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. 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 1 are 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	Gilotrif® (afatinib), Iressa® (gefitinib), Tagrisso® (osimertinib), or Tarceva® (erlotinib)
	EGFR exon 20 T790M alterations	Tagrisso® (osimertinib)
	ALK rearrangements	Alecensa® (alectinib), 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)
Melanoma	*BRAF* V600E	Tafinlar® (dabrafenib) or Zelboraf® (vemurafenib)
	BRAF V600E and V600K	Mekinist® (trametinib) or Cotellic® (cobimetinib) in combination with 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) or Rubraca® (rucaparib)
Cholangiocarcinoma	*FGFR2* fusions and select rearrangements	Pemazyre™ (pemigatinib)
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)
Solid tumors	TMB ≥ 10 mutations per megabase	Keytruda® (pembrolizumab)

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 by FoundationOne®CDx (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]Multiple references listed in https://www.mycancergenome.org/content/disease/breast-cancer/ERBB2/238/) report the frequency of HER2 overexpression as 20% in breast cancer. 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 CNAs including *ERBB2*.
- Clinical performance of Tagrisso® (osimertinib) in patients with an *EGFR* exon 20 T790M mutation detected with an allele fraction <5% is ongoing and has not been established.
- Concordance with other validated methods for CNA (with the exception of *ERBB2* amplifications and *BRCA1/2* homozygous deletions) and gene rearrangement (with the exception of *ALK*) detection has not been demonstrated and will be provided in the post-market setting. Confirmatory testing using a clinically validated assay should be performed for all CNAs and rearrangements not associated with CDx claims noted in Table 1 of the Intended Use, but used for clinical decision making.
- The MSI-H/MSS designation by FMI FoundationOne®CDx (F1CDx) test is based on genome wide analysis of 95 microsatellite loci and not based on the 5 or 7 MSI loci described in current clinical practice guidelines. Refer https://www.accessdata.fda.gov/cdrh_docs/pdf17/P170019B.pdf for additional details on methodology. The threshold for MSI-H/MSS was determined by analytical concordance to comparator assays (IHC and PCR) using uterine, cecum and colorectal cancer FFPE tissue. Patients with microsatellite status of "Cannot Be Determined" should be retested with an orthogonal (alternative) method. The clinical validity of the qualitative MSI designation has not been established.
- 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.

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 a single DNA extraction method 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 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*	

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 six 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

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
- 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			■	■	■			■						

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
Chest wall														
Cholangiocarcinoma										**				
Colon														
Diaphragm														
Duodenum			*											
Ear			*											
Endometrium			*											
Esophagus														
Fallopian Tube														
Gallbladder														
Gastro-esophageal junction														
Head and Neck														
Kidney														
Larynx			*											
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														

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
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													

Genes	Substitutions	Insertion/Deletions	CNAs	Rearrangements	Precision	LoD	NGS Concordance	Inter-lab Concordance	In Silico Study	DNA Extraction		Guard Band	Interfering Substances
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	■		■			■	■	■	■	■	■		
CD22	■	■			■	■			■				
CD274	■	■	■					■	■	■	■		
CD70									■				
CD74									■				
CD79A		■				■			■				
CD79B	■								■	■	■		
CDC73	■			■				■	■			■	■
CDH1	■	■			■	■	■	■	■	■	■		
CDK12	■	■	■	■	■	■	■	■	■	■	■		
CDK4	■		■		■		■	■	■	■	■		■
CDK6	■								■				■
CDK8	■		■		■	■	■	■	■			■	
CDKN1A	■	■					■	■	■	■	■		
CDKN1B	■	■				■		■	■				
CDKN2A	■	■	■	■	■	■	■	■	■	■	■	■	■
CDKN2B	■		■		■	■		■	■	■	■	■	■

