# SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

## I. GENERAL INFORMATION

| | |
|---|---|
| Device Generic Name: | Next generation sequencing oncology panel, somatic or germline variant detection system |
| Device Trade Name: | FoundationOne® CDx (F1CDx) |
| Device Procode: | PQP |
| Applicant's Name and Address: | Foundation Medicine, Inc.<br>400 Summer Street<br>Boston, MA 02210 |
| Date(s) of Panel Recommendation: | None |
| Premarket Approval Application (PMA) Number: | P170019/S060 |
| Date of FDA Notice of Approval: | May 27, 2026 |

The original PMA (P170019) for FoundationOne CDx (F1CDx) was approved on November 30, 2017, for the detection of genetic alterations in patients who may benefit from one of eighteen FDA-approved therapies for non-small cell lung cancer (NSCLC), melanoma, breast cancer, colorectal cancer (CRC), and ovarian cancer. Subsequently, additional PMA supplements were approved for expanding the indications for use of F1CDx since the original approval. See Section VII for more details.

The current supplement was submitted to expand the intended use of F1CDx to include a companion diagnostic (CDx) indication for the detection of HRR gene alterations (*ATM, ATR, BRCA1, BRCA2, CDK12, CHEK2, FANCA, MLH1, MRE11A, NBN, PALB2, RAD51C*) in metastatic castrate-resistant prostate cancer (mCRPC) patients who may benefit from treatment with TALZENNA® (talazoparib) in combination with XTANDI® (enzalutamide).

## II. INDICATIONS FOR 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 |
|---|---|---|
| Breast cancer | *AKT1* E17K;<br>*PIK3CA* R88Q, N345K, C420R, E542K, E545A, E545D, E545Q, E545K, E545G, Q546E, Q546K, Q546R, Q546P, M1043V, M1043I, H1047Y, H1047R, H1047L, and G1049R;<br><br>and *PTEN* alterations | TRUQAP™ (capivasertib) in combination with FASLODEX® (fulvestrant) |
| | *ERBB2* (HER2) amplification | HERCEPTIN® (trastuzumab) |
| | | KADCYLA® (ado-trastuzumab emtansine) |
| | | PERJETA® (pertuzumab) |
| | *PIK3CA* mutations | ITOVEBI™ (inavolisib) in combination with palbociclib and fulvestrant |
| | *PIK3CA* mutations (C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y) | PIQRAY® (alpelisib) |
| Cholangiocarcinoma | *FGFR2* fusions and select rearrangements | PEMAZYRE® (pemigatinib) |
| Colorectal cancer | *KRAS/NRAS* wild-type (absence of mutations in exon 2 (codons 12 and 13), exon 3 (codons 59 and 61), and exon 4 (codons 117 and 146)) | ERBITUX® (cetuximab) |
| | | VECTIBIX® (panitumumab) |
| Melanoma | *BRAF* V600 mutation-positive | TECENTRIQ® (atezolizumab) in combination with COTELLIC® (cobimetinib) and ZELBORAF® (vemurafenib) |
| | *BRAF* V600E | BRAF Inhibitor Approved by FDA* |
| | *BRAF* V600E and V600K | BRAF/MEK Inhibitor Combinations Approved by FDA* |
| | | MEKINIST® (trametinib) |

| Tumor Type | Biomarker(s) Detected | Therapy |
|---|---|---|
| Non-small cell lung cancer (NSCLC) | *ALK* rearrangements | ALECENSA® (alectinib) |
| | | ALUNBRIG® (brigatinib) |
| | | XALKORI® (crizotinib) |
| | | ZYKADIA® (ceritinib) |
| | *BRAF* V600E | BRAFTOVI® (encorafenib) in combination with MEKTOVI® (binimetinib) |
| | | TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib) |
| | *EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations | EGFR Tyrosine Kinase Inhibitors (TKI) Approved by FDA* |
| | *EGFR* exon 20 T790M alterations | TAGRISSO® (osimertinib) |
| | *MET* single nucleotide variants (SNVs) and indels that lead to MET exon 14 skipping | TABRECTA® (capmatinib) |
| | | TEPMETKO® (tepotinib) |
| | *ROS1* fusions | ROZLYTREK® (entrectinib) |
| Ovarian cancer | *BRCA1, BRCA2* alterations | LYNPARZA® (olaparib) |
| Pediatric low-grade glioma | *BRAF* V600 mutation-positive and *BRAF* fusions | OJEMDA™ (tovorafenib) |
| Prostate cancer | *BRCA1, BRCA2* alterations | AKEEGA® (niraparib + abiraterone acetate) |
| | | LYNPARZA® (olaparib) in combination with abiraterone |
| | Homologous Recombination Repair (HRR) gene (*BRCA1, BRCA2*, *ATM, ATR, CDK12, CHEK2, FANCA, MLH1, MRE11A, NBN, PALB2, RAD51C*) alterations | TALZENNA® (talazoparib) in combination with XTANDI® (enzalutamide) |
| | 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 | MSI-High | KEYTRUDA® (pembrolizumab) |
| | *NTRK1/2/3* fusions | ROZLYTREK® (entrectinib) |
| | | VITRAKVI® (larotrectinib) |
| | *RET* fusions | RETEVMO® (selpercatinib) |
| | TMB ≥ 10 mutations per megabase | KEYTRUDA® (pembrolizumab) |

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

## III. CONTRAINDICATIONS

There are no known contraindications.

## IV. WARNINGS AND PRECAUTIONS

The warnings/precautions and limitations are included in the FoundationOne®CDx assay labeling.

## V. DEVICE DESCRIPTION

FoundationOne CDx (F1CDx) is performed exclusively at Foundation Medicine, Inc. owned and operated sites. The assay includes reagents, software, instruments, and procedures for testing DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tumor samples.

F1CDx uses DNA extracted from FFPE tumor samples. The assay employs two extraction methods (either DNAx or CoExtraction, an automated DNA/RNA co-extraction methodology) for DNA extraction from routine FFPE biopsy or surgical resection specimens; 50-1000 ng of DNA will undergo whole-genome shotgun library construction and hybridization-based capture of all coding exons from 309 cancer-related genes, one promoter region, one non-coding (ncRNA), and select intronic regions from 34 commonly rearranged genes, 21 of which also include the coding exons (refer to Table 2 and Table 3 for the complete list of genes included in F1CDx). In total, the assay detects alterations in a total of 324 genes. Using the Illumina® NovaSeq 6000, 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). Rearrangements in one of the targeted genes included in Table 3 may be reported along with their uniquely identified genomic partners, which can be any gene in the genome even if not explicitly targeted by the assay. Additionally, genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) are reported.

**Table 2. Genes with full coding exonic regions included in F1CDx 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, a promoter region, and an ncRNA gene**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *ALK introns 18, 19* | *BRCA1 introns 2, 7, 8, 12, 16, 19, 20* | *ETV4 introns 5, 6* | *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)* | *NOTCH2 intron 26* | *PDGFRA introns 7, 9, 11* | *ROS1 introns 31-35* | *TERC ncRNA* |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | *introns 6-11* | | | | |
| *BCR introns 8, 13, 14* | *CD74 introns 6- 8* | *ETV6* introns 5, 6* | *FGFR2 intron 1, 17* | *MSH2 intron 5* | *NTRK1 introns 8-10* | *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*

**Test Output**
The output of the test includes:

Category 1: 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

Genomic findings other than those listed in Table 1 of the intended use statement (i.e., Categories 2 and 3) are not prescriptive or conclusive for labeled use of any specific therapeutic product.

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

- Specimen Preparation Instructions
- Shipping Instructions
- Return Shipping Label

**Instruments**
The F1CDx assay is intended to be performed with serial number-controlled instruments as indicated in Table 4, below. All instruments are qualified by Foundation Medicine, Inc. (FMI) under FMI's Quality System.

**Table 4. Instruments for use with the F1CDx assay**

| Instrument |
|---|
| Hamilton STAR/STARlet Liquid Handling Workstation |
| Covaris LE220-plus Focused ultrasonicator |
| Thermo Fisher Scientific KingFisher™ Flex with 96 Deep-well Head |
| Illumina® NovaSeq 6000 System |

**Test Process**

All assay reagents included in the F1CDx assay process are qualified by FMI and are compliant with the medical device Quality System Regulation (QSR).

**A. Specimen Collection and Preparation**

Formalin-fixed, paraffin-embedded (FFPE) tumor specimens are collected and prepared following standard pathology practices. FFPE specimens may be received either as unstained slides or as an FFPE block.

Prior to starting the assay, a Hematoxylin and Eosin (H&E) stained slide is prepared, and then reviewed by a board-certified pathologist to confirm disease ontology and to ensure that adequate tissue (0.6 $mm^3$), tumor content (≥ 20% tumor) and sufficient nucleated cells are present to proceed with the assay.

**B. DNA Extraction**

DNAx Extraction Method

Specimens passing pathology review are queued for DNA extraction which begins with lysis of cells from FFPE tissue by digestion with a proteinase K buffer followed by automated purification using the 96-well KingFisher™ FLEX Magnetic Particle Processor.

After completion of DNA extraction, double-stranded DNA (dsDNA) is quantified by the Quant-iT™ PicoGreen® fluorescence assay using the provided lambda DNA standards (Invitrogen) prior to Library Construction (LC). The sample must yield a minimum of 55 ng of genomic DNA to ensure sufficient DNA for quality control (QC) and to proceed with LC.

CoEx Extraction Method

Specimens passing pathology review are queued for nucleic acid extraction which begins with placement of the FFPE samples into an AutoLys tube, where using a pre-programmed automated method, the AutoLys STAR adds RNA digestion and proteinase K solutions. The RNA containing lysate is removed for downstream RNA extraction using the KingFisher RNA extraction process.

The AutoLys Tubes containing partially digested tissue then receive DNA Lysis solution on the AutoLys STAR and are placed into a Vortemp for digestion. The sample is then centrifuged to separate sample-associated paraffin from the lysate, and returned to the AutoLys STAR where the lysate is transferred to a KingFisher dKF plate. The dKF plate is loaded onto the Hamilton STAR for automated addition of DNA binding buffer and magnetic beads, and DNA isolation is performed using the KingFisher Flex. The DNA samples are then transferred to matrix tubes on DNAE plates using Hamilton STAR before proceeding to DNA quantification.

After completion of DNA extraction, double-stranded DNA (dsDNA) is quantified by the Quant-iT™ PicoGreen® fluorescence assay using the provided lambda DNA standards (Invitrogen) prior to Library Construction (LC). The sample must yield a

minimum of 55 ng of genomic DNA to ensure sufficient DNA for quality control (QC) and to proceed with LC.

**C. Library Construction**

Library Construction (LC) begins with the normalization of DNA to 50-1000 ng. The normalized DNA samples are randomly sheared (fragmented) to ~200 bp by adaptive focused acoustic sonication using the Covaris LE220 before purification with a 1.8X volume of AMPure® XP Beads (Agencourt®). Solid-phase reversible immobilization (SPRI) purification and subsequent library construction with the NEBNext® reagents (custom-filled kits by New England Biolabs), including mixes for end repair, dA addition and ligation, are performed in 96-well plates (Eppendorf) on the Bravo Benchbot (Agilent) or Hamilton Microlab STAR/STARlet Liquid Handling Workshop using the "with-bead" protocol to maximize reproducibility and library yield. Indexed (6 bp barcodes) sequencing libraries are polymerase chain reaction (PCR) amplified with HiFi™ (Kapa) for 10 cycles, and subsequently 1.8X SPRI purified. Purification and dilution for QC are performed.

Following LC, a QC procedure is performed by quantifying single-stranded DNA (ssDNA) from purified libraries using the Quant-iT™ PicoGreen® ssDNA Assay Kit (Life Technologies) read on a Molecular Devices Multimode SpectraMax M2 plate Reader. Libraries yielding insufficient sequencing library are failed.

**D. Hybrid Capture**

Hybrid Capture (HC) begins with normalization of each library to 500-2000 ng. Normalized samples then undergo solution hybridization which is performed using a > 50-fold molar excess of a pool of individually synthesized 5'-biotinylated DNA 120 bp oligonucleotides. The baits target ~1.8 Mb of the human genome including all coding exons of 309 cancer-related genes, introns or non-coding regions of 35 genes, plus > 3,500 single nucleotide polymorphisms (SNPs) located throughout the genome. Baits are designed by tiling overlapping 120 bp DNA sequence intervals covering target exons (60 bp overlap) and introns (20 bp overlap), with a minimum of three baits per target; SNP targets are allocated one bait each. Intronic baits are filtered for repetitive elements[2] as defined by the UCSC Genome RepeatMasker track.

After hybridization, the library-bait duplexes are captured on paramagnetic MyOne™ streptavidin beads (Invitrogen), and off-target material is removed by washing one time with 1X SSC at 25°C and four times with 0.25X SSC at 55°C. The PCR master mix is added to directly amplify (12 cycles) the captured library from the washed beads.[3] After 12 cycles of amplification, the samples are 1.8X SPRI purified. Purification and dilution for QC are performed.

QC for HC is performed by measuring dsDNA yield using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies) read on a Molecular Devices Multimode SpectraMax M2 plate Reader. Captured libraries yielding less than 140 ng of sequencing library are failed.

## E. Sequencing

Sequencing is performed using on-board cluster generation using patterned flow cell technology to generate monoclonal clusters from a single DNA template followed by sequencing using sequencing by synthesis (SBS) chemistry on the Illumina NovaSeq 6000. Fluorescently labeled 3′-blocked deoxynucleotide triphosphates (dNTPs) together with a polymerase are incorporated through the flow cell to create a growing nucleotide chain that is excited by a laser. A camera captures the emission color of the incorporated base and then is cleaved off. The terminator is then removed to allow the nucleotide to revert to its natural form and to allow the polymerase to add another base to the growing chain. A new pool of fluorescently labeled 3′-blocked dNTPs are added with each new sequencing cycle. The color changes for each new cycle as a new base is added to the growing chain. This method allows for millions of discrete clusters of clonal copies of DNA to be sequenced in parallel.

