FoundationOne®Heme Technical Information

CLINICAL BACKGROUND

Molecular diagnostics are routinely used to understand the molecular mechanism of an individual's haematologic malignancy to support diagnosis and determine the patient's prognosis.[1]

Advances in genomic research over recent years have improved our knowledge such that we now understand that cancer is a genomic disease, with molecular alterations fuelling its progression. The Catalogue of Somatic Mutations in Cancer (COSMIC) database now holds data for >28,000 whole genomes from >1 million samples and describes >4 million coding mutations.[2]

By profiling tumours to identify somatic alterations, physicians may be able to individualise treatment by matching a patient with the most suitable targeted therapy for their cancer.[3] This approach may also be valuable in the treatment of cancers driven by gene rearrangements and fusions, such as haematologic malignancies, sarcomas, and paediatric cancers.[1,4–6]

METHODS

FoundationOne®Heme is a Comprehensive Genomic Profile that applies a next-generation sequencing with hybrid capture-based target enrichment approach to identify somatic genomic alterations in genes known to be unambiguous drivers of haematologic malignancies (leukaemias, lymphomas, and myelomas) and sarcomas. Each profile simultaneously sequences the complete coding region of 406 genes, as well as selected introns of 31 genes involved in rearrangements. FoundationOne®Heme also interrogates the RNA sequence (complementary DNA; cDNA)* of 265 commonly rearranged genes to better identify gene fusions (including de novo and rare gene fusions). In addition to detecting rearrangements, FoundationOne®Heme detects all classes of genomic alterations, including base substitutions, insertion and deletions (indels), and copy number alterations (CNAs), using a small, routine clinical sample.

All FoundationOne®Heme samples are simultaneously profiled for tumour mutation burden (TMB) status.[7] TMB is determined by measuring the number of somatic mutations occurring in sequenced genes on the FoundationOne®Heme profile and extrapolating to the genome as a whole.[7]

*cDNA (complementary DNA) is synthetic DNA that has been synthesised by reverse transcriptase using mRNA (messenger RNA) as a template.

FOUNDATIONONE®HEME TECHNICAL INFORMATION		BASE SUBSTITUTIONS[1,8]	INSERTIONS AND DELETIONS[1,8]	COPY NUMBER ALTERATIONS[1,8]	REARRANGEMENTS[1]
Sensitivity		>99%† (MAF ≥5%)	97%† (MAF ≥10%; 1–40bp)	>95%† (homozygous deletions; or amplifications ≥8 copies)	>98% (gene fusions; 10% tumour fraction)
Specificity (PPV)		>99%†	>99%†	>99%†	>98%
Concordance TMB		>90% (≥20% tumour nuclei)‡[7,9]			
Typical median depth of coverage	**DNA**	500[1]			
	RNA	3M unique pairs[1]			
Sample requirements		≥80 µm tissue, of which a minimum of 20% is of malignant origin, on 16 unstained slides plus 1 H&E slide or in an FFPE block.§[10]			
Turn-around time		21-day average‖			

REPORTING

The FoundationOne®Heme report may reveal alterations that may lead to treatment options for physicians and their patients to consider. Results are provided in a report available via a link sent in a secure email and via Foundation Medicine Online.¶

When a clinically relevant alteration is found, the first page of the report identifies the specific gene and alteration(s) and for each alteration, provides a list of targeted therapies or clinical trials relevant to the patient's cancer.

The report offers a curation of peer-reviewed studies and other publicly available evidence compiled by Foundation Medicine's computational bioinformatics scientists, molecular biologists, cancer genomic scientists and pathologists. This may include associations between the presence of a specific genomic alteration (or wild-type status) and one or more therapies which may have clinical benefit (or may lack of clinical benefit). Alterations designated to be variants of unknown significance (VUS) are listed at the back of the report in the VUS section.

In some cases, pertinent negatives are displayed on the front of the report; these are genes that have no alterations identified but are particularly relevant to the specific cancer type (e.g. *ABL1* in chronic myelogenous leukaemia and acute lymphoid leukaemia, *IDH2* in acute myeloid leukaemia).

TMB status is reported on the front page for all cancer types. TMB status may be reported as TMB-High (≥20 Muts/Mb), TMB-Intermediate (6–19 Muts/Mb) or TMB-Low (≤5 Muts/Mb). 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'.

