FOUNDATIONONE® CDx

FoundationOne® CDx Technical Information

CLINICAL BACKGROUND

The aim of personalised medicine is to identify and help match treatments with patients who are most likely to experience a favourable benefit-risk outcome with a selected therapy.[1] Companion diagnostics — tests that provide information that is essential for the safe and effective use of a corresponding therapeutic product — are increasingly becoming an indispensable part of personalised medicine.[2,3] The use of companion diagnostics is likely to rapidly continue to increase in the future as more diagnostic-partnered therapies are launched and with increased application to disease areas.[1,3]

METHODS

FoundationOne CDx is the first broad companion diagnostic with FDA-approval for all solid tumours.[4] This Comprehensive Genomic Profile (CGP) platform applies next-generation sequencing *in vitro* diagnostics with a hybrid capture-based target enrichment approach and whole-genome shotgun library construction, to identify all four classes of somatic genomic alterations, including substitutions, insertions and deletions, copy number alterations (CNAs), and select rearrangements, to a typical median depth of coverage of >500× (with >99% of exons at coverage >100×).[4]

In total, FoundationOne CDx detects alterations in 324 genes, including 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.[4]

All FoundationOne CDx samples are simultaneously profiled for tumour mutational burden (TMB) and microsatellite instability (MSI) status.[4] The MSI-H/MSS designation by FoundationOne CDx is based on genome wide analysis of 95 microsatellite loci and not based on the 5 or 7 MSI loci described in current clinical practice guidelines.[4] The threshold for MSI-H/MSS was determined by analytical concordance to comparator (IHC and PCR) using uterine, cecum and colorectal cancer FFPE tissue.[4] TMB by FoundationOne CDx is defined based on counting the total number of all synonymous and non-synonymous variants present at ≥5% allele frequency (after filtering) and reported as mutations per megabase (mut/Mb).[4]

FFPE: formalin-fixed paraffin-embedded; **IHC**: immunohistochemistry; **MSI-H**: microsatellite instability-high, **MSS**: microsatellite instability-stable; **PCR**: polymerase chain reaction.

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INTENDED USE

FoundationOne CDx is intended for use as a companion diagnostic test to identify patients that may benefit from treatment following detection of specific genomic alterations in 5 approved indications, including non-small cell lung cancer (NSCLC), colorectal cancer (CRC), melanoma, breast cancer and ovarian cancer, in accordance with approved therapeutic labelling and oncology guidelines.[4]

In addition to its use as a companion diagnostic, FoundationOne CDx may be used to provide tumour mutation profiling by physicians in accordance with approved guidelines in oncology for patients with solid tumours.[4]

Companion diagnostic indications[4]:

INDICATIONS	BIOMARKER	FDA-APPROVED THERAPY
NSCLC	*EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations	afatinib, gefitinib, or erlotinib
	EGFR exon 20 T790M alterations	osimertinib
	ALK rearrangements	alectinib, crizotinib, or ceritinib
	BRAF V600E	dabrafenib in combination with trametinib
Melanoma	*BRAF* V600E	dabrafenib or vemurafenib
	BRAF V600E or V600K	trametinib or cobimetinib, in combination with vemurafenib
Breast Cancer	*ERBB2* (*HER2*) amplification	trastuzumab, ado-trastuzumab-emtansine or pertuzumab
CRC	*KRAS* wild-type (absence of mutations in codons 12 and 13)	cetuximab
	KRAS wild-type (absence of mutations in exons 2, 3, and 4) and *NRAS* wild-type (absence of mutations in exons 2, 3, and 4)	panitumumab
Ovarian Cancer	*BRCA1/2* alterations	rucaparib

Current as of March, 2018.