Genes	Substitutions	Insertion/Deletions	CNAs	Rearrangements	Precision	LoD	NGS Concordance	Inter-lab Concordance	In Silico Study	DNA Extraction		Guard Band	Interfering Substances
CDKN2C													
CEBPA													
CHEK1													
CHEK2													
CIC													
CREBBP													
CRKL													
CSF1R													
CSF3R													
CTCF													
CTNNA1													
CTNNB1													
CUL3													
CUL4A													
CXCR4													
CYP17A1													
DAXX													
DDR1													
DDR2													
DIS3													
DNMT3A													
DOT1L													
EED													
EGFR													
EP300													
EPHA3													
EPHB1													
EPHB4													
ERBB2													
ERBB3													
ERBB4													
ERCC4													
ERG													
ERRFI1													
ESR1													
ETV4													
ETV5													
ETV6													
EWSR1													
EZH2													
EZR													
FAM46C													
FANCA													
FANCC													
FANCG													
FANCL													
FAS													
FBXW7													
FGF10													
FGF12													
FGF14													
FGF19													

Genes	Substitutions	Insertion/Deletions	CNAs	Rearrangements	Precision	LoD	NGS Concordance	Inter-lab Concordance	In Silico Study	DNA Extraction		Guard Band	Interfering Substances
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	■	■	■		■	■	■	■	■	■	■		■
IKBKE	■	■	■		■	■		■	■				
IKZF1	■		■		■	■	■	■	■				
INPP4B	■				■	■		■	■	■	■		
IRF2	■				■			■	■				
IRF4									■				
IRS2	■	■	■		■	■		■	■	■	■	■	
JAK1	■	■	■	■	■	■	■	■	■	■	■		
JAK2	■	■	■		■		■	■	■	■	■		
JAK3	■	■	■			■	■	■	■	■	■		
JUN	■					■			■				
KDM5A	■	■	■		■	■		■	■			■	
KDM5C	■			■	■	■		■	■				
KDM6A	■	■	■		■	■	■	■	■	■	■		
KDR	■	■	■		■	■	■	■	■	■	■	■	
KEAP1	■	■			■	■		■	■	■	■		■
KEL	■				■			■	■	■	■		■

Genes	Substitutions	Insertion/Deletions	CNAs	Rearrangements	Precision	LoD	NGS Concordance	Inter-lab Concordance	In Silico Study	DNA Extraction		Guard Band	Interfering Substances
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	■	■	■	■	■	■	■	■	■	■	■	■	
MYCL	■	■	■		■			■	■				
MYCN	■	■					■	■	■				
MYD88	■						■	■	■				
NBN	■	■	■		■	■	■	■	■	■	■		
NF1	■	■	■	■	■	■	■	■	■	■	■	■	
NF2	■	■			■		■	■	■				
NFE2L2	■	■				■		■	■	■	■		
NFKBIA	■	■	■			■		■	■			■	
NKX2-1	■	■	■			■	■	■	■			■	
NOTCH1	■	■		■	■	■	■	■	■	■	■	■	■
NOTCH2	■	■	■		■		■	■	■			■	■
NOTCH3	■	■			■	■		■	■	■	■	■	■
NPM1			■					■	■				
NRAS	■						■	■	■				
NT5C2		■				■			■				
NTRK1	■	■	■		■	■	■	■	■	■	■		■

Genes	Substitutions	Insertion/Deletions	CNAs	Rearrangements	Precision	LoD	NGS Concordance	Inter-lab Concordance	In Silico Study	DNA Extraction		Guard Band	Interfering Substances
NTRK2													
NTRK3													
NUTM1													
P2RY8													
PALB2													
PARK2													
PARP1													
PARP2													
PARP3													
PAX5													
PBRM1													
PDCD1													
PDCD1LG2													
PDGFRA													
PDGFRB													
PDK1													
PIK3C2B													
PIK3C2G													
PIK3CA													
PIK3CB													
PIK3R1													
PIM1													
PMS2													
POLD1													
POLE													
PPARG													
PPP2R1A													
PPP2R2A													
PRDM1													
PRKAR1A													
PRKCI													
PTCH1													
PTEN													
PTPN11													
PTPRO													
QKI													
RAC1													
RAD21													
RAD51													
RAD51B (RAD51L1)													
RAD51C													
RAD51D (RAD51L3)													
RAD52													
RAD54L													
RAF1													
RARA													
RB1													
RBM10													
REL													
RET													