## F. Sequence Analysis

Sequence data is analyzed using proprietary software developed by FMI. Sequence data are mapped to the human genome (hg19) using Burrows-Wheeler Aligner (BWA) v0.5.9.[4] PCR duplicate read removal and sequence metric collection are performed using Picard 1.47 (http://picard.sourceforge.net) and SAMtools 0.1.12a.[5] Local alignment optimization is performed using Genome Analysis Toolkit (GATK) 1.0.4705.[6] Variant calling is performed only in genomic regions targeted by the test.

Base substitution detection is performed using a Bayesian methodology, which allows for the detection of novel somatic alterations at low mutant allele frequency (MAF) and increased sensitivity for alterations at hotspot sites through the incorporation of tissue-specific prior expectations.[7] Reads with low mapping (mapping quality < 25) or base calling quality (base calls with quality ≤ 2) are discarded. Final calls are made at MAF ≥ 5% (MAF ≥ 1% at hotspots).

To detect indels, *de novo* local assembly in each targeted exon is performed using the de-Bruijn approach.[8] Key steps are:

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

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

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

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

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

Tumor mutational burden (TMB) is measured by counting all synonymous and non-synonymous substitution and indel variants present at 5% allele frequency or greater and filtering out potential germline variants according to published databases of known germline polymorphisms including Single Nucleotide Polymorphism database (dbSNP) and Exome Aggregation Consortium (ExAC). Additional germline alterations still present after database querying are assessed for potential germline status and filtered out using a somatic-germline/zygosity (SGZ) algorithm. Furthermore, known and likely driver mutations are filtered out to exclude bias of the data set. The resulting mutation number is then divided by the coding region corresponding to the number of total variants counted, or 793 kb. The resulting number is communicated as mutations per Mb unit (mut/Mb).

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

## G. Report Generation

Approved results are annotated by automated software with CDx relevant information and are merged with patient demographic information prior to approval and release by the laboratory director or designee.

## H. Internal Process Controls Related to the System

### Positive Control

Each assay run includes a control sample run in duplicate. The control sample contains a pool of ten HapMap cell lines and is used as a positive mutation detection control. One hundred (100) different germline SNPs present across the entire targeted region are required to be detected by the analysis pipeline. If SNPs are not detected as expected, this results in a QC failure, as it indicates a potential processing error.

### Sensitivity Control

The HapMap control pool used as the positive control is prepared to contain variants at 5%-10% MAF which must be detected by the analysis pipeline to ensure the expected sensitivity for each run.

### Negative Control

Samples are barcoded molecularly at the LC stage. Only reads with a perfect molecular barcode sequence are incorporated into the analysis. The Analysis Pipeline includes an algorithm that analyzes the SNP profile of each specimen to identify potential contamination that may have occurred prior to molecular barcoding and can detect contamination lower than 1%.

## I. Variant Classification

**Biomarker Rules for SNVs and indels that lead to *MET* exon 14 skipping**
An SNV or indel in *MET* shall be considered to result in skipping of exon 14 if one or more of the following criteria are met:

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

**Homologous Recombination Repair (HRR) Genes**
A clinical report is provided to the ordering physician for each F1CDx test performed at Foundation Medicine, Inc. Each report is generated and reviewed by an internal team consisting of clinical bioinformatics analysts, scientists, curators, and pathologists for mutations positive for the therapies identified. Each sample is assessed for mutations in the HRR genes detailed in Tables 5 and 6. For these genes, both deleterious and suspected deleterious mutations in short variant, copy number alteration, and rearrangement variant classes are determined by an in-house software pipeline. Alterations listed in the COSMIC database and homozygous deletions are considered deleterious. Suspected deleterious mutations include truncating events (i.e., splice, frameshift, and nonsense alterations), as well as large rearrangements that disrupt the coding sequence. The COSMIC check is a second layer of check for HRR positive suspected deleterious alterations. All splice, nonsense, and frameshift alterations in HRR genes are considered biomarker positive and would be considered as suspected deleterious mutations (or "likely" status in FMI reporting rules). If these mutations are additionally reported in COSMIC, they would be listed as deleterious mutations (or "known" status in FMI reporting).

The F1CDx assay is intended as an aid in selecting prostate cancer patients with deleterious or suspected deleterious HRR variants, identified by the rules below in Table 5, and who may be eligible for treatment with Lynparza® (olaparib).

**Table 5. Biomarker definition for HRR gene (*BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D*, and *RAD54L*) Alterations for Olaparib treatment**

| Gene | Variant Class | Alteration Type | Description* |
|---|---|---|---|
| *BRCA1*<br>*BRCA2*<br>*ATM* | Short Variant | Nonsense, frameshift or splice site | Any deleterious nonsense, frameshift, or splicing event that spans or occurs within ±2 bases of |

| | | | |
|---|---|---|---|
| *BARD1*<br>*BRIP1*<br>*CDK12*<br>*CHEK1*<br>*CHEK2*<br>*FANCL*<br>*PALB2*<br>*RAD51B*<br>*RAD51C*<br>*RAD51D*<br>*RAD54L* | | | the intron/exon junction |
| | | Missense or non-frameshift | Any of the mutations listed in Tables 7-9 for *ATM, BRCA1,* and *BRCA2* |
| | Copy Number Alteration | Homozygous copy number loss | Deleterious homozygous copy number loss of one or more exons |
| | Rearrangement | Rearrangement | Any rearrangement that disrupts protein function |

*For *BRCA2*, truncating mutations must occur upstream of bases encoding amino acid 3326. Additionally, the frameshift mutation T367fs*13 in *FANCL* is ineligible. All short variants must occur in the canonical transcript.

The specific deleterious mutation (DM) and suspected deleterious mutation (SDM) missense mutations or non-frameshift mutations for *BRCA1, BRCA2,* and *ATM* are shown in Table 7-9, below. However, any missense or non-frameshift mutations in the other HRR genes would not be considered HRR positive.

The F1CDx assay is also intended as an aid in selecting prostate cancer patients with deleterious or suspected deleterious HRR variants, identified by the rules below in Table 6, and who may be eligible for treatment with TALZENNA® (talazoparib) in combination with XTANDI® (enzalutamide).

**Table 6. Biomarker definition for HRR gene (*BRCA1, BRCA2, ATM, ATR, CDK12, CHEK2, FANCA, MLH1, MRE11A, NBN, PALB2,* and *RAD51C*) Alterations for Talazoparib in combination with Enzalutamide treatment**

| Gene | Variant Class | Description |
|---|---|---|
| *BRCA1*<br>*BRCA2*<br>*ATM*<br>*ATR*<br>*CDK12*<br>*CHEK2*<br>*FANCA*<br>*MLH1*<br>*MRE11A*<br>*NBN* | Short Variant | Any nonsense, frameshift, or splice site alteration[1]<br>• Any splice alterations within the splice donor or acceptor site[2]<br>• For BRCA2, truncating mutations must occur upstream of bases encoding amino acid 3326<br>Any of the additional mutations listed in Tables 7 – 11 |
| | Copy Number | Homozygous deletion of one or more exons, regardless of transcript |

| | | |
|---|---|---|
| *PALB2*<br>*RAD51C* | Rearrangement | Any inactivating rearrangement, regardless of transcript |

[1] Missense mutations in the start codon (except for those in tables 7-11) and short variant deletions spanning from upstream of the start codon (annotated as M1?) are biomarker negative.
[2] The splice site is defined as the first or last two bases of the intron (except for those in tables 7-11).

**Table 7. List of short variants in *ATM**

| | | | | |
|---|---|---|---|---|
| M1I | E2039K | A2622V | F2827C | splice site 331+5G>A |
| M1L | A2067D | D2625_A2626>EP | R2832C | splice site 8418+5_8418+8delGTGA |
| M1T | R2227C | V2662D | S2855_V2856>RI | |
| P292L | Y2470D | D2708N | D2913Y | |
| D2016G | A2524P | V2716A | R3008C | |
| R2032K | R2547_S2549del | G2765S | R3008H | |

**Table 8. List of short variants in *BRCA1**

| | | | | |
|---|---|---|---|---|
| M1I | C47S | T1685A | W1718C | P1812A |
| M1R | C47Y | T1685I | S1722F | A1823T |
| M1T | C61Y | H1686R | V1736A | V1833M |
| M1V | C61G | V1688del | V1736G | W1837C |
| M18K | C61S | M1689R | G1738E | W1837G |
| M18T | C64G | T1691I | G1738R | W1837R |
| L22S | C64R | T1691K | D1739G | V1838E |
| C24R | C64Y | D1692Y | D1739V | Y1853C |
| I26N | C64W | D1692H | G1748D | splice site 212+3A>G |
| T37K | R71G | D1692N | R1753T | splice site 213-11T>G |
| T37R | R71K | A1693del | K1759N | splice site 213-12A>G |
| C39R | R71M | C1697R | L1764P | splice site 302-3C>G |
| C39Y | R71T | R1699Q | I1766S | splice site 4675+3A>T |
| C39G | S770L | R1699W | I1766N | splice site 4986+3G>C |
| C39W | R1495K | L1705P | G1770V | splice site 4986+5G>A |
| H41R | R1495M | G1706E | M1775K | splice site 4986+6T>C |
| C44F | R1495T | G1706R | M1775R | splice site 4986+6T>G |
| C44S | E1559K | A1708E | L1780P | splice site 5074+3A>G |
| C44Y | E1559Q | V1714G | C1787S | splice site 5194-12G>A |
| C47F | A1623G | S1715N | C1787_G1788>SD | splice site 5406+4A>G |
| C47R | S1655F | S1715R | G1788V | |

**Table 9. List of short variants in BRCA2***

| | | | | |
|---|---|---|---|---|
| M1R | K1530N | R2659G | D2723G | D3095E |
| M1I | R2336H | R2659K | D2723H | D3095G |

| | | | | |
|---|---|---|---|---|
| M1V | R2336P | R2659T | D2723V | N3124I |
| M1T | R2336L | Y2660D | G2724W | N3187K |
| D23N | T2412I | E2663K | Y2726C | splice site 316+4delA |
| D23Y | L2510P | E2663V | G2748D | splice site 316+5G>A |
| S142I | R2602T | S2670L | R2784W | splice site 8487+3A>G |
| S142N | H2623R | I2675V | G2793R | splice site 8754+3G>C |
| V159M | W2626C | L2686P | Q2829R | splice site 8754+4A>G |
| G173R | W2626R | L2688P | A2911E | splice site 8754+5G>A |
| V211I | I2627F | T2722K | E3002K | splice site 8954-5A>G |
| V211L | L2647P | T2722R | R3052W | |
| Y600C | L2653P | D2723A | G3076V | |

**Table 10. List of Short Variants in CHEK2**

| | |
|---|---|
| R117G | K373E |
| G151S | A392V |

**Table 11. List of Short Variants in *FANCA, MLH1, RAD51C***

| Gene | Short Variant |
|---|---|
| *FANCA* | F320L, F1263del |
| *MLH1* | R265C |
| *RAD51C* | L219S |

**Note: This biomarker definition includes ATM and BRCA1/2 inactivating mutation lists that may change over time as a result of periodic review of new evidence, in accordance with FDA-approved variant classification procedures.*

**Biomarker Rules for Rearrangements that Lead to *NTRK1*, *NTRK2*, or *NTRK3* Fusions:**

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

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

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

Unidentified partners (encoded as N/A) or LINC non-coding partners are also considered CDx biomarker negative.

**Biomarker Rules for ALK Rearrangements:**
Rearrangements in *ALK* shall be considered CDx biomarker positive if the following criterion is met:

- Any oncogenic *ALK* rearrangement whose breakpoint occurs within ALK intron 19 or whose partner gene is EML4.

**Biomarker Rules for *FGFR2* Fusions and Select Rearrangements:**
Rearrangements in *FGFR2* shall be considered CDx biomarker positive if the following criteria are met:

- The rearrangement event involved *FGFR2* and a literature-derived known partner gene regardless of strand or frame,
- The rearrangement event involved *FGFR2* and a novel partner gene that is both in-frame and in-strand,
- Any *FGFR2* rearrangement with one breakpoint in the hotspot region (intron 17-18) and the other breakpoint in the intergenic region or within another gene. This rule excludes 3' duplications of only exon 18,
- Intragenic duplication of kinase domain (exon 9-17).

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

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

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

**Biomarker Rules for *RET* Fusions:**
Fusions in *RET* shall be considered CDx biomarker positive if the following criteria are met:

- Any fusion event involving *RET* and another protein-coding gene
- *RET* and the partner gene must be in the same 5'-3' orientation
- *RET* must be on the 3' end of the detected rearrangement
- The *RET* breakpoint must occur before the start of the kinase domain (amino acids 724-1016)

**Biomarker Rule for AKT1, PIK3CA, and PTEN Alterations**:
Alterations in *AKT1, PIK3CA,* and/or *PTEN* are considered Companion biomarker positive if the following criteria are met.

**Table 12. Biomarker definition for *AKT1, PIK3CA,* and *PTEN***

| Gene (Transcript) | Alteration Type | Protein/Transcript Effect |
|---|---|---|
| *AKT1* (NM_001014431) | SNV | E17K |
| *PIK3CA* (NM_006218) | SNV | R88Q, N345K, C420R, E542K, E545A, E545D, E545Q, E545K, E545G, Q546E, Q546K, Q546R, Q546P, M1043V, M1043I, H1047Y, H1047R, H1047L, and G1049R |
| *PTEN* (NM_000314) | SNV and indels | C124R, G129E, R130Q, C136R, S170R, R173C, C124S, G129V, R130G C136Y, G129R, R130L, R130P |
| | | Any nonsense, frameshift, or splice site alteration<br>• Missense mutations in the start codon and short variant deletions spanning from upstream of the start codon (annotated as M1?) are included in this category |
| | Copy Number Alteration | Homozygous deletion (HD) represents a deletion of one or more exons regardless of transcript in both alleles |
| | Rearrangement (RE) | Any rearrangement that disrupts protein function, regardless of transcript<br>• Intragenic events including duplications of only part of the gene, deletions, or inversions.<br>• Translocations, deletions, or inversions where one breakpoint is in PTEN and the other breakpoint is in another gene or intergenic region. |

**Biomarker Rule for *BRAF* V600 mutations and *BRAF* fusions:**
*BRAF* V600 and *BRAF* fusion alterations are considered companion diagnostic biomarker positive if the following criteria are met.

