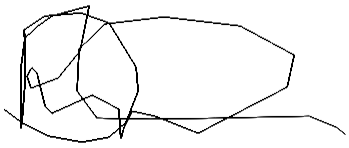
HMF-IVDD-275
OncoAct user manual

Approval Quality Manager:



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Amendment history				
Version	**Description of the change**	**Section / Page nr.**	**reviewed by**	**Issue date**
1.0	First Issue	All	PR	02/06/2021 04:21 PM
2.0	Update for OncoAct v5.33-1.0	All	SvdB	15/11/2023 14:01

Table of Contents

Instructions for use IVDR device:
Hartwig Medical OncoAct

Online version: https://www.oncoact.nl/manual

1 Identification

An OncoAct report can be identified by the following aspects:

- Hartwig Medical Foundation logo in the top left corner on all pages of the report.
- Title 'Hartwig Medical OncoAct' in the top-center of all pages of the report.
- Signature of the Director Hartwig Medical Foundation on the last page of the report.

2 Label

Device Hartwig Medical OncoAct

Manufacturer
Hartwig Medical Foundation
Science Park 408
1098 XH Amsterdam
www.hartwigmedicalfoundation.nl

(01)8720299486058
(8012)v5.33-1.0

Instructions for use are supplied in electronic form instead of paper form.
URL: www.oncoact.nl/manual
Email: diagnosticssupport@hartwigmedicalfoundation.nl
Device with internet access, web browser and PDF reader required for reading the manual. Paper instructions for use can be requested at no additional cost by contacting us using the indicated e-mail address and will be delivered within 7 days.

3 Intended purpose

OncoAct is an in vitro diagnostic (IVD) medical device consisting of software that analyses whole genome sequencing data for cancer diagnostics and treatment decision making purposes. It detects and measures all types of oncology related DNA-based genomic events and genomic characteristics (biomarkers) that can be relevant for diagnosis and treatment decision making of cancer patients using whole genome DNA sequencing data derived from non-formalin fixated tumor and reference biomaterial. Analytical results can be quantitative as well as qualitative. The product of the software that is delivered to the customer involves a report that presents an overview of oncology related genomic events and characteristics (biomarkers) including links to associated treatments and possible clinical studies. OncoAct is only made available to registered clinicians or other registered medical experts who have requested the IVD test, to facilitate and/or support diagnosis and treatment decision making for cancer patients. The intended clinical use of OncoAct are cancer patients that seek systemic treatment and for whom the biomaterials, tumor material with sufficient tumor cells and a reference sample, can be collected safely.

4 Intended users

4.1 IVD users

Bioinformaticians and clinical molecular biologists in pathology working for Hartwig Medical Foundation are the intended users of OncoAct in terms of data analysis and reporting (analytical use).

4.2 Registered clinicians and other registered medical experts

Registered clinicians and other registered medical experts working in oncology in hospitals are users of the results (the findings) that are displayed in the OncoAct report (clinical use). The medical experts will use the results in the process of treatment decision making, in dialogue with other specialists (e.g., in molecular tumor boards).

5 Test principle

Whole Genome Sequencing can be performed to generate a complete picture of oncology related genomic events and characteristics (biomarkers). Besides analyzing Whole Genome Sequencing data of the tumor (generated by sequencing DNA originating from tumor material), Whole Genome Sequencing data is also analyzed of normal cells (generated by sequencing DNA originating from healthy non-tumor material from the same individual). This results in a comprehensive analysis, including:

- Discovery of (somatic) small variants (~<50 bp), as well as information about the copy number, biallelic and if a variant is a hotspot or driver.
- Tumor characteristics: tumor purity and ploidy
- Gains and losses of genes
- Gene fusions
- Homozygous disruptions
- Gene disruptions
- Viral insertions and detected non-integrated viruses
- Homologous recombination deficiency score
- Microsatellite status
- Pharmacogenetics for DPYD and UT1GA1 gene
- Molecular Tissue of Origin prediction
- Tumor mutational load and tumor mutational burden
- Genomic based treatment approaches: high level evidence and clinical studies
- Graphical overview of all events found within the tumor

The contents of the report, containing all the above information, gives the registered medical expert the opportunity to personalize the treatment of this patient for his or her specific cancer.