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

Genes	Substitutions	Insertion/Deletions	CNAs	Rearrangements	Precision	LoD	NGS Concordance	Inter-lab Concordance	In Silico Study	DNA Extraction		Guard Band	Interfering Substances
XRCC2													
ZNF217													
ZNF703													

2.1 Concordance – Comparison to an Orthogonal Method

The detection of alterations by the FoundationOne®CDx (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 breast cancer samples were analyzed to determine concordance specific to *PIK3CA* base substitutions
- 26 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 NSCLC samples were analyzed to determine concordance for detection of qualifying *MET* exon 14 base substitutions and indels
- 120 samples were analyzed to determine concordance specific to HRR alterations (including base substitutions, indels, rearrangements and homozygous deletions)
- 218 samples were analyzed to determine concordance with a CLIA validated whole exome sequencing (WES) assay for detection of TMB ≥ 10 mutations per megabase

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 platform 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 are also summarized in Table 6. 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]*	NPA [95% CI]*
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%]
***PIK3CA* substitutions in Breast Cancer**	53	0	0	48	100.00% [93.3%-100.0%]	100.00% [92.6%-100.0%]
FGFR2* fusions*	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%]	99.2% [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%]

*The 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.
** PPA and NPA were adjusted using a prevalence of 9.6% to account for sampling differential.

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%]).

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 8 below.

Table 8. 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%

The qualitative output for MSI (MSI-H vs. MSS) in the F1 LDT and F1CDx were evaluated. PPA, NPA and Overall Percent Agreement (OPA) of MSI status between the two assays was calculated for all 165 samples. Of the 165 samples, 5 were MSI-H by F1 LDT and 160 were MSS by F1 LDT; there was one discordant sample observed. The discordant sample was called MSS by F1 LDT and MSI-H by F1CDx. After manual review, the discordant case had an MSI score close to the threshold used to classify MSI status. PPA was 100% with a 95% confidence interval (95% CI) of 47.8-100%, NPA was 99.5% with a 95% CI of 96.6%-99.98% and OPA was 99.4% with a 95% CI of 96.7%-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 9a below.

Table 9a. 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 9b and 9c below.

Table 9b. 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 9c. 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 10 below.

Table 10. 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 11). 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 11. 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 12 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 12. Interfering substance evaluated.

Substances	Level	# Samples	# Replicates/Sample
No Interferent	–	5	2
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) calling as a qualitative biomarker. The analysis included 19 retrospective samples from 3 previous studies and all acceptance criteria were met.

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

In this study, 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 154 samples had alterations representative of CDx associated alterations as well as exemplar alterations in a variety of genomic contexts, as shown in Tables 13 and 14 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 13. Sample set selection for CDx validation.

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)
MET	8[4]	SNVs and indels that lead to Exon 14 skipping	NSCLC
HRR Genes	47	Base Substitutions, Indels, Rearrangements, Homozygous Deletions	Prostate
TMB	46[4]	TMB ≥ 10 mutations per megabase	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)

Table 14. 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	-	-

Alteration Type	Number of Unique Samples	Alteration Size	Genomic Context
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 15 and 16 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 15. 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	95% CI Upper Limit
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%

Table 16. Positive and negative call rates per sample for platform variants (N=717).

Alteration Type(s) Assessed		exact 95% CI			exact 95% CI	
	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%

Alteration Type(s) Assessed		exact 95% CI			exact 95% CI	
	PC Rate*	Lower	Upper	NC Rate**	Lower	Upper
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

For the assessment of MSI, 100% agreement was observed, with a lower limit of 99.7% and upper limit of 100%. For TMB determination, thirteen 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.