**Table 13. Biomarker definition for *BRAF* V600X and *BRAF* fusion alterations**

| Gene (Transcript) | Variant Type | Biomarker rules |
|---|---|---|
| *BRAF* (NM_004333) | Short Variant | Any missense alteration resulting in V600X[1] |
| | Rearrangement | Any fusion event that involves *BRAF* and another protein-coding gene and meets all the following criteria:<br>• *BRAF* and the partner gene must be in the same 5'-3' orientation |

| | | |
|---|---|---|
| | | • *BRAF* must be on the 3' end of the detected fusion<br>• The *BRAF* breakpoint must occur after the autoinhibitory domain and before the kinase domain |

[1]X refers to any single amino acid change resulting from a missense alteration.

## VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are FDA-approved companion diagnostic (CDx) alternatives for the detection of genetic alterations using FFPE tumor specimens, as listed in Table 1 of the F1CDx intended use statement. The approved CDx tests are listed in Table 14 below; for additional details see FDA List of Cleared or Approved Companion Diagnostic Devices at: https://www.fda.gov/medical-devices/vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-vitro-and-imaging-tools. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

**Table 14. List of FDA approved CDx assays for genes targeted by F1CDx**

| | Device | Company | Technology | Therapy | Indication |
|---|---|---|---|---|---|
| ***HER2-Amplification*** | PathVysion HER-2 DNA Probe Kit | Abbott Molecular, Inc. | FISH | HERCEPTIN (trastuzumab) | Breast cancer |
| | PATHWAY Anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody | Ventana Medical Systems, Inc. | IHC | HERCEPTIN (trastuzumab) | Breast cancer |
| | InSite HER-2/neu Kit | Biogenex Laboratories, Inc. | IHC | HERCEPTIN (trastuzumab) | Breast cancer |
| | SPOT-Light HER2 CISH Kit | Life Technologies, Inc. | CISH | HERCEPTIN (trastuzumab) | Breast cancer |
| | Bond Oracle HER2 IHC System | Leica Biosystems | IHC | HERCEPTIN (trastuzumab) | Breast cancer |
| | HER2 CISH pharmDx Kit | Dako Denmark A/S | CISH | HERCEPTIN (trastuzumab) | Breast cancer |
| | INFORM HER2 Dual ISH DNA Probe Cocktail | Ventana Medical Systems, Inc. | Dual ISH | HERCEPTIN (trastuzumab) | Breast cancer |
| | HercepTest | Dako Denmark A/S | IHC | HERCEPTIN (trastuzumab)<br>PERJETA (pertuzumab)<br>KADCYLA (ado-trastuzumab emtansine) | Breast cancer<br>Gastric or Gastroesophageal junction adenocarcinoma |

| | Device | Company | Technology | Therapy | Indication |
|---|---|---|---|---|---|
| | HER2 FISH pharmDx Kit | Dako Denmark A/S | FISH | HERCEPTIN (trastuzumab) PERJETA (pertuzumab) KADCYLA (ado-trastuzumab emtansine) | Breast cancer Gastric or Gastroesophageal junction adenocarcinoma |
| ***BRAF-V600E and V600K*** | THxID BRAF Kit | bioMerieux | PCR | MEKINIST (tramatenib) | Melanoma |
| | cobas 4800 BRAF V600 Mutation Test | Roche Molecular Systems, Inc. | PCR | COTELLIC (cobimetinib) ZELBORAF (vemurafenib) | Melanoma |
| ***BRAF-V600E*** | cobas 4800 BRAF V600 Mutation Test | Roche Molecular Systems, Inc. | PCR | ZELBORAF (vemurafenib) | Melanoma |
| | THxID BRAF Kit | bioMerieux | PCR | TAFINLAR (dabrafenib) | Melanoma |
| | Oncomine Dx Target Test | Life Technologies, Inc. | NGS | TAFINLAR (dabrafenib) MEKINIST (trametinib) | NSCLC |
| | *therascreen* BRAF V600E RGQ PCR Kit | QIAGEN | PCR | BRAFTOVI (encorafenib) Erbitux (cetuximab) | CRC |
| ***NRAS*** | Praxis Extended RAS Panel | Illumina, Inc. | NGS | VECTIBIX (panitumumab) | CRC |
| ***KRAS*** | cobas KRAS Mutation Test | Roche Molecular Systems, Inc. | PCR | ERBITUX (cetuximab) VECTIBIX (panitumumab) | CRC |
| | *therascreen* KRAS RGQ PCR Kit | QIAGEN | PCR | ERBITUX (cetuximab) VECTIBIX (panitumumab) | CRC |
| | Praxis Extended RAS Panel | Illumina, Inc. | NGS | VECTIBIX (panitumumab) | CRC |
| ***ALK – fusion*** | Vysis ALK Break Apart FISH Probe Kit | Abbott Molecular, Inc. | FISH | XALKORI (crizotinib) | NSCLC |
| | ALK (D5F3) CDx Assay | Ventana Medical Systems, Inc. | IHC | XALKORI (crizotinib) | NSCLC |

| | Device | Company | Technology | Therapy | Indication |
|---|---|---|---|---|---|
| ***EGFR – Exon 19 deletions & L858R*** | cobas EGFR Mutation Test v2 | Roche Molecular Systems, Inc. | PCR | TARCEVA (erlotinib)<br>TAGRISSO (osimertinib)<br>IRESSA (gefitinib) | NSCLC |
| | *therascreen* EGFR RGQ PCR Kit | QIAGEN | PCR | GILOTRIF (afatinib)<br>IRESSA (gefitinib) | NSCLC |
| | Oncomine Dx Target Test | Life Technologies, Inc. | NGS | IRESSA (gefitinib) | NSCLC |
| ***EGFR T790M*** | cobas EGFR Mutation Test v2 | Roche Molecular Systems, Inc. | PCR | TAGRISSO (osimertinib) | NSCLC |
| ***BRCA1/2*** | BRACAnalysis CDx | Myriad Genetic Laboratories, Inc. | NGS | LYNPARZA (olaparib)<br><br>LYNPARZA (olaparib)-treatment/ maintenance<br><br>TALZENNA (talazoparib) | Breast, pancreatic, and prostate cancers<br>Ovarian cancer<br><br>Breast cancer |
| | Myriad myChoice® CDx | Myriad Genetic Laboratories, Inc. | NGS | ZEJULA (niraparib) or Lynparza (olaparib) | Ovarian cancer |
| ***PIK3CA*** | *therascreen* PIK3CA RGQ PCR Kit | QIAGEN | PCR | PIQRAY (alpelisib) | Breast cancer |
| ***ROS1*** | Oncomine Dx Target Test | Life Technologies, Inc. | NGS | XALKORI (crizotinib) | NSCLC |
| ***RET*** | Oncomine Dx Target Test | Life Technologies, Inc. | NGS | RETEVMO (selpercatinib<br><br>GAVRETO (pralsetinib) | NSCLC and Thyroid Cancer<br><br>NSCLC |

**Abbreviations:** FISH – fluorescence in situ hybridization; IHC – immunohistochemistry; CISH chromogenic in situ hybridization; ISH – in situ hybridization; PCR – polymerase chain reaction; NGS – next generation sequencing.

## VII. MARKETING HISTORY

The F1CDx Premarket Approval (PMA) was originally approved on November 30, 2017, by FDA (P170019) and is commercially available in the U.S. since March 30, 2018. The approved PMA supplements that affected the Intended Use are listed in Table 15.

**Table 15: Marketing History**

| Submission No. | Date of Approval | Biomarker/Update | Patient Population | Drug |
|---|---|---|---|---|
| P170019/S004 | July 1, 2019 | *BRCA1/2* alterations | Ovarian Cancer | LYNPARZA® (olaparib) |
| P170019/S005 | April 10, 2019 | genomic loss of heterozygosity (LOH) | Ovarian Cancer | N/A |
| P170019/S006 | December 3, 2019 | *PIK3CA* alterations | Breast Cancer | PIQRAY® (alpelisib) |
| P170019/S008 | July 1, 2019 | *EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations | Non-Small Cell Lung Cancer | TAGRISSO® (osimertinib) |
| P170019/S011 | May 6, 2020 | *MET* single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping | Non-Small Cell Lung Cancer | TABRECTA® (capmatinib) |
| P170019/S013 | April 17, 2020 | *FGFR2* fusions | Cholangiocarcinoma | PEMZYRE® (pemigatinib) |
| P170019/S015 | May 19, 2020 | mutations in homologous recombination repair (HRR) genes | metastatic castration resistant prostate cancer (mCRPC) | LYNPARZA® (olaparib) |
| P170019/S016 | June 16, 2020 | high tumor mutational burden (TMB) at the cut-off of 10 mutations per megabase (mut/Mb) | Solid Tumors | KEYTRUDA® (pembrolizumab) |
| P170019/S017 | October 23, 2020 | *NTRK1, NTRK2, or NTRK3* fusions | Solid Tumors | VITRAKVI® (larotrectinib) |
| P170019/S021 | May 28, 2021 | *FGFR2* Fusion/Rearrangements | Cholangiocarcinoma | TRUSELTIQ® (infigratinib) |
| P170019/S022 | July 21, 2021 | Additional variants to *BRCA1* and *BRCA2* | Ovarian Cancer | LYNPARZA® (olaparib) or RUBRACA® (rucaparib) |
| | | Additional variants to *BRCA1, BRCA2* and *ATM* | Prostate Cancer | LYNPARZA® (olaparib) |
| P170019/S023 | June 30, 2021 | *ALK* Rearrangements | Non-Small Cell Lung Cancer | ALUNBRIG® (brigatinib) |
| P170019/S025 | November 10, 2021 | *BRAF* V600E Alterations | Melanoma | BRAF Inhibitor Monotherapy |

| Submission No. | Date of Approval | Biomarker/Update | Patient Population | Drug |
|---|---|---|---|---|
| | | | | Group Claim |
| | | *BRAF* V600E or V600K Alterations | Melanoma | BRAF/MEK Inhibitor Combination Group Claim |
| P170019/S029 | February 18, 2022 | Microsatellite Instability High (MSI-H) Status | Solid Tumors | KEYTRUDA® (Pembrolizumab) |
| P170019/S030 | January 19, 2022 | *BRAF* V600 Mutation-Positive | Unresectable Or Metastatic Melanoma | TECENTRIQ ® (atezolizumab) In Combination with Cobimetinib and Vemurafenib |
| P170019/S033 | March 16, 2022 | *EGFR* Exon 19 Deletions or *EGFR* Exon 21 L858R Mutations | Non-Small Cell Lung Cancer | Any One of The FDA-Approved EGFR Tyrosine Kinase Inhibitors (TKI) |
| P170019/S014 | June 7, 2022 | *NTRK1, NTRK2, or NTRK3* fusions | Solid Tumors | ROZLYTREK® (entrectinib) |
| | | *ROS1* fusions | NSCLC | |
| P170019/S042 | August 11, 2023 | *BRCA1, BRCA2* alterations | Prostate Cancer | AKEEGA® (niraparib + abiraterone acetate) |
| P170019/S043 | October 6, 2023 | *RET* fusions | Solid Tumors | RETEVMO® (selpercatinib) |
| P170019/S039 | October 11, 2023 | *BRAF* V600E | NSCLC | BRAFTOVI® (encorafenib) in combination with MEKTOVI® (binimetinib) |
| P170019/S048 | November 16, 2023 | *AKT1, PIK3CA, PTEN* alterations | Breast Cancer | TRUQAP™ (capivasertib) in combination with FASLODEX® (fulvestrant) |
| P170019/S052 | August 30, 2024 | *BRCA1*, *BRCA2* alterations | Prostate Cancer | LYNPARZA® (olaparib) in combination with abiraterone |
| P170019/S054 | January 16, 2025 | *BRAF* V600 mutations and *BRAF* fusions | Pediatric low-grade glioma | OJEMDA® (tovorafenib). |
| P170019/S067 | May 12, 2026 | *MET* single nucleotide variants (SNVs) and indels that lead to MET exon 14 skipping | NSCLC | TEPMETKO® (tepotinib) |

## VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently, inappropriate patient management decisions. Patients with false positive results may undergo treatment with one of the therapies listed in the above intended use statement without clinical benefit and may experience adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment with the indicated therapy. There is also a risk of delayed results, which may lead to delay of treatment with the indicated therapy. For the specific adverse events related to the approved therapeutics, please see the approved drug product labels.

## IX. SUMMARY OF NON-CLINICAL STUDIES

### A. Laboratory Studies

The primary evidence supporting the performance of F1CDx for detecting HRR gene (*ATM, ATR, BRCA1, BRCA2, CDK12, CHEK2, FANCA, MLH1, MRE11A, NBN, PALB2, RAD51C*) alterations in metastatic castration-resistant prostate cancer patients was from data generated using intended use specimens across the validation studies. In addition to the existing platform-level validation results (P170019), as well as validation results for some HRR genes (*ATR, BRCA1, BRCA2, CDK12, CHEK2, PALB2, RAD51C*; P170019/S015), refer to Section IX.A. in P170019 and P170019/S015 Summary of Safety and Effectiveness Data, respectively, analytical concordance, limit of blank (LoB), intermediate precision, and site-to-site precision and confirmation of limit of detection (LoD) studies were conducted to support the indication for additional HRR gene (*ATR, FANCA, MLH1, MRE11A, NBN*) alterations. Table 16 below includes a list of genes/variant types that were represented in the key analytical validation studies, in accordance with their prevalence in mCRPC, to support performance of the assay for detection of the variant types in the HRR genes (*ATR, FANCA, MLH1, MRE11A, NBN*) that were not evaluated in P170019/S015.

