Hartwig MEDICAL FOUNDATION

Hartwig Medical OncoAct

OncoAct tumor WGS report

Summary

PRIMARY TUMOR LOCATION

Skin

PRIMARY TUMOR TYPE

Melanoma

The information regarding the primary tumor location and type, and the information related to the biopsy, is based on information received from the originating hospital.

Summary of most relevant findings

- Molecular tissue of origin prediction: Melanoma (likelihood: 99.6%).
- TERT (c.-125_-124delCCinsTT) promoter mutation.
- CDKN2A (p.Ala68fs, p.Gly83fs) inactivation.
- BRAF (p.Val600Glu) activating mutation, possible indication for BRAF and/or MEK inhibitors (clinical trial).
- PTEN (copies: 0) loss, possible indication for PI3K inhibitors (clinical trial).

An overview of all detected cancer associated DNA aberrations can be found in the report

Further interpretation of these results within the patient's clinical context is required by a clinician with support of a molecular tumor board.

Tumor characteristics

Tumor purity	99%
Molecular tissue of origin prediction	Melanoma (99.6%)
Tumor mutational burden status	Low (13.7)
Microsatellite status	MSS (0.1)
HR Status	Proficient (0)
Virus	NONE

Genomic alterations in cancer genes

Genes with driver mutation	BRAF, CDKN2A, TERT
Amplified gene(s)	NONE
Deleted gene(s)	PTEN
Homozygously disrupted genes	NONE
Gene fusions	NONE

HOSPITAL PATIENT ID
reportingId

HOSPITAL PATHOLOGY ID
pathologyNumber

HOSPITAL SAMPLE LABEL
hospitalSampleLabel

COHORT
cohort

REPORT DATE
01-Jan-2024

NAME
initials surname (M)

DATE OF BIRTH
01-Jan-1900

REQUESTED BY
studyPI

HOSPITAL
officialHospitalName

BIOPSY LOCATION
Skin

BIOPSY SUBLOCATION
Other/unknown

BIOPSY LATERALISATION
-

BIOPSY FROM PRIMARY TUMOR
yes

Pharmacogenetics

GENE	FUNCTION
DPYD	Normal Function
UGT1A1	Normal Function

HLA Alleles

GENE	GERMLINE ALLELE
HLA-A	A*01:01
HLA-B	B*40:02 | B*08:01
HLA-C	C*07:01 | C*03:04

Germline results

Data concerning cancer predisposition genes may be requested by a clinical geneticist after the patient has given informed consent.

Genomic based therapy approaches

High level evidence

DRUG TYPE	TUMOR TYPE SPECIFIC	MATCH	LEVEL	RESPONSE	GENOMIC EVENT
BRAF Inhibitor	Yes	Hotspot, Codon 600	A	▲	BRAF p.V600E
BRAF Inhibitor,MEK inhibitor (Pan),MEK1 Inhibitor,MEK2 Inhibitor	Yes	Hotspot, Codon 600	A	▲	BRAF p.V600E
MEK inhibitor (Pan),MEK1 Inhibitor,MEK2 Inhibitor	Yes	Hotspot	A	▲	BRAF p.V600E
MEK inhibitor (Pan),MEK1 Inhibitor,MEK2 Inhibitor,RAF Inhibitor (Pan)	Yes	Codon 600	A	▲	BRAF p.V600E
RAF Inhibitor (Pan)	Yes	Hotspot, Codon 600	A	▲	BRAF p.V600E
Akt Inhibitor (Pan)	No	Deletion	B	▲	PTEN partial loss
PIK3CB inhibitor	No	Deletion	C	▲	PTEN partial loss

Tumor type specific clinical studies (NL)

TRIAL	MATCH	GENOMIC EVENT
COLUMBUS-AD	Hotspot	BRAF p.V600E
DRUP	Activation, Codon 600	BRAF p.V600E
	Deletion, Inactivation	PTEN partial loss
EBIN (EORTC-1612-MG)	Codon 600	BRAF p.V600E
KN-8701	Activation, Hotspot	BRAF p.V600E
NASAM	Hotspot	BRAF p.V600E

Potential eligibility for DRUP is dependent on tumor type details therefore patients with certain tumor types may not be eligible for the DRUP study.

