 95% CI: 0.16, 0.77), respectively.

For the sensitivity analysis to c for the clinical efficacy of alpelisib in combination with fulvestrant for the *PIK3CA* CDx-positive population, the hazard ratio estimates ranged from 0.43 to 0.44. The upper bounds of the 95% confidence intervals for the corresponding hazard ratios were all below 1.0. Sensitivity analysis against the missing CDx results demonstrated the robustness of the efficacy analysis.

Table 45. Clinical efficacy on progression free survival in the CTA1-enrolled CDx-positive patients (Primary analysis set, CTA1-enrolled).

Progression free survival (months)	Alpelisib 300mg qd + Fulv N=56	Placebo qd + Fulv N=52	HR(95% CI) Alpelisib 300mg qd + Fulv / Placebo qd + Fulv (1)
No of events (%)	41 (73.2)	41 (78.8)	0.52 (0.29, 0.93)
PD (%)	39 (69.6)	41 (78.8)	
Death (%)	2 (3.6)	0	
No of censored (%)	15 (26.8)	11 (21.2)	
Median (95% CI) (2)	11.2 (8.3, 18.5)	5.5 (1.9, 10.9)	

(1) Hazard ratio (HR) estimated using Cox regression model. The model is adjusted by the identified baseline clinical covariates, as well as the covariates that are imbalanced between treatment and control. The model is stratified by the two stratification factors: presence of lung and/or liver metastases, previous treatment with any CDK4/6 inhibitor.

CI: Wald Confidence Interval.

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

-CDx results obtained on deviation are treated as missing.

Table 46. Clinical efficacy on progression free survival in the CTA2-enrolled (CTA2+, CDx+) patients (Primary analysis set, CTA2-enrolled).

Progression free survival (months)	Alpelisib 300mg qd + Fulv N=42	Placebo qd + Fulv N=48	HR(95% CI) Alpelisib 300mg qd + Fulv / Placebo qd + Fulv (1)
No of events (%)	19 (45.2)	36 (75.0)	0.35 (0.16, 0.77)
PD (%)	18 (42.9)	31 (64.6)	
Death (%)	1 (2.4)	5 (10.4)	
No of censored (%)	23 (54.8)	12 (25.0)	
Median (95% CI) (2)	10.9 (5.6, NE)	4.2 (2.1, 7.4)	

(1) Hazard ratio (HR) estimated using Cox regression model. The model is adjusted by the identified baseline clinical covariates, as well as the covariates that are imbalanced between treatment and control. The model is stratified by the two stratification factors: presence of lung and/or liver metastases, previous treatment with any CDK4/6 inhibitor.

CI: Wald Confidence Interval.

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

-CDx results obtained on deviation are treated as missing.

Conclusions

The data from this study support reasonable assurance of the safety and effectiveness of the F1CDx assay when used to aid clinicians in identifying breast cancer patients with *PIK3CA* alterations who may be eligible for treatment with alpelisib.

3.12 Clinical evaluation of *FGFR2* rearrangement detection for treating Cholangiocarcinoma (CCA) patients with pemigatinib

The clinical performance of F1CDx for detecting *FGFR2* fusions and rearrangements in CCA patients who may benefit from treatment with pemigatinib was established with clinical data generated from the Incyte trial INCB 54828-202, and a clinical bridging study to establish concordance between the confirmatory clinical trial assay (CTA) and the F1CDx assay.

3.12.1 Summary of the Clinical Study – INCB 54828-202 (FIGHT-202)

Study INCB 54828-202 is a prospective, multicenter, open-label, Phase II study in participants with previously treated, advanced/metastatic or surgically unresectable cholangiocarcinoma, including participants with *FGFR2*-rearranged cholangiocarcinoma. The primary endpoint of Study INCB 54828-202 was the objective response rate (ORR) in participants with *FGFR2*-rearranged cholangiocarcinoma

to determine whether treatment with pemigatinib is safe and effective. Participants in Study INCB 54828-202 were assigned to cohorts for statistical analysis based on tumor *FGF/FGFR* status as determined by the FMI F1 CTA: Cohort A included participants with *FGFR2* fusions and select rearrangements in cholangiocarcinoma, and Cohorts B and C included participants with other cholangiocarcinoma molecular subtypes. Eligible participants received pemigatinib on a 2-weeks-on/1-week-off schedule at a starting dose of 13.5 mg once a day. Treatment continued until documented disease progression or unacceptable toxicity.

In the trial, a patient whose tumor harbors an *FGFR2* rearrangement containing an intact kinase domain was defined as eligible under the following conditions:

- *FGFR2* rearrangements with a literature-derived known partner gene regardless of strand or frame,
- *FGFR2* rearrangements in the same 5' to 3' orientation and in frame with a novel partner gene,
- *FGFR2* rearrangements with one breakpoint in the hotspot region (intron 17-exon 18) and the other breakpoint in an intergenic region or within another gene. This rule excludes 3' duplications of only exon 18,
- Intragenic duplication of the kinase domain (exon 9-17)

3.12.2 Accountability of the PMA Cohorts

A total of 146 participants with previously treated, advanced/metastatic or surgically-unresectable cholangiocarcinoma were enrolled in Study INCB 54828-202. Based on tumor sample testing from the FMI F1 CTA, 145 participants were included in the efficacy evaluable population after one participant was not able to be confirmed by the F1 CTA. The 145 participants were assigned to one of the following cohorts for statistical analyses:

- Cohort A: 107 participants with *FGFR2* fusions/rearranged cholangiocarcinoma
- Cohort B: 20 participants with other *FGF/FGFR* alterations
- Cohort C: 18 participants with tumors negative for *FGF/FGFR* alterations

The efficacy of PEMAZYRE was determined in cohort A (107) patients with locally advanced unresectable or metastatic cholangiocarcinoma whose disease had progressed on or after at least 1 prior therapy and who had an *FGFR2* gene fusion or non-fusion rearrangement, as determined by the clinical trial assay. Qualifying in-frame fusions and other rearrangements were predicted to have a breakpoint within intron 17/exon 18 of the *FGFR2* gene leaving the *FGFR2* kinase domain intact.

3.12.3 Efficacy Evaluation

3.12.3.1 Clinical efficacy results in Intent to Treat population

The major efficacy outcome measures were overall response rate (ORR) and duration of response (DoR) as determined by an independent review committee (IRC) according to RECIST v1.1. The results of this study are shown in Table 47 below.

Table 47. Efficacy results in FIGHT-202 trial.

Efficacy Parameter	PEMAZYRE N = 107
ORR (95% CI)	36% (27, 45)
Complete response	2.8%
Partial response	33%

3.12.3.2 Summary of the Clinical Bridging Study

Following testing by the F1 CTA, residual DNA for patients in INCB 54828-202 was banked to support the clinical bridging study testing with the F1CDx assay. The safety and effectiveness of F1CDx for detecting *FGFR2* rearrangements in CCA patients who may benefit from treatment with pemigatinib was demonstrated in a retrospective analysis of residual DNA from patients enrolled in the INCB 54828-202 trial. Residual DNA was available for 108 patients screened with the CTA (80 in Cohort A, 14 in Cohort B,

10 in Cohort C, and 4 screen failures). in addition to 73 *FGFR2* rearrangement-negative specimens for a total of 181 positive and negative F1CDx evaluable samples included in the analysis. A bridging study was conducted to assess the clinical efficacy of F1CDx in identifying *FGFR2* rearrangement positive patients for treatment with pemigatinib and the concordance between *FGFR2* rearrangement status (mutant and non-mutant) tested with the CTA and F1CDx in the efficacy evaluable population. Of the evaluable specimens in cohort A (n=80), the most common finding was *FGFR2-BICC1* [27% (22/80)] in the evaluable set. Patients also had rearrangements without an identifiable partner gene. All of the biomarker positive cases in the F1CDx *FGFR2* CCA Clinical Bridging Study had breakpoints in the *FGFR2* hotspot region, intron 17 – exon 18. (Figure 1)

Clinical efficacy results in the CDx-positive population

Clinical utility of F1CDx was evaluated by estimation of clinical efficacy in the *FGFR2* rearranged, CTA-enrolled population based on the primary objective of ORR per central review per RECIST v1.1 criteria. Sensitivity analysis, using the multiple imputation method, was performed to evaluate the robustness of the clinical efficacy estimate against the 27 missing CDx results from Cohort A and 14 missing results from cohort B and C combined. The ORR for the F1CDx *FGFR2*-rearrangement-positive population estimated by the bridging study was 37.50% and aligns with the ORR for the CTA *FGFR2*-rearrangement-positive population, which was 35.51% (Table 48). Sensitivity analysis, using the multiple imputation method, was performed to evaluate the robustness of the clinical efficacy estimate against the 27 missing CDx results from the efficacy evaluable population (Cohort A). The distribution of *FGFR2* fusions in the trial that were available for bridging is shown in Figure 7 below.

Table 48. Summary of ORR in different subpopulations for completed data.

Population	CTA+	CTA+ and F1CDx+	CTA+ and F1CDx-
n	107	80	0
ORR	35.51%	37.50%	N/A
95% 2-sided exact CIs	[26.50%,45.35%]	[26.92%,49.04%]	N/A

Note: Given the NPA=1, the efficacy of F1CDx *FGFR2* rearrangement positives can be estimated from the (CTA+, F1CDx+) group.

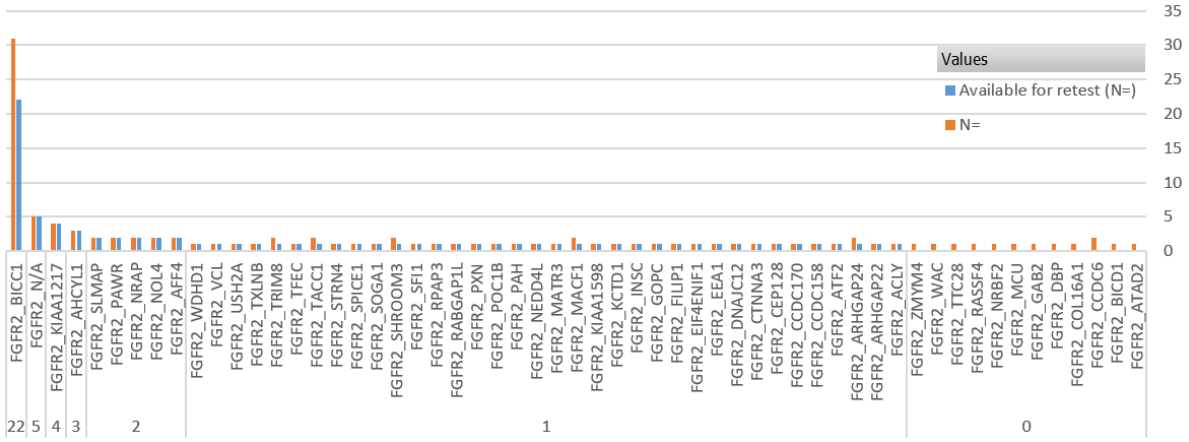


Figure 7. Distribution of *FGFR2* fusions and rearrangements in Cohort A in support of efficacy.

3.12.3.3 Safety Analysis

The F1CDx assay is not expected to directly cause actual or potential adverse effects, but test results may directly impact patient treatment risks.

3.12.3.4 Clinical Concordance

Patients with valid F1CDx results together with FMI archived samples were used to demonstrate concordance of F1CDx to the CTA. Retrospective testing with F1CDx yielded 181 CDx-evaluable results used for further analysis (84 positive and 97 negative). Agreement between F1CDx and the CTA was demonstrated. The PPA, NPA, OPA, adjusted PPV, and adjusted NPV all exhibited 100% agreement between the F1CDx assay and the F1 CTA.

3.12.3.5 Conclusions

The data from this study support reasonable assurance of the safety and effectiveness of the F1CDx assay when used to aid clinicians in identifying CCA patients with *FGFR2* fusions and rearrangements who may be eligible for treatment with pemigatinib.

3.13 Clinical evaluation of *MET* exon 14 classification for treating NSCLC patients with capmatinib

The clinical performance of F1CDx for detecting SNVs and indels that lead to *MET* exon 14 skipping in NSCLC patients who may benefit from treatment with capmatinib was established with clinical data generated from the Novartis trial CINC280A2201 (GEOMETRY-mono 1), and a clinical bridging study to establish concordance between the enrollment clinical trial assay (CTA) and the F1CDx assay.