**Table 16. Variants Used in Three Key Analytical Validation Studies**

| | LoD | | | | Precision | | | | Accuracy | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HD | RE | ID | SUB | HD | RE | ID | SUB | HD | RE | ID | SUB |
| *ATR* | 0 | 2 | 1 | 1 | 0 | 2 | 1 | 1 | 0 | 0 | 0 | 0 |
| *FANCA* | 1 | 1 | 1 | 0 | 2 | 2 | 2 | 0 | 16 | 2 | 11 | 14 |
| *MLH1* | 1 | 2 | 2 | 0 | 1 | 2 | 2 | 0 | 0 | 0 | 0 | 0 |
| *MRE11A* | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| *NBN* | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |

HD: homozygous deletion
RE: rearrangement
ID: insertion/deletion
SUB: base substitutions
The prevalence of HD and RE alterations in these genes ranged from 0.00% to 1.64% in the TALAPRO-2 trial and an external publicly available prostate cancer database that uses another NGS-based technology.
The prevalence of ID and SUB alterations in these genes ranged from 0.17% to 2.20% in the TALAPRO-2 trial and an external publicly available prostate cancer database that uses another NGS-based technology.

Prostate cancer samples evaluated in the analytical validation studies were extracted with DNAx extraction method using the HiSeq (LoB, LoD, intermediate precision, analytical concordance studies) or the NovaSeq (site-to-site precision study) sequencer configuration. The F1CDx device was updated to include addition of an automated DNA/RNA CoExtraction methodology (CoEx method) to enable isolation of DNA and RNA from the same FFPE tumor specimens. The F1CDx was authorized for DNA only when using the CoEx method. Analytical validation studies were leveraged from P170019/S036 to demonstrate the comparable performance between the two nucleic acid extraction methods. The comparability studies included a total of 8 prostate cancer samples harboring HRR gene (*ATM, CHEK2, PALB2*) alterations. Additionally, the analytical validation studies included 18 samples carrying HRR gene (*BRCA1, BRCA2*) alterations from non-prostate indications. The observed PPA for HRR alterations was 100% when using the DNAx method as reference. The F1CDx device was also updated to replace the Illumina HiSeq 4000 sequencer with the Illumina NovaSeq 6000 sequencer. Analytical validation studies were conducted to demonstrate the comparable performance between the two sequencer configurations, using samples from prostate cancer to provide evidence of the robust detection of HRR gene alterations utilizing the NovaSeq configuration. 9 prostate cancer samples harboring RE and HD alterations in *ATR, FANCA, MLH1, MRE11A,* and *NBN* genes were included in this study. The observed PPA for HRR alterations was 100%.

The F1CDx analysis pipeline was modified after the analytical and clinical validation studies were completed. The analytical and clinical validation provided to support the detection of HRR gene alterations in metastatic castration-resistant prostate cancer by F1CDx were performed using the analysis pipeline versions that have undergone further iteration with modification in the final device design. To demonstrate that the F1CDx analysis pipeline changes continue to support the performance for which the test is approved, regression testing using the most current analysis pipeline version will be performed post-market to confirm the robust performance for F1CDx for the detection of HRR gene alterations (see section XIII).

**1. Analytical Accuracy/Concordance**

**Comparison to an Orthogonal Method**

An analytical accuracy study was conducted previously as part of P170019/S015 to demonstrate the concordance between F1CDx and an externally validated NGS assay (evNGS) for the detection of HRR genes *BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D* and *RAD54L*) alterations.

An additional analytical accuracy study was conducted in this sPMA to demonstrate the concordance between F1CDx and an externally validated NGS assay (evNGS) for the detection of *FANCA* gene alterations. The concordance study was conducted using forty-one (41) biomarker-positive samples sourced from banked DNA derived from FFPE prostate cancer samples tested in FMI's clinical lab, five (5) biomarker-positive

samples selected from relevant clinical trials, and 110 biomarker-negative samples randomly selected from banked residual DNA samples tested in FMI's clinical lab and any relevant clinical trial samples that met the inclusion criteria.

In total, 156 samples were processed in the concordance study with representation of *FANCA* base substitutions, insertions, deletions, rearrangements, and homozygous deletions. Of the 156 samples, eight (8) samples were excluded from the primary analysis because they failed to meet F1CDx processing QC metrics. All 156 samples passed the sample QC metrics for the evNGS assay, however, 25 of the 156 samples were identified to have low tumor content or low sample quality.

A total of 148 samples were included in the concordance analysis. The F1CDx and evNGS results for the detection of FANCA alterations using evNGS as the comparator assay are provided in the contingency table in Table 17. The PPA and NPA using the evNGS assay results as the reference were calculated without adjusting for the distribution of samples selected using F1CDx, positive predictive value (PPV) and negative predictive value (NPV) were also estimated conditional on F1CDx. The statistical analysis in Table 17 showed a positive predictive value (PPV) of 88.37% with 95% CI (75.52%, 94.93%) and a negative predictive value (NPV) of 98.10% with 95% CI (93.32%, 99.48%), and a positive percent agreement (PPA) of 95% with 95% CI (83.50%, 98.62%) and a negative percent agreement (NPA) of 95.37% with 95% CI (89.62%, 98.01%) unadjusted for prevalence.

**Table 17. Contingency Table Comparing the Detection of *FANCA* alterations by F1CDx and evNGS.**

| | **evNGS+** | **evNGS-** | **Invalid** | **Total** | |
|---|---|---|---|---|---|
| **F1CDx+** | 38 | 5 | 0 | 43 | PPV: 88.37% [95%CI*:75.52%, 94.93%] |
| **F1CDx-** | 2 | 103 | 0 | 105 | NPV:98.10%[95% CI*: 93.32%, 99.48%] |
| **Invalid** | 3 | 5 | 0 | 8 | |
| **Total** | 40 | 108 | 0 | 148 | |
| | PPA:95.00% [95%CI*:83.50%, 98.62%] | NPA:95.37% [95% CI*: 89.62%, 98.01%] | | | |

*95% 2-sided confidence intervals (CI) were calculated using the Wilson score method

There were 7 discordant calls between F1CDx and evNGS test results. Of the 7 calls, the following was observed:

- Two samples were detected by the evNGS, but reported as biomarker negative by F1CDx. One sample was flagged for low tumor purity by F1CDx, and the other sample was flagged by F1CDx for noisy Copy Number Alteration (CNA)

and detected by F1CDx but removed as an artifact post-curation due to the copy number noise observed in the sample.

- Two samples were detected by F1CDx as *FANCA* loss and detected as deep copy loss by the evNGS but reported as biomarker negative taking a more stringent approach, because the SNP analysis could not confirm if the copy loss was biallelic.
- One sample was detected by F1CDx as *FANCA* loss but detected as LOH by the evNGS based on SNP analysis.
- One sample was detected by F1CDx as *FANCA* insertion and the evNGS detected an adjacent insertion of the same length but in the intronic region.
- One sample was detected by F1CDx as *FANCA* rearrangement but not detected by the evNGS due to insufficient coverage near the exon boundary for this sample.

## 2. Analytical Sensitivity

### a. Limit of Blank (LoB)

A LoB study was conducted for HRR gene alterations in *ATR, FANCA, MLH1, MRE11A,* and *NBN*. The LoB of zero was confirmed by testing six biomarker negative prostate cancer samples. Of the 60 replicates, 100% produced valid results and no HRR gene alterations were detected across the sample replicates that were evaluated. Therefore, the false positive rate for calling alterations was determined to be 0.00%.

The LoB was also confirmed as zero for HRR gene alterations in *BRCA1, BRCA2, ATM, BRIP1, BARD1, CDK12, CHEK1, CHEK2, FANCL, PALB2, PPP2R2A, RAD518, RAD51C, RAD51D,* and *RAD54L* in a previous study conducted as part of the approved PMA P170019/S015, demonstrating a false-positive rate of 0.00% for detecting HRR gene alterations in biomarker-negative FFPE tissue samples from patients with prostate cancer.

### b. Limit of Detection (LoD)

The F1CDx LoD for the detection of HRR gene alterations was established through confirmation of platform-based LoD and within CDx-specific LoD studies.

The median platform LoD for HRR gene alterations was established through a previously conducted study for the approved PMA P170019. The LoD for HRR gene alterations (*ATR*, *FANCA*, *MLH1*, *MRE11A*, and *NBN)* was confirmed through a precision study that used samples at or near 1x LoD, as detailed in section 2b below. A summary of the previously established LoD and confirmed LoD results is provided in Table 18 below.

**Table 18. Previously Established LoD and Confirmed LoD Results (including *ATR*, FANCA, MLH1, MRE11A, and *NBN*)**

| | Previously Established Median Platform LoD (% variant allele frequency (VAF) for SVs, chimeric reads for REs, and % tumor purity (TP) for HDs) | Confirmed LoD (%VAF for SVs, chimeric reads for REs, and %TP for HDs) |
|---|---|---|
| **HRR gene base substitutions** | 8.30% | 8.30%-9.76% |
| **HRR gene indels** | 8.20%[1]; 13.70%[2] | 9.25%-15.79% |
| **HRR gene homozygous deletions** | 33.40% | 50.76%-63.30% |
| **HRR gene rearrangements** | 17.23 | 19.48-21.87 |

[1]Indels at non-homopolymer context, including insertions up to 42bp and deletions up to 276bp
[2]Indels at homopolymer context (6bp repeat)
SV: short variant (base substitution, insertion, deletion); RE: rearrangement; HD: homozygous deletion

An additional LoD study was executed to determine the LoD of the F1CDx assay for detection of *FANCA* rearrangements in prostate cancer. DNA from one prostate cancer sample harboring *FANCA* rearrangements selected from the FMI DNA archives was assessed at five chimeric read levels (30, 15, 10, 5, 2.5) with 20 replicates tested for each dilution level, except for the highest titration level (30 reads), where only 14 replicates were tested. The LoD was determined to be 23.15 chimeric reads for *FANCA* rearrangements using the probit method with an estimated 95% probability of detection.

**3. Precision**

**a. Intermediate Precision for *FANCA* alterations**

An intermediate precision study was performed to evaluate precision for calling of *FANCA* alterations using DNA derived from four unique FFPE prostate cancer samples at or near LoD. The samples selected for testing are presented in Table 19.

**Table 19. List of Samples**

| Sample ID | Targeted alterations | TP (%) | Supporting read pairs for RE, TP (%) for HD or VAF (%) for SVs | Defined Alteration LoD |
|---|---|---|---|---|
| 1 | *FANCA_PRKACA* likely truncation | 61.9 | 37 reads | Platform Level LoD for RE: 16.85-22.38 average reads<br>HRR gene LoD for RE: 14.3-39.3 average reads |
| 2 | *FANCA_ZNF778* likely truncation | 20 | 20 reads | |
| 3 | *FANCA* known homozygous deletion | 29.8 | 29.8% TP | Platform Level LoD for HD: TP (%). 33.4.<br>HRR gene LoD for HD: TP (%) 23.9 |
| 4[1] | W911fs*31 likely frameshift | 41.6 | 13.96% VAF | Platform level for likely indel: MAF (%) 6.0-10.2 |

[1]Source samples were titrated with biomarker-negative DNA to the desired VAF (1.0X to 2.5X LoD)

Each sample was tested in duplicate for each run/plate. Two runs were performed with 2 reagent lots on 3 sequencers (2 replicates x 2 runs x 2 reagent lots x 3 sequencers). A total of 108 replicates were processed for this study; however, data from 12 sample replicates processed on 2 of the plates were not included in the study analysis due to an incorrect reagent lot being used. Out of the planned 96 sample replicates, 92 sample replicates were processed with 90 sample replicates successfully passing LC to sequencing. Reproducibility results are detailed in Table 20.

**Table 20. Reproducibility for Targeted *FANCA* Alterations**

| Target Alteration | Average Observed MAF[1](%)/ TP[2](%)/reads[3] | Median Platform LoD | # of Positive Replicates | # of Valid Replicates | Reproducibility (%) | 95% 2-sided Score CIs (%) | Fold LoD (x) |
|---|---|---|---|---|---|---|---|
| *FANCA_PRKACA* _truncation | 21 | 17.23 | 23 | 23 | 100.00 | [85.69, 100.00] | 1.22 |
| *FANCA_ZNF778* _truncation | 12.88[4] | 17.23 | 21 | 24 | 87.50 | [69.00, 95.66] | 0.75 |
| *FANCA*_loss | 26.76 | 33.40 | 20 | 20 | 100.00 | [83.89, 100.00] | 0.80 |
| *FANCA*_2730_2731delCT | 13.37 | 8.20 | 22 | 22 | 100.00 | [85.13, 100.00] | 1.63 |

[1]MAF is applicable for short variants
[2]TP is applicable for copy number variants
[3]Reads is applicable for rearrangements
[4]Reads value was set as 0 for non-detected replicates

One sample (target variant *FANCA_ZNF778*_truncation) had point estimate reproducibility less than 90%. Upon investigation, it was noticed that the pipeline detected the targeted variant for all three (3) discordant replicates, but the variant was filtered due to low read support.

To evaluate repeatability, valid results from each of the replicates within a specific sequencer, reagent lot, and run for each sample were calculated as fraction of concordant pairs (i.e., same biomarker status of the two (2) replicates from the same plate). The results are shown in Table 21 below.

**Table 21. Repeatability of Target *FANCA* Alteration Detection**

| Source Sample | Target Alteration | # of Agreed Pairs | # of Valid Pairs | Repeatability (%) | 95% 2-sided Score CIs (%) |
|---|---|---|---|---|---|
| 1 | *FANCA_PRKACA* _truncation | 11 | 11 | 100.00 | [74.12, 100.00] |
| 2 | *FANCA_ZNF778*_truncation | 9 | 12 | 75.00 | [46.77, 91.11] |

| | | | | | |
|---|---|---|---|---|---|
| 3 | *FANCA*_loss | 10 | 10 | 100.00 | NA[1] |
| 4 | *FANCA*_2730_2731delCT | 10 | 10 | 100.00 | NA[1] |

[1]CI not provided for sample size ≤10.

**b. Site-to-Site Precision**

To evaluate the performance of HRR gene alteration detection [REs, Short Variant (SV)s, and HDs], a site-to-site precision study using 16 prostate cancer samples harboring HRR gene alterations (biomarker-positive samples) in *ATR*, *FANCA*, *MLH1*, *MRE11A*, and *NBN* at 1x -1.5x LoD was performed. Table 22 below lists the biomarker-positive samples harboring targeted alterations (SVs and REs) that required titration. Samples were titrated with biomarker negative diluent DNA extracted from prostate cancer FFPE samples to achieve the targeted VAF and chimeric reads. Table 23 lists the biomarker-positive samples harboring SVs and HDs that did not require titration. Precision of F1CDx was assessed by chimeric reads for REs, by VAF for SVs, and by TP for HDs close to the LoD. In total, 384 replicates were evaluated for this study.