CONCORDANCE DATA

Follow-on companion diagnostic claims are based on a non-inferiority statistical testing approach using the enrichment design presented in the clinical paper by Li M, 2016.[2] All studies passed the acceptance criteria specific in each study protocol[4,5]:

BIOMARKER	PPA*	NPA*	COMPARATOR METHOD
***EGFR* exon 19 deletions and L858R**	98.1% (106/108)	99.4% (153/154)	cobas® *EGFR* mutation test v2
***EGFR* T790M**	98.9% (87/88)	86.1% (93/108)	cobas® *EGFR* mutation test v1 cobas® *EGFR* mutation test v2
***ALK* rearrangements**	92.9% (78/84)	100% (75/75)	Ventana *ALK* (D5F3) CDx assay Vysis *ALK* Break-Apart FISH Probe Kit
KRAS	100% (173/173)	100% (154/154)	therascreen® *KRAS* RGQ PCR kit
***ERBB2* (*HER2*) amplifications**	89.4% (101/113)	98.4% (180/183)	Dako *HER2* FISH PharmDx® kit
***BRAF* V600**	99.4% (166/167)	89.6% (121/135)†	cobas® *BRAF* V600 mutation test
***BRAF* V600E**	99.3% (149/150)	99.2% (121/122)	cobas® *BRAF* V600 mutation test
***BRAF* V600 dinucleotide**‡	96.3% (26/27)	100% (24/24)	THxID® *BRAF* kit

FoundationOne CDx has also established high concordance with FoundationOne® CGP. In an assay of 165 samples, FoundationOne CDx demonstrated a PPA of 98.3% overall, including 98.8% in short variants, 94.3% in CNAs and 100% in rearrangements.[6]

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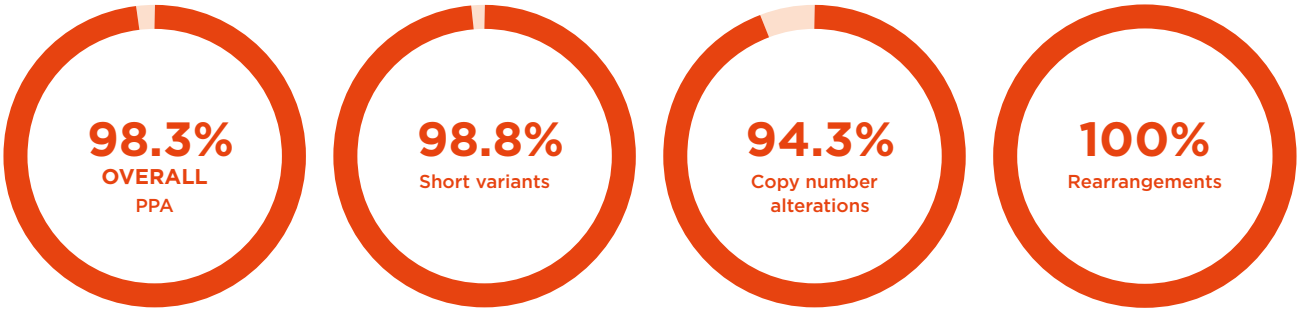

* The reference standard used to calculate PPA and NPA is defined as the consensus calls between the two comparator methods (i.e. PPA being when FoundationOne CDx and the comparator method(s) identified genomic alterations in mutated patients and NPA being when FoundationOne CDx and the comparator method(s) did not identify genomic alterations in non-mutated patients).

† Sensitivity of dinucleotide detection of *BRAF* V600K and V600E was found to be significantly reduced in cobas® test, in particular for samples in which FoundationOne CDx detected the dinucleotides to be of lower than 40% mutant allele frequency, leading to low NPA values.

‡ A study using the THxID® *BRAF* kit (bioMérieux) was conducted with samples with *BRAF* V600 dinucleotide mutation detected by FoundationOne CDx and *BRAF* V600 negative samples to provide a better evaluation of V600 dinucleotide concordance.

CGP: comprehensive genomic profiling; **NPA**: negative percent agreement; **PPA**: positive percent agreement.

WARNINGS AND PRECAUTIONS

- Genomic alterations reported may include somatic (not inherited) or germline (inherited) alterations; however, the test does not distinguish between germline and somatic alterations. The test does not provide information about susceptibility.[4]
- Biopsy may pose a risk to the patient when archival tissue is not available for use with the assay. The patient's physician should determine whether the patient is a candidate for biopsy.[4]
- Reflex testing to an alternative FDA-approved companion diagnostic should be performed for patients who have an *ERBB2* amplification result detected with copy number equal to 4 (baseline ploidy of tumour +2) for confirmatory testing. While this result is considered negative by FoundationOne CDx, in a clinical concordance study with an FDA-approved FISH test, 70% (7 out of 10 samples) were positive, and 30% (3 out 10 samples) were negative by the FISH test with an average ratio of 2.3. The frequency of *ERBB2* copy number 4 in breast cancer is estimated to be approximately 2%.[§4]