VARIANTS OF UNKNOWN SIGNIFICANCE (VUS)

Sometimes an alteration may be detected that 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 FoundationOne®Heme report in the event they may become clinically meaningful in the future.

EQUIVOCAL

A copy number alteration 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®Heme 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®Heme analytical methodology has identified the presence of the alteration in <10% of the assayed tumour DNA.

† Substitution, indels, and CNAs were validated by reanalysing 47 samples previously profiled with FoundationOne®, in which 169 alterations were identified in 55 genes common to both FoundationOne® and FoundationOne®Heme assays (102 substitutions, 59 indels, and 8 CNAs; 10 low-frequency subclonal variants were excluded from the analysis). The concordance between the 2 sets of results was 99.4% (168/169).[1]
‡ Based on concordance between FoundationOne® CGP and WES analysis data (26/29).
§ For liver specimens, a minimum of 40% of tumour nuclei is required.[10]
‖ As measured from the date the Foundation Medicine laboratory receives a sample that meets requirements.
¶ Please contact your local Customer Services team to set up an account on Foundation Medicine Online.

FFPE: formalin-fixed paraffin-embedded; **H&E slide**: haematoxylin and eosin-stained slide; **MAF**: mutant allele frequency; **PPV**: positive predictive value; **TMB**: tumour mutation burden; **WES**: whole exome sequencing.

FOUNDATION**ONE**®**HEME CURRENT GENE LIST**#

FoundationOne®Heme is a comprehensive genomic profile that interrogates the entire coding sequence of 406 genes as well as selected introns of 31 genes involved in rearrangements, and utilises RNA to interrogate 265 genes known to be somatically altered in haematologic malignancies and sarcomas.

DNA GENE LIST: ENTIRE CODING SEQUENCE (BASE SUBSTITUTIONS, INDELS, COPY NUMBER ALTERATIONS)