3.13.1 Summary of the Clinical Study – CINC280A2201 (GEOMETRY-mono 1)

GEOMETRY-mono 1 is a prospectively designed, multicenter, open-label, single arm Phase II study of oral cMET inhibitor (capmatinib) in adult patients with *EGFR* wild-type (wt), advanced NSCLC. The primary objective was to assess overall response rate (ORR) by a BIRC assessment to determine whether treatment with capmatinib is effective. Patients have been enrolled into multiple cohorts of the study, out of which the bridging study was focused on the fully-enrolled *MET* exon 14 deletion positive Cohorts 4 and 5b. Cohort 4 only enrolled pretreated (second and third line) *MET* exon 14 deleted patients, and Cohort 5b only enrolled treatment-naïve *MET* exon 14 deleted patients. Patients were screened for enrollment into Cohorts 4 and 5b for *MET* exon 14 deletion status using a *MET* exon 14 deletion reverse-transcriptase PCR (RT-PCR) CTA. After initial patient screening, clinical samples were stored for retrospective testing. GEOMETRY-mono 1 is an ongoing trial that was initiated on June 11, 2015 with first patient first visit (FPFV). Patients receive 400 mg of capmatinib orally twice daily in tablet form. Dose adjustments for capmatinib are permitted for safety concerns. Efficacy is evaluated every six weeks from the first day of treatment until RECIST 1.1 disease progression.

3.13.2 Summary of the Clinical Bridging Study

The safety and effectiveness of F1CDx for detecting SNVs and indels that lead to *MET* exon 14 skipping in NSCLC patients who may benefit from treatment with capmatinib was demonstrated in a retrospective analysis of samples from patients enrolled in the GEOMETRY-mono 1 trial. A bridging study was conducted to assess the clinical efficacy of F1CDx in identifying patients positive for SNVs and indels that lead to *MET* exon 14 skipping for treatment with capmatinib and the concordance between *MET* exon 14 deletion status tested with the CTA and F1CDx in the intent-to-test population. Retrospective testing with F1CDx was done for patients from Cohorts 4 and 5b, and a random selection of *MET* exon 14 deletion negative patients. The retrospective testing population consisted of 204 patients (78 *MET* exon 14 deletion positive patients, and 126 *MET* exon 14 deletion negative patient samples), originally tested by the *MET* exon 14 CTA for patient selection.

3.13.3 Safety Analysis

The F1CDx assay is not expected to directly cause actual or potential adverse effects, but test results may directly impact patient treatment risks.

3.13.4 Accountability of the PMA Cohorts

A total of 3036 patients were screened for trial eligibility from 152 investigational sites across 25 countries. 2551 patients within the original 3036 were screened for *MET* exon 14 deletion by the CTA. Within that screened population, 2295 patients produced valid positive and negative CTA results. As of April 15, 2019, a total of 334 patients had been enrolled into all available cohorts. Of the patients whose samples produced valid CTA results, 97 were enrolled into Cohorts 4 and 5b of the GEOMETRY-mono 1 trial, with 69 and 28 patients respectively. *MET* exon 14 deletion negative patients were not enrolled in the GEOMETRY-mono 1 trial. Available samples from *MET* exon 14 deletion negative patients were evaluated for the bridging study, including 130 randomly selected CTA-negative patients. Out of the 130 CTA-negative samples, 93 were randomly assigned to Cohort 4 and 37 to Cohort 5b. Of the 227 positive and negative samples (97 positive and 130 negative), retrospective testing with F1CDx was performed for 204 CTA-tested patient samples that met the F1CDx sample testing criteria (78 positive and 126 negative). The F1CDx testing yielded 198 CDx-evaluable results and six (6) invalid results for the CDx and CTA concordance analysis.

Sensitivity analyses were conducted with all 227 samples to determine the impact of missing F1CDx results on concordance and efficacy results, which included 19 positive patient samples not tested due to failing to meet the F1CDx minimum tissue sample requirements, laboratory error and/or not meeting quality control metrics.

3.13.5 Clinical Concordance

The primary concordance analysis was conducted on 204 samples (78 positive and 126 negative). Agreement between F1CDx and the CTA was demonstrated. The point estimates of PPA, NPA and OPA between F1CDx and the CTA, shown in Table 49, were calculated with and without invalid CDx results, using the CTA results as reference for the CTA-enrolled patients.

Table 49. Agreement between F1CDx and CTA based on CTA results in combined cohorts by F1CDx sample requirements.

		Without CDx "Invalid"		With CDx "Invalid"	
	Measure of agreement	**Percent agreement % (n/N)**	**95% CI (1)**	**Percent agreement % (n/N)**	**95% CI (1)**
Cohort 4 and Cohort 5b	PPA	98.6 (72/ 73)	(92.6, 100)	92.3 (72/ 78)	(84.0, 97.1)
	NPA	100 (125/125)	(97.1, 100)	99.2 (125/126)	(95.7, 100)
	OPA	99.5 (197/198)	(97.2, 100)	96.6 (197/204)	(93.1, 98.6)

N: The total number of patients. It is the denominator for percentage (%) calculation.
n: Number of patients with agreement between CTA and CDx.
(1) The 95% CI calculated using Clopper-Pearson method

3.13.6 Efficacy Evaluation

GEOMETRY- mono 1 clinical efficacy results

The GEOMETRY-mono 1 clinical trial met the primary objective, demonstrating a high ORR as assessed by BIRC. Treatment with capmatinib was considered efficacious in both Cohort 4 (second and third line) and Cohort 5b (treatment-naive) as demonstrated by an ORR per BIRC of 40.6% (95% CI: 28.9, 53.1) and of 67.9% (95% CI: 47.6, 84.1), respectively (Table 50 below). Robustness of the data was further confirmed by the supportive analysis of ORR by Investigator assessment, ORR for the PFS and for key subgroups.

Table 50. Treatment-naïve and previously treated *MET*-skipping positive locally advanced or metastatic NSCLC - efficacy results in patients treated with capmatinib in GEOMETRY-mono 1

Efficacy Parameter	Previously Treated (Cohort 4) N = 69	Treatment-Naïve by (Cohort 5b) N = 28
Overall Response Rate[a], % (95% CI)[b]	40.6 (28.9, 53.1)	67.9 (47.6, 84.1)
Complete Response (CR), n (%)	0	3.6%
Partial Response (PR), n (%)	40.6%	64.3%

[a] Determined by RECIST v1.1.
[b] Clopper and Pearson exact binomial 95% CI.

Clinical efficacy results in the CDx-positive population
Clinical utility of F1CDx 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. Baseline demographic and disease characteristics were compared between the CDx evaluable and CDx unevaluable within all enrolled CTA-positive patients in Cohorts 4 and 5b. Clinical efficacy of capmatinib in patients with SNVs and indels that lead to *MET* exon 14 skipping with valid CDx results and after imputing missing CDx results were similar between the CDx-positive and CTA-positive patient groups in the GEOMETRY-mono 1 trial. Table 51 shows the efficacy results in CTA enrolled CDx-positive patients, while detailed efficacy results are available in Tables 16 and 17 of the SSED.

Table 51. Summary of clinical efficacy results by test method and sample set

Test Method	Cohort 4 ORR with 95% CI	Cohort 5b ORR with 95% CI
F1CDx	44.2% (30.6 – 58.7%)	70% (45.7 – 88.1 %)
CTA	40.6% (28.9 – 53.1%)	67.9% (47.6 – 84.1%)

3.13.7 Conclusions
The data from this study support reasonable assurance of the safety and effectiveness of the F1CDx assay when used to aid clinicians in identifying NSCLC patients with SNVs and indels that lead to *MET* exon 14 skipping who may be eligible for treatment with capmatinib.

3.14 Clinical evaluation of HRR gene alterations for treating prostate cancer patients with olaparib
The clinical performance of F1CDx for determination of the mutation status of the HRR gene panel was established based on confirmed FMI F1CDx subgroup results, which were derived from tumor analysis results using the CLIA HRR CTA in the clinical study D081DC00007 (PROfound).

Study Design
PROfound was a Phase III, randomised, open-label, multicentre trial to assess the efficacy and safety of olaparib monotherapy in patients with metastatic castration-resistant prostate cancer (mCRPC) that have qualifying homologous recombination repair (HRR) gene mutations that were predicted to be deleterious or suspected deleterious (known or predicted to be detrimental/lead to loss of function) who have failed prior treatment with a new hormonal agent (NHA).

Patients were randomised in a 2:1 ratio to the treatments as specified below:

- Olaparib tablets orally 300 mg bd
- Investigators choice of NHA with either enzalutamide 160 mg orally once daily (od) or abiraterone acetate 1000 mg orally qd with prednisone 5 mg orally bd (prednisolone was permitted for use instead of prednisone, if necessary)

Eligible patients were those with HRRm mCRPC, who had progressed following prior treatment with an NHA. All patients must have had a qualifying HRR mutation assessed via the FMI CLIA HRR CTA to be randomised. Qualifying HRR gene mutations were *BRCA1, BRCA2* and *ATM* for Cohort A, and *BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, PPP2R2A, RAD51B, RAD51C, RAD51D* and *RAD54L* for Cohort B.

Note: Although patients with PPP2R2A gene mutations were enrolled in the trial, Lynparza is not indicated for the treatment of patients with this gene mutation because of lack of response, and a numerical decrement in both rPFS and OS compared to enzalutamide or abiraterone.

Safety Analysis

The F1CDx assay is not expected to directly cause actual or potential adverse effects, but test results may directly impact patient treatment risks.

Efficacy Evaluation

PROfound met its primary objective, demonstrating a statistically significant improvement in rPFS as assessed by BICR with olaparib 300 mg bd compared with investigators choice of NHA in Cohort A. Specifically, the PROfound efficacy data with olaparib demonstrated:

- A statistically significant improvement in rPFS as assessed by BICR with olaparib 300 mg bd compared with investigators choice of NHA in Cohort A, with a 66% reduction in the risk of BICR-confirmed radiological disease progression or death and a prolongation of median progression free interval of 3.8 months with olaparib vs investigators choice of NHA. The rPFS outcome in the confirmed FMI F1CDx subgroup (HR 0.33 [95% CI 0.24, 0.46]) was consistent with the Full Analysis Set (FAS) (HR 0.34 [95% CI 0.25, 0.47]).

Table 52. Summary of analysis of rPFS based on BICR (Cohort A).

Analysis group:	Full Analysis Set		Confirmed FMI F1CDx Subgroup	
	Olaparib 300 mg bd (N=162)	Investigators choice of NHA (N=83)	Olaparib 300 mg bd (N=157)	Investigators choice of NHA (N=83)
n (%) of events[a]	106 (65)	68 (82)	101 (64)	68 (82)
Treatment effect				
Median rPFS (95% CI) [months]	7.4 (6.24, 9.33)	3.6 (1.91, 3.71)	7.4 (6.87, 9.33)	3.6 (1.91, 3.71)
HR (95% CI)[b]	0.34 (0.25, 0.47)		0.33 (0.24, 0.46)	
2-sided p-value[c]	<0.0001		<0.0001	

[a] Progression, as assessed by BICR, was defined by RECIST 1.1 and/or PCWG-3 or death (by any cause in the absence of progression) regardless of whether the patient withdrew from randomised therapy or received another anticancer therapy prior to progression.

[b] The HR and CI were calculated using a Cox proportional hazards model adjusted for the variables selected in the primary pooling strategy (prior taxane use and measurable disease in Cohort A). The Efron approach was used for handling ties. An HR <1 favours olaparib 300 mg bd.

[c] The analysis was performed using the log-rank test stratified by the variables selected in the primary pooling strategy (prior taxane use and measurable disease in Cohort A) using the Breslow method for handling ties.

bd twice daily; BICR blinded independent central review; CI confidence interval; FAS full analysis set; HR hazard ratio; NHA new hormonal agent; PCWG-3 Prostate Cancer Working Group 3; RECIST Response Evaluation Criteria in Solid Tumours; rPFS radiological progression-free survival.

- There was a statistically significant improvement in confirmed radiological ORR by BICR for patients in Cohort A with measurable disease at baseline in the olaparib arm compared with the investigators´ choice of NHA arm. The efficacy in the confirmed FMI F1CDx subgroup showed a similar performance as compared to the Full Analysis Set.

Table 53. Confirmed radiological objective response rate, logistic regression based on BICR (EFR; Cohort A).

Analysis group	Treatment group	N	Number (%) of patients with response[a]	Comparison between groups
				2-sided p-value[b]
Full Analysis Set	Olaparib 300 mg bd	84	28 (33.3)	
	Investigators choice of NHA	43	1 (2.3)	<0.0001
Confirmed FMI F1CDx Subgroup	Olaparib 300 mg bd	84	27 (33.8)	
	Investigators choice of NHA	43	1 (2.3)	<0.0001

[a] Radiological objective response rate determined based on BICR assessed RECIST 1.1 and bone scan data (using all scans regardless of whether they were scheduled or not) in patients with measurable disease. Response required confirmation. Radiological objective response rate compared using logistic regression (PROC GENMOD) adjusting for previous taxane use as a covariate.

[b] Where the number of patients with a response was ≥5, a 1-sided p-value was calculated based on twice the change in log-likelihood resulting from the addition of the treatment factor to the model that contains the specified covariates. Where the number of patients with a response was <5, the 2-sided p-value was calculated based on the mid p-value modification of the Fisher's exact test.