LIMITATIONS

- For *in vitro* diagnostic use.[4]
- Genomic findings other than those listed for intended use (see table on page 2 of this document) are not prescriptive or conclusive for labelled use of any specific therapeutic product.[4]
- A negative result does not rule out the presence of a mutation below the limits of detection of the assay.[4]
- Samples with <25% tumour may have decreased sensitivity for the detection of CNAs, including *ERBB2*.[4]
- Clinical performance of Tagrisso® (osimertinib) in patients with an *EGFR* exon 20 T790M mutation detected with an allele fraction <5% is ongoing and has not been established.[4]
- Concordance with other validated methods for CNA (with exception of *ERBB2*) and gene rearrangement (with exception of *ALK*) detection has not been demonstrated and will be provided in the post-market setting.[4] Confirmatory testing using a clinically validated assay should be performed for all CNAs and rearrangements not associated with those listed for intended use with FoundationOne CDx (see table on page 2 of this document) but used for clinical decision making.[4]

[§] Multiple references listed in https://www.mycancergenome.org/content/disease/breast-cancer/ERBB2/238/ report the frequency of *HER2* overexpression as 20% in breast cancer. Based on the FoundationOne CDx *HER2* CDx concordance study, approximately 10% of *HER2* amplified samples had copy number 4. Thus, total frequency is conservatively estimated to be approximately 2%.[4]

CNA: copy number alteration.

REPORTING

The FoundationOne CDx report is designed to highlight alterations that may lead to additional treatment options for physicians and their patients to consider. The output of FoundationOne CDx profiling includes:

- **Category 1**: Companion diagnostic (CDx) claims for intended use
- **Category 2**: Cancer mutations with evidence of clinical significance
- **Category 3**: Cancer mutations with potential clinical significance

Results are provided in a report available via a link sent in a secure email and via Foundation Medicine Online.[¶]

The FoundationOne CDx report has a simplified format that features 3 sections:

1. **FDA-approved content**
2. **Professional services**
3. **Appendix**

When a clinically relevant genomic alteration is found in any one of the current gene list (next page of this document), the first page of the report identifies the specific gene and alteration(s), including genomic alterations associated with companion diagnostic claims that inform eligibility for associated therapies and other tumour-profiling results that can be used for treatment management according to oncology guidelines.

An interpretation of the gene and specific alteration, as well as corresponding therapeutic implications specific to the patient's tumour, are provided within the body of the report. This may incorporate analyses of peer-reviewed studies and other publicly available information identified by Foundation Medicine. These analyses may include associations between a genomic alteration (or lack of alteration) and one or more therapies identified as having potential clinical benefit (or potential lack of clinical benefit), as well as clinical trials enrolling patients with this genomic profile.

In some cases, pertinent negatives are displayed on the front of the report; these are genes that have no alterations but are particularly relevant to the specific tumour type (e.g. *KRAS* in colon cancer, *EGFR* in lung cancer). Alterations designated to be variants of unknown significance (VUS) are listed at the back of the report in the VUS section.

MSI status is reported on the front page as MSI-High, MSI-Intermediate or MSI-Stable. If the report is affected by certain quality metrics (e.g. poor sample quality due to low tumour purity or contamination), the MSI status may be listed as 'Cannot Be Determined'.

TMB status is reported on the front page for all solid tumours found to be TMB-High (≥20 Muts/Mb) status. In select tumour types including NSCLC, melanoma, urothelial carcinoma, colorectal cancer, and endometrial cancer, TMB status may also be reported as TMB-Intermediate (6–19 Muts/Mb) or TMB-Low (≤5 Muts/Mb). In these cases, TMB status can be found in the VUS section. If the report is affected by certain quality metrics (e.g. poor sample quality due to low tumour purity or contamination), the TMB status may be listed as 'Cannot Be Determined'. Not all qualified reports will list TMB in this way.