The iClusion database is used to annotate genomic events for potential clinical study eligibility. Please note that clinical study eligibility depends on multiple patient and tumor characteristics of which only the genomic events are considered in this report.

The Clinical Knowledgebase (CKB) is used to annotate genomic events with clinical evidence. Only evidence of level A (FDA approved therapy and/or guidelines), level B (late clinical trials), and/or level C (early clinical trials) are reported. Evidence items of level D (case reports and preclinical evidence) are not reported. The response symbol ▲ means that the evidence is responsive. The resistent symbol ▼ means that the evidence is resistant. The abbreviation P (mentioned after the response symbol) indicates the evidence is predicted responsive/resistent (meaning, the evidence data are limited but a potenial response/resistence is suggested). More details about CKB can be found in their Glossary Of Terms.

If the evidence matching is based on a mutation, but this is not a hotspot (see table Tumor specific variants under Genomic events), evidence should be interpreted with extra caution.
If the evidence matching is based on an amplification, evidence that corresponds with ‘overexpression’ of that gene is also matched. The same rule applies for deletions and 'underexpression'.

Genomic events (1/2)

Tumor purity & ploidy

Tumor purity	99%
Average tumor ploidy	3.1

Tumor specific variants

GENE	POSITION	VARIANT	READ DEPTH	COPIES	TVAF	BIALLELIC	HOTSPOT	DRIVER
BRAF	7:140453136	c.1799T>A (p.Val600Glu)	**150** / 221	6	68%	No	Yes	High
CDKN2A (p14ARF)	9:21971153	c.246_247delCG (p.Gly83fs)	**99** / 99	2	100%	Yes	Near	High
CDKN2A (p16)	9:21971153	c.203_204delCG (p.Ala68fs)	**99** / 99	2	100%	Yes	Near	High
TERT	5:1295228	c.-125_-124delCCinsTT	**56** / 65	2	87%	Yes	Yes	High
SF3B1	2:198266779	c.2153C>T (p.Pro718Leu)	**74** / 111	3	67%	No		Low
TP63	3:189604330	c.1497G>T (p.Met499Ile)	**47** / 112	4	42%	No		Low

Variant annotation is by default based on the canonical transcript. In case another transcript is more commonly used in routine practice, this annotation is also provided.

Tumor specific gains & losses

CHROMOSOME	REGION	GENE	TYPE	MIN COPIES	MAX COPIES	CHROMOSOME ARM COPIES
10	q23.31	PTEN	partial loss	0	2	2

Tumor specific gene fusions

NONE

Tumor specific homozygous disruptions

Complete loss of wild type allele

NONE

Tumor specific gene disruptions

LOCATION	GENE	DISRUPTED RANGE	TYPE	CLUSTER ID	DISRUPTED COPIES	UNDISRUPTED COPIES
10q23.31	PTEN	Intron 5 -> Intron 6	DEL	68	2	0

Genomic events (2/2)

Tumor specific viral insertions

NONE

Pharmacogenetics

GENE	GENOTYPE	FUNCTION	LINKED DRUGS	SOURCE
DPYD	*1_HOM	Normal Function	5-Fluorouracil;Capecitabine;Tegafur	PHARMGKB
UGT1A1#	*1_HOM	Normal Function	Irinotecan	PHARMGKB

#Note that we do not separately call the *36 allele. Dutch clinical guidelines consider the *36 allele to be clinically equivalent to the *1 allele.