- There was a statistically significant improvement in rPFS as assessed by BICR for olaparib-treated patients compared with investigators choice of NHA-treated patients in Cohort A+B, with a 51% reduction in the risk of radiological disease progression or death and a prolongation of median progression-free interval of 2.3 months with olaparib vs investigators choice of NHA (HR=0.49; 95% CI 0.38, 0.63; p<0.0001; median rPFS 5.8 months vs 3.5 months, respectively, for FAS and confirmed FMI F1CDx subgroup).

Table 54. Summary of analysis of rPFS based on BICR (Cohort A+B).

Analysis group:	Full Analysis Set		Confirmed FMI F1CDx Subgroup	
	Olaparib 300 mg bd (N=256)	Investigators choice of NHA (N=131)	Olaparib 300 mg bd (N=248)	Investigators choice of NHA (N=128)
n (%) of events[a]	180 (70.3)	99 (75.6)	172 (69.4)	96 (75.0)
Treatment effect				
Median rPFS (95% CI) [months]	5.8 (5.52, 7.36)	3.5 (2.20, 3.65)	6.2 (5.52, 7.36)	3.5 (2.10, 3.65)
HR (95% CI)[b]	0.49 (0.38, 0.63)		0.49 (0.38, 0.63)	
2-sided p-value[c]	<0.0001		<0.0001	

[a] Progression, as assessed by BICR, was defined by RECIST 1.1 and/or PCWG-3 or death (by any cause in the absence of progression) regardless of whether the patient withdrew from randomised therapy or received another anticancer therapy prior to progression.

[b] The HR and CI were calculated using a Cox proportional hazards model adjusted for the variables selected in the primary pooling strategy (prior taxane use and measurable disease in Cohort A+B). The Efron approach was used for handling ties. An HR <1 favours olaparib 300 mg bd.

[c] The analysis was performed using the log-rank test stratified by the variables selected in the primary pooling strategy (prior taxane use and measurable disease in Cohort A+B) using the Breslow method for handling ties.

bd twice daily; BICR blinded independent central review; CI confidence interval; FAS full analysis set; HR hazard ratio; NHA new hormonal agent; PCWG-3 Prostate Cancer Working Group 3; RECIST Response Evaluation Criteria in Solid Tumours; rPFS radiological progression-free survival.

- In Cohort A, the interim OS data indicate a trend for OS benefit in olaparib -treated patients compared with investigators choice of NHA-treated patients, with a median OS improvement of 3.4 months in the olaparib- arm vs the investigators choice of NHA arm (HR=0.64; 95% CI 0.43, 0.97; p=0.0173; median OS 18.5 months vs 15.1 months, respectively).
- The olaparib safety and tolerability profile in this study was consistent with that observed in previous studies of olaparib.

3.15 Clinical Evaluation of pembrolizumab in TMB-H solid tumors

Summary of the Clinical Study – KEYNOTE-158

The clinical performance of F1CDx for detecting TMB-H (defined as TMB $\geq$ 10 mutations per megabase) and the efficacy of KEYTRUDA (pembrolizumab) were investigated in a prospectively-planned retrospective analysis of 10 cohorts (A through J) of patients with various previously treated unresectable or metastatic solid tumors with high tumor mutational burden (TMB) who were enrolled in a multicenter, non-randomized, open-label trial, KEYNOTE-158 (NCT02628067). The trial excluded patients who previously received an anti-PD-1 or other immune-modulating monoclonal antibody, or who had an autoimmune disease, or a medical condition that required immunosuppression. Patients received KEYTRUDA 200 mg intravenously every 3 weeks until unacceptable toxicity or documented disease progression. Assessment of tumor status was performed every 9 weeks for the first 12 months and every 12 weeks thereafter.

The statistical analysis plan pre-specified ≥ 10 and ≥ 13 mutations per megabase using F1CDx as cutpoints to assess TMB. Testing of TMB was blinded with respect to clinical outcomes. The major efficacy outcome measures were ORR and DoR in the patients who have received at least one dose of KEYTRUDA as assessed by BICR according to RECIST v1.1, modified to follow a maximum of 10 target lesions and a maximum of 5 target lesions per organ.

In KEYNOTE-158, 1,050 patients (Cohorts A through J) were included in the efficacy analysis population. TMB was analysed in the therapeutic efficacy (TE) subset of 790 patients with sufficient tissue for testing based on testing requirements for the investigational F1CDx assay. Of the 790 patients, 102 (13%) had tumors identified as TMB-H (defined as a TMB ≥ 10 mutations per megabase). Among the 102 TMB-H patients, the study population characteristics were: median age of 61 years (range: 27 to 80), 34% age 65 or older; 34% male; 81% White; and 41% ECOG PS of 0 and 58% ECOG PS of 1. TMB was also analysed in the device validation (DV) population of 719 patients using the final F1CDx assay. Of the 719 patients, 91 (13%) had tumor identified as TMB-H (≥ 10 mutations per megabase) and the study population characteristics were: median age of 60 years (range: 27 to 80), 35% age 65 or older; 34% male; 81% White; and 41% ECOG PS of 0 and 58% ECOG PS of 1.

Efficacy results for the therapeutic efficacy (TE) (n=102) and device validation (DV) (n=91) populations are summarized in Table 55.

Table 55. Efficacy results for patients with TMB-H (≥ 10 mut/Mb) cancer in KEYNOTE-158.

Endpoint	KEYTRUDA 200 mg every 3 weeks	
	Therapeutic Efficacy Population n=102*	Device Validation Population n=91*
Objective Response Rate		
ORR (95% CI)	29% (21, 39)	33% (24, 44)
Complete response rate	4%	4%
Partial response rate	25%	29%
Duration of Response		
Median in months (range)	NR (2.2+, 34.8+)†	NR (2.2+, 34.8+)†
% with duration ≥6 months	87%	87%
% with duration ≥12 months	57%	57%
% with duration ≥24 months	50%	50%

* Median follow-up time of 11.1 months for TE population, and 13.4 months for DV population.
† Based on patients (n=30) with a response by independent review
\+ Denotes ongoing
NR = not reached

ORR was assessed by tumor type, and the results were similar in the TE and DV populations. Efficacy results per tumor type are shown for the TE and DV populations in Tables 56 and 57, respectively. ORR was generally higher in the TMB-H population for most tumor types than in the non-TMB-H population.

Table 56. Summary of best objective response per tumor type in TE population.

Tumor Type*	TMB ≥10 mut/Mb				TMB <10 mut/Mb				ORR Ratio‡
	N	n	%	95% CI†	N	n	%	95% CI†	TMB ≥10 mut/Mb vs. TMB <10 mut/Mb
Overall	102	30	29	(21, 39)	688	43	6	(5, 8)	4.7
Anal	14	1	7	(0.2, 34)	75	8	11	(5, 20)	0.7
Neuroendocrine	5	2	40	(5, 85)	82	1	1	(0, 7)	32.8
Endometrial	15	7	47	(21, 73)	67	4	6	(2, 15)	7.8
Cervical	16	5	31	(11, 59)	59	7	12	(5, 23)	2.6
Vulvar	12	2	17	(2, 48)	59	2	3	(0, 12)	4.9
Small Cell Lung	34	10	29	(15, 47)	42	4	10	(3, 23)	3.1
Mesothelioma	1	0	0	(0, 98)	84	9	11	(5, 19)	0.0
Thyroid	2	2	100	(16, 100)	78	3	4	(1, 11)	26.0
Salivary	3	1	33	(1, 91)	79	3	4	(1, 11)	8.8

* No TMB-H patients were identified in the cholangiocarcinoma cohort
† Based on binomial exact confidence interval method.
‡ ORR ratios were calculated prior to rounding the objective response values shown in this table

Table 57. Summary of best objective response per tumor type in DV population.

Tumor Type	TMB >=10 mut/Mb				TMB <10 mut/Mb				ORR Ratio
	N	n	%	95% CI†	N	n	%	95% CI†	TMB >=10 mut/Mb vs. TMB <10 mut/Mb
Overall	91	30	33	(24, 44)	628	41	7	(5, 9)	5.0
Anal	14	1	7	(0.2, 34)	73	8	11	(5, 20)	0.7
Neuroendocrine	5	2	40	(5, 85)	73	1	1	(0, 7)	29.2

Endometrial	15	7	47	(21, 73)	64	3	5	(1, 13)	10.0
Cervical	15	5	33	(12, 62)	52	6	12	(4, 23)	2.9
Vulvar	10	2	20	(3, 56)	52	2	4	(0.5, 13)	5.2
Small Cell Lung	26	10	38	(20, 59)	30	4	13	(4, 31)	2.9
Mesothelioma	1	0	0	(0, 98)	80	9	11	(5, 20)	0.0
Thyroid	2	2	100	(16, 100)	75	3	4	(1, 11)	25.0
Salivary	3	1	33	(1, 91)	74	3	4	(1, 11)	8.2

* No TMB-H patients were identified in the cholangiocarcinoma cohort
† Based on binomial exact confidence interval method.
‡ ORR ratios were calculated prior to rounding the objective response values shown in this table

The KEYNOTE-158 results indicate that pembrolizumab monotherapy provides clinically meaningful ORR and DoR in previously treated participants with TMB-H solid tumors across cancer types who have no satisfactory alternative treatment options.

3.16 Clinical evaluation of VITRAKVI (larotrectinib) in patients with solid tumors with *NTRK1, NTRK2, NTRK3* fusions

Summary of Clinical Studies

The clinical validity of FoundationOne®CDx (F1CDx) for detecting *NTRK1, NTRK2,* or *NTRK3* fusions in patients with solid tumors who may benefit from treatment with larotrectinib was demonstrated in a clinical bridging study that consisted of the retrospective analysis of specimens from patients enrolled in the LOXO-TRK-14001 (Bayer 20288, NCT02122913), -15002 (Bayer 20289, NAVIGATE, NCT02576431), and -15003 (Bayer 20290, SCOUT, NCT02637687) clinical trials (referred to as 14001, 15002, and 15003, respectively) supplemented with *NTRK* fusion negative samples from the FMI clinical archive. Study 14001 is an on-going, multicenter, open-label, Phase 1 dose escalation study in adult patients with advanced solid tumors (all comers) unselected for *NTRK* gene fusion cancer. Following the dose escalation portion of the study, a dose expansion was initiated for patients with documented TRK fusion cancer and for patients who the Investigator believed might benefit from a highly selective TRK inhibitor. Study 15002 is an on-going multi-center, open-label, Phase 2 “basket” study in patients age 12 and older with recurrent advanced solid tumors with a documented *NTRK* gene fusion as assessed by an outside laboratory. Finally, Study 15003 is an on-going multi-center, open-label, Phase 1/2 study in pediatric patients aged from birth to 21 years with advanced solid or primary central nervous system (CNS) tumors.

NTRK fusion status to determine patient eligibility for enrollment was performed using local clinical trial assay (LCTAs) that included DNA next generation sequencing (NGS), RNA NGS, fluorescent in situ hybridization (FISH), and reverse transcriptase- polymerase chain reaction (RT-PCR) methods. The majority of 105 clinical trial patients with known *NTRK* fusion status enrolled into the trials had been tested with NGS methods (92%); 51% of the 105 patients had been tested with DNA NGS methods and 41% with RNA NGS methods. Of the 105 clinical trial patients, 78 patients were *NTRK* fusion positive and 27 were *NTRK* fusion negative. The assessment of efficacy of larotrectinib was based on the first 55 patients with solid tumors with an *NTRK* gene fusion enrolled across the three clinical trials. The primary endpoint was overall response rate (ORR) according to independent review committee assessment using RECIST v1.1 criteria. The ORR of the 55 patient set was 75%, 95% CI: [61%, 85%].

Accountability of the PMA Cohort

Of the 78 *NTRK* fusion positive patients and 27 *NTRK* fusion negative patients enrolled in 14001, 15002, and 15003, 45 patients and 24 patients, respectively, had samples available for testing with F1CDx for a total of 69 samples. Of the 69 samples, 67 samples had valid results and were used to support the clinical concordance analysis. Two(2) samples had invalid results due to failing F1CDx input criteria or low tumor purity. Of the 55 *NTRK* fusion positive patients in the efficacy set, 32 had samples available testing with F1CDx. F1CDx testing yielded 31 valid results to support the F1CDx efficacy analysis. One sample was invalid due to failing F1CDx input criteria.

In addition to clinical trial samples, 206 supplemental *NTRK* fusion negative samples as determined by the DNA NGS FoundationOne LDT were provided from the FMI clinical archive to support the clinical concordance study. Of these 206 samples that were re-tested on F1CDx, 203 samples had valid results.