6 Input data limitations

The input data for the IVD test should be Whole Genome Sequencing data (tumor and reference) that fulfils the following criteria:

- The tumor and reference data are from the same individual
- The tumor data is generated using non-formalin fixated tumor material with a minimal tumor-cell percentage of 20% (determined by standard pathology procedures or molecular analysis)
- The reference data is generated from healthy non-tumor materials
- The tumor and reference data are not contaminated with data from other sources including other individuals (e.g. stem cell transplantation) or a mixture of tumor and reference data (e.g. leukemia)
- The data is generated using the Illumina TruSeq nano or verified equivalent quality* library preparation kit with a NovaSeq 6000 or verified equivalent quality* sequencer with read length 2 x 150/151 bp **quality must have been verified using Hartwig distributed test samples.*
- The data has a minimal quality value (Q30) of 85%
- The reference data has a minimal yield of 100 Gbases after the removal of reads with a lower than 85% quality value (Q30)
- The tumor data has a minimal yield of 300 Gbases after the removal of reads with a lower than 85% quality value (Q3)
- The data is submitted in FASTQ format
- The data is submitted together with all relevant identifiers and the primary tumor location and type

7 Calculations and interpretations of results

The software includes several different software items (tools) with different calculations to approximate the biological truth. Therefore, results should be interpreted with caution, and should be used solely as supporting evidence for diagnosis and treatment decision making by registered medical experts.

7.1 Interpretation of reports

7.1.1 Types of reports

There are 6 different versions of the OncoAct DNA analysis report, all serving different purposes:

Type	**Purpose**	**Link to Hartwig documentation code:**
OncoAct WGS tumor report	Reporting for input data that passes every quality check in the IVD test (the input data fulfilled all criteria as described under 6)	HMF-FOR-080
Reports when quality checks were not successful (the input data did not fulfill the set criteria as described under 6):		
OncoAct tumor WGS report - low purity analysis	Reporting for input data that does not pass the tumor purity quality check in the IVD test, and the IVD test could therefore only be performed with lower performance (the input data did not fulfill the purity criterium (“the tumor data is generated using fresh	*HMF-FOR-209*

	tumor material with a minimal tumor-cell percentage of 20%") as described under 6, but reporting of test results is still desirable with a disclaimer that the results should be interpreted with extra caution)	
OncoAct tumor WGS report - failed tumor analysis	Reporting for input data, where the data from the tumor does not pass the quality checks in the IVD test, and therefore no results for the tumor could be generated (the input data for the tumor did not fulfill the criteria as described under 6, but reporting of test results for the reference is still desirable with a disclaimer that only limited results are available)	*HMF-FOR-083*
OncoAct tumor WGS report - failed analysis	Reporting for input data that does not pass the quality checks in the IVD test, and therefore no results could be generated (the input data did not fulfill the criteria as described under 6)	*HMF-FOR-082*

7.1.1.1 OncoAct tumor WGS report

The OncoAct tumor WGS report is given out when the input data passed all quality control checks and reliable results were generated with the IVD test. At the end of this user manual an example OncoAct tumor WGS report is added with explanations about all the different sections, see 11 appendix: OncoAct Tumor WGS report manual.

7.1.1.2 OncoAct tumor WGS report – low purity analysis

Similar report as the OncoAct tumor WGS report (described above), but with a disclaimer that the results should be interpreted with extra caution.

7.1.1.3 OncoAct tumor WGS report – failed tumor analysis

Limited report with only results of the IVD test for the reference input data. The report also contains a description of the reason for the failure of the analysis of the tumor input data.

7.1.1.4 OncoAct tumor WGS report – failed analysis

One page report without results of the IVD test, and only describing the reason for the failure of the analysis of the input data.