HLA Alleles

GENE	GERMLINE ALLELE	GERMLINE COPIES	TUMOR COPIES	NUMBER SOMATIC MUTATIONS*	INTERPRETATION: PRESENCE IN TUMOR
HLA-A	A*01:01	2	4	None	Yes
HLA-B	B*08:01	1	2	None	Yes
	B*40:02	1	2	None	Yes
HLA-C	C*03:04	1	2	None	Yes
	C*07:01	1	2	None	Yes

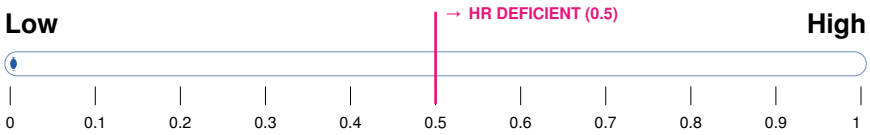
*When phasing is unclear, the mutation will be counted in both alleles as 0.5. Copy number of detected mutations can be found in the tumor specific variants table.

Tumor genomic profiles (1/2)

Homologous recombination status

Proficient 0

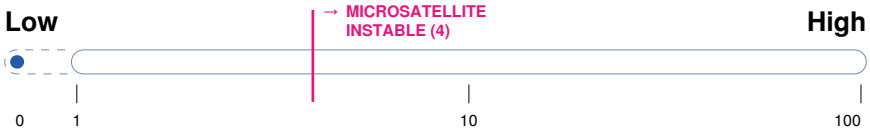
The homologous recombination (HR) deficiency score is determined by a WGS signature-based classifier for comparing the observed profile with signatures found across HR deficient (HRD) samples. Tumors with a score < 0.5 are considered HR proficient, tumors with a score ≥ 0.5 are considered HRD.



Microsatellite status

Stable 0.1

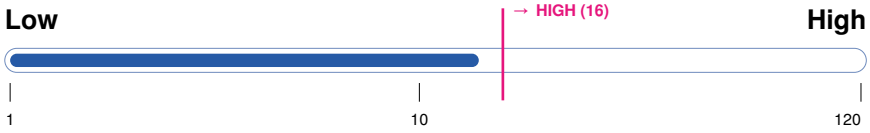
The microsatellite stability score represents the number of somatic insertions and deletions in (short) repeat sections across the whole genome of the tumor per Mb and is a good marker for instability in microsatellite repeat regions. Tumors with a score < 4.0 are considered microsatellite stable (MSS), tumors with a score ≥ 4.0 are considered microsatellite instable (MSI).



Tumor mutational burden

Low 13.7

The tumor mutational burden score represents the number of all somatic variants across the whole genome of the tumor per Mb. Patients with a mutational burden over 16 could be eligible for immunotherapy studies.