A supplementary analysis was conducted to evaluate the intermediate precision for demonstrating the repeatability and reproducibility in detecting TMB-H status (≥ 10 mutations per megabase) as a CDx biomarker in 46 pan tumor FFPE specimens. Repeatability and reproducibility results are 99.54% (95% CI [98.39%, 99.89%]) and 99.72% (95% CI [99.18%, 99.94%]), respectively, for the TMB cut-off of 10 mutations per megabase.

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)

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 fifteen (15) CDx biomarkers are summarized in Tables 17-1 and 17-2 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. In addition, LoD of MSI-H was also evaluated. The LoD for representative alterations detected by the F1CDx platform is summarized in Tables 18-1 and 18-2.

Table 17-1. Summary of LoD for alterations associated with CDx claims (short variants). LoD is based on Allele Fraction.

Alteration	LoD[1] Allele Fraction (%) (100% 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 100% 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 17-2. Summary of analytical sensitivity based on tumor purity for biomarkers associated with CDx claims.

Alteration	Tumor Purity (%) (100% 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 Caclculated
HRR gene homozygous deletions[8]	23.9%[7]	Not Calculated
TMB ≥ 10 mutations per megabase[8]	28.16%[7]	Not Calculated

[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 100% 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.

Table 18-1. 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 18-2. 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%
MSI-High	3	8.3%-15.8%

[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%. LoD from the hit rate approach is defined as the lowest level with 100% hit rate (worst scenario)
[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.

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 19 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 20). 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 19. 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 20. 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 21 provides a summary of concordance across replicates. A study with an additional ten samples will be completed post-market.

Table 21. 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 ($\geq$ 10 mutations per megabase) calling. The analysis included 35 retrospective samples and all acceptance criteria were met.

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 22-1 through 22-4.

Table 22-1. 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 22-2. 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%)

Process	Input Level	# of sample failures	Variant Type	# of concordant successes	# of variant comparisons	Success Rate (95% CI) (Number of Concordant comparisons)
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 22-3. 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%)
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 22-4. 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%)

Process	Input Level	# of sample failures	Variant Type	# of concordant successes	# of variant comparisons	Success Rate (95% CI) (Number of Concordant comparisons)
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 3.1-3.6 and summarized in Section 3.7 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 FDA-approved CDxs (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, 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 3.8-3.14 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.

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 23 below.

Table 23. 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 24.

Table 24. 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[2,3,4], 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 25.

Table 25. 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).

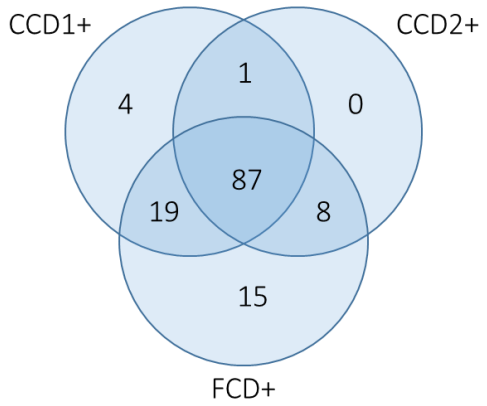


Figure 2. Venn Diagram for *EGFR* T790M-positive samples.

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%) is ongoing and has not been established.