VARIANTS OF UNKNOWN SIGNIFICANCE (VUS)

Often an alteration is detected in the patient's tumour, but that specific alteration has not yet been adequately characterised in the scientific literature at the time the report was issued, and/or the genomic context of these alterations makes their significance unclear. We choose to include these variants in the report in the event that they may become clinically meaningful in the future.

EQUIVOCAL

A CNA denoted as 'Amplification—equivocal' implies that the sequencing data provide some, but not unambiguous, signal that the copy number exceeds the threshold for copy number events assigned to the relevant gene. The threshold used in FoundationOne CDx for identifying copy number amplification is 5 for *ERBB2* and 6 for all other genes.

An alteration denoted as 'Loss—equivocal' implies that the sequencing data provide some, but not unambiguous, signal of homozygous deletion of the gene in question.

SUBCLONAL

An alteration designated as subclonal signifies that the FoundationOne CDx analytical methodology has identified the presence of the alteration in <10% of the assayed tumour DNA.

[¶] Please contact your local customer services team to set up an account on Foundation Medicine Online.

FOUNDATIONONE CDx CURRENT GENE LIST

In total, FoundationOne CDx detects alterations in 324 genes, including all coding exons from 309 cancer-related genes, one promoter region, one ncRNA, and select intronic regions from 34 commonly rearranged genes, 21 of which also include the coding exons.[4]

CURRENT GENE LIST								
ABL1	*ACVR1B*	*AKT1*	*AKT2*	*AKT3*	*ALK*	*ALOX12B*	*AMER1 (FAM123B)*	*APC*
AR	*ARAF*	*ARFRP1*	*ARID1A*	*ASXL1*	*ATM*	*ATR*	*ATRX*	*AURKA*
AURKB	*AXIN1*	*AXL*	*BAP1*	*BARD1*	*BCL2*	*BCL2L1*	*BCL2L2*	*BCL6*
BCOR	*BCORL1*	*BRAF*	*BRCA1*	*BRCA2*	*BRD4*	*BRIP1*	*BTG1*	*BTG2*
BTK	*C11orf30 (EMSY)*	*CALR*	*CARD11*	*CASP8*	*CBFB*	*CBL*	*CCND1*	*CCND2*
CCND3	*CCNE1*	*CD22*	*CD274 (PD-L1)*	*CD70*	*CD79A*	*CD79B*	*CDC73*	*CDH1*
CDK12	*CDK4*	*CDK6*	*CDK8*	*CDKN1A*	*CDKN1B*	*CDKN2A*	*CDKN2B*	*CDKN2C*
CEBPA	*CHEK1*	*CHEK2*	*CIC*	*CREBBP*	*CRKL*	*CSF1R*	*CSF3R*	*CTCF*
CTNNA1	*CTNNB1*	*CUL3*	*CUL4A*	*CXCR4*	*CYP17A1*	*DAXX*	*DDR1*	*DDR2*
DIS3	*DNMT3A*	*DOT1L*	*EED*	*EGFR*	*EP300*	*EPHA3*	*EPHB1*	*EPHB4*
ERBB2	*ERBB3*	*ERBB4*	*ERCC4*	*ERG*	*ERRFI1*	*ESR1*	*EZH2*	*FAM46C*
FANCA	*FANCC*	*FANCG*	*FANCL*	*FAS*	*FBXW7*	*FGF10*	*FGF12*	*FGF14*
FGF19	*FGF23*	*FGF3*	*FGF4*	*FGF6*	*FGFR1*	*FGFR2*	*FGFR3*	*FGFR4*
FH	*FLCN*	*FLT1*	*FLT3*	*FOXL2*	*FUBP1*	*GABRA6*	*GATA3*	*GATA4*
GATA6	*GID4 (C17orf39)*	*GNA11*	*GNA13*	*GNAQ*	*GNAS*	*GRM3*	*GSK3B*	*H3F3A*
HDAC1	*HGF*	*HNF1A*	*HRAS*	*HSD3B1*	*ID3*	*IDH1*	*IDH2*	*IGF1R*
IKBKE	*IKZF1*	*INPP4B*	*IRF2*	*IRF4*	*IRS2*	*JAK1*	*JAK2*	*JAK3*
JUN	*KDM5A*	*KDM5C*	*KDM6A*	*KDR*	*KEAP1*	*KEL*	*KIT*	*KLHL6*
KMT2A (MLL)	*KMT2D (MLL2)*	*KRAS*	*LTK*	*LYN*	*MAF*	*MAP2K1 (MEK1)*	*MAP2K2 (MEK2)*	*MAP2K4*
MAP3K1	*MAP3K13*	*MAPK1*	*MCL1*	*MDM2*	*MDM4*	*MED12*	*MEF2B*	*MEN1*
MERTK	*MET*	*MITF*	*MKNK1*	*MLH1*	*MPL*	*MRE11A*	*MSH2*	*MSH3*