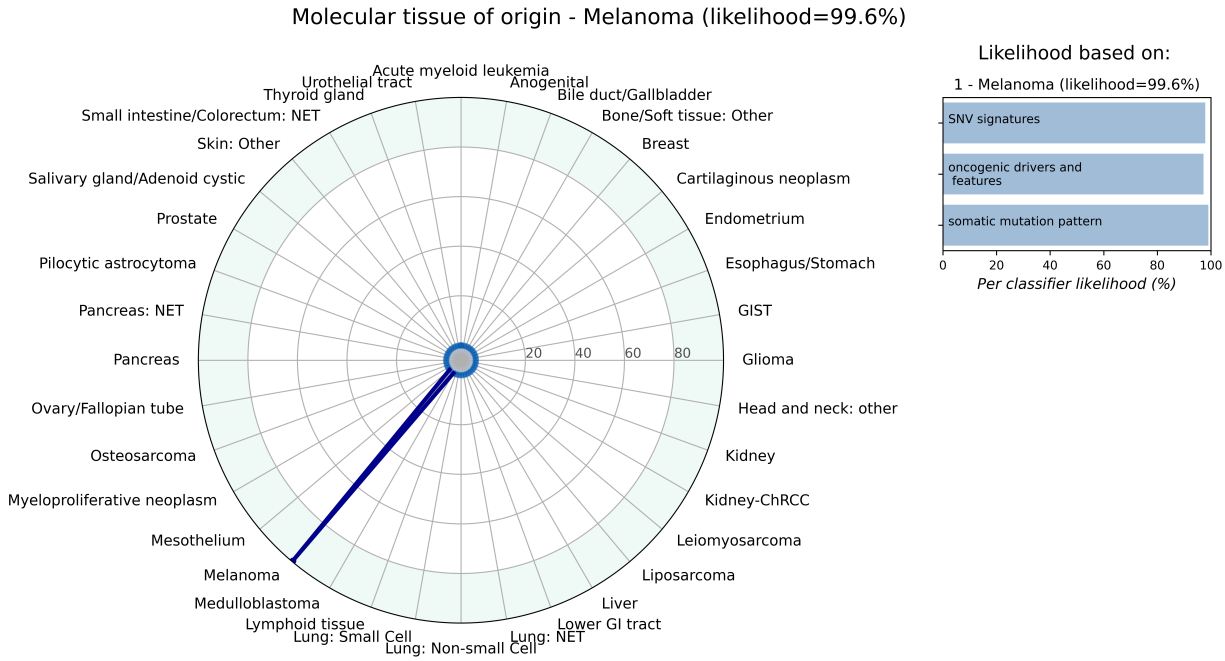
Tumor mutational load

183

The tumor mutational load represents the total number of somatic missense variants across the whole genome of the tumor.

Tumor genomic profiles (2/2)