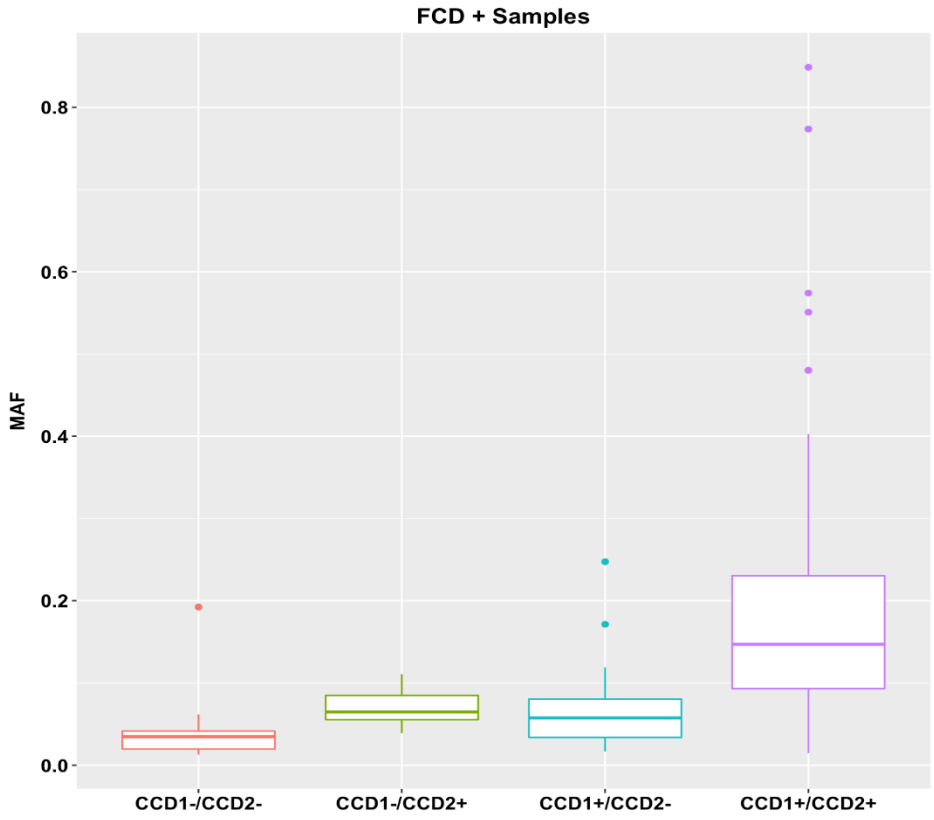


Figure 3. Distribution of MAF in F1CDx+ (FCD) samples.

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 26 below.

Table 26. 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[5,6]. 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 27 below.

Table 27. 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.

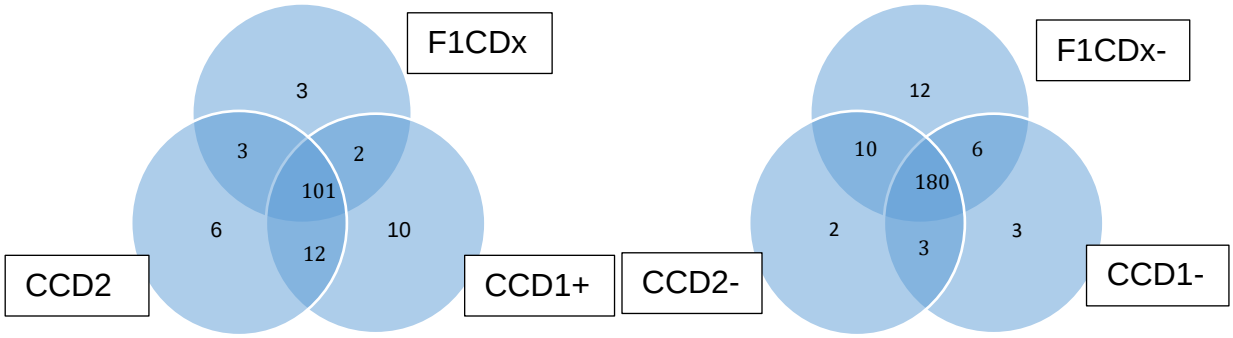


Figure 4. Venn Diagrams for *ERBB2*-amplification positive (left panel) and negative (right panel) samples.

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 28. 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 29 below.

Table 29. 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.