**Table 22. List of Biomarker-Positive Samples That Required Titration**

| Sample ID | Disease Ontology | Variant Type | Alteration |
|---|---|---|---|
| 1 | Prostate acinar adenocarcinoma | RE | *ATR-PARK2* truncation |
| 2 | Prostate acinar adenocarcinoma | RE | *ATR-N/A** truncation |
| 3 | Prostate acinar adenocarcinoma | RE | *MLH1-USP38* truncation |
| 4 | Prostate undifferentiated carcinoma | RE | *RAF1-MLH1* truncation |
| 5 | Prostate acinar adenocarcinoma | RE | *MRE11A-SEPT10* truncation |
| 6 | Prostate acinar adenocarcinoma | RE | *NBN-N/A** truncation |
| 7 | Prostate acinar adenocarcinoma | RE | *NBN-NBN* deletion |
| 8 | Prostate acinar adenocarcinoma | RE | *NBN-N/A** truncation |
| 9 | Prostate acinar adenocarcinoma | SV (indel) | *ATR*_c. 1883_1884AT>CACAAG |
| 10 | Prostate neuroendocrine carcinoma | SV (insertion) | *FANCA*_c. 3558_3559insG |
| 11 | Prostate acinar adenocarcinoma | SV (deletion) | *MLH1*_c. 2245delC |
| 12 | Prostate neuroendocrine carcinoma | SV (insertion) | *MLH1*_c. 1489_1490insC |
| 13 | Prostate acinar adenocarcinoma | SV (substitution) | *MRE11A_c.178 3+1G>A* |

*An "-*N/A*" partner gene indicates that the partner gene was not identified/ represents intragenic breakpoints

**Table 23. List of Biomarker-Positive Samples That Did Not Require Titration (Neat)**

| Sample ID | Disease Ontology | Variant Type | Alteration |
|---|---|---|---|
| 14 | Prostate acinar adenocarcinoma | *HD* | *MLH1* loss (exons 19 of 19) |
| 15 | Prostate acinar adenocarcinoma | HD | *FANCA* loss (exons 43 of 43) |
| 16 | Prostate acinar adenocarcinoma | SV (substitution) | *ATR_* c. 3659T>A (substitution) |

The study evaluated 16 biomarker-positive samples, at two (2) laboratory locations in Cambridge, MA, and Morrisville, NC. For each sample, 24 replicates were processed; the variables examined in the study included different sites, reagent lots, and library construction start days.

The 16 samples harboring HRR gene alterations in *ATR, FANCA, MLH1, MRE11A,* and *NBN* were processed in the precision study at ~1-1.5x LoD, and at a challenging DNA input (close to 50 ng).

Reproducibility was evaluated in the prostate cancer samples by processing replicates from the same source sample, under conditions where one factor was changed at a time. The alterations had to be detected in each replicate of the source sample and meet the biomarker definition to be considered a positive call. The point estimates and 95% two-sided score CIs for reproducibility of each sample are detailed in Table 24. 14 out of 16 samples had variants ≥1x LoD and 2 samples had variants below LoD.

**Table 24. Reproducibility for All Targeted Variants**

| Alteration | Variant Type | Observed Average %MAF[1] /Reads[2] /%TP[3] | Median Platform LoD | # of Positive Replicates | # of Valid Replicates | Reproducibility (%). | 95% Two-sided Score CI (%) | Fold LoD (x) |
|---|---|---|---|---|---|---|---|---|
| *ATR_1883_1884AT>CACAAG* | SV | 9.53 | 8.20 | 24 | 24 | 100.00 | [86.20, 100.00] | 1.16 |
| *ATR_3659T>A* | SV | 8.48 | 8.30 | 24 | 24 | 100.00 | [86.20, 100.00] | 1.02 |
| *ATR-PARK2* | RE | 21.87 | 17.23 | 22 | 23 | 95.65 | [79.01, 99.23] | 1.27 |
| *FANCA_3558_3559insG* | SV | 9.25 | 8.20 | 24 | 24 | 100.00 | [86.20, 100.00] | 1.13 |
| *FANCA_loss* | HD | 63.30 | 33.40 | 24 | 24 | 100.00 | [86.20, 100.00] | 1.90 |
| *MLH1_1489_1490insC* | SV | 15.79 | 13.70 | 24 | 24 | 100.00 | [86.20, 100.00] | 1.15 |
| *MLH1_2245delC* | SV | 9.41 | 8.20 | 24 | 24 | 100.00 | [86.20, 100.00] | 1.15 |
| *MLH1_loss* | HD | 50.76 | 33.40 | 24 | 24 | 100.00 | [86.20, 100.00] | 1.52 |
| *MLH1-RAF1* | RE | 21.71 | 17.23 | 24 | 24 | 100.00 | [86.20, 100.00] | 1.26 |
| *MLH1-USP38* | RE | 20.08 | 17.23 | 24 | 24 | 100.00 | [86.20, 100.00] | 1.17 |

| Alteration | Variant Type | Observed Average %MAF[1] /Reads[2] /%TP[3] | Median Platform LoD | # of Positive Replicates | # of Valid Replicates | Reproducibility (%). | 95% Two-sided Score CI (%) | Fold LoD (x) |
|---|---|---|---|---|---|---|---|---|
| *MRE11A_17 83+1G>A* | SV | 9.76 | 8.30 | 24 | 24 | 100.00 | [86.20, 100.00] | 1.18 |
| *MRE11A-SEPT10* | RE | 19.48 | 17.23 | 22 | 23 | 95.65 | [79.01, 99.23] | 1.13 |
| *NBN-N/A* | RE | 20.33 | 17.23 | 23 | 24 | 95.83 | [79.76, 99.26] | 1.18 |
| *NBN-N/A* | RE | 18.62 | 17.23 | 21 | 24 | 87.50 | [69.00, 95.66] | 1.08 |
| *ATR-N/A* | RE | 15.54 | 17.23 | 21 | 24 | 87.50 | [69.00, 95.66] | 0.90 |
| *NBN-NBN* | RE | 13.29 | 17.23 | 20 | 24 | 83.33 | [64.15, 93.32] | 0.77 |

[1]MAF is applicable for short variants
[2]Reads is applicable for rearrangements
[3]TP is applicable for copy number variants
An "-*N/A*" partner gene indicates that the partner gene was not identified/ represents intragenic breakpoints

One sample with a targeted NBN-N/A RE tested at 1.08x LoD demonstrated reproducibility of 87.50%. This sample had three replicates with evidence for the variant observed but filtered due to insufficient supporting read evidence.
One sample with a targeted ATR-N/A RE and one sample with a targeted NBN-NBN RE demonstrated reproducibility <95%. These two samples had observed reads below the median platform LoD.

Repeatability was assessed by processing samples with two replicates from the same source sample and plate within each site, reagent lot, and LC start day. The result was considered to be in agreement if the duplicate replicates processed under identical conditions had the same detection status for the targeted variants. The repeatability results for each targeted variant are detailed in Table 25 below.

**Table 25. Repeatability for All Targeted Variants**

| Alteration | Variant Type | # of Agree Pairs | # of Valid Pairs | Repeatability (%) | 95% Two-sided Score CI (%) |
|---|---|---|---|---|---|
| *ATR_1883_1884AT>CACAAG* | SV | 12 | 12 | 100.00 | [75.75, 100.00] |
| *ATR_3659T>A* | SV | 12 | 12 | 100.00 | [75.75, 100.00] |
| *ATR-PARK2* | RE | 10 | 11 | 90.91 | [62.26, 98.38] |
| *FANCA_3558_3559insG* | SV | 12 | 12 | 100.00 | [75.75, 100.00] |
| *FANCA_loss* | HD | 12 | 12 | 100.00 | [75.75, 100.00] |
| *MLH1_1489_1490insC* | SV | 12 | 12 | 100.00 | [75.75, 100.00] |
| *MLH1_2245delC* | SV | 12 | 12 | 100.00 | [75.75, 100.00] |
| *MLH1_loss* | HD | 12 | 12 | 100.00 | [75.75, 100.00] |
| *MLH1-RAF1* | RE | 12 | 12 | 100.00 | [75.75, 100.00] |

| Alteration | Variant Type | # of Agree Pairs | # of Valid Pairs | Repeatability (%) | 95% Two-sided Score CI (%) |
|---|---|---|---|---|---|
| *MLH1-USP38* | RE | 12 | 12 | 100.00 | [75.75, 100.00] |
| *MRE11A 178 3+1G>A* | SV | 12 | 12 | 100.00 | [75.75, 100.00] |
| *MRE11A-SEPT10* | RE | 10 | 11 | 90.91 | [62.26, 98.38] |
| *NBN-N/A* | RE | 11 | 12 | 91.67 | [64.61, 98.51] |
| *NBN-N/A* | RE | 9 | 12 | 75.00 | [46.77, 91.11] |
| *ATR-N/A* | RE | 9 | 12 | 75.00 | [46.77, 91.11] |
| *NBN-NBN* | RE | 8 | 12 | 66.67 | [39.06, 86.19] |

Three samples harboring RE demonstrated repeatability <95%. One sample was an NBN-N/A RE tested close to LoD (1.08x LoD). The other two samples, one sample with an ATR-N/A RE and one sample with an NBN-NBN RE, were tested below LoD (0.90 and 0.77x LoD, respectively).

### B. Animal Studies

No animal studies were conducted using the F1CDx assay.

### C. Additional Studies

No additional studies were conducted using the F1CDx assay.

## X. SUMMARY OF PRIMARY CLINICAL STUDY

The clinical performance of F1CDx for identification of prostate cancer patients with HRR gene alterations who may benefit from treatment with TALZENNA® (talazoparib) in combination with XTANDI® (enzalutamide) was demonstrated based on results from the TALAPRO-2 clinical trial.

A summary of the clinical study design is presented below:

### A. TALAPRO-2 Study Design

TALAPRO-2 is an international, double-blinded, placebo-controlled, randomized Phase 3 study of talazoparib in combination with enzalutamide compared with placebo in combination with enzalutamide in patients with mCRPC, where no systemic cancer treatments have been initiated after documentation of CRPC with the exception of androgen deprivation therapy (ADT) and first-generation anti-androgen agents. Part 1 was open-label and non-randomized and evaluated the safety, tolerability, and pharmacokinetics (PK) of talazoparib in combination with enzalutamide. The primary objective of Part 1 was to determine the starting dose of talazoparib in combination with enzalutamide to be used in Part 2. Part 2 was randomized, double-blinded, and placebo-controlled and evaluated the safety and efficacy of talazoparib in combination with enzalutamide compared with placebo in combination with enzalutamide. The major efficacy outcome measure of Part 2 was radiographic progression-free survival (rPFS)

evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1 and Prostate Cancer Working Group (PCWG3) (bone) criteria, assessed by Blinded Independent Central Review (BICR). An additional efficacy outcome measure was Overall Survival (OS). Part 2 of the study included 2 cohorts and were 1:1 randomized to talazoparib in combination with enzalutamide versus placebo in combination with enzalutamide.

**Part 2 Cohort 1**
The first cohort (Cohort 1) enrolled 805 mCRPC patients unselected for HRR status (referred to as an all-comers population). Although patients were unselected for HRR status, HRR deficiency assessment prior to randomization was required for stratification.

**Part 2 Cohort 2**
After enrollment for Cohort 1 was closed, enrollment into the study continued but was restricted to patients with HRR deficiencies (Cohort 2). Cohort 2 enrolled 230 patients harboring HRR gene alterations that were likely to sensitize the patient's tumor to poly (adenosine diphosphate [ADP]-ribose) polymerase (PARP) inhibition (HRR-deficient).

Patients with HRR gene alterations were identified prior to randomization by central testing of tumor tissue or blood samples (liquid biopsy) using primarily the F1CDx and/or the F1LCDx assays.
Of the 805 mCRPC patients enrolled into Cohort 1, 169 patients were HRR-deficient and thus, there were 399 HRR-deficient patients from Cohort 1 and Cohort 2.

**Clinical Validation Study Design**
The clinical effectiveness of TALZENNA® in combination with XTANDI® among patients with HRR gene alterations as determined by F1CDx was demonstrated through a clinical validation study using specimens from the TALAPRO-2 clinical trial. The clinical validation study was conducted to: (1) evaluate the efficacy of talazoparib in combination with enzalutamide in mCRPC patients with HRR gene alterations as determined by F1CDx and (2) assess the robustness of the efficacy evaluated with sensitivity analyses that account for the uncertainty due to missing data for the F1CDx biomarker status.

The clinical validation for F1CDx was based on 399 HRR-deficient patients from TALAPRO-2, which included clinical trial patients harboring HRR gene alterations from Cohorts 1 and 2. The clinical performance for F1CDx was assessed through the primary efficacy analysis of HRR gene alteration positive patients as determined by F1CDx.

1. **Clinical Inclusion and Exclusion Criteria**

   **Inclusion criteria:**
   Enrollment in the TALAPRO-2 study was limited to patients who met the following key inclusion criteria:

   - At least 18 years of age. For Japan, at least 20 years of age.

- Histologically or cytologically confirmed adenocarcinoma of the prostate without small cell or signet cell features. If the patient does not have a prior histological diagnosis, a baseline de novo biopsy must be used to confirm the diagnosis and to support biomarker analysis.
- Asymptomatic or mildly symptomatic metastatic castration resistant prostate cancer (mCRPC).
- For enrollment into Part 2 only (optional in Part 1): assessment of DNA damage repair (DDR) mutation status by prospective analysis of blood (liquid biopsy), or tissue (de novo or archival tissue), or historical analysis (with Sponsor pre-approval), of most recent tumor tissue per FoundationOne® testing. (Note: for patients enrolling in Part 1, DDR deficiency testing is optional).
- For enrollment into Part 2 only (optional for Part 1): Unless prohibited by local regulations or ethics committee decision, consent to a saliva sample collection for retrospective sequencing of the same DDR genes tested on tumor tissue and blood (liquid biopsy), or a subset thereof, and to serve as a germline control in identifying tumor mutations.
- Surgically or medically castrated, with serum testosterone ≤50 ng/dL (≤1.73 nmol/L) at screening. Ongoing androgen deprivation therapy with a gonadotropin releasing hormone (GnRH) agonist or antagonist for patients who have not undergone bilateral orchiectomy must be initiated at least 4 weeks before Day 1 (Part 1) or randomization (Part 2) and must continue throughout the study.
- Metastatic disease in bone documented on bone scan or in soft tissue documented on CT/MRI scan.
- Progressive disease at study entry in the setting of medical or surgical castration.