Molecular tissue of origin prediction

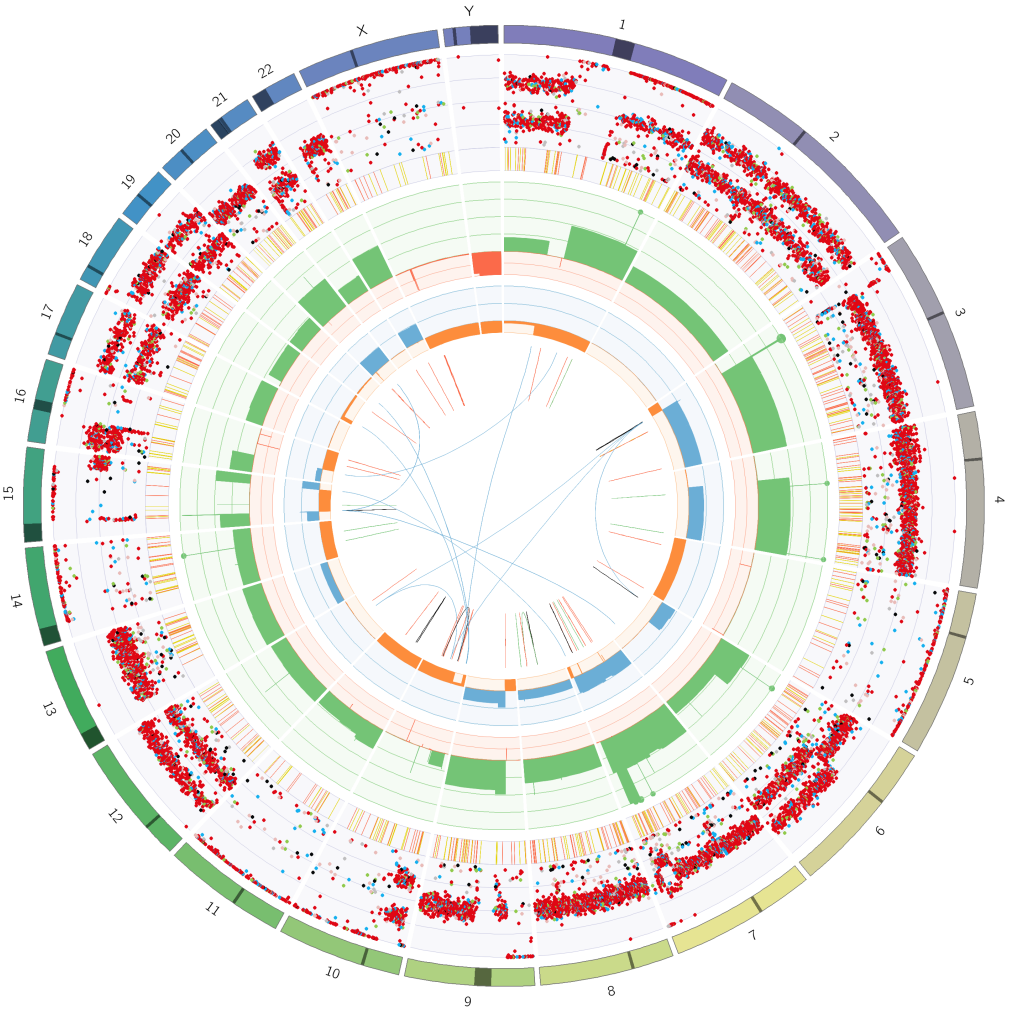


The title shows the conclusion of the prediction of the molecular tissue of origin. If none of the similarity predictions has a likelihood ≥ 80%, no reliable conclusion can be drawn ('results inconclusive').

The left plot shows the likelihoods (similarity) for all the origin types analyzed by the molecular tissue of origin prediction tool. Only when the likelihood is ≥ 80% (a peak in the green outer band of the plot), a reliable prediction (with > 90% accuracy) can be drawn. Lower likelihoods (< 80%) suggest there is similarity with that tissue of origin, but this is less strong and there is lower confidence.

The right plot(s) shows the breakdown of the strongest predicted likelihood(s) into the contribution of the 1) SNV types (related to those used in Cosmic signatures), 2) driver landscape and passenger characteristics (e.g. tumor-type specific drivers), and 3) somatic mutation pattern (mutation distribution across the genome).

CIRCOS plot



The outer first circle shows the chromosomes. The darker shaded areas represent large gaps in the human reference genome: i.e. regions of centromeres, heterochromatin & missing short arms.

The second circle shows all tumor specific variants (incl. exon, intron and intergenic regions) and are divided into an outer ring of single nucleotide polymorphism (SNP) allele frequencies and an inner ring of short insertion/deletion (INDEL) locations. Variant allele frequencies have been corrected for tumor purity and scale from 0 to 100%. Each dot represents a single variant and are colored according to the type of base change (e.g. C>T/G>A in red) and are in concordance with the coloring used in Alexandrov et al. 2013 Nature paper that describes the use of mutational signatures. INDELs are colored yellow and red for insertions and deletions respectively.

The third circle shows all observed tumor purity adjusted copy number changes, including both focal and chromosomal events. Copy number losses are indicated in red, green shows regions of copy number gain. The scale ranges from 0 (complete loss) to 6 (high level gains). If the absolute copy number is > 6 it is shown as 6 with a green dot on the diagram.

The fourth circle represents the observed 'minor allele copy numbers' across the chromosome. The range of the chart is from 0 to 3. The expected normal minor allele copy number is 1, and anything below 1 is shown as a loss and represents a LOH event (orange). Minor allele copy numbers above 1 indicate amplification events of both A and B alleles at the indicated locations (blue).

The innermost circle displays the observed structural variants within or between the chromosomes. Translocations are indicated in blue, deletions in red, insertions in yellow, tandem duplications in green and inversions in black.

Report explanation (1/2)

Details on the report in general

This report is created using NovaSeq 6000 (Illumina) WGS analysis, which data is processed using Hartwig MedicalOncoAct® software and reporting. The OncoAct WGS specification sheet can be downloaded here: https://www.oncoact.nl/specsheetOncoActWGS. All activities are performed under ISO17025 accreditation (RVA, L633).