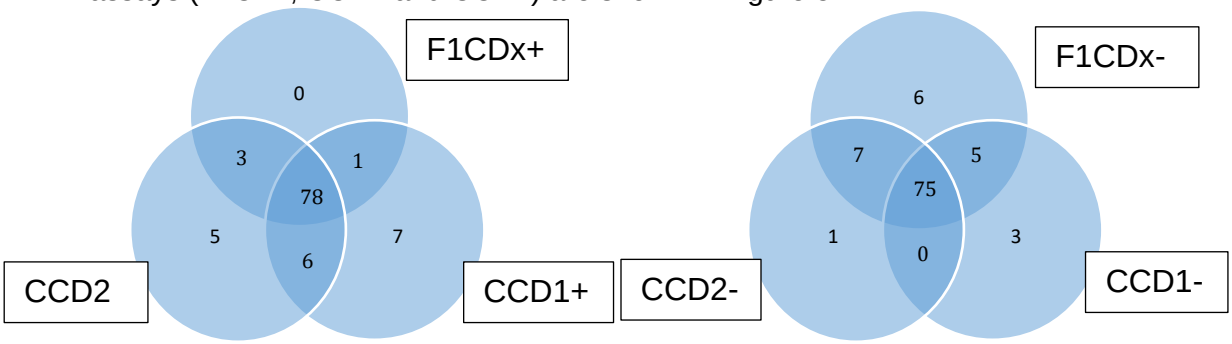


Figure 5. Venn Diagrams for *ALK*-rearrangement positive (left panel) and negative (right panel) samples.

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 30. 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%]).

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 31 below.

Table 31. 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 32. 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 32. 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 33 below.

Table 33. 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 34.

Table 34. 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 35. 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 35. 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 36. 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

3.7 Summary of Clinical Concordance Studies

A summary of clinical concordance study results is included in Table 37 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 37. 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
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.

3.8 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 38 below.

Table 38. 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
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

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

3.9.1 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.

3.9.2 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 diferences 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.

3.9.3 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 39 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 39. 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.

3.10 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.

3.10.1 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.

3.10.2 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 40 and Table 41 below.

Table 40. 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 41. Agreement between CDx and CTA2 based on the CTA2 results (Concordance analysis set for CTA2).