FoundationOne®CDx Clinical Bridging Study for *NTRK*

A clinical bridging study was conducted to assess the clinical effectiveness of F1CDx in identifying *NTRK1, NTRK2,* or *NTRK3* fusion positive patients for treatment with larotrectinib, and to assess the concordance between *NTRK* fusion positive samples tested with the LCTAs and F1CDx. F1CDx was used to retrospectively test the available patient samples from studies 14001, 15002, and 15003 (N = 69) and the supplemental *NTRK* fusion negative samples (N = 206).

Safety Analysis

The F1CDx assay is not expected to directly cause actual or potential adverse effects, but test results may directly impact patient treatment risks. Refer to Drugs@FDA for complete safety information on VITRAKVI® (larotrectinib)

Effectiveness Results

Concordance Analysis

The concordance analysis between the F1CDx and the LCTAs using the clinical trial samples and supplemental negatives is shown in Table 58.

Table 58: Concordance between the F1CDx and LCTA methods for detection of *NTRK* gene fusions based on the LCTA results (all patients tested by CDx)

	Excluding CDx invalid results		Including CDx invalid results	
Measure of Agreement	**% Agreement (N)**	**95% CI [(a)]**	**% Agreement (N)**	**95% CI [(a)]**
Positive percent agreement	84.1% (37/44)	69.9% -93.4%	82.2% (37/45)	67.9% -92.0%
Negative percent agreement	100.0% (226/226)	98.4% -100.0%	98.3% (226/230)	95.6% -99.5%
Overall percent agreement	97.4% (263/270)	94.7% -99.0%	95.6% (263/275)	92.5% -97.7%

Abbreviations: CDx = Companion Diagnostic; CI = Confidence Interval; LCTA= Local Clinical Trial Assay; *NTRK* = Neurotrophic Tyrosine Kinase.
The LCTA inferred *NTRK*3 gene fusions were considered fusion positive.
a The 95% CI was calculated using the Clopper-Pearson exact method.

A sensitivity analysis against the 34 missing CDx results was conducted to assess the robustness of the agreement analysis. Missing CDx results for the LCTA fusion positive patients were imputed using a logistic regression model including 10 covariates (race, ethnicity, age group, stage of disease at initial diagnosis, prior cancer systemic treatments, prior cancer related surgery, ECOG performance status, *NTRK* fusion gene, LCTA method sample substrate, and binary clinical response to larotrectinib). Agreement estimates, including the imputed values, were PPA= 78.3%, 95% CI [64.4%, 89.9%] and NPA=100% (Table 59). The method of calculation for the 95% confidence interval accounted for both within and between imputation variance.

Table 59. Concordance between the CDx and LCTA methods for detection of NTRK gene fusions including imputed values in LCTA fusion positive patients with missing CDx results

Measure of Agreement	% Agreement	95% CI (a)
PPA	78.3%	64.4%, 89.9%
NPA	100.0%	100.0%, 100.0%
OPA	94.4%	90.5%, 97.4%

[a] The 95% CI was calculated based on multiple imputation (MI) Boot pooled sample method.

The F1CDx assay showed high concordance with the DNA NGS LCTA methods with PPA = 95%, 95% CI [75%, 100%] and NPA = 100%, 95% CI [98%, 100%] (Table 60).

Table 60. Concordance between the CDx and DNA NGS LCTA methods for detection of *NTRK* gene fusions based on LCTA results and excluding invalid results

Measure of Agreement	% Agreement (N)	95% CI [(a)]
PPA	95.0% (19/20)	75.1%, 99.9%
NPA	100.0% (221/221)	98.3%, 100.0%
OPA	99.6% (240/241)	97.7%, 100.0%

[a] The 95% CI was calculated based on Clopper-Pearson exact method.

However, the positive concordance of F1CDx with RNA NGS methods was lower (PPA = 70%, 95% CI [46%, 88%]) (Table 61).

Table 61. Concordance between the CDx and RNA NGS LCTA methods for detection of NTRK gene fusions based on LCTA results and excluding invalid results

Measure of Agreement	% Agreement (N)	95% CI [(a)]
PPA	70.0% (14/20)	45.7%, 88.1%
NPA	100.0% (4/4)	39.8%, 100.0%
OPA	75.0% (18/24)	53.3%, 90.2%

[a] The 95% CI was calculated based on Clopper-Pearson exact method.

F1CDx was concordant with FISH and RT-PCR based on testing of 5 samples (Table 62). Due to the low sample counts, agreement measures were not calculated.

Table 62. Contingency table comparing NTRK fusion detection results between the CDx and the FISH and RT-PCR LCTA methods (all patients tested by CDx)

F1CDx result by test method	LCTA positive	LCTA negative
FISH		
CDx Positive	3	0
CDx Negative	0	1
Total	3	1
RT-PCR		
CDx Positive	1	0
CDx Negative	0	0
Total	1	0

FISH = Fluorescence in Situ Hybridization; RT-PCR = ReverseTranscriptase-Polymerase Chain Reaction.

A total of 7 of the 275 samples tested with the F1CDx assay showed discordant results between F1CDx and the LCTAs. All 7 discordant results were *NTRK* fusion positive by the LCTA and fusion negative by F1CDx. Of the seven (7) discordant results, six (6) had been tested with an RNA NGS LCTA method and one (1) with an DNA NGS LCTA method.

The discordances between the RNA NGS LCTA methods and F1CDx can be explained by to differences in technology used to detect *NTRK1/2/3* fusions, as well as an expected degree of measurement error by the LCTAs and F1CDx. *NTRK* often presents complex genomic rearrangement events with a variety of breakpoints spanning multiple introns. This complexity of rearrangement events presents certain limitations for targeted DNA sequencing. F1CDx was designed to focus on hotspot introns that are repeatedly described in the literature, which means rare and complex breakpoints may not be captured by F1CDx baiting. The DX1 bait-set used by the F1CDx assay includes the coding regions of *NTRK1, NTRK2*, and *NTRK3* and select introns from these genes, however no introns within *NTRK3* are baited and the *NTRK1* intron 8, and *NTRK2* intron 12 are not fully baited. While the most common fusion partner of *NTRK3, ETV6*,

has several introns baited which allows for detection *ETV6-NTRK3* fusions, *EVT6* intron 5 is also not fully baited. A portion of fusion events between these two genes are likely being undetected as a result of DX1 also not baiting intron 4 of *ETV6*.

Of the seven (7) discordant patients, four (4) patients had complete or partial response, supporting that these four (4) samples were most likely true positives. Investigation findings concluded that F1CDx did not detect the fusion events in six (6) of the discordances for one of two reasons: 1) F1CDx does not bait the intron where the breakpoint occurred, or 2) the rearrangement event was too complex to be fully baited by F1CDx, and therefore the full picture of the event was not captured. The remaining one (1) discordant case could have been explained by time of sample collection and testing, since the sample tested by F1CDx was from a sample collected at a different timepoint than used for the LCTA test.

Clinical Efficacy Results

Clinical effectiveness of F1CDx was evaluated by estimation of clinical efficacy in the F1CDx-positive, LCTA-positive population. Clinical outcome was assessed by independent review committee using RECIST 1.1 criteria. Efficacy of larotrectinib in the F1CDx positive, LCTA-positive population was 77% (95% CI: [56%, 91%]) overall response rate (see Table 63). This is comparable to the efficacy for the NDA filing, where larotrectinib demonstrated an estimated 75% (95% CI: [61%, 85%]) overall response rate in the NDA efficacy population. Of the 26 F1CDx-positive patients in the efficacy set, six (6) (23%) patients had achieved a complete response and 14 (54%) had received a partial response with larotrectinib therapy (see Table 63).

Table 63. Primary efficacy results: the best overall response and overall response rate for *NTRK* fusion positive patients by LCTA and CDx results in the efficacy analysis set

Clinical outcome	LCTA fusion positive (N=55)	CDx fusion positive and LCTA fusion positive (N=26)	CDx fusion Negative and LCTA fusion positive (N=5)	CDx fusion results missing and LCTA fusion positive (N=24)
ORR% (95% CI[a])	75% (61%, 85%)	77% (56%, 91%)	80% (28%, 99%)	71% (49%, 87%)
Complete response	12 (22%)	6 (23%)	2 (40%)	4 (17%)
Partial response	29 (53%)[b]	14 (54%)	2 (40%)	13 (54%)[b]
Duration of Response[c]	**N=41**	**N=20**	**N=4**	**N=17**
Range (months)	1.6, 33.2	1.6, 20.3	3.7, 23.6	2.7, 33.2
% with duration ⩾ 6 months	73.2%	80.0%	50.0%	70.6%
% with duration ⩾ 9 months	63.4%	65.0%	50.0%	64.7%
% with duration ⩾ 12 months	39.0%	25.0%	50.0%	52.9%

[a] The 95% confidence interval was calculated using the Clopper-Pearson exact method.
[b] Includes one pediatric patient with unresectable infantile fibrosarcoma who underwent resection following partial response and who remained disease-free at data cutoff.
[c] Includes patients with ongoing response after data cutoff.

Twenty-four (24) patients have missing CDx results (i.e., 43.6% of the PAS population have missing results). Sensitivity analysis against the 24 missing CDx results was conducted to assess the robustness of the clinical efficacy analysis for the F1CDx positive patients. Missing CDx results for the LCTA fusion positive patients in the efficacy set were imputed 100 times using a logistic regression model including 9 covariates based on the missing at random (MAR) assumption.

Covariates identified included covariates imbalanced between the CDx evaluable and CDx non-evaluable sets, covariates associated with the F1CDx results and covariates associated with patient clinical response to larotrectinib. 9 covariates were used in the imputation model for the efficacy sensitivity analysis: race, ethnicity, age group, gender, stage of disease at initial diagnosis, ECOG performance status, *NTRK* fusion gene, LCTA method sample substrate, and binary clinical response to larotrectinib. Clinical efficacy, including the imputed CDx results, was ORR=74%, 95% CI [59%, 89%] (Table 64) and was similar to the results of the primary efficacy analysis (ORR=77%, 95% CI [56%, 91%]) (Table 63). However, it should be noted that the clinical effectiveness of F1CDx to identify patients with solid tumors with *NTRK1, NTRK2* or *NTRK3* fusions who may benefit from larotrectinib treatment is based on ~56% of the efficacy population.

Table 64. Sensitivity analysis for overall response rate by CDx result for NTRK fusion positive patients including imputed values for missing CDx results in the efficacy analysis set

Clinical Outcome	CDx fusion positive and LCTA fusion positive	CDx fusion negative and LCTA fusion positive
ORR% (95% CI [(a)])	74% (59% - 89%)	78% (46% - 100%)

[a] The 95% confidence interval was calculated based on MI Boot pooled sample method.

Sensitivity analysis was performed to estimate ORR in the total F1CDx positive population including the F1CDx positive, LCTA positive and the F1CDx positive, LCTA negative subpopulations. To assess the potential impact of the F1CDx positive, LCTA negative portion of the F1CDx positive intended use population on clinical effectiveness, 206 *NTRK* negative samples by the FoundationOne LDT were selected from the FMI clinical archive along with 24 *NTRK* negative clinical trial samples available for testing were used to obtain a NPA that was representative for the LCTAs used to enroll patients in the larotrectinib trials. Since the estimated NPA (PPV) is 100%, the ORR for the F1CDx positive population is the same as the ORR for the F1CDx positive and LCTA positive population. However, the NPA estimate between F1CDx and LCTA is subject to uncertainty and could be biased given that the majority of the *NTRK* fusion negative patients, in both the full population and those whose samples were available for testing with F1CDx had been tested using DNA NGS methods (>70%). FoundationOne LDT was the most commonly used DNA based NGS method that was used for *NTRK* fusion status determination for patients in the larotrectinib clinical trials. The same assay was also used to select the supplemental negative samples in the clinical bridging study as the representative LCTA. Therefore, the estimated NPA could be subject to bias.

Sensitivity analysis to determine the minimum PPV that will lead to an ORR of 30% at the lower bound of the two-sided 95% confidence interval for the CDx positive population was performed. This analysis was conducted to determine the NPA corresponding to this tipping point PPV by assuming fixed prevalence of NTRK fusion *(*0.32%)[12] and PPA (84%) observed from the concordance analysis to demonstrate the robustness of the study results.

For each value of c (the scaling factor for the assumed ORR (LCTA negative/F1CDx positive)), the tipping point PPV that led to an ORR of the F1CDx positive population with the lower bound of the two-sided 95% confidence interval at 30% was determined. When c is greater than or equal to 0.85, indicating the ORR in the LCTA negative/F1CDxpositive population is close to the ORR in the LCTA positive/F1CDxpositive population, the two-sided 95% lower confidence limit (LCL) of ORR is always greater than 30% so there is no tipping point of PPV. At all values of PPV (and NPA), the two-sided 95% LCL is > 30%. At c values between 0 and 0.8, a tipping point PPV ranges from 99.5% to 88.6% and NPA ranges from 100% to 99.97%.