The OncoAct WGS user manual can be downloaded here: https://www.oncoact.nl/manual.

The analyses are performed using reference genome version GRCh37 (made available by the Genome Reference Consortium).

The genes and related gene transcripts used for reporting can be downloaded from the resources. In general the canonical transcripts as defined by Ensembl are used.

Genomic event detection in samples with lower tumor purity is less sensitive. The likelihood of failing to detect potential events increases in case of a low (implied) tumor purity (< 20%).

The implied tumor purity is the percentage of tumor cells in the tumor material based on analysis of whole genome data.

Details on the reported genomic based therapy approaches

The Clinical Knowledgebase (CKB) is used to annotate genomic events with clinical evidence. The evidence is gathered from CKB without further checks or interpretation. More details about CKB can be found in their Glossary Of Terms.

The iClusion database is used to annotate genomic events for potential clinical study eligibility. The studies are gathered from the iClusion database without further checks or interpretation.

Details on the tumor specific variants

The 'Read depth' indicates the raw number of reads supporting the variant versus the total number of reads on the mutated position.

The 'Copies' field indicates the number of alleles present in the tumor on this particular mutated position.

The 'tVAF' field indicates the variant allele frequency corrected for the implied tumor purity.

The 'Biallelic' field indicates whether the variant is present across all alleles in the tumor (and is including variants with loss-of-heterozygosity).

The 'Hotspot' field indicates whether a variant is part of the most sensitive calling tier used in the analyses. The tiers are determined based on different knowledge databases including CIViC, DoCM,and CGI.

The 'Driver' field indicates the driver probability on gene level and is calculated using data in the Hartwig Medical Database. A variant in a gene with high driver likelihood is likely to be positively selected during the oncogenic process.

The external ClinVar database is used to determine the pathogenicity of observed germline variants.

Details on the reported tumor specific gains & losses

The lowest copy number value along the exonic regions of the canonical transcript is determined as a measure for the gene's copy number.

Copy numbers are corrected for the implied tumor purity and represent the number of copies in the tumor DNA.

Any gene with < 0.5 copies along the entire canonical transcript is reported as a full loss. Any gene where only a part along the canonical transcript has < 0.5 copies is reported as a partial loss.
Any gene with ≥ 3 times the average tumor ploidy in copies along the entire canonical transcript is reported as a full gain. Any gene where only a part of the canonical transcript has ≥ than 3 times the average tumor ploidy in copies is reported as a partial gain.

Details on the reported tumor specific gene fusions

The canonical, or otherwise longest transcript that is validly fused, is reported.

Reporting of fusions is restricted to a selection of known fusions and a selection of pre-defined fusions where one partner is promiscuous in either the 5' or 3' position. The full list of fusions can be downloaded from the resources.

The 'Driver' field is set to high in case the fusion is a known fusion, or a fusion where the promiscuous partner is fused in an exon range that is typically observed in literature.

All other fusions get assigned a low driver likelihood.

Details on the reported tumor specific homozygous / gene disruptions

Genes are reported as being disrupted when their canonical transcript has been disrupted.

The range of the disruption is indicated by the intron/exon/promoter region of the break point and the direction the disruption faces.

The type of disruption can be INV (inversion), DEL (deletion), DUP (duplication), INS (insertion), SGL (single) or BND (translocation).

A gene for which no wild type exists anymore in the tumor DNA due to disruption(s) is reported in a separate section called 'homozygous disruptions'.

Report explanation (2/2)

Details on the reported tumor specific viral insertions

The NCBI viral reference database is used in the analyses to annotate and classify viral insertions. Reporting of viral insertions is restricted to a selection of clinically relevant viruses (HPV, MCV, HBV, EBV and HHV-8). Viral insertions are only reported when genomic integration of the virus in the tumor is detected or when the percentage of the viral genome that is covered is > 90% and the coverage of the virus genome is higher than the expected mean coverage of the tumor. For reporting of EBV both of the conditions should be met.