**Exclusion criteria:**
Patients were <u>not</u> permitted to enroll in the TALAPRO-2 study if they met any of the following key exclusion criteria:

- Any prior systemic cancer treatment initiated in the non-metastatic CRPC or mCRPC disease state. (ADT and first-generation anti-androgens received in the CRPC disease state are NOT exclusionary).
- Patients whose only evidence of metastasis is adenopathy below the aortic bifurcation.
- Prior treatment with second-generation androgen receptor inhibitors (enzalutamide, apalutamide, and darolutamide), a PARP inhibitor, cyclophosphamide, or mitoxantrone for prostate cancer.
- Prior treatment with platinum-based chemotherapy within 6 months (from the last dose) prior to Day 1 (Part 1) or randomization (Part 2), or any history of disease progression on platinum-based therapy within 6 months (from the last dose).
- Treatment with cytotoxic chemotherapy which includes but is not limited to docetaxel, biologic therapy including sipuleucel-T, or radionuclide therapy received in the castration-sensitive prostate cancer is NOT

exclusionary if discontinued in the 28 days prior to Day 1 (Part 1) or randomization (Part 2). Prior treatment with abiraterone in the castration-sensitive settings is not exclusionary if discontinued prior to randomization. Hormonal therapy (eg, bicalutamide, nilutamide, flutamide, estrogens) are not exclusionary if discontinued prior to randomization. Prednisone>10 mg/day (or equivalents) is exclusionary.

- Treatment with any investigational agent within 4 weeks before Day 1 (Part 1) or randomization (Part 2).
- Prior treatment with opioids for pain related to either primary prostate cancer or metastasis within 28 days prior to Day 1 (Part 1) or randomization (Part 2).
- Current use of potent P-gp inhibitors within 7 days prior to Day 1 (Part 1) or randomization (Part 2).
- Known or suspected brain metastasis or active leptomeningeal disease.
- Symptomatic or impending spinal cord compression or cauda equina syndrome.
- Any history of myelodysplastic syndrome, acute myeloid leukemia, or prior malignancy *except* for any of the following:
    - Carcinoma in situ or non melanoma skin cancer.
    - Any prior malignancies ≥3 years before randomization with no subsequent evidence of recurrence or progression regardless of the stage.
    - Stage 0 or Stage 1 cancer <3 years before randomization that has a remote probability of recurrence or progression in the opinion of the investigator.

**2. Follow-up Schedule**

Patients were followed-up in accordance with the TALAPRO-2 study safety follow-up and long-term follow-up schedules. The F1CDx clinical validation study involved only testing and analysis of tissue tumor FFPE samples; as such, no additional patient subject follow-up was conducted with regard to the diagnostic study.

**3. Clinical Endpoints**

Clinical efficacy of talazoparib in combination with enzalutamide in mCRPC patients with HRR gene alterations by the F1CDx assay was evaluated by the following primary efficacy endpoint: rPFS, defined as the time from the date of randomization to first objective evidence of radiographic progression as assessed in soft tissue per RECIST 1.1 or in bone (upon subsequent confirmation) per PCWG3 guidelines by BICR, or death, whichever occurred first. The primary efficacy analysis set included F1CDx+ HRR-deficient patients, who were HRR gene alteration positive by the F1CDx assay based on the F1CDx biomarker definition for HRR gene alterations.

## B. Accountability of PMA Cohort

The clinical validation study was based on the combined HRR-deficient population in the TALAPRO-2 study which consisted of 399 HRR-deficient patients (169 from Cohort 1 and 230 from Cohort 2). Of these 399 HRR-deficient patients, a total of 302 patients had valid results by F1CDx and 97 were not tested or did not have valid results by F1CDx. Out of the 302 patients with F1CDx valid results, 247 were HRR+ by F1CDx and 55 were HRR- by F1CDx (but positive by other enrolling assays).

## C. Study Population Demographics and Baseline Parameters

The summary of selected demographic, patient and disease characteristics for the F1CDx HRR-deficient (n=247) population is shown in Table 26.

**Table 26. Demographic and patient characteristics for F1CDx HRR-deficient population**

| Baseline/Demographic Characteristics | Summary Statistics/Categorical Level | Treatment Group (N=132) | Placebo Group (N=115) | P-value |
|---|---|---|---|---|
| Age | min | 41 | 44.00 | 0.827 |
| | Q1 | 64.00 | 63.00 | |
| | median | 68.00 | 70.00 | |
| | mean | 68.98 | 69.31 | |
| | Q3 | 76.00 | 76.00 | |
| | max | 90.00 | 90.00 | |
| | SD | 9.06 | 8.98 | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| Age group | < 65 | 37 (28.03%) | 35 (30.43%) | 0.947 |
| | >= 75 | 39 (29.55%) | 33 (28.70%) | |
| | 65 to < 75 | 56 (42.42%) | 47 (40.87%) | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| BMI | min | 18.60 | 18.40 | 0.370 |
| | Q1 | 24.00 | 24.45 | |
| | median | 27.20 | 27.85 | |
| | mean | 27.63 | 28.08 | |
| | Q3 | 29.95 | 30.87 | |
| | max | 43.50 | 43.20 | |
| | SD | 4.55 | 5.06 | |
| | (Missing) | 0 (0.00%) | 1 (0.87%) | |
| Baseline use of a bone protecting agent | No | 112 (84.85%) | 98 (85.22%) | 1.000 |
| | Yes | 20 (15.15%) | 17 (14.78%) | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |

| Baseline/Demographic Characteristics | Summary Statistics/Categorical Level | Treatment Group (N=132) | Placebo Group (N=115) | P-value |
|---|---|---|---|---|
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Baseline circulating tumor cells (CTC) count (continuous) | min | 0.00 | 0.00 | 0.521 |
| | Q1 | 0.00 | 0.00 | |
| | median | 1.00 | 1.00 | |
| | mean | 46.30 | 22.32 | |
| | Q3 | 13.75 | 11.00 | |
| | max | 1218.00 | 457.00 | |
| | SD | 161.29 | 69.39 | |
| | (Missing) | 26 (19.70%) | 20 (17.39%) | |
| Baseline circulating tumor cells (CTC) count (categorical with cutoff = 5) | <5 | 64 (48.48%) | 60 (52.17%) | 0.772 |
| | >=5 | 42 (31.82%) | 35 (30.43%) | |
| | (Missing) | 26 (19.70%) | 20 (17.39%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Baseline circulating tumor cells (CTC) count (categorical with cutoff = 0) | >0 | 65 (49.24%) | 55 (47.83%) | 0.667 |
| | 0 | 41 (31.06%) | 40 (34.78%) | |
| | (Missing) | 26 (19.70%) | 20 (17.39%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Distribution of disease at screening involved: bone | No | 11 (8.33%) | 22 (19.13%) | 0.023 |
| | Yes | 117 (88.64%) | 93 (80.87%) | |
| | (Missing) | 4 (3.03%) | 0 (0.00%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Distribution of disease at screening involved: lymph node | No | 73 (55.30%) | 64 (55.65%) | 0.897 |
| | Yes | 55 (41.67%) | 51 (44.35%) | |
| | (Missing) | 4 (3.03%) | 0 (0.00%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Distribution of disease at screening involved: other soft tissue | No | 113 (85.61%) | 106 (92.17%) | 0.391 |
| | Yes | 15 (11.36%) | 9 (7.83%) | |
| | (Missing) | 4 (3.03%) | 0 (0.00%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Distribution of disease at screening involved: visceral disease (liver) | No | 123 (93.18%) | 113 (98.26%) | 0.451 |
| | Yes | 5 (3.79%) | 2 (1.74%) | |
| | (Missing) | 4 (3.03%) | 0 (0.00%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| | No | 111 (84.09%) | 106 (92.17%) | 0.213 |

| Baseline/Demographic Characteristics | Summary Statistics/Categorical Level | Treatment Group (N=132) | Placebo Group (N=115) | P-value |
|---|---|---|---|---|
| Distribution of disease at screening involved: visceral disease (lung) | Yes | 17 (12.88%) | 9 (7.83%) | |
| | (Missing) | 4 (3.03%) | 0 (0.00%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Distribution of disease at screening involved: visceral disease (lung or liver) | No | 106 (80.30%) | 106 (92.17%) | 0.034 |
| | Yes | 22 (16.67%) | 9 (7.83%) | |
| | (Missing) | 4 (3.03%) | 0 (0.00%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Disease localization at screening | Bone only | 50 (37.88%) | 49 (42.61%) | 0.008 |
| | Both bone and soft tissue | 67 (50.76%) | 44 (38.26%) | |
| | None | 4 (3.03%) | 0 (0.00%) | |
| | Soft tissue only | 11 (8.33%) | 22 (19.13%) | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Eastern Cooperative Oncology Group (ECOG) performance status | 0 | 87 (65.91%) | 74 (64.35%) | 0.894 |
| | 1 | 45 (34.09%) | 41 (35.65%) | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Geographic region | Asia | 30 (22.73%) | 22 (19.13%) | 0.854 |
| | European Union/GBR | 59 (44.70%) | 56 (48.70%) | |
| | North America | 14 (10.61%) | 14 (12.17%) | |
| | Rest of the world | 29 (21.97%) | 23 (20.00%) | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Gleason score category 1 | High [8-10] | 105 (79.55%) | 81 (70.43%) | 0.088 |
| | Low [2-4] | 1 (0.76%) | 0 (0.00%) | |
| | Medium [5-7] | 23 (17.42%) | 31 (26.96%) | |
| | (Missing) | 3 (2.27%) | 3 (2.61%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Gleason score category 2 | <=6 | 5 (3.79%) | 3 (2.61%) | 0.223 |
| | 7 | 19 (14.39%) | 28 (24.35%) | |
| | 8 | 29 (21.97%) | 25 (21.74%) | |
| | 9-10 | 76 (57.58%) | 56 (48.70%) | |
| | (Missing) | 3 (2.27%) | 3 (2.61%) | |

| Baseline/Demographic Characteristics | Summary Statistics/Categorical Level | Treatment Group (N=132) | Placebo Group (N=115) | P-value |
|---|---|---|---|---|
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Histopathological classification | Adenocarcinoma | 132 (100.00%) | 114 (99.13%) | 0.466 |
| | Adenocarcinoma with neuroendocrine features | 0 (0.00%) | 1 (0.87%) | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Number of bone metastases at screening | 0 | 19 (14.39%) | 23 (20.00%) | 0.762 |
| | 1-4 | 47 (35.61%) | 38 (33.04%) | |
| | 5-9 | 29 (21.97%) | 23 (20.00%) | |
| | 10-20 | 16 (12.12%) | 16 (13.91%) | |
| | >20 | 21 (15.91%) | 15 (13.04%) | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Baseline pain score by Brief Pain Inventory-Short Form (BPI-SF) | 0-1 | 94 (71.21%) | 64 (55.65%) | 0.012 |
| | 2-3 | 38 (28.79%) | 51 (44.35%) | |
| | >3 | 0 (0.00%) | 0 (0.00%) | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Baseline serum Prostate-Specific Antigen (PSA) (ng/mL) | min | 0.31 | 0.10 | 0.412 |
| | Q1 | 6.22 | 8.15 | |
| | median | 15.07 | 18.00 | |
| | mean | 89.69 | 68.99 | |
| | Q3 | 61.35 | 56.55 | |
| | max | 3412.00 | 1055.00 | |
| | SD | 324.61 | 155.72 | |
| | (Missing) | 2 (1.52%) | 0 (0.00%) | |
| Race | ASIAN | 31 (23.48%) | 23 (20.00%) | 0.904 |
| | BLACK OR AFRICAN AMERICAN | 4 (3.03%) | 3 (2.61%) | |
| | MULTIPLE | 0 (0.00%) | 1 (0.87%) | |
| | NATIVE HAWAIIAN OR OTHER PACIFIC ISLANDER | 1 (0.76%) | 0 (0.00%) | |

| Baseline/Demographic Characteristics | Summary Statistics/Categorical Level | Treatment Group (N=132) | Placebo Group (N=115) | P-value |
|---|---|---|---|---|
| | WHITE | 91 (68.94%) | 77 (66.96%) | |
| | (Missing) | 5 (3.79%) | 11 (9.57%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Race group | ASIAN | 31 (23.48%) | 23 (20.00%) | 0.755 |
| | NON-ASIAN | 96 (72.73%) | 81 (70.43%) | |
| | (Missing) | 5 (3.79%) | 11 (9.57%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Renal impairment at baseline | Mild (60-89) | 64 (48.48%) | 50 (43.48%) | 0.451 |
| | Moderate (30-59) | 14 (10.61%) | 10 (8.70%) | |
| | Normal (>=90) | 50 (37.88%) | 53 (46.09%) | |
| | (Missing) | 4 (3.03%) | 2 (1.74%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Time from primary diagnosis to randomization in months | min | 5.59 | 7.16 | 0.260 |
| | Q1 | 14.92 | 17.51 | |
| | median | 22.29 | 26.25 | |
| | mean | 39.70 | 41.97 | |
| | Q3 | 51.78 | 53.91 | |
| | max | 216.57 | 182.21 | |
| | SD | 39.98 | 37.52 | |
| | (Missing) | 4 (3.03%) | 7 (6.09%) | |
| TNM stage at diagnosis: M | M0 | 54 (40.91%) | 48 (41.74%) | 0.749 |
| | M1 | 32 (24.24%) | 19 (16.52%) | |
| | M1A | 3 (2.27%) | 4 (3.48%) | |
| | M1B | 27 (20.45%) | 27 (23.48%) | |
| | M1C | 4 (3.03%) | 5 (4.35%) | |
| | MX | 12 (9.09%) | 10 (8.70%) | |
| | (Missing) | 0 (0.00%) | 2 (1.74%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| TNM stage at diagnosis: N | N0 | 45 (34.09%) | 46 (40.00%) | 0.592 |
| | N1 | 62 (46.97%) | 50 (43.48%) | |
| | NX | 25 (18.94%) | 18 (15.65%) | |
| | (Missing) | 0 (0.00%) | 1 (0.87%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| TNM stage at diagnosis: T | T1 | 2 (1.52%) | 8 (6.96%) | 0.174 |
| | T2 | 32 (24.24%) | 21 (18.26%) | |

