FOUNDATIONONE® CDx

Technical Information and Test Overview

Clinical Background

Cancers have long been categorized and treated based on the anatomic site of origin of the cancer, e.g., lung, breast, colon, skin, etc. Increasingly, oncologists and pathologists are also focusing on the genomic alterations, in the genes that drive a cancer.

As we understand more about these underlying DNA alterations, cancer may be treated with targeted therapies that are designed to be specific to those changes in a patient's tumour with the hope that they may be less toxic and more effective than traditional cytotoxic treatments.

Methods

FoundationOne®CDx is a comprehensive† Genomic Profile that applies next-generation sequencing to identify 4 classes of mutations across all genes known to be drivers of solid tumours (Table 1) as well as genomic signatures including tumour mutational burden (TMB) and microsatellite instability (MSI). The test simultaneously sequences the coding region of

324 cancer-related genes often rearranged or altered in cancer to a typical median depth of coverage of greater than 500X. Each covered read represents a unique DNA fragment to enable the highly sensitive and specific detection of genomic alterations that occur at low frequencies due to tumour heterogeneity, low tumour purity and small tissue samples. FoundationOne CDx detects 4 classes of genomic alterations, including base substitutions, insertions and deletions (indels), copy number alterations (CNAs) and rearrangements using a small, routine FFPE sample (including core or fine needle biopsies).[1]

Reporting

Clinically relevant genomic alterations are associated with either a therapy that is currently marketed in the US, or a mechanism-driven clinical trial.[1] If a clinically relevant alteration is found in any one of the genes on the current gene list, the report will identify the gene and alteration and will provide an interpretation that is specific to the patient's tumour.

The genes listed on the front page of the report are found to have one or more clinically relevant alterations. All other genes are not found to have any clinically relevant alterations. In some cases, pertinent negatives are displayed on the front of the report; these are genes that have no alterations but are particularly relevant for the specific tumour type (e.g., KRAS in colon cancer, EGFR in lung cancer). The complete list of genes that are tested appears in the "Current Gene List" table on page 2, in the appendix of each FoundationOne CDx report.

Table 1

Technical Information	Base Substitutions[1]	Indels[1]	Copy Number Alterations[1]	Rearrangements
Sensitivity	>99% MAF* ≥5%	>97% MAF* ≥10%	>95% CN ≥8 or 0 ≥30% tumour nuclei	≥90%[2] >99% for ALK fusion[3] ≥20% tumour nuclei
Specificity (PPV**)	>99%	>99%	>99%	>99%
Typical Median depth of coverage (each covered read is of a unique DNA fragment to enable detection of alterations at low frequency)	500[1]			
Sample requirements	≥40 µm tissue, of which a minimum of 20% is of malignant origin, on 8 to 10 unstained slides or in an FFPE block. Needle biopsy is also acceptable.			
Turn-around time	14 day average***			

* Mutant Allele Frequency as defined in Frampton *et al*
** Positive Predictive Value as defined in Frampton *et al*
*** As measured from the date the Foundation Medicine laboratory receives a sample that meets requirements.

Often an alteration is detected in one of the genes included on FoundationOne CDx, but that specific alteration has not yet been adequately characterized in the scientific literature. We include these variants in the report so that they may be acted upon in the future should clinical evidence emerge.

Equivocal

Designation signifies when there is some, but not unambiguous, evidence of amplification or homozygous loss of a gene.

Subclonal

Designation signifies that the FoundationOne CDx analytical methodology has identified the presence of the alteration in less than 10% of the estimated tumour DNA.

FoundationOne CDx Includes Genes That Are Commonly Tested for in All Solid Tumours

FoundationOne CDx is a comprehensive† and fully informative genomic profiling service that can reveal 4 classes of actionable alterations, including those in cancer-driving genes that are rarely or never tested for in solid tumours. The FoundationOne CDx report may reveal alterations that lead to additional treatment options for physicians and their patients to consider.[1]

Note: The data presented above refers to the version of the FoundationOne Panel studied in Frampton *et al.*

Variants of Unknown Significance (VUS)

* The analytic validation of FoundationOne CDx, based on a prior version of the assay (236 genes, 19 select rearrangements) was published in Nature Biotechnology[1] and established the performance specifications required to deliver the high level of accuracy routinely obtained for 4 classes of genomic alteration by FoundationOne CDx.

1. Frampton *et al.* Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nature Biotech* 2013 Nov.
2. Based on analysis of coverage and re-arrangement structure in the COSMIC database for solid tumour fusion genes where alteration prevalence could be established, complemented by detection of exemplar rearrangements in cell line titration experiments.
3. Based on ALK re-arrangement concordance analysis vs. a standard clinical FISH assay.
4. Current as of Dec 12, 2017.

† Comprehensive Genomic Profiling with FoundationOne CDx involves:
- Next generation sequencing of the 324 cancer-related genes known to be clinically relevant to solid tumours
- Uniform coverage across the entire coding sequence of each gene plus select introns from 36 genes frequently rearranged in cancer
- Use of hybrid-capture technology to identify base substitutions, insertions/deletions, copy number alterations and rearrangements

Current Gene List[4] (as at October 2018)

FoundationOne CDx identifies 4 classes of alterations in each of the genes listed below[1] and now also reports Tumour Mutational Burden (TMB) measurements and Microsatellite Instability (MSI) status.

As a pan-cancer test, FoundationOne CDx is designed to interrogate the entire coding sequence of 324 cancer-related genes often rearranged or altered in cancer. These genes are known to be somatically altered in solid cancers based on recent scientific and clinical literature.

Genes with full coding exonic regions: For detection of substitutions, insertion-deletions and copy-number alterations

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*
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*						

**Compared to the FoundationOne CDx gene list.*

Select rearrangements: Genes from select intronic regions for the detection of gene rearrangements

ALK	*BCL2*	*BCR*	*BRAF*	*BRCA1*	*BRCA2*	*CD74*	*EGFR*	*ETV4*
ETV5	*ETV6*	*EWSR1*	*EZR*	*FGFR1*	*FGFR2*	*FGFR3*	*KIT*	*KM2A (MLL)*
MSH2	*MYB*	*MYC*	*NOTCH2*	*NTRK1*	*NTRK2*	*NUTM1*	*PDGFRA*	*RAF1*
RARA	*RET*	*ROS1*	*RSPO2*	*SDC4*	*SLC34A2*	*TERC**	*TERT (promoter only)†*	
TMPRSS2								

**TERC is a non-coding RNA gene. †TERT is a gene with a promoter region.*

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