ABL1	ACTB	AKT1	AKT2	AKT3	ALK	AMER1 (FAM123B or WTX)	APC	APH1A
AR	ARAF	ARFRP1	ARHGAP26 (GRAF)	ARID1A	ARID2	ASMTL	ASXL1	ATM
ATR	ATRX	AURKA	AURKB	AXIN1	AXL	B2M	BAP1	BARD1
BCL10	BCL11B	BCL2	BCL2L2	BCL6	BCL7A	BCOR	BCORL1	BIRC3
BLM	BRAF	BRCA1	BRCA2	BRD4	BRIP1 (BACH1)	BRSK1	BTG2	BTK
BTLA	C11 orf30 (EMSY)	CAD	CALR	CARD11	CBFB	CBL	CCND1	CCND2
CCND3	CCNE1	CCT6B	CD22	CD274 (PDL1)	CD36	CD58	CD70	CD79A
CD79B	CDC73	CDH1	CDK12	CDK4	CDK6	CDK8	CDKN1B	CDKN2A
CDKN2B	CDKN2C	CEBPA	CHD2	CHEK1	CHEK2	CIC	CIITA	CKS1B
CPS1	CREBBP	CRKL	CRLF2	CSF1R	CSF3R	CTCF	CTNNA1	CTNNB1
CUX1	CXCR4	DAXX	DDR2	DDX3X	DNM2	DNMT3A	DOT1L	DTX1
DUSP2	DUSP9	EBF1	ECT2L	EED	EGFR	ELP2	EP300	EPHA3
EPHA5	EPHA7	EPHB1	ERBB2	ERBB3	ERBB4	ERG	ESR1	ETS1
ETV6	EXOSC6	EZH2	FAF1	FAM46C	FANCA	FANCC	FANCD2	FANCE
FANCF	FANCG	FANCL	FAS (TFRSF6)	FBXO11	FBXO31	FBXW7	FGF10	FGF14
FGF19	FGF23	FGF3	FGF4	FGF6	FGFR1	FGFR2	FGFR3	FGFR4
FHIT	FLCN	FLT1	FLT3	FLT4	FLYWCH1	FOXL2	FOXO1	FOXO3
FOXP1	FRS2	GADD45B	GATA1	GATA2	GATA3	GID4 (C17orf39)	GNA11	GNA12
GNA13	GNAQ	GNAS	GPR124	GRIN2A	GSK3B	GTSE1	HDAC1	HDAC4
HDAC7	HGF	HIST1H1C	HIST1H1D	HIST1H1E	HIST1H2AC	HIST1H2AG	HIST1H2AL	HIST1H2AM
HIST1H2BC	HIST1H2BJ	HIST1H2BK	HIST1H2BO	HIST1H3B	HNF1A	HRAS	HSP90AA1	ICK
ID3	IDH1	IDH2	IGF1R	KBKE	IKZF1	IKZF2	IKZF3	IL7R
INHBA	INPP4B	INPP5D (SHIP)	IRF1	IRF4	IRF8	IRS2	JAK1	JAK2
JAK3	JARID2	JUN	KAT6A (MYST3)	KDM2B	KDM4C	KDM5A	KDM5C	KDM6A
KDR	KEAP1	KIT	KLHL6	KMT2A (MLL)	KMT2C (MLL3)	KMT2D (MLL2)	KRAS	LEF1
LRP1B	LRRK2	MAF	MAFB	MAGED1	MALT1	MAP2K1	MAP2K2	MAP2K4
MAP3K1	MAP3K14	MAP3K6	MAP3K7	MAPK1	MCL1	MDM2	MDM4	MED12
MEF2B	MEF2C	MEN1	MET	MIB1	MITF	MKI67	MLH1	MPL
MRE11A	MSH2	MSH3	MSH6	MTOR	MUTYH	MYC	MYCL (MYCL1)	MYCN
MYD88	MYO18A	NCOR2	NCSTN	NF1	NF2	NFE2L2	NFKBIA	NKX2-1
NOD1	NOTCH1	NOTCH2	NPM1	NRAS	NT5C2	NTRK1	NTRK2	NTRK3
NUP93	NUP98	P2RY8	PAG1	PAK3	PALB2	PASK	PAX5	PBRM1
PC	PCBP1	PCLO	PDCD1	PDCD11	PDCD1LG2 (PDL2)	PDGFRA	PDGFRB	PDK1
PHF6	PIK3CA	PIK3CG	PIK3R1	PIK3R2	PIM1	PLCG2	POT1	PPP2R1A
PRDM1	PRKAR1A	PRKDC	PRSS8	PTCH1	PTEN	PTPN11	PTPN2	PTPN6 (SHP-1)
PTPRO	RAD21	RAD50	RAD51	RAF1	RARA	RASGEF1A	RB1	RELN
RET	RHOA	RICTOR	RNF43	ROS1	RPTOR	RUNX1	S1PR2	SDHA
SDHB	SDHC	SDHD	SERP2	SETBP1	SETD2	SF3B1	SGK1	SMAD2
SMAD4	SMARCA1	SMARCA4	SMARCB1	SMC1A	SMC3	SMO	SOCS1	SOCS2
SOCS3	SOX10	SOX2	SPEN	SPOP	SRC	SRSF2	STAG2	STAT3
STAT4	STAT5A	STAT5B	STAT6	STK11	SUFU	SUZ12	TAF1	TBL1XR1
TCF3 (E2A)	TCL1A (TCL1)	TET2	TGFBR2	TLL2	TMEM30A	TMSB4XP8 (TMSL3)	TNFAIP3	TNFRSF11A
TNFRSF14	TNFRSF17	TOP1	TP53	TP63	TRAF2	TRAF3	TRAF5	TSC1
TSC2	TSHR	TUSC3	TYK2	U2AF1	U2AF2	VHL	WDR90	WHSC1 (MMSET or NSD2)
WISP3	WT1	XBP1	XPO1	YY1AP1	ZMYM3	ZNF217	ZNF24 (ZSCAN3)	ZNF703
ZRSR2								

SELECT DNA REARRANGEMENTS

ALK	BCL2	BCL6	BCR	BRAF	CCND1	CRLF2	EGFR	EPOR
ETV1	ETV4	ETV5	ETV6	EWSR1	FGFR2	IGH	IGK	IGL
JAK1	JAK2	KMT2A (MLL)	MYC	NTRK1	PDGFRA	PDGFRB	RAF1	RARA
RET	ROS1	TMPRSS2	TRG					