| Baseline/Demographic Characteristics | Summary Statistics/Categorical Level | Treatment Group (N=132) | Placebo Group (N=115) | P-value |
|---|---|---|---|---|
| | T3 | 56 (42.42%) | 54 (46.96%) | |
| | T4 | 21 (15.91%) | 14 (12.17%) | |
| | TX | 21 (15.91%) | 17 (14.78%) | |
| | (Missing) | 0 (0.00%) | 1 (0.87%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| TNM stage at study entry: M | M0 | 2 (1.52%) | 0 (0.00%) | 0.405 |
| | M1 | 65 (49.24%) | 53 (46.09%) | |
| | M1A | 6 (4.55%) | 11 (9.57%) | |
| | M1B | 49 (37.12%) | 38 (33.04%) | |
| | M1C | 10 (7.58%) | 9 (7.83%) | |
| | (Missing) | 0 (0.00%) | 4 (3.48%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| TNM stage at study entry: N | N0 | 50 (37.88%) | 43 (37.39%) | 0.876 |
| | N1 | 57 (43.18%) | 45 (39.13%) | |
| | NX | 25 (18.94%) | 23 (20.00%) | |
| | (Missing) | 0 (0.00%) | 4 (3.48%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| TNM stage at study entry: T | T0 | 9 (6.82%) | 3 (2.61%) | 0.413 |
| | T1 | 1 (0.76%) | 3 (2.61%) | |
| | T2 | 22 (16.67%) | 14 (12.17%) | |
| | T3 | 39 (29.55%) | 30 (26.09%) | |
| | T4 | 17 (12.88%) | 16 (13.91%) | |
| | TX | 44 (33.33%) | 45 (39.13%) | |
| | (Missing) | 0 (0.00%) | 4 (3.48%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |
| Type of progression at study entry | Bone only | 7 (5.30%) | 6 (5.22%) | 0.698 |
| | Bone+soft tissue | 2 (1.52%) | 5 (4.35%) | |
| | PSA only | 69 (52.27%) | 60 (52.17%) | |
| | PSA+bone | 24 (18.18%) | 22 (19.13%) | |
| | PSA+bone+soft tissue | 12 (9.09%) | 6 (5.22%) | |
| | PSA+soft tissue | 14 (10.61%) | 10 (8.70%) | |
| | Soft tissue only | 4 (3.03%) | 6 (5.22%) | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| | Total | 132 (100.00%) | 115 (100.00%) | |

| Baseline/Demographic Characteristics | Summary Statistics/Categorical Level | Treatment Group (N=132) | Placebo Group (N=115) | P-value |
|---|---|---|---|---|
| Weight | min | 45.90 | 52.00 | 0.371 |
| | Q1 | 72.56 | 70.75 | |
| | median | 80.50 | 82.20 | |
| | mean | 82.89 | 84.09 | |
| | Q3 | 88.78 | 93.60 | |
| | max | 134.50 | 140.00 | |
| | SD | 16.66 | 17.53 | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |

In the clinical validation study, baseline characteristics were compared between F1CDx evaluable and F1CDx unevaluable populations. The F1CDx unevaluable population included patients with positive HRR gene alteration status by non-F1CDx assay [F1LCDx, historical F1/F1CDx, or Interactive Web Response System (IWRS)] prior to randomization per the biomarker definition for clinical trial enrollment and had unevaluable HRR gene alteration status by F1CDx. The demographics and disease characteristics for the F1CDx evaluable and F1CDx unevaluable patients are provided in Table 27. Differences in characteristics of the F1CDx evaluable and F1CDx unevaluable populations were identified by covariate analysis. The covariates that showed significant differences between the two groups at a significance level of =0.2 were considered to be imbalanced between the F1CDx evaluable and the F1CDx unevaluable groups for enrolled patients.

**Table 27. Demographic and Baseline Characteristics for F1CDx evaluable and unevaluable populations**

| Baseline or Demographic Characteristics | Summary Statistics/ Categorical Level | F1CDx Evaluable (n=302) | F1CDx Unevaluable (n=97) | P- value |
|---|---|---|---|---|
| Previous treatment status | No Prior NHT or Taxane | 200 (66.23%) | 50 (51.55%) | 0.011 |
| | Prior NHT or Taxane | 102 (33.77%) | 47 (48.45%) | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| Age | min | 41 | 47 | 0.727 |
| | Q1 | 64 | 65 | |
| | median | 70 | 70 | |
| | mean | 69.85 | 69.61 | |
| | Q3 | 76 | 74 | |
| | max | 90 | 86 | |
| | SD | 8.78 | 7.45 | |

| Baseline or Demographic Characteristics | Summary Statistics/ Categorical Level | F1CDx Evaluable (n=302) | F1CDx Unevaluable (n=97) | P- value |
|---|---|---|---|---|
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| Age group | < 65 | 78 (25.83%) | 23 (23.71%) | 0.328 |
| | >= 75 | 94 (31.13%) | 24 (24.74%) | |
| | 65 to < 75 | 130 (43.05%) | 50 (51.55%) | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| BMI | min | 18.4 | 17.1 | 0.900 |
| | Q1 | 24.48 | 23.85 | |
| | median | 27.4 | 27.7 | |
| | mean | 27.87 | 28.28 | |
| | Q3 | 30.5 | 31.55 | |
| | max | 44.6 | 59.4 | |
| | SD | 4.72 | 6.16 | |
| | (Missing) | 2 (0.66%) | 2 (2.06%) | |
| Baseline use of a bone protecting agent | No | 259 (85.76%) | 80 (82.47%) | 0.419 |
| | Yes | 43 (14.24%) | 17 (17.53%) | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| Baseline circulating tumor cells (CTC) count (continuous) | min | 0 | 0 | 0.512 |
| | Q1 | 0 | 0 | |
| | median | 1 | 1.5 | |
| | mean | 35.78 | 38.74 | |
| | Q3 | 12 | 14 | |
| | max | 1218 | 963 | |
| | SD | 135.47 | 144.8 | |
| | (Missing) | 57 (18.87%) | 31 (31.96%) | |
| Baseline circulating tumor cells (CTC) count (categorical with cutoff = 5) | <5 | 156 (51.66%) | 41 (42.27%) | 0.886 |
| | >=5 | 89 (29.47%) | 25 (25.77%) | |
| | (Missing) | 57 (18.87%) | 31 (31.96%) | |
| Baseline circulating tumor cells (CTC) count (categorical with cutoff = 0) | >0 | 141 (46.69%) | 41 (42.27%) | 0.574 |
| | 0 | 104 (34.44%) | 25 (25.77%) | |
| | (Missing) | 57 (18.87%) | 31 (31.96%) | |
| Distribution of disease at screening involved: bone | No | 42 (13.91%) | 17 (17.53%) | 0.418 |
| | Yes | 253 (83.77%) | 80 (82.47%) | |
| | (Missing) | 7 (2.32%) | 0 (0.00%) | |
| Distribution of disease at screening involved: lymph node | No | 164 (54.30%) | 52 (53.61%) | 0.814 |
| | Yes | 131 (43.38%) | 45 (46.39%) | |

| Baseline or Demographic Characteristics | Summary Statistics/ Categorical Level | F1CDx Evaluable (n=302) | F1CDx Unevaluable (n=97) | P- value |
|---|---|---|---|---|
| | (Missing) | 7 (2.32%) | 0 (0.00%) | |
| Distribution of disease at screening involved: other soft tissue | No | 265 (87.75%) | 84 (86.60%) | 0.357 |
| | Yes | 30 (9.93%) | 13 (13.40%) | |
| | (Missing) | 7 (2.32%) | 0 (0.00%) | |
| Distribution of disease at screening involved: visceral disease (liver) | No | 285 (94.37%) | 92 (94.85%) | 0.541 |
| | Yes | 10 (3.31%) | 5 (5.15%) | |
| | (Missing) | 7 (2.32%) | 0 (0.00%) | |
| Distribution of disease at screening involved: visceral disease (lung) | No | 259 (85.76%) | 84 (86.60%) | 0.727 |
| | Yes | 36 (11.92%) | 13 (13.40%) | |
| | (Missing) | 7 (2.32%) | 0 (0.00%) | |
| Distribution of disease at screening involved: visceral disease (lung or liver) | No | 252 (83.44%) | 81 (83.51%) | 0.627 |
| | Yes | 43 (14.24%) | 16 (16.49%) | |
| | (Missing) | 7 (2.32%) | 0 (0.00%) | |
| Disease localization at screening | Bone only | 120 (39.74%) | 37 (38.14%) | 0.569 |
| | Both bone and soft tissue | 133 (44.04%) | 43 (44.33%) | |
| | None | 6 (1.99%) | 0 (0.00%) | |
| | Soft tissue only | 43 (14.24%) | 17 (17.53%) | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| Eastern Cooperative Oncology Group (ECOG) performance status | 0 | 194 (64.24%) | 52 (53.61%) | 0.072 |
| | 1 | 108 (35.76%) | 45 (46.39%) | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| Geographic region | Asia | 60 (19.87%) | 20 (20.62%) | 0.183 |
| | European Union/GBR | 151 (50.00%) | 42 (43.30%) | |
| | North America | 31 (10.26%) | 18 (18.56%) | |

| Baseline or Demographic Characteristics | Summary Statistics/ Categorical Level | F1CDx Evaluable (n=302) | F1CDx Unevaluable (n=97) | P- value |
|---|---|---|---|---|
| | Rest of the world | 60 (19.87%) | 17 (17.53%) | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| Gleason score category 1 | High [8-10] | 222 (73.51%) | 73 (75.26%) | 0.917 |
| | Low [2-4] | 1 (0.33%) | 0 (0.00%) | |
| | Medium [5-7] | 71 (23.51%) | 22 (22.68%) | |
| | (Missing) | 8 (2.65%) | 2 (2.06%) | |
| Gleason score category 2 | <=6 | 10 (3.31%) | 4 (4.12%) | 0.936 |
| | 7 | 62 (20.53%) | 18 (18.56%) | |
| | 8 | 67 (22.19%) | 21 (21.65%) | |
| | 9-10 | 155 (51.32%) | 52 (53.61%) | |
| | (Missing) | 8 (2.65%) | 2 (2.06%) | |
| Histopathological classification | Adenocarcinoma | 300 (99.34%) | 97 (100.00%) | 1.000 |
| | Adenocarcinoma with neuroendocrine features | 2 (0.66%) | 0 (0.00%) | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| Number of bone metastases at screening | 0 | 56 (18.54%) | 18 (18.56%) | 0.157 |
| | 1-4 | 104 (34.44%) | 22 (22.68%) | |
| | 5-9 | 61 (20.20%) | 20 (20.62%) | |
| | 10-20 | 39 (12.91%) | 18 (18.56%) | |
| | >20 | 42 (13.91%) | 19 (19.59%) | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| Baseline pain score by Brief Pain Inventory-Short Form (BPI-SF) | 0-1 | 197 (65.23%) | 61 (62.89%) | 0.281 |
| | 2-3 | 105 (34.77%) | 35 (36.08%) | |
| | >3 | 0 (0.00%) | 1 (1.03%) | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| Baseline serum Prostate-Specific Antigen (PSA) (ng/mL) | min | 0.1 | 0.04 | 0.360 |
| | Q1 | 6.53 | 7.8 | |

| Baseline or Demographic Characteristics | Summary Statistics/ Categorical Level | F1CDx Evaluable (n=302) | F1CDx Unevaluable (n=97) | P- value |
|---|---|---|---|---|
| | median | 16.95 | 21 | |
| | mean | 79.35 | 61.03 | |
| | Q3 | 62.2 | 56.29 | |
| | max | 3412 | 713.2 | |
| | SD | 242.7 | 113.89 | |
| | (Missing) | 2 (0.66%) | 0 (0.00%) | |
| Race | ASIAN | 63 (20.86%) | 21 (21.65%) | 0.660 |
| | BLACK OR AFRICAN AMERICAN | 8 (2.65%) | 3 (3.09%) | |
| | MULTIPLE | 1 (0.33%) | 0 (0.00%) | |
| | NATIVE HAWAIIAN OR OTHER PACIFIC ISLANDER | 1 (0.33%) | 1 (1.03%) | |
| | WHITE | 213 (70.53%) | 60 (61.86%) | |
| | (Missing) | 16 (5.30%) | 12 (12.37%) | |
| Race group | ASIAN | 63 (20.86%) | 21 (21.65%) | 0.658 |
| | NON-ASIAN | 223 (73.84%) | 64 (65.98%) | |
| | (Missing) | 16 (5.30%) | 12 (12.37%) | |
| Renal impairment at baseline | Mild (60-89) | 143 (47.35%) | 33 (34.02%) | 0.066 |
| | Moderate (30-59) | 29 (9.60%) | 12 (12.37%) | |
| | Normal (>=90) | 122 (40.40%) | 49 (50.52%) | |
| | (Missing) | 8 (2.65%) | 3 (3.09%) | |
| Time from primary diagnosis to randomization in months | min | 5.49 | 5.85 | 0.348 |
| | Q1 | 16.41 | 18.73 | |
| | median | 26.07 | 30.13 | |
| | mean | 43.85 | 48.8 | |
| | Q3 | 57.42 | 66.5 | |