Conclusions

The data from this study support reasonable assurance of the safety and effectiveness of the F1CDx assay when used to aid clinicians in identifying solid tumor patients harboring *NTRK1, NTRK2,* or *NTRK3* fusions that may be treated with VITRAKVI® larotrectinib.

3.17 Clinical evaluation of *FGFR2 Rearrangements* for treating patients with cholangiocarcinioma with infigratinib

The clinical performance of F1CDx for detecting *FGFR2* fusions and rearrangements in patients with previously treated cholangiocarcinoma who may benefit from treatment with infigratinib was established with clinical data generated from QED Therapeutics Study CBGJ398X2204 and a clinical bridging study to establish concordance between the clinical trial assays (CTAs) and the F1CDx assay.

3.17.1 Summary of the Clinical Study – QED CBGJ398X2204

Study CBGJ398X2204 (NCT02150967) is a prospective, multicenter, open-label, Phase 2 study of oral infigratinib in adult patients with previously treated, unresectable locally advanced or metastatic cholangiocarcinoma with *FGFR2* genetic alterations, including *FGFR2* gene fusions or rearrangements. A total of 122 patients were screened and enrolled into Cohort 1 as of 31 March 2020, which was the data cutoff for the clinical bridging study. Within the enrolled Cohort 1 population, 108 patients with *FGFR2* fusions or rearrangements (Interim Analysis Set 2) comprised the primary efficacy analysis set. The primary objective of the clinical study was to evaluate the efficacy of single-agent infigratinib in patients with locally advanced or metastatic cholangiocarcinoma as measured by overall response rate (ORR) and supported by duration of reponse (DoR), assessed by blinded independent central review (BICR) according to RECIST v1.1. The study also sought to evaluate the efficacy of infigratinib as measured by overall response assessed by investigator, progression free survival, best overall response, overall survival, disease control as assessed by investigator and by central imaging review as per RECIST 1.1 as secondary objectives. Additionally, CBGJ398X2204 aimed to characterize the safety, tolerability and pharmacokinetic profile of infigratinib. CBGJ398X2204 is an ongoing study that was initiated on 23 July 2014 with first patient first visit (FPFV). Patients received infigratinib as an oral monotherapy at 125 mg once daily for 21 consecutive days followed by 7 days off therapy, in 28-day cycles. Treatment continued until documented disease progression or unacceptable toxicity.

The assessment of efficacy was conducted using the Interim Analysis Set 2 for Cohort 1 of Study CBGJ398X2204 that consists of subjects who were enrolled in Cohort 1 with *FGFR2* gene fusions or rearrangements and received at least one dose of infigratinib (n=108). Patients were enrolled into the study based on molecular pre-screening results from a variety of clinical trial assays (CTAs) that detected *FGFR2* gene fusions or rearrangements.

3.17.2 Accountability of the PMA Cohorts

The first patient was enrolled in Study CBGJ398X2204 on 23 July 2014 (first patient first visit (FPFV)); a total of 122 patients were screened and enrolled into Cohort 1 as of 31 March 2020, the data cutoff for the clinical bridging study. Within the enrolled Cohort 1 population, the primary efficacy analysis set is comprised of 108 patients identified as having *FGFR2* rearrangements (Interim Analysis Set 2).

Of the 108 *FGFR2* rearrangement positive patient specimens available from CBGJ398X2204, 69 produced F1CDx-evaluable results. Nine (9) samples were screened for CBGJ398X2204 using F1CDx, so these banked samples were excluded from retrospective testing and concordance analysis. *FGFR2* negative samples used to support this clinical validation were drawn from the 14 patients in Cohort 1 who did not have FGFR2 rearrangements, yielding 11 F1CDx-evaluable results. In addition, 91 F1CDx-evaluable samples from *FGFR2* rearrangement negative cholangiocarcinoma patients identified from the FMI clinical database were included. All of the samples included in the clinical bridging study were

required to meet minimum sample input requirements. In total, 102 *FGFR2* rearrangement negative samples were F1CDx-evaluable and included in the clinical validation.

Retrospective F1CDx testing ultimately yielded 162 CDx-evaluable results (60 *FGFR2* rearrangement positive, 102 *FGFR2* rearrangement negative) which were used for the CDx and CTA concordance analysis. Clinical efficacy was evaluated in all *FGFR2* rearrangement positive samples and sensitivity analysis was performed to evaluate the impact of any missing results.

3.17.3 Summary of the Clinical Bridging Study

The aim of the bridging study was to determine the concordance between *FGFR2* rearrangement results from the enrolling CTAs generated at the time of patient screening for CBGJ398X2204 and the *FGFR2* rearrangement results from the retrospective analysis by F1CDx. The study was also conducted to establish the clinical utility of F1CDx in identifying patients positive for *FGFR2* rearrangements who may benefit from treatment with infigratinib.

A patient whose tumor harbors an *FGFR2* rearrangement containing an intact kinase domain is defined as eligible under the following conditions:

- *FGFR2* rearrangements with a literature-derived known partner gene regardless of strand or frame,
- *FGFR2* rearrangements in the same 5' to 3' orientation and in frame with a novel partner gene,
- *FGFR2* rearrangements with one breakpoint in the hotspot region (intron 17-exon 18) and the other breakpoint in an intergenic region or within another gene. This rule excludes 3' duplications of only exon 18,
- Intragenic duplication of the kinase domain (exon 9-17)

Retrospective testing with F1CDx was done for patients from Cohort 1 who received at least one dose of infigratinib prior to 31 March 2020. The bridging study population consisted of 213 patients (108 *FGFR2* rearrangement positive patients, and 105 *FGFR2* rearrangement negative patient samples).

Concordance between F1CDx and the CTA was demonstrated with the CDx-evaluable patient population from the CBGJ398X2204 study that produced valid F1CDx results. Clinical utility of F1CDx was evaluated by estimation of clinical efficacy in the CTA-enrolled *FGFR2* rearrangement positive patient population as assessed by the primary objective of ORR. Baseline demographic and disease characteristics were compared between the CDx-evaluable and CDx-unevaluable populations within all enrolled CTA-positive patients.

3.17.4 Clinical Concordance

A total of 162 samples were evaluated in the concordance evaluation between the CTAs and F1CDx. The concordance analysis population consisted of the F1CDx-evaluable samples, less 9 samples where F1CDx had been the enrolling CTA.

Concordance was evaluated using the results of PPA, NPA, PPV and NPV. The PPA and NPA, adjusted for a prevalence of 9.6% for *FGFR2* rearrangements in the cholangiocarcinoma population (based on clinical cholangiocarcinoma samples observed in the FMI database), with corresponding 95% 2-sided Wilson score CIs using the CTA as reference, were established as 96.67% (95% CI 88.64-99.08%) and 100.00% (95% CI 96.37-100.00%), respectively. The PPV and NPV, adjusted for a prevalence of 9.6% for FGFR2 rearrangements in the cholangiocarcinoma population (based on clinical cholangiocarcinoma samples observed in the FMI database) were established as 100.00% (95% 2-sided score CI 93.79-100.00%) and 99.65% (95% 2-sided bootstrap percentile CI 99.09-100.00%), respectively.

The results of the agreement analysis demonstrated concordance between the F1CDx assay and the CTA.

3.17.5 Efficacy Evaluation

3.17.5.1 Clinical efficacy results in Study CBGJ398X2204 Cohort 1 – previously treated CCA with an FGFR2 fusion or rearrangement

The primary efficacy outcome measures were overall response rate (ORR) and duration of response (DoR), as determined by blinded independent central review (BICR) according to Response Evaluation Criteria in Solid Tumors (RECIST) v1.1. Treatment with infigratinib was considered efficacious in Cohort 1 (previously treated CCA with an *FGFR2* fusion or rearrangement) as demonstrated by an ORR per BICR of 23.1% (95% CI: 15.6, 32.2) (Table 65 below). Robustness of the data was further confirmed by the supportive analysis of ORR and DoR by investigator assessment and for key subgroups.

Table 65: Efficacy Results from CBGJ398X2204 Study per BICR Assessment

Outcome measurement	Infigratinib, N=108
ORR (95% CI)	23.1 (15.6, 32.2)
Complete response, n (%)	1 (1%)
Partial response, n (%)	24 (22.2%)

The study CBJ398X2204 results indicate that infigratinib monotherapy provides clinically meaningful ORR and DoR in previously treated patients with unresectable locally advanced or metastatic cholangiocarcinoma with an *FGFR2* gene fusion or rearrangement.

3.17.5.2 Clinical efficacy results in the CDx-positive population

The ORR was evaluated in patients identified as being *FGFR2* rearrangement positive by the CTAs and F1CDx. This population consisted of 67 patients, of which 19 were identified as responders, as assessed by BICR review according to RECIST v1.1.

The clinical efficacy for the F1CDx-positive population ($ORR_{(\delta CDx+)}$) was calculated by a weighted sum of the clinical efficacy of patients enrolled by the F1CDx assay, denoted as ORR_{F1CDx}, and the clinical efficacy bridged from patients enrolled by the CTA assay, denoted as ORR_{bridge}. Table 66 summarizes the results of this analysis. The clinical efficacy of the F1CDx-positive population was 28.07% (95% CI 17.22-38.92%) which aligns closely with the ORR of the CTA-positive population at 23.15% (95% CI 16.20-31.94%).

Table 66. Bridging Study Estimated Drug Efficacy for F1CDx+ on Observed Data

ORR_{F1CDx} (%)	ORR_{bridge} (%)	$ORR(\widehat{\delta}_{CDx+})$ (%)	95% 2-sided CIs (%)
33.33	27.59	28.07	(17.22, 38.92)

The ORR in the CDx-positive CTA-positive population (i.e., double positive) was 28.36% (95% 2-sided score CIs: 18.97%-40.09%).

Sensitivity analysis, using the multiple imputation method was performed to assess the robustness of clinical efficacy in the CDx-positive population against the 39 missing CDx results. The sensitivity results

were very close to the observed clinical efficacy of the CTA (23.15%), which demonstrates the robustness of the clinical efficacy analysis. The 95% 2-sided CIs for all four of the imputation methods contained the observed clinical efficacy for the F1CDx positive population, which demonstrates the ability of F1CDx to identify patients most likely to benefit from treatment with infigratinib.

3.17.5.3 Safety Analysis

The F1CDx assay is not expected to directly cause actual or potential adverse effects, but test results may directly impact patient treatment risks.

3.17.6 Conclusions

The data from this study support reasonable assurance of the safety and effectiveness of the F1CDx assay when used to aid clinicians in identifying CCA patients with *FGFR2* fusions and rearrangements who may be eligible for treatment with infigratinib.

3.18 Clinical Evaluation of KEYTRUDA (pembrolizumab) in patients with MSI-H solid tumors

A clinical bridging study using 444 patient samples from KEYNOTE-158 Cohort K (n=321) and KEYNOTE-164 (n=123) was conducted to establish the clinical validity of the F1CDx as a CDx for pembrolizumab in MSI-H solid tumors. KEYNOTE-158 is an ongoing multicenter, global, open-label trial of KEYTRUDA in patients with multiple types of advanced (unresectable and/or metastatic) cancers who have failed prior therapy. All patients enrolled in this study had a histologically or cytologically documented, advanced solid tumor that was incurable and for which prior standard first-line treatment had failed. Patients had progressed on or were intolerant to therapies that are known to provide clinical benefit. All patients received pembrolizumab 200 mg Q3W. All patients enrolled in this study had a histologically or cytologically documented, advanced solid tumor that was incurable and for which prior standard first-line treatment had failed. Patients had progressed on or were intolerant to therapies that are known to provide clinical benefit. All patients received pembrolizumab 200 mg Q3W. KEYNOTE-158 Cohort K enrolled patients with unresectable or metastatic MSI-H/dMMR solid tumors (except CRC). KEYNOTE-164 is a multicenter, multicohort, single arm, open-label trial designed to evaluate the efficacy of pembrolizumab in previously treated patients with unresectable or metastatic MSI-H/dMMR CRC tumors. Local IHC or PCR assays were primarily used to enroll KEYNOTE-158 Cohort K and KEYNOTE-164 participants. All participants received pembrolizumab 200 mg Q3W.

Primary objectives of KEYNOTE-158 Cohort K and KEYNOTE-164 were to evaluate ORR per RECIST 1.1 (assessed by central imaging) to pembrolizumab. Secondary objectives included assessment of DOR, PFS and OS in the pembrolizumab treated participants. The data cut-off date for the clinical efficacy analyses for KEYNOTE-158 was October 05, 2020 and September 09, 2019 for KEYNOTE 164.

Samples from KEYNOTE-158 Cohorts A-J patients (patients in rare tumor non-CRC cohorts who had failed prior therapy and tumor bank samples were additionally tested with F1CDx to determine the NPA of the F1CDx vs. CTA. Sample accounting is included in Table 67.