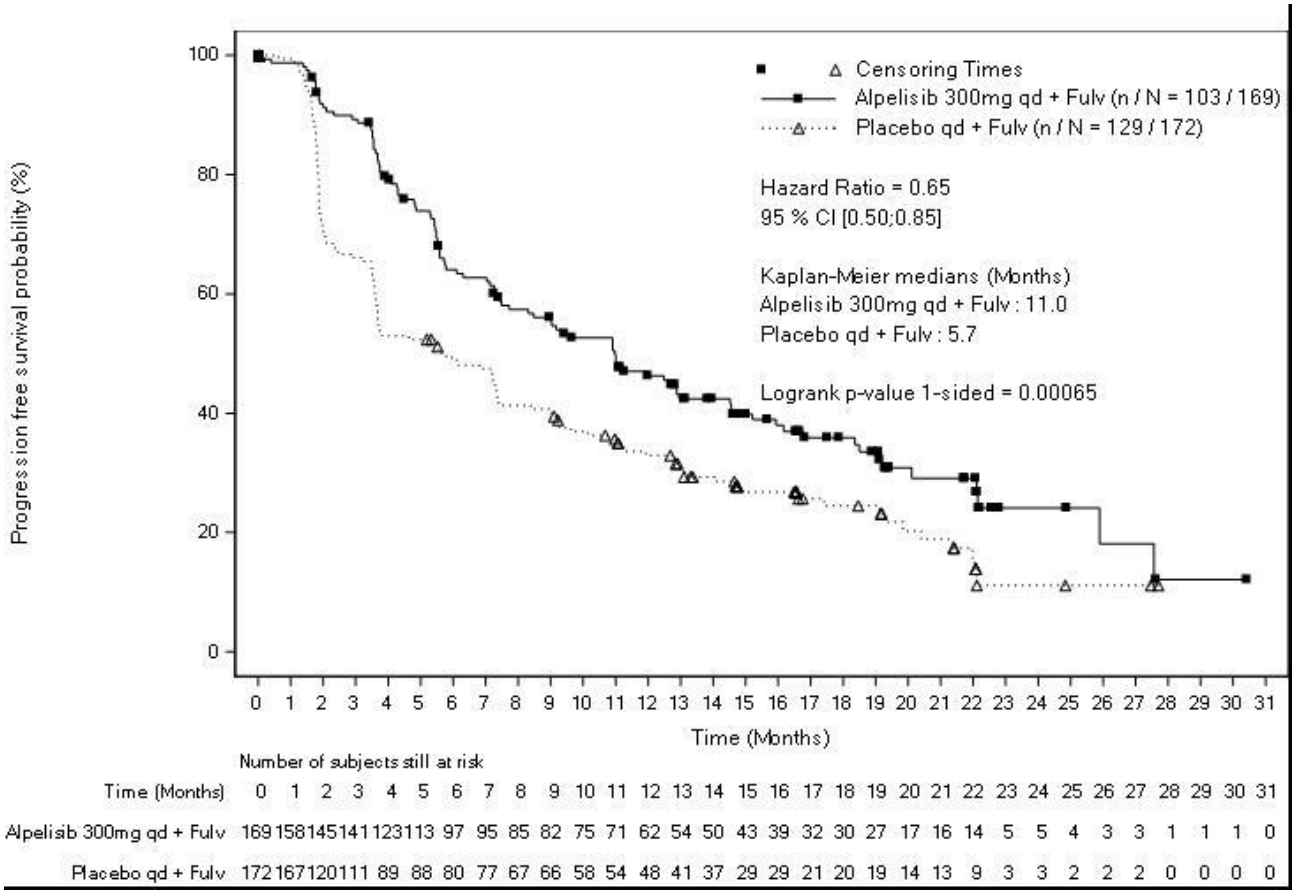
	Without invalid CDx results		With invalid CDx results	
Measure of Agreement	**Percent Agreement (N)**	**95% CI (1)**	**Percent Agreement (N)**	**95% CI (1)**
PPA	91.6% (197/215)	(87.1%, 95.0%)	90.4% (197/218)	(85.7%, 93.9%)
NPA	98.8% (162/164)	(95.7%, 99.9%)	97.0% (162/167)	(93.2%, 99.0%)
OPA	94.7% (359/379)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

	Without invalid CDx results		With invalid CDx results	
Measure of Agreement	**Percent Agreement (N)**	**95% CI (1)**	**Percent Agreement (N)**	**95% CI (1)**

(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 42 and Table 43 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 42. 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 43. 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.

3.11 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.

3.11.1 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, *FGFR2* candidate fusions and rearrangements were defined as the following:

- An *FGFR2* rearrangement predicted to be a fusion: Breakpoint is within the *FGFR2* intron 17/ exon 18 hotspot and the gene partner is known in the literature, in strand with *FGFR2;* or is a novel partner that is predicted to be in strand and in frame with *FGFR2*.
- An *FGFR2* rearrangement, which cannot be conclusively predicted to be a fusion: Breakpoint is within the *FGFR2* intron 17/exon 18 hotspot but the partner gene is out of frame or out of strand with exon 17 of *FGFR2*. Alternatively, the downstream end of the breakpoint may be in an intergenic region and not within another gene (designated as partner n/a).

3.11.2 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.

3.11.3 Efficacy Evaluation

3.11.3.1 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 44 below.

Table 44. Efficacy results in FIGHT-202 trial.

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

3.11.3.2 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 45). 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 45. 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.