| Baseline or Demographic Characteristics | Summary Statistics/ Categorical Level | F1CDx Evaluable (n=302) | F1CDx Unevaluable (n=97) | P- value |
|---|---|---|---|---|
| | max | 216.57 | 204.09 | |
| | SD | 41.27 | 46.08 | |
| | (Missing) | 16 (5.30%) | 4 (4.12%) | |
| TNM stage at diagnosis: M | M0 | 123 (40.73%) | 45 (46.39%) | 0.850 |
| | M1 | 63 (20.86%) | 20 (20.62%) | |
| | M1A | 10 (3.31%) | 1 (1.03%) | |
| | M1B | 64 (21.19%) | 20 (20.62%) | |
| | M1C | 10 (3.31%) | 3 (3.09%) | |
| | MX | 29 (9.60%) | 7 (7.22%) | |
| | (Missing) | 3 (0.99%) | 1 (1.03%) | |
| TNM stage at diagnosis: N | N0 | 110 (36.42%) | 42 (43.30%) | 0.385 |
| | N1 | 132 (43.71%) | 35 (36.08%) | |
| | NX | 55 (18.21%) | 18 (18.56%) | |
| | (Missing) | 5 (1.66%) | 2 (2.06%) | |
| TNM stage at diagnosis: T | T0 | 0 (0.00%) | 2 (2.06%) | 0.139 |
| | T1 | 13 (4.30%) | 7 (7.22%) | |
| | T2 | 65 (21.52%) | 15 (15.46%) | |
| | T3 | 134 (44.37%) | 42 (43.30%) | |
| | T4 | 41 (13.58%) | 16 (16.49%) | |
| | TX | 46 (15.23%) | 14 (14.43%) | |
| | (Missing) | 3 (0.99%) | 1 (1.03%) | |
| TNM stage at study entry: M | M0 | 2 (0.66%) | 0 (0.00%) | 0.444 |
| | M1 | 140 (46.36%) | 43 (44.33%) | |
| | M1A | 24 (7.95%) | 4 (4.12%) | |
| | M1B | 104 (34.44%) | 35 (36.08%) | |
| | M1C | 26 (8.61%) | 14 (14.43%) | |
| | MX | 1 (0.33%) | 0 (0.00%) | |
| | (Missing) | 5 (1.66%) | 1 (1.03%) | |
| TNM stage at study entry: N | N0 | 109 (36.09%) | 39 (40.21%) | 0.517 |
| | N1 | 128 (42.38%) | 43 (44.33%) | |
| | NX | 59 (19.54%) | 14 (14.43%) | |
| | (Missing) | 6 (1.99%) | 1 (1.03%) | |
| TNM stage at study entry: T | T0 | 13 (4.30%) | 4 (4.12%) | 0.330 |
| | T1 | 5 (1.66%) | 5 (5.15%) | |
| | T2 | 48 (15.89%) | 10 (10.31%) | |
| | T3 | 82 (27.15%) | 25 (25.77%) | |
| | T4 | 42 (13.91%) | 17 (17.53%) | |
| | TX | 106 (35.10%) | 35 (36.08%) | |
| | (Missing) | 6 (1.99%) | 1 (1.03%) | |

| Baseline or Demographic Characteristics | Summary Statistics/ Categorical Level | F1CDx Evaluable (n=302) | F1CDx Unevaluable (n=97) | P- value |
|---|---|---|---|---|
| Type of progression at study entry | Bone only | 14 (4.64%) | 4 (4.12%) | 0.851 |
| | Bone+soft tissue | 8 (2.65%) | 1 (1.03%) | |
| | PSA only | 151 (50.00%) | 46 (47.42%) | |
| | PSA+bone | 61 (20.20%) | 19 (19.59%) | |
| | PSA+bone+soft tissue | 21 (6.95%) | 10 (10.31%) | |
| | PSA+soft tissue | 34 (11.26%) | 14 (14.43%) | |
| | Soft tissue only | 13 (4.30%) | 3 (3.09%) | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |
| Weight | min | 45.9 | 45 | 0.577 |
| | Q1 | 72.62 | 73.5 | |
| | median | 81.32 | 83.5 | |
| | mean | 83.74 | 85.45 | |
| | Q3 | 91.95 | 97 | |
| | max | 140 | 177.7 | |
| | SD | 16.64 | 20.51 | |
| | (Missing) | 0 (0.00%) | 0 (0.00%) | |

## D. Safety and Effectiveness Results

### 1. Safety Results

The safety with respect to treatment with talazoparib in combination with enzalutamide was addressed during the review of the talazoparib in combination with enzalutamide NDA (No. NDA 211651/S-010) and is not addressed in detail in this Summary of Safety and Effectiveness Data. The evaluation of safety was based on the analysis of adverse events (AEs), clinical laboratory evaluations, physical examinations, and vital signs. Please refer to Drugs@FDA for complete safety information on TALZENNA® (talazoparib) in combination with XTANDI® (enzalutamide).

### 2. Effectiveness Results

The effectiveness of F1CDx to identify patients with HRR gene alterations who may benefit from treatment with talazoparib in combination with enzalutamide is

supported by the evaluation of the mCRPC patient population enrolled in TALAPRO-2 harboring HRR gene alterations. The efficacy analysis and sensitivity analysis of missing results are presented below.

### i. Clinical Efficacy Results

The TALAPRO-2 study demonstrated that treatment with talazoparib in combination with abiraterone resulted in a clinically meaningful and statistically significant improvement in rPFS compared with placebo + enzalutamide in the HRR-deficient (HRRm) population (n=399).

The clinical validity of F1CDx for the detection of HRR gene alterations in mCRPC patients was based on the F1CDx+ subset based on the CDx biomarker definition (n=247) within the HRR-deficient population from the TALAPRO-2 study. As presented in Table 28, in the F1CDx HRRm population, the median rPFS was not estimable (NE) (95% two-sided CI: [27.40, NE]) in the experimental treatment group and 13.80 months (95% two-sided CI: [10.97, 16.62]) in the placebo group as estimated by the Kaplan-Meier method. The estimated HR based on stratified Cox regression models and the Breslow tie handling method was 0.30 (95% two-sided CI: [0.20, 0.46]) comparing the treatment group versus the placebo group. Patients without HRR alterations as determined by F1CDx (n=55) did not derive benefit from talazoparib + enzalutamide when compared to placebo + enzalutamide.

**Table 28. Efficacy analyses for F1CDx HRRm population in TALAPRO-2**

| | **Total HRRm population (n=399)** | | **F1CDx + HRRm population (n=247)** | | **F1CDx- HRRm population (n=55)** | |
|---|---|---|---|---|---|---|
| | **Talazoparib/ enzalutamide** N =200 | **Placebo/ enzalutamide** N = 199 | **Talazoparib/ enzalutamide** N = 132 | **Placebo/ enzalutamide** N = 115 | **Talazoparib/ enzalutamide** N=23 | **Placebo/ enzalutamide** N=32 |
| rPFS Events, n (%) | 66 (33) | 104 (52) | 39 (30) | 68 (59) | 9 (39) | 10 (31) |
| Median rPFS (95% CI), months | NE(21.9, NE) | 13.8 (11.0, 16.7) | NE (27.40, NE) | 13.8 (10.97, 16.62) | 13.80 (11.04, NE) | 21.32 (15.44 , NE) |
| Hazard ratio (95% CI)[a] | 0.45 (0.33, 0.61) | | 0.30 (0.20, 0.46) | | 1.49 (0.58, 3.83) | |

### ii. Sensitivity Analysis for Missing CDx Results

A sensitivity analysis was conducted to assess the robustness of the clinical efficacy results accounting for the patients with missing F1CDx status. Samples were considered unevaluable if they had positive HRR gene alteration status by non-F1CDx assay (F1LCDx, historical F1/F1CDx, or

Interactive Web Response System IWRS) prior to randomization per the biomarker definition for clinical trial enrollment and had unevaluable HRR gene alteration status by F1CDx.
Following imputation, the HR estimated for rPFS based on the Cox proportional hazards model using the complete dataset was 0.37 (95% CI: [0.35, 0.40]) in the HRRm population as determined by F1CDx testing.

**3. Pediatric Extrapolation**

Patients aged 18 years of older were eligible for enrollment into the TALAPRO-2 study if they fulfilled other inclusion criteria. The youngest patient enrolled in the study was 41 years of age. For the TALAPRO-2 study, data from adult patients aged 41 years and older are considered generalizable to the pediatric population aged 18-21 years and can be relied on to establish safety and effectiveness within the 18-21 years age group.

## XI. FINANCIAL DISCLOSURE

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included one investigator who was a full-time or part-time employee of the sponsor and the investigators had disclosable financial interests/arrangements as defined in 21 CFR 54.2(a), (b), (c) and (f) and described below:

- Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: [0]
- Significant payment of other sorts: [0]
- Proprietary interest in the product tested held by the investigator: [1]
- Significant equity interest held by investigator in sponsor of covered study: [0]

The applicant has adequately disclosed the financial interest/arrangements with clinical investigators. Statistical analyses were conducted by FDA to determine whether the financial interests/arrangements had any impact on the clinical study outcome. The information provided does not raise any questions about the reliability of the data.

## XII. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA supplement was not referred to the Molecular and Clinical Genetics Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

## XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

### A. Effectiveness Conclusions

The effectiveness of F1CDx to identify prostate cancer patients with HRR gene alterations who may benefit from TALZENNA® (talazoparib) in combination with XTANDI® (enzalutamide) treatment is supported by the results from the clinical validation study. This study was performed using specimens from patients enrolled in the TALAPRO-2 clinical trial with HRR gene altered status. The data from the analytical validation and clinical validation studies support the reasonable assurance of safety and effectiveness of the F1CDx assay when used in accordance with the indications for use. Data from the TALAPRO-2 trial show that patients who had HRR gene alterations, received benefit from treatment with talazoparib in combination with enzalutamide and support the addition of this CDx indication to F1CDx. The clinical validation study demonstrated the ability of F1CDx to detect HRR gene altered patients that benefit from TALZENNA® (talazoparib) in combination with XTANDI® (enzalutamide).

### B. Safety Conclusions

The risks of the device are based on data collected in the analytical studies conducted to support PMA approval as described above. The F1CDx assay is an in vitro diagnostic test, which involves testing of DNA extracted from FFPE tumor tissue. The assay can be performed using DNA extracted from existing (archival) tissue samples routinely collected as part of the diagnosis and patient care.

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently, inappropriate patient management decisions in cancer treatment. Patients with false positive results may undergo treatment with one of the therapies listed in Table 1 of the intended use statement without clinical benefit and may experience adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment with the indicated therapy. There is also a risk of delayed results, which may lead to delay of treatment with the indicated therapy.

### C. Benefit-Risk Determination

The probable benefits of F1CDx in identification of HRR gene alterations for treatment with TALZENNA® (talazoparib) in combination with XTANDI® (enzalutamide) are based on data collected in the TALAPRO-2 clinical trial and the clinical validation study. The clinical benefit of the F1CDx assay for the selection of prostate cancer patients with HRR gene alterations was demonstrated in a clinical validation study using samples from patients enrolled in the TALAPRO-2 clinical study. Clinical outcome of HRR gene altered patients by F1CDx shows that the estimated HR in the F1CDx+ population was 0.30 (95% two-sided CI: [0.20, 0.46] comparing talazoparib in combination with enzalutamide versus placebo in combination with enzalutamide. The results demonstrate better rPFS and reduced risk

of disease progression or death in patients who received treatment with TALZENNA® (talazoparib) in combination with XTANDI® (enzalutamide). This supports the probable benefit of F1CDx in selecting HRR gene altered patients for treatment with TALZENNA® (talazoparib) in combination with XTANDI® (enzalutamide).

There is potential risk associated with the use of this device, mainly due to 1) false positive, false negatives, or failure to provide a result, and 2) incorrect interpretation of test results by the user. The risks of the F1CDx assay are associated with the potential mismanagement of patients resulting from false results of the test. Patients who are determined to be false positive by the test may be exposed to TALZENNA® (talazoparib) in combination with XTANDI® (enzalutamide), a drug that may lead to adverse events without probable benefit or may have delayed access to treatments that could be more beneficial. A false negative result may prevent a patient from accessing a potentially beneficial drug.

The risk of false results is partially mitigated by clinical and analytical studies presented above. The representation of certain HRR gene rearrangements and homozygous deletions were limited in the analytical validation studies and may not be detected consistently by F1CDx. To address this, we modified one of the existing limitations to mitigate the risks of this test:

Certain gene fusions, homozygous deletions, and rearrangements including but not limited to BRCA1, BRCA2, CDK12 and indels in PALB2 were not adequately represented in analytical validations studies and may not be detected consistently by F1CDx.

The clinical and analytical performance of the device included in this submission demonstrate that the assay is expected to perform with reasonable accuracy, mitigating the potential for false results. In addition, to supplement the premarket data, some post-market studies are planned as summarized in Section XIV, below.

1. Patient Perspective
   This submission either did not include specific information on patient perspectives or the information did not serve as part of the basis of the decision to approve or deny the PMA for this device.

In conclusion, given the available information above, the data support that for the F1CDx assay, and the indications noted in the intended use statement, the probable benefits outweigh the probable risks.

## D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the analytical and clinical studies support the performance of F1CDx as an

aid for the identification of HRR gene alterations in patients with prostate cancer for whom TALZENNA® (talazoparib) in combination with XTANDI® (enzalutamide) may be indicated.

## XIV. CDRH DECISION

CDRH issued an approval order for the PMA (P170019/S060) on May 27, 2026.

Foundation Medicine, Inc. (FMI) must provide the following software information in a post-approval report within 6 months of approval of this PMA supplement:

FMI will provide evidence, acceptable to FDA, that performance expectations with the currently deployed Genomics Platform Analysis Pipeline (AP), including the version at the time of the submission of your sPMA (v3.37.0), are representative of the performance in the clinical and analytical validation studies in P170019/S060. Such evidence may include regression testing using the clinical and analytical datasets to perform in silico reanalysis of the results obtained in the clinical and analytical validation studies in this supplement and confirmation that there is little or no deviation in the quality metrics for each of the samples to support the accuracy and precision of F1CDx remains the same.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality Management System (QMSR) regulation (21 CFR 820).

## XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.

## XVI. REFERENCES


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