SELECT GENE FUSIONS

ABI1	ABL1	ABL2	ACSL6	AFF1	AFF4	ALK	ARHGAP26 (GRAF)	ARHGEF12
ARID1A	ARNT	ASXL1	ATF1	ATG5	ATIC	BCL10	BCL11A	BCL11B
BCL2	BCL3	BCL6	BCL7A	BCL9	BCOR	BCR	BIRC3	BRAF
BTG1	CAMTA1	CARS	CBFA2T3	CBFB	CBL	CCND1	CCND2	CCND3
CD274 (PDL1)	CDK6	CDX2	CHIC2	CHN1	CIC	CIITA	CLP1	CLTC
CLTCL1	CNTRL (CEP110)	COL1A1	CREB3L1	CREB3L2	CREBBP	CRLF2	CSF1	CTNNB1
DDIT3	DDX10	DDX6	DEK	DUSP22	EGFR	EIF4A2	ELF4	ELL
ELN	EML4	EP300	EPOR	EPS15	ERBB2	ERG	ETS1	ETV1
ETV4	ETV5	ETV6	EWSR1	FCGR2B	FCRL4	FEV	FGFR1	FGFR1OP
FGFR2	FGFR3	FLI1	FNBP1	FOXO1	FOXO3	FOXO4	FOXP1	FSTL3
FUS	GAS7	GLI1	GMPS	GPHN	HERPUD1	HEY1	HIP1	HIST1H4I
HLF	HMGA1	HMGA2	HOXA11	HOXA13	HOXA3	HOXA9	HOXC11	HOXC13
HOXD11	HOXD13	HSP90AA1	HSP90AB1	IGH	IGK	IGL	IKZF1	IL21R
IL3	IRF4	ITK	JAK1	JAK2	JAK3	JAZF1	KAT6A (MYST3)	KDSR
KIF5B	KMT2A (MLL)	LASP1	LCP1	LMO1	LMO2	LPP	LYL1	MAF
MAFB	MALT1	MDS2	MECOM	MKL1	MLF1	MLLT1 (ENL)	MLLT10 (AF10)	MLLT3
MLLT4 (AF6)	MLLT6	MN1	MNX1	MSI2	MSN	MUC1	MYB	MYC
MYH11	MYH9	NACA	NBEAP1 (BCL8)	NCOA2	NDRG1	NF1	NF2	NFKB2
NIN	NOTCH1	NPM1	NR4A3	NSD1	NTRK1	NTRK2	NTRK3	NUMA1
NUP214	NUP98	NUTM2A	OMD	P2RY8	PAFAH1B2	PAX3	PAX5	PAX7
PBX1	PCM1	PCSK7	PDCD1LG2 (PDL2)	PDE4DIP	PDGFB	PDGFRA	PDGFRB	PER1
PHF1	PICALM	PIM1	PLAG1	PML	POU2AF1	PPP1CB	PRDM1	PRDM16
PRRX1	PSIP1	PTCH1	PTK7	RABEP1	RAF1	RALGDS	RAP1GDS1	RARA
RBM15	RET	RHOH	RNF213	ROS1	RPL22	RPN1	RUNX1	RUNX1T1 (ETO)
RUNX2	SEC31A	SEPT5	SEPT6	SEPT9	SET	SH3GL1	SLC1A2	SNX29 (RUNDC2A)
SRSF3	SS18	SSX1	SSX2	SSX4	STAT6	STL	SYK	TAF15
TAL1	TAL2	TBL1XR1	TCF3 (E2A)	TCL1A (TCL1)	TEC	TET1	TFE3	TFG
TFPT	TFRC	TLX1	TLX3	TMPRSS2	TNFRSF11A	TOP1	TP63	TPM3
TPM4	TRIM24	TRIP11	TTL	TYK2	USP6	WHSC1 (MMSET or NSD2)	WHSC1L1	YPEL5
ZBTB16	ZMYM2	ZNF384	ZNF521					

#Current as of February 16th, 2017.

The analytic validation of FoundationOne®Heme, based on a prior version of the FoundationOne®Heme assay (405 genes for DNA, 265 genes for RNA), was published in *Blood*[1] and established that the performance specifications obtained by FoundationOne®Heme for all classes of genomic alteration were concordant with the previously validated FoundationOne® assay in 47 clinical samples.

This updated version of FoundationOne®Heme met these performance specifications by demonstrating high concordance with genomic profiles of clinical specimens previously profiled on the validated version of FoundationOne®.

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