Table 67: F1CDx Sample Accounting Across Cohorts

STUDY	F1CDx Tested	F1CDx Evaluable
All	1664	1189
KN158 Cohort K	168	104
KN158 Cohort A to J	1006	716

KN164	81	62
Tumor Bank	409	307

Together 28.47% of samples, 473 out of 1664, failed to provide F1CDx valid results. Of these 473, 132 did not provide F1CDx valid results due to MSI QC failures.

Concordance Analysis

Table 68 shows concordance between the CTA and F1CDx evaluable samples across solid tumors There were 15 CTA non-evaluable samples (14 from KEYNOTE-158 Cohort A-J and 1 tumor bank) out of 1189 F1CDx evaluable samples and these samples were therefore excluded from the concordance analyses, leaving 1,174 for the analyses.

Table 68: Concordance between CTA and F1CDx

Tumor Type	N	CTA+ F1CDx+	CTA+ F1CDx-	CTA- F1CDx+	CTA- F1CDx-	PPA % (95% CI)	NPA % (95% CI)	OPA % (95% CI)
All	1174	134	58	7	975	69.8 (63.0, 75.8)	99.3 (98.5, 99.7)	94.5 (93.0, 95.6)
Colorectal	151	60	13	0	78	82.2 (71.9, 89.3)	100.0 (95.3, 100.0)	91.4 (85.8, 94.9)
Endometrial	101	28	4	5	64	87.5 (71.9, 95.0)	92.8 (84.1, 96.9)	91.1 (83.9, 95.2)
Gastric	66	11	3	0	52	78.6 (52.4, 92.4)	100.0 (93.1, 100.0)	95.5 (87.5, 98.4)
Ovarian	10	7	3	0	0	70.0 (39.7, 89.2)	NA	70.0 (39.7, 89.2)
Others	846	28	35	2	781	44.4 (32.8, 56.7)	99.7 (99.1, 99.9)	95.6 (94.0, 96.8)

CTA-Positive includes samples from KEYNOTE-158 Cohorts A-K and Tumor Bank
CTA-Negative includes sample from KEYNOTE-158 Cohorts A-J and Tumor Bank

Demographic Characteristics

For the clinical device bridging study, baseline characteristics were compared between the CTA positive, F1CDx evaluable and F1CDx non-evaluable populations.

As noted above, the CTA-positive population consists of 444 patients, 321 from KEYNOTE-158 Cohort K and 123 from KEYNOTE-164. Among these 444 patients, the baseline characteristics were: median age of 59 years, 36% ≥ 65 years of age; 46% male; 78% White, 13% Asian, and 4% Black; and 44% had an ECOG PS of 0 and 56% had an ECOG PS of 1. Ninety-three percent (93%) of patients had metastatic disease. Sixty-two percent (62%) of patients received 2 or more prior lines of therapy.

Clinical Efficacy Results

The clinical validity of F1CDx for the detection of MSI-H status in patients with solid tumors was based on estimation of clinical efficacy in the F1CDx-positive, CTA-positive population. The major efficacy outcome measure was ORR per RECIST 1.1 (assessed by central imaging). ORR for the CTA positive, F1CDx positive/CTA positive, F1CDx negative/CTA positive, and F1CDx missing/CTA positive are presented in Table 69.

Table 69: Efficacy Results in KEYNOTE-164 and KEYNOTE-158 Cohort K Combined

Clinical outcome	CTA positive (N=444)	F1CDx positive and CTA positive (N=107)	F1CDx negative and CTA positive (N=58)	F1CDx result missing and CTA positive (N=279)
ORR% (95% CI[(a)])	31.8% (27.4, 36.3)	43.0% (33.5, 52.9)	12.1% (5.0, 23.3)	31.5% (26.1, 37.3)
Complete response	38 (8.6%)	13 (12.1%)	2 (3.4%)	23 (8.2%)
Partial response	103 (23.2%)[b]	33 (30.8%)	5 (8.6%)	65 (23.3%)
Duration of Response	**N=141**	**N=46**	**N=7**	**N=88**
Median in months (range)	NR (2.1+ - 51.1+)	NR (3.7+ - 46.2+)	15.1 (6.1+ - 32.2+)	NR (2.1+ - 51.1+)
% with duration ⩾ 6 months	129 (95.6)	43 (95.6)	7 (100.0)	79 (95.2)
% with duration ⩾ 12 months	104 (90.1)	36 (88.6)	4 (83.3)	64 (91.4)

(a) Based on binomial exact confidence interval method.

Database Cutoff Date:
KEYNOTE 164: September 09, 2019,
KEYNOTE 158: October 05, 2020

The ORR in the CTA-positive population was 31.8% (141/444), (95% CI: 27.4, 36.3). There were 53 CTA-positive participants who also had F1CDx results with partial or complete responses. Among them 86.8% (46/53) were positive by F1CDx (95% CI: 74.7, 94.5). There were 112 CTA-positive participants who also had F1CDx results with no responses. Among the 112 CTA positive patients who did not respond to KEYTRUDA, only 54.5% (61/112) were positive by F1CDx (95% CI: 44.8, 63.9). Taken together, F1CDx has a higher percent of positive results among participants with responses than among participants without responses [difference between 86.8% (46/53) and 54.5% (61/112) was 32.3% with 95% CI: (17.6, 44.6)].

The ORR in F1CDx-positive/CTA-positive participants was 43.0% (46/107), (95% CI: 33.5, 52.9). The ORR in F1CDx-negative/CTA-positive participants was 12.1% (7/58), (95% CI: 5.0, 23.3). The ORR in F1CDx-positive/CTA-positive participants was higher than the ORR in F1CDx-negative/CTA-positive participants [difference between 43.0% (46/107) and 12.1% (7/58) was 30.9% with 95% CI: (16.7, 42.8)].

The similarity of the ORR for the CTA-positive population (n=444) overall (31.8%, 95% CI: 27.4, 36.3) and for those missing a valid F1CDx result (n=279; 31.5%, 95% CI: 26.1, 37.3) suggests no overt imbalance in efficacy effect of pembrolizumab between patients on whom the F1CDx was or was not obtained.

Sensitivity Analysis

Sensitivity analyses with regard to missing values were conducted to evaluate the robustness of the ORR estimates in consideration of the subjects with missing/invalid CDx results and the missing F1CDx-positive, CTA-negative population that was not enrolled and evaluated by KEYNOTE-158 Cohort K and KEYNOTE-164 clinical studies.

To evaluate the impact of missing/invalid F1CDx results, the distribution of patients for baseline covariates, disease characteristics, tumor organ system, and tumor types was compared among the CTA-positive population, the F1CDx-evaluable/CTA-positive subpopulation, and F1CDx-missing CTA-positive subpopulation. A multiple imputation method was utilized to account for patients with missing or non-evaluable F1CDx MSI tumor status (n=279). The imputation model

included the clinical outcome and covariates that are considered predictive of missingness of the F1CDx tumor status and showing some predictive value of the F1CDx tumor status.

The clinical efficacy (ORR) for the F1CDx-positive subjects in the device intended use population was estimated under different assumed scenarios based on observed and imputed F1CDx results.

For the F1CDx-positive, CTA-negative population that was not enrolled and evaluated by KEYNOTE-158 Cohort K and KEYNOTE-164 clinical studies, bridging equations that involved an ORR attenuation factor that ranges from 0 (assume full attenuation of the efficacy in CTA-negative/F1CDx-positive) to 1 (assume no attenuation of the efficacy in CTA-negative/F1CDx-positive compared to the observed ORR in F1CDx-positive patients in the efficacy population) were used for the clinical efficacy analysis in this missing population.

Sensitivity analysis considering the NPA and assuming different CTA positivity rates in the F1CDx intended use population, which ranged 2-5%, were investigated to assess influence on the efficacy estimated for the intended use, i.e., F1CDx positive subjects. These sensitivity analyses demonstrated the robustness of the clinical efficacy estimate from the primary analysis.

Subgroup Analyses
Response to KEYTRUDA for the CTA positive, F1CDx-positive/CTA-positive, F1CDx-negative/CTA-positive and F1CDx-missing/CTA-positive the F1CDx patients was analyzed by primary tumor type. Within the F1CDx positive patients for the efficacy set, 13 tumor types were represented.
Response rates by tumor types are included in Table 70.

Table 70: ORR Estimates per tumor type in Subpopulations by F1CDx Status

Tumor Type	Responder (n) / Subpopulation (N), ORR% (95% CI)			
	CTA-Positive (N=444)	F1CDx-Positive and CTA-Positive (N=107)	F1CDx-Negative and CTA-Positive (N=58)	F1CDx-result missing and CTA-Positive (N=279)
CRC	42/123, 34.1% (25.8, 43.2)	19/48, 39.6% (25.8 54.7)	1/13, 7.7% (0.2, 36.0)	22/62, 35.5% (23.7, 48.7)
Non-CRC	99/321, 30.8% (25.8, 36.2)	27/59, 45.8% (32.7, 59.2)	6/45, 13.3% (5.1, 26.8)	66/217, 30.4% (24.4, 37.0)
Endometrial	33/68, 48.5% (36.2, 61.0)	5/17, 29.4% (10.3, 56.0)	3/4, 75.0% (19.4, 99.4)	25/47, 53.2% (38.1, 67.9)
Gastric	13/42, 31.0% (17.6, 47.1)	6/11, 54.5% (23.4, 83.3)	0/3, 0.0% (0.0, 70.8)	7/28, 25.0% (10.7, 44.9)
Ovarian	8/24, 33.3% (15.6, 55.3)	5/7, 71.4% (29.0, 96.3)	0/3, 0.0% (0.0, 70.8)	3/14, 21.4% (4.7, 50.8)
Small Intestine	12/25, 48.0% (27.8, 68.7)	3/9, 33.3% (7.5, 70.1)	NA	9/16, 56.3% (29.9, 80.2)
Cholangio carcinoma	9/22, 40.9% (20.7, 63.6)	3/4, 75.0% (19.4, 99.4)	1/3, 33.3% (0.8, 90.6)	5/15, 33.3% (11.8, 61.6)
Breast	1/11, 9.1% (0.2, 41.3)	1/1, 100.0% (2.5, 100)	0/1, 0.0% (0.0, 97.5)	0/9, 0.0% (0.0, 33.6)

Pancreatic	4/22, 18.2% (5.2, 40.3)	2/2, 100.0% (15.8, 100.0)	0/2, 0.0% (0.0, 84.2)	2/18, 11.1% (1.4, 34.7)
Brain	1/17, 5.9% (0.1, 28.7)	NA	1/2, 50.0% (1.3, 98.7)	0/15, 0.0% (0.0, 21.8)
Sarcoma	3/14, 21.4% (4.7, 50.8)	NA	0/7, 0.0% (0.0, 41.0)	3/7, 42.9% (9.9, 81.6)
Neuro endocrine	2/12, 16.7% (2.1, 48.4)	NA	0/6, 0.0% (0.0, 45.9)	2/6, 33.3% (4.3, 77.7)
Other	13/64, 20.3% (11.3, 32.2)	2/8, 25.0% (3.2, 65.1)	1/14, 7.1% (0.2, 33.9)	10/42, 23.8% (12.1, 39.5)

Database Cutoff Date:
KEYNOTE 164: September 9, 2019,
KEYNOTE 158: October 5, 2020

In the two (2) tumor types with the highest MSI-H prevalence across solid tumors, the ORR in F1CDx-positive/CTA-positive subpopulation CRC (n=48) and endometrial cancer (n=17) were 39.6% (95% CI: 25.8, 54.7) and 29.4% (95% CI: 10.3, 56.0) respectively. For non-CRC tumors combined, the ORR in F1CDx-positive/CTA-positive subpopulation (n=59) was 45.8% (95% CI: 32.7, 59.2).

While the point estimate for ORR in endometrial cancer patients with F1CDx-positive/CTA-positive status is lower than the point estimate for CTA positive patients, efficacy data from KEYNOTE 158 Cohort D, which enrolled endometrial cancer patients provides additional efficacy data for the F1CDx positive endometrial cancer population. There were 17 KEYNOTE-158 Cohort D patients that were determined to be F1CDx MSI-H; Seven out of the 17 F1CDx MSI-H (positive) patients were responders, and thus the ORR in these 17 F1CDx-positive patients was 41.2% (95% CI: 18.4, 67.1).

Effectiveness Conclusions

The data from the analytical validation and clinical device bridging studies support the reasonable assurance of safety and effectiveness of the F1CDx assay when used in accordance with the indications for use as an aid in identifying MSI-H status in patients with solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options who may benefit from treatment with KEYTRUDA. Data from the KEYNOTE-158 and KEYNOTE 164 trials with data cut-off dates of October 05, 2020 and September 09, 2019, respectively, demonstrate that patients who had MSI-H status received benefit from treatment with KEYTRUDA and support the addition of the proposed CDx indication to F1CDx.