Current as of December 12, 2017.[4]

CURRENT GENE LIST (CONTINUED)								
MSH6	*MST1R*	*MTAP*	*MTOR*	*MUTYH*	*MYC*	*MYCL (MYCL1)*	*MYCN*	*MYD88*
NBN	*NF1*	*NF2*	*NFE2L2*	*NFKBIA*	*NKX2-1*	*NOTCH1*	*NOTCH2*	*NOTCH3*
NPM1	*NRAS*	*NT5C2*	*NTRK1*	*NTRK2*	*NTRK3*	*P2RY8*	*PALB2*	*PARK2*
PARP1	*PARP2*	*PARP3*	*PAX5*	*PBRM1*	*PDCD1 (PD-1)*	*PDCD1LG2 (PD-L2)*	*PDGFRA*	*PDGFRB*
PDK1	*PIK3C2B*	*PIK3C2G*	*PIK3CA*	*PIK3CB*	*PIK3R1*	*PIM1*	*PMS2*	*POLD1*
POLE	*PPARG*	*PPP2R1A*	*PPP2R2A*	*PRDM1*	*PRKAR1A*	*PRKCI*	*PTCH1*	*PTEN*
PTPN11	*PTPRO*	*QKI*	*RAC1*	*RAD21*	*RAD51*	*RAD51B*	*RAD51C*	*RAD51D*
RAD52	*RAD54L*	*RAF1*	*RARA*	*RB1*	*RBM10*	*REL*	*RET*	*RICTOR*
RNF43	*ROS1*	*RPTOR*	*SDHA*	*SDHB*	*SDHC*	*SDHD*	*SETD2*	*SF3B1*
SGK1	*SMAD2*	*SMAD4*	*SMARCA4*	*SMARCB1*	*SMO*	*SNCAIP*	*SOCS1*	*SOX2*
SOX9	*SPEN*	*SPOP*	*SRC*	*STAG2*	*STAT3*	*STK11*	*SUFU*	*SYK*
TBX3	*TEK*	*TET2*	*TGFBR2*	*TIPARP*	*TNFAIP3*	*TNFRSF14*	*TP53*	*TSC1*
TSC2	*TYRO3*	*U2AF1*	*VEGFA*	*VHL*	*WHSC1 (MMSET)*	*WHSC1L1*	*WT1*	*XPO1*
XRCC2	*ZNF217*	*ZNF703*						

SELECT REARRANGEMENTS								
ALK	*BCL2*	*BCR*	*BRAF*	*BRCA1*	*BRCA2*	*CD74*	*EGFR*	*ETV4*
ETV5	*ETV6*	*EWSR1*	*EZR*	*FGFR1*	*FGFR2*	*FGFR3*	*KIT*	*KMT2A (MLL)*
MSH2	*MYB*	*MYC*	*NOTCH2*	*NTRK1*	*NTRK2*	*NUTM1*	*PDGFRA*	*RAF1*
RARA	*RET*	*ROS1*	*RSPO2*	*SDC4*	*SLC34A2*	*TERC*#	*TERT*##	*TMPRSS2*

#ncRNA gene. ##promoter region only.

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Please confirm with the local Roche office for the availability of this product.

References: **1**. Scheerens H *et al. Clin Transl Sci* 2017;10:84–92. **2**. Li M. *Stat Biopharm Res* 2016;8:355–63. **3**. Agarwal A *et al. Pharmacogen Personal Med* 2015;8:99–110. **4**. FoundationOne®CDx Full Specification Information. **5**. Sun J *et al. J Thorac Oncol* 2017 12:S2118–19. **6**. Sun J *et al.* 2017 poster presentation.

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