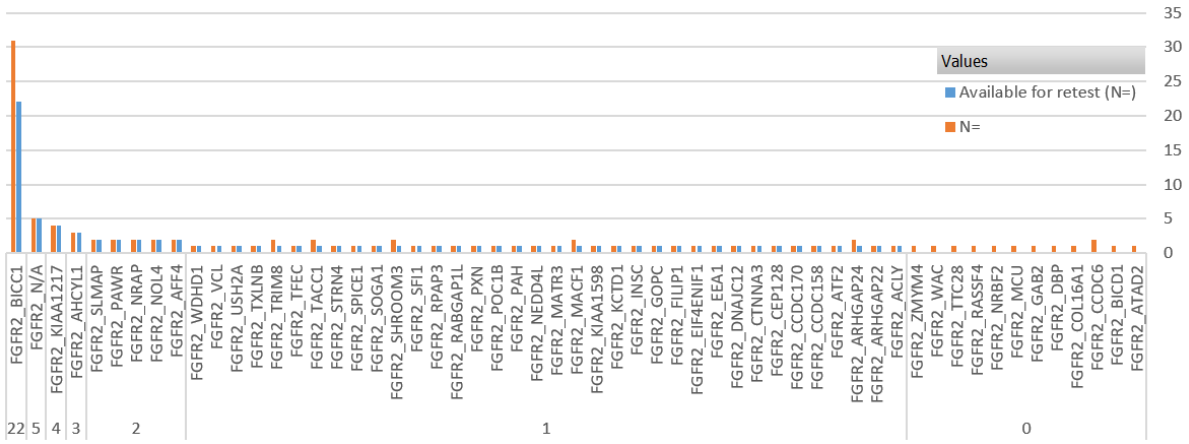


Figure 7. Distribution of *FGFR2* fusions and rearrangements in Cohort A in support of efficacy.

3.11.3.3 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.

3.11.3.4 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.

3.11.3.5 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.

3.12 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.

3.12.1 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.

3.12.2 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.

3.12.3 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.

3.12.4 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.

3.12.5 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 46, were calculated with and without invalid CDx results, using the CTA results as reference for the CTA-enrolled patients.

Table 46. 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

3.12.6 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 47 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 47. 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 48 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 48. 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%)

3.12.7 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.

3.13 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 49. 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 50. 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)	<0.0001
	Investigators choice of NHA	43	1 (2.3)	
Confirmed FMI F1CDx Subgroup	Olaparib 300 mg bd	84	27 (33.8)	<0.0001
	Investigators choice of NHA	43	1 (2.3)	

[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 51. 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.

3.14 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 52.

Table 52. 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 53 and 54, respectively. ORR was generally higher in the TMB-H population for most tumor types than in the non-TMB-H population.

Table 53. 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 54. 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.

4. References

1. Li M. Statistical Methods for Clinical Validation of Follow-On Companion Diagnostic Devices via an External
2. 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).
3. 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).
4. 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).

5. 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
6. 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

Revision Table:

Revision Number	Reason for Change
1	Initial Release.
2	Updated to reflect two approved indications: (1) indication in NSCLC for EGFR exon 19 deletions and EGFR exon 21 L858R alterations in association with osimertinib and (2) indication in breast cancer for PIK3CA alterations in association with alpelisib.
3	Updates include: Addition of Section 3.10 Clinical Bridging Study data to support PIK3CA indication in breast cancer, addition of Summary of Clinical Concordance Study Table 3.7., and revision of table headers within Section 2.1-2.3 for additional clarity.
4	Update to reflect approved indication in cholangiocarcinoma for *FGFR2* fusions and select rearrangements associated with pemigatinib.
5	Updated to reflect approved indication in NSCLC for *MET* single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping in association with capamatinib.
6	Updated to reflect approved indication in prostate cancer for HRR gene alterations associated with olaparib.
7	Updated to reflect approved indication in pan-tumor cancer for TMB ≥ 10 mutations per megabase associated with pembroluzimab.