Safety Conclusions

The F1CDx assay is an *in vitro* diagnostic test, which involves testing of DNA extracted from FFPE tumor tissue. The assay can be performed using DNA extracted from existing (archival) tissue samples routinely collected as part of the diagnosis and patient care. The risks of the device are assessed based on data collected in the clinical study conducted to support PMA approval as described above. Risks of the F1CDx assay are associated with failure of the device to perform as expected or failure to correctly interpret test results and, subsequently, inappropriate patient management decisions in cancer treatment.

Patients with false positive results may undergo treatment with KEYTRUDA without clinical benefit and may experience adverse reactions associated with KEYTRUDA therapy. Patients with false negative results may not be considered for treatment with KEYTRUDA.

There is also a risk of delayed results, which may lead to delay of treatment with KEYTRUDA when patients receive an MSI "Cannot be Determined" result by the F1CDx assay. Patients with MSI "Cannot be Determined" result due an FB-MSI score >0.0041 and <0.0124, should be re-tested with a validated orthogonal (alternative) method as these MSI scores represent a range of scores with low reliability. The likelihood of a patient receiving this result is 3.29% within solid tumors. Patients with an MSI- "Cannot Be Determined" result due to a post-sequencing quality control (QC) failure should consider re-testing with FoundationOneCDx or a validated orthogonal (alternative) method, if clinically appropriate. The likelihood of a patient receiving an invalid result for MSI status which takes into account samples failing to meet QC criteria defined within pathology review, DNA extraction, Library Construction, and Hybrid Capture, genomic analysis and MSI QC specific criteria is approximately 21.5% to 28.5% within solid tumors as observed in within the F1CDx clinical commercial testing and device clinical bridging study.

Refer to Drugs@FDA for complete safety information on pembrolizumab (KEYTRUDA).

3.18.1 Post-Approval Study to Support the Clinical Effectiveness of F1CDx for the Treatment with KEYTRUDA

An additional post approval study was conducted to evaluate the clinical effectiveness of F1CDx by retrospectively testing 41 patient samples from patients with non-CRC solid tumors with MSI-H/dMMR status as determined by CTAs enrolled and treated with KEYTRUDA in Keynote 158 cohort K. Of these 41 patient samples, 31 patients had F1CDx results that passed QC criteria, i.e., yielded evaluable/valid results with F1CDx. Of the 31 patients with F1CDx evaluable results, 21 were confirmed by F1CDx as MSI-H. The remaining 10 patients did not have MSI-H status confirmed by F1CDx; 8 patients had MSS status and 2 had MSI-cannot be determined status due to MSI scores being >0.0041 but <0.0124. The ORR in the 21 patients with confirmed MSI-H by F1CDx was 42.9%; 95% CI: 21.8, 66.0. The ORR in the 10 patients that did not have MSI-H status confirmed by F1CDx was 30%; 95% CI: 6.7, 65.2. The ORR in the F1CDx non-evaluable population, i.e., had invalid results by F1CDx, was 10%; 95%CI:0.3, 44.5. The results of the post approval study further confirmed the clinical effectiveness for F1CDx to identify patients with solid tumors with MSI-H status that may benefit from KEYTRUDA therapy.

3.19 Clinical Evaluation of ROZLYTREK (entrectinib) in Solid Tumor Patients with *NTRK* Fusions

The clinical effectiveness of F1CDx for detecting *NTRK1*, *NTRK2*, *NTRK3* (NTRK) fusions in solid tumors of patients who may benefit from treatment with entrectinib was demonstrated in a retrospective analysis of specimens from patients enrolled in clinical trials ALKA-372-001 (ALKA), RXDX-101-01 (STARTRK-1), and RXDX-101-02 (STARTRK-2).

3.19.1 Summary of the Clinical Studies

ALKA was a phase 1 dose escalation study of entrectinib in adult patients with advanced metastatic solid tumors. STARTRK-1 was a phase 1, multicenter, open-label study of entrectinib in adult patients with locally advanced or metastatic cancer confirmed to be positive for *NTRK1*, *NTRK2*, *NTRK3*, or NSCLC patients harboring *ROS1* fusions. STARTRK-2 is an open-label, multicenter basket study of entrectinib for the treatment of patients with solid tumors that harbor *NTRK1*, *NTRK2*, *NTRK3*, or NSCLC patients *ROS1* rearrangements.

3.19.2 Accountability of the PMA Cohort

A total of 330 unique samples were evaluated, including 52 clinical trial samples and 278 procured samples. Initially, the clinical bridging study included 54 *NTRK* efficacy evaluable patient samples, as well as 20 additional *NTRK* patients who were enrolled between Dec 1, 2017 and Apr 30, 2018. Ultimately, 41 *NTRK* efficacy evaluable patient samples and 11 additional *NTRK* patients who were enrolled between Dec 1, 2017 and Apr 30, 2018 had sufficient material to support clinical bridging.

3.19.3 Summary of the Clinical Bridging Study

The bridging study was conducted to assess: 1) concordance between the local clinical trial assays (CTAs) and F1CDx ; and 2) estimate the overall response rate (ORR) in the efficacy population (CTA-positive population) for entrectinib treatment among clinical study participants whose tumor samples met the biomarker criteria, as determined by retrospective testing with the F1CDx.

3.19.4 Clinical Concordance

A total of 265 samples were evaluated in the concordance evaluation between F1CDx and the CTAs which included a wide range of assays, including RNA-based assays. The concordance analysis population consisted of the F1CDx-evaluable samples.

Concordance was evaluated using the results of PPA and NPA. The PPA and NPA, presented in **Table 71**, were established as 63.6%% (CI 46.6-77.8%) and 100.00% (CI 98.4-100.00%), respectively.

Table 71. Concordance for *NTRK* Fusions between F1CDx and the CTAs

		CTAs		
		Detected	Not Detected	Total
F1CDx	Detected	21	0	21
	Not Detected	12	232	244
	Total	33	232	265
Agreement Statistics with CTAs as comparator		PPA_{CTAs} 63.6% [46.6%, 77.8%]*	NPA_{CTAs} 100.00% [98.4%, 100.0%]*	

* Confidence intervals calculated using the Wilson score method

3.19.5 Efficacy Evaluation

3.19.5.1 Clinical efficacy results in the CDx-positive population for solid tumor patients with *NTRK1, 2, 3* fusions treated with entrectinib

The clinical efficacy of entrectinib in the pivotal clinical trial was measured in Overall Response Rate (ORR) with either confirmed complete response (CR) or partial response (PR) based on Blinded Independent Central Review (BICR). Only clinical samples with clinical outcome data were used in this analysis.

The ORR for all 74 samples of this study (CTA+) population was 62.2% (46/74) with Exact 2-sided 95% CI [50.1, 73.2]. Seventeen (17) patients (17/21) were CTA+ and exhibited F1CDx *NTRK*-positive results. The ORR for this population was 81.0% with Exact 2-sided 95% CI [58.1, 94.6]. Eleven (11) patients were CTA+ but F1CDx *NTRK*-negative. The ORR for this population was 36.4% (4/11) with Exact 2-sided 95% CI [10.9, 69.2]. Forty-two (42) patients were CTA+ but without a F1CDx *NTRK* result. The ORR for this population was 59.5% (25/42) with Exact 2-sided 95% CI [43.3, 74.4], as summarized in Table 72.

Table 72. Efficacy by *NTRK* Status in Biomarker Subgroups

Clinical outcome	CTA positive popuation (N=74)	F1CDx positive and CTA positive (N=21)	F1CDx negative and CTA positive (N=11)	F1CDx result missing and CTA positive (N=42)
ORR% [95% CI*]	62.2% [50.1, 73.2]	81.0 [58.1, 94.6]	36.4 [10.9, 69.2]	59.5 [43.3, 74.4]
Complete response	5 (6.8%)	3 (14.3%)	0 (0%)	2 (4.8%)
Partial response	41 (55.4%)	14 (66.7%)	4 (36.4%)	23 (54.8%)
Number of responders	**N=46**	**N=17**	**N=4**	**N=25**
Duration of Response				
Median in months (range)	7.4 (1.4-26.0)	9.2 (1.9-22.1)	11.1 (1.4-26.0)	7.1 (2.8-25.9)
% with duration ≥ 6 months	54.3% (25/46)	52.9% (9/17)	75% (3/4)	52% (13/25)
% with duration ≥ 9 months	43.5% (20/46)	52.9% (9/17)	75% (3/4)	32% (8/25)
% with duration ≥ 12 months	30.4% (14/46)	35.3% (6/17)	50% (2/4)	24% (6/25)

***Exact 2-sided 95% CI reported**

3.19.5.2 Safety Analysis

The F1CDx assay is not expected to directly cause actual or potential adverse effects, but test results may directly impact patient treatment risks.

3.19.5.3 Conclusions

The data from this study support reasonable assurance of the safety and effectiveness of the F1CDx assay when used to aid clinicians in identifying solid tumor patients with *NTRK1, 2,* or *3* fusions who may be eligible for treatment with entrectinib.

3.20 Clinical Evaluation of ROZLYTREK (entrectinib) in NSCLC Patients with *ROS1* Fusions

The clinical effectiveness of F1CDx for detecting *ROS1* fusions in NSCLC patients who may benefit from treatment with entrectinib was demonstrated in a retrospective analysis of specimens from patients enrolled in clinical trials ALKA-372-001 (ALKA), RXDX-101-01 (STARTRK-1), and RXDX-101-02 (STARTRK-2).

3.20.1 Summary of the Clinical Studies

ALKA was a phase 1 dose escalation study of entrectinib in adult patients with advanced metastatic solid tumors. STARTRK-1 was a phase 1, multicenter, open-label study of entrectinib in adult patients with locally advanced or metastatic cancer confirmed to be positive for *NTRK1*, *NTRK2*, *NTRK3*, or NSCLC patients harboring *ROS1* fusions. STARTRK-2 is an open-label, multicenter basket study of entrectinib for the treatment of patients with solid tumors that harbor *NTRK1*, *NTRK2*, *NTRK3*, or NSCLC patients *ROS1* rearrangements.

3.20.2 Accountability of the PMA Cohort

A total of 395 unique samples were evaluated, including 85 clinical trial samples and 310 procured samples. Initially, the clinical bridging study included 51 ROS1 NSCLC efficacy evaluable samples, as well as 41 additional ROS1-positive, ROS1 inhibitor-naive NSCLC patients with measurable disease who had insufficient follow-up (<12 months) at time of the NDA submission and an additional 67 ROS1 NSCLC patients who were enrolled prior to October 31, 2018. In total, clinical outcome data from 159 patients enrolled before October 31, 2018 based on the May 1, 2019 clinical data cutoff date were planned for use in the bridging analysis. Ultimately 85 of these clinical trial samples were available to support the clinical bridging analysis; 16 ROS1 NSCLC efficacy evaluable samples, 21 additional ROS1-positive, ROS1 inhibitor-naive NSCLC patients with measurable disease who had insufficient follow-up (<12 months) at time of the NDA submission and an additional 48 ROS1 NSCLC patients who were enrolled prior to October 31, 2018.

3.20.3 Summary of the Clinical Bridging Study

The clinical efficacy analysis was performed by analyzing the concordance between F1CDx and the enrollment CTAs, followed by the imputation of the missing F1CDx result to then determine the clinical outcome of the *ROS1*-rearrangement positive population identified with F1CDx.

The *ROS1* clinical efficacy population (n=51) consisted of nine (9) patients from ALKA, seven (7) from STARTRK-1, and 35 patients from STARTRK-2. *ROS1* positivity was determined by NGS in 71% and by FISH in 29% of the study patient population. Fifty-five percent (55%) had central laboratory confirmation of *ROS1* positivity using the study clinical trial assay (CTA). The ORR of the *ROS1*-positive NDA population was 78%. The 95% Confidence Interval (CI) was [65%, 89%].

3.20.4 Clinical Concordance

A total of 291 samples were evaluated in the concordance evaluation between F1CDx and the CTAs which included a wide range of assays, including RNA-based assays. The concordance analysis population consisted of the F1CDx-evaluable samples.

Concordance was evaluated using the results of PPA and NPA. The PPA and NPA, presented in **Table 73**, were established as 73.9%% (95% CI 59.7-84.4%) and 99.2% (95% CI 97.1-99.8%), respectively.

Table 73. Concordance for *ROS1* Fusions between F1CDx and the CTAs

		CTAs		
		Detected	Not Detected	Total
F1CDx	Detected	34	2	36
	Not Detected	12	243	255
	Total	46	245	291
Agreement Statistics with CTAs as comparator		PPA_{CTAs} 73.9% [59.7%, 84.4%]	NPA_{CTAs} 99.2% [97.1%, 99.8%]	

* Confidence intervals calculated using the Wilson score method

3.20.5 Efficacy Evaluation

3.20.5.1 Clinical efficacy results in the CDx-positive population for NSCLC patients with *ROS1* fusions treated with entrectinib

The clinical efficacy of entrectinib in the pivotal clinical trial was measured in Overall Response Rate (ORR) with either confirmed complete response (CR) or partial response (PR) based on Blinded Independent Central Review (BICR). Only clinical samples with clinical outcome data were used in this analysis.