Details on the reported pharmacogenetics

The pharmacogenetic haplotypes are reported based on germline analysis. The PharmGKB database is used to annotate the observed haplotypes. Details on the pharmacogenetic haplotypes and links to related treatment adjustments can be downloaded from the resources.

The called haplotypes for a gene are the simplest combination of haplotypes that perfectly explains all of the observed variants for that gene. If no combination of haplotypes in the panel can perfectly explain the observed variants, then 'Unresolved Haplotype' is called.

Wild type is assumed when no variants are observed.

Details on the reported HLA Alleles

HLA Class I types (HLA-A, HLA-B and HLA-C) are reported based on germline analysis, but also the tumor status of each of those alleles is indicated (somatic mutations, complete loss, and/or allelic imbalance).

The IMGT/HLA database is used as a reference set of Human MHC class I alleles. HLA typing is done to 4-digits, which means it uniquely identifies a specific protein, but ignores synonymous variants (6 digits) and intronic differences (8 digits).

Sample details & disclaimers

Sample details

The samples have been sequenced at **Hartwig Medical Foundation, Science Park 408, 1098XH Amsterdam**.

The hospital patient ID is: **reportingId** and the pathology tissue ID is: **pathologyNumber**.

The results in this report have been obtained between **01-Jan-2023** and **01-Jan-2024**.

This analysis is performed on the tumor sample as arrived on **01-Jan-2023** with barcode **normal**.

This analysis is performed on the reference sample as arrived on **01-Jan-2023** with barcode **referenceSampleBarcode**.

The results stated in this report are based on the tested tumor and reference sample.

This experiment is performed according to lab procedures: **ISO017 v4.1-SNP028 v5.0-PREP041 v4.0**

This report is addressed to: **studyPI, officialHospitalName, hospitalPostalCode hospitalCity**.

Disclaimers

The data on which this report is based is generated from tests that are performed under NEN-EN-ISO/IEC-17025:2017 TESTING L633 accreditation and have passed all internal quality controls.

This report is generated using the molecular pipeline version **5.33** and OncoAct reporting pipeline version **1.0.**

(basic) UDI-DI: **(01)8720299486058(8012)v5.33-1.0.**

This report was generated automatically and checked by a trained Clinical Molecular Biologist in Pathology (KMBP)**.**

The ‘primary tumor location’ and ‘primary tumor type’ have influence on the clinical evidence/study matching. No check is performed to verify the received information.

The conclusion of this report is based solely on the results of the whole genome sequencing of the received biomaterials, and the additional primary tumor location and type information received from the hospital. Further interpretation of these results within the patient’s clinical context is required by a clinician with support of a molecular tumor board.

Based on a implied tumor purity of at least 20%, the test has a sensitivity of > 95% for detection of tumor specific variants, tumor specific gains and losses, tumor specific gene fusions and tumor specific gene/homozygous disruptions.

Hartwig Medical Foundation is not responsible for the content of all external data sources used to do the analyses and generate this report. Hartwig Medical Foundation is not liable and cannot be held accountable for any incorrectness, incompleteness or error of any other kind in these data sources, or the external software used to harmonize and curate these data sources.

Based on the Dutch Act on Exceptional Medical Treatments (in Dutch: ‘Wet op de bijzondere medische verrichten’) Stichting Hartwig Medical Foundation is not allowed to provide genetic counseling and therefore will not share specific germline information, unless otherwise instructed and on explicit request of a hospital that is authorised to provide genetic counseling to individual patients.

For feedback or complaints please contact **qualitysystem@hartwigmedicalfoundation.nl.**

For questions about the contents of this report, please contact **diagnosticssupport@hartwigmedicalfoundation.nl.**

— End of report —

TESTING
RvA L 633

Edwin Cuppen,
Director Hartwig Medical Foundation