The ORR in the CTA-positive population was 67.3% (107/159), (95% CI: 59.4, 74.5). Thirty-four (34) patients (34/46) were CTA+ and exhibited F1CDx *ROS1*-positive results. The ORR for this population was 64.7% (22/34) with Exact 2-sided 95% CI [46.5, 80.3]. Twelve (12) patients were CTA+ but F1CDx *ROS1*-negative. The ORR for this population was 58.3% (7/12) with the Exact 2-sided 95% CI [27.7, 84.8].

One-hundred thirteen (113) patients were CTA+ but without a F1CDx *ROS1* result. The ORR for this population was 69.0% (78/113) with Exact 2-sided 95% CI [59.6, 77.4], as summarized in Table 74.

Table 74. Efficacy by *ROS1* Status in Biomarker Subgroups

Clinical outcome	CTA positive population (N=159)	F1CDx positive and CTA positive (N=34)	F1CDx negative and CTA positive (N=12)	F1CDx result missing and CTA positive (N=113)
ORR% [95% CI*]	67.3% [59.4, 74.5]	64.7 [46.5, 80.3]	58.3 [27.7, 84.8]	69.0 [59.6, 77.4]
Complete response	14 (8.8%)	3 (8.8%)	0 (0%)	11 (9.7%)
Partial response	93 (58.5%)	19 (55.9%)	7 (58.3%)	67 (59.3%)
Number of responsders	**N=107**	**N=22**	**N=7**	**N=78**
Duration of Response				
Median in months (range)	9.5 (1.8-42.3)	10.1 (1.9-24.6)	9.5 (3.5-24.6)	9.5 (1.8-42.3)
% with duration ≥ 9 months	61.7% (66/107)	72.7% (16/22)	57.1% (4/7)	59.0% (46/78)
% with duration ≥ 12 months	41.1% (44/107)	36.4% (8/22)	42.9% (3/7)	42.3% (33/78)
% with duration ≥ 18 months	19.6% (21/107)	4.5% (1/22)	14.3% (1/7)	24.4% (19/78)

***Exact 2-sided 95% CI reported**

3.20.5.2 Safety Analysis

The F1CDx assay is not expected to directly cause actual or potential adverse effects, but test results may directly impact patient treatment risks.

3.20.6 Conclusions

The data from this study support reasonable assurance of the safety and effectiveness of the F1CDx assay when used to aid clinicians in identifying NSCLC patients with *ROS1* fusions who may be eligible for treatment with entrectinib.

3.21 Clinical Evaluation of AKEEGA® (niraparib + abiraterone acetate) in metastatic castration resistant prostate (mCRPC) Patients with *BRCA1, BRCA2* Alterations

The clinical performance of F1CDx for detection of *BRCA1, BRCA2* alterations in mCRPC patients who may benefit from AKEEGA was established with clinical data generated from F1CDx in the clinical study 64091742PCR3001 (hereafter referred to as MAGNITUDE).

3.21.1 Summary of the Clinical Study – 64091742PCR3001 (MAGNITUDE, PCR3001)

The MAGNITUDE trial is a Phase 3, randomized, double-blinded, placebo-controlled, multicenter study of niraparib in combination with abiraterone acetate (AA) and prednisone versus abiraterone acetate and prednisone (AAP) for treatment of subjects with metastatic prostate cancer. Participants in study PCR3001 were assigned to cohorts based on HRR alteration status as determined by tissue and plasma assays. During the prescreening phase, subjects were required to submit both blood and tumor (archival or recently collected) samples for determination of HRR gene alteration status. A total of 423 patients were screened and enrolled into Cohort 1. Within the enrolled Cohort 1 population, 229 patients with *BRCA1 and BRCA2* alterations detected by the tissue and/or plasma assays comprised the analysis set for for this study. The primary efficacy endpoint was radiographic progression-free survival (rPFS) as assessed by the blinded independent central review (BICR) committee.

The assessment of efficacy was conducted using the *BRCA* subgroup from Cohort 1 of the MAGNITUDE study that consists of subjects who were enrolled in Cohort 1 with *BRCA1 and BRCA2* alterations.

3.21.2 Accountability of the PMA Cohort

Of the 423 HRR+ patients enrolled in Cohort 1, 229 patients with *BRCA1 and BRCA2* alterations were detected by at least one assay (either tissue, plasma, or both), which comprised the *BRCA* subgroup. Of

the 229 *BRCA* subgroup samples, 179 produced F1CDx-evaluable results. Within this subgroup, 161 patients were *BRCA1 or BRCA2* alteration positive by F1CDx and 18 were *BRCA1 and BRCA2* alteration negative by F1CDx. The remaining 50 patients did not have a valid F1CDx result.

3.21.3 Efficacy Evaluation

The clinical efficacy for each subgroup (i.e., F1CDx *BRCA*+, F1CDx *BRCA*-, F1CDx invalid results, and not tested with F1CDx) within the analysis set was evaluated in terms of rPFS. The median rPFS was estimated with Kaplan-Meier estimates. The hazard ratio (HR) and log(HR) were calculated by both stratified and multivariate Cox regression for each subgroup. A sensitivity analysis was performed to evaluate the robustness of the clinical efficacy accounting for uncertainty due to missing data.

The estimated median rPFS for the F1CDx *BRCA*+ group was 18.43 months (95% CI: [16.13, NA]) for the treatment arm and 10.87 months (95% CI [8.31, 13.80]) for the control arm. The median rPFS for the F1CDx *BRCA*- group was 11.04 months in the treatment arm. Among the control arm, 2 radiographic progression events occurred out of 6 patients; the rPFS probability did not drop below 50% and the median rPFS could not be estimated. For the F1CDx invalid results group, the median rPFS was 14.98 months in the treatment arm compared to 16.43 months in the control arm. Within the not tested by F1CDx group, only 1 radiographic progression event was observed out of 9 patients in the treatment arm; the rPFS probability did not drop below 50% and thus the median rPFS could not be estimated. The median rPFS in the control arm was 8.34 months.

Table 75: *BRCA* Subgroup Efficacy Summary in the F1CDx *BRCA*+ and F1CDx *BRCA*- Populations

Population	No. of Patients		No. of Events		Median rPFS (months) [§]	
	Niraparib + AAP	Placebo + AAP	Niraparib + AAP	Placebo + AAP	Niraparib + AAP	Placebo + AAP
F1CDx *BRCA*+	76	85	30	51	18.43 [16.13, NA[§§]]	10.87 [8.31,13.80]
F1CDx *BRCA* -	12	6	9	2	11.04	NA[§§§]
F1CDx invalid results	17	18	5	10	14.98	16.43
Not tested by F1CDx	9	6	1	3	NA[§§§§]	8.34

[§] 95% CIs of the median rPFS were provided for group F1CDx BRCA+.
[§§] The 95% CI upper bound cannot be estimated because the upper bound of the confidence band for rPFS probability did not drop below 50%.
[§§§] Two radiographic progression events occurred out of 6 patients. Due to the low frequency of the events (i.e., 2/6), the rPFS probability did not drop below 50% and thus the median could not be estimated. The rPFS of the two events were 4.57 months and 8.21 months.
[§§§§] One radiographic progression event occurred out of nine patients at 2.00 months. Due to low frequency of the events (1/9), the rPFS probability did not drop below 50% and thus the median could not be estimated.

Stratified and multivariate Cox regression models were built. The primary model was a stratified Cox regression model with the planned treatment as the only covariate and two pre-specified strata in drug SAP including prior AAP use and past taxane-based chemo exposure. Gene mutation group based on enrolling assay (*BRCA1/2*+ vs others) was not used because all patients were *BRCA1/2*+.

Table 76 presents the estimated HRs among different subgroups (F1CDx *BRCA*+, F1CDx *BRCA*-, F1CDx invalid results, and not tested with F1CDx). The HR by stratified Cox regression for the F1CDx *BRCA*+ group was 0.45 (95% CI: [0.28,0.71]), which suggested a 55% reduction in the risk of radiographic progression when using niraparib+AAP compared with placebo+AAP. The upper bound of the 95% CI is 0.71 and is less than 1 indicating superior clinical efficacy in the treatment arm compared to the control arm with statistical significance. The HR was 3.34 (95% CI: [0.33,33.79]) for the F1CDx *BRCA* group, 0.89 (95% CI: [0.27,2.93]) for the F1CDx invalid results group and 0.19 (95% CI: [0.02,2.07]) for the not tested with F1CDx group.

Table 76 Estimations of HR and log(HR) by Stratified Cox in the *BRCA* Subgroup Population

Population	HR between treatment and control arms	95% CI for HR between treatment and control arms
F1CDx *BRCA*+	0.45	[0.28,0.71]
F1CDx *BRCA*-	3.34	[0.33,33.79]
F1CDx invalid results	0.89	[0.27,2.93]
Not tested by F1CDx	0.19	[0.02,2.07]

3.21.4 Sensitivity Analysis – Impact of F1CDx Unevaluable Set (F1CDx invalid results + not tested by F1CDx)

A sensitivity analysis was performed to evaluate the robustness of the clinical efficacy estimated accounting for the F1CDx-unevaluable (F1CDx invalid results and not tested by F1CDx) results for enrolled patients from the the analysis set.

The F1CDx unevaluable results were imputed using a multivariate logistic regression model based on the missing at random (MAR) assumption. The rPFS and HR were re-estimated for the F1CDx *BRCA*+ population based on imputed data with a bootstrapping multiple imputation method. The median rPFS was estimated to be 16.56 months (95% CI: [14.98, 21.95]) in the treatment arm and 10.87 months (95% CI: [8.31,13.67]) in the placebo arm, comparable to the primary efficacy results with 18.43 months for the treatment arm and 10.87 months for the placebo arm (Table 75). The HR was 0.44 (95% CI: [0.16,0.73]) by stratified Cox regression, which is comparable to the observed HR of 0.45 by stratified Cox regression. The robustness of the clinical efficacy analysis was demonstrated by accounting for the missingness of F1CDx testing results.

3.21.5 Safety Analysis

The F1CDx assay is not expected to directly cause actual or potential adverse effects, but test results may directly impact patient treatment risks.

3.21.6 Conclusions

The data from this study supports reasonable assurance of the safety and effectiveness of the F1CDx assay when used to aid clinicians in identifying prostate cancer patients with *BRCA1*, *BRCA2* alterations who may benefit from treatment with AKEEGA (Niraparib + Abiraterone acetate).

4. References

1. Li M. Statistical Methods for Clinical Validation of Follow-On Companion Diagnostic Devices via an External Concordance Study, J Biopharm Stat 26:6, 1067-107
2. Li H. & Durbin, R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics 26, 589-595 (2010).
3. Li H., Handsaker B., Wysoker A., et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25, 2078-2079 (2009).
4. DePristo MA., Banks E., Poplin R., et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet 43, 491-498 (2011).
5. Forbes SA, Bindal N., Bamford S., et al. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. Nucleic Acids Res 39, D945-950 (2011).
6. Compeau PE., Pevzner PA., Tesler, G. How to apply de Bruijn graphs to genome assembly. Nat Biotechnol 29, 987-991 (2011).
7. Kimura H., Ohira T., Uchida O., et al. Analytical performance of the cobas *EGFR* mutation assay for Japanese non-small-cell lung cancer. Lung Cancer 83, 329-333 (2014).
8. Lopez-Rios F., Angulo B., Gomez B., et al. Comparison of molecular testing methods for the detection of *EGFR* mutations in formalin-fixed paraffin-embedded tissue specimens of non-small cell lung cancer. J Clin Pathol 66, 381–385 (2013).

9. Wong AT, To RM, Wong CL, et al. Evaluation of 2 Real-Time PCR Assays for In Vitro Diagnostic Use in the Rapid and Multiplex Detection of *EGFR* Gene Mutations in NSCLC. Diagn Mol Pathol 22, 138-43 (2013).

10. Press et al., HER2 Gene Amplification Testing by Fluorescent In Situ Hybridization (FISH): Comparison of the ASCO-College of American Pathologists Guidelines With FISH Scores Used for Enrollment in Breast Cancer International Research Group Clinical Trials, J Clin Oncol 34:3518-3528, 2016

11. Dowsett, M. et al, Disease-Free Survival According to Degree of HER2 Amplification for Patients Treated with Adjuvant Chemotherapy With or Without 1 Year of Trastuzumab: The HERA Trial, J Clin Oncol 27:2962-2969, 2009

12. Okamura R, Boichard A, Kato S, Sicklick JK, Bazhenova L, Kurzrock R. Analysis of NTRK Alterations in Pan-Cancer Adult and Pediatric Malignancies: Implications for NTRK Targeted Therapeutics. JCO Precis Oncol. 2018;2018:10.1200/PO.18.00183.

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