7.2 Recommendations for quality control procedures

No quality control procedures are needed to be performed by the user. However, registered medical experts need to be competent (correct education and training) for the interpretation of molecular diagnostic test results in general and the OncoAct report in specific.

7.3 Analytical performance

The OncoAct software includes several different outputs. The analytical performance claims of the different outputs are based on the validations and verifications that were done in the Quality Management System (ISO17025; accredited since 2017). Below an overview of all the analytical performance claims and the performance in the validations and/or verifications:

Feature	*#*	*Performance claim*	*Method validation*	*Performance found*	*Evidence documentation available at Hartwig (can be viewed on request)*
OncoAct analytical applicability	1	OncoAct is applicable for input data (tumor and reference) fulfilling all set criteria as described under 6	Comparison to current 'standard-of-care' in clinical practice	The available analytical evidence demonstrates that this claim is met	HMF-VAL-051 Validation of molecular T% test; HMF-VAL-063 Validation of average tumor ploidy; HMF-VAL-074 Clinical Validation of OncoAct
OncoAct analytical sensitivity for somatic genomic events	2	For input data (tumor and reference) fulfilling all criteria as described under 6, the sensitivity for the detection of somatic: SNVs, MNVs and indels, structural variants (with fusions and homozygous disruptions), and gene copy number changes should be 95% or higher	See claims 3 (SNVs, MNVs and indels), 4 (structural variants, with 5 (fusions) and 6 (homozygous disruptions)) and 7 (gene copy number changes)	See claims 3 (SNVs, MNVs and indels), 4 (structural variants, with 5 (fusions) and 6 (homozygous disruptions)) and 7 (gene copy number changes)	HMF-VAL-074 Clinical Validation of OncoAct; See claims 3 (SNVs, MNVs and indels), 4 (structural variants, with 5 (fusions) and 6 (homozygous disruptions)) and 7 (gene copy number changes)
Analytical sensitivity and positive predictive value/specificity for somatic SNVs, MNVs and indels	3	Sensitivity and positive predictive value/specificity for the detection of SNVs, MNVs and indels should both be over 95% compared to current standard of care tests	Comparison to current 'standard-of-care' in clinical practice	The available analytical evidence demonstrates that this claim is met – the original validation showed: sensitivity = 99.2%, specificity = 95.8%, in recent comparisons with standard of care tests a sensitivity of 99.2% is found	HMF-VAL-061 Validation of SNV-MNV-INDEL mutations using WGS; HMF-VAL-045 Validation of WGS based variants by smMIP, HMF-VAL-065 Validation of SAGE 2.2, https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988
Analytical sensitivity and specificity somatic fusions from structural variants	4	Sensitivity and specificity for the detection of fusions from structural variants should be over 95% compared to current standard of care tests	Comparison to current 'standard-of-care' in clinical practice	The available analytical evidence demonstrates that this claim is met – the original validation showed: sensitivity = 93%, specificity = 95%, in recent comparisons with standard of care tests a sensitivity of 97.7% is found	HMF-VAL-066 Validation of structural variant analysis; HMF-VAL-060 Validation of fusion gene readout using WGS, https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988
Analytical concordance somatic (homozygous)	5	Concordance for the detection of (homozygous) disruptions from	Comparison to current 'standard-of-care' in clinical practice (although	14 of the 16 samples were concordant; for 2 of the 16 samples discordant results	HMF-VAL-066 Validation of structural variant analysis; HMF-VAL-068

disruptions from structural variants		structural variants should be over 99% compared to current standard of care tests, or another explanation should be found	the current test looks at a different mechanism so is not fully comparable)	were found but this was due to the difference in test type (and no mistakes).	Validation of homozygous disruption readout
Analytical concordance for somatic gene copy number changes	7	Concordance for the detection of gene copy number changes should be over 95% compared to current standard of care tests	Comparison to current 'standard-of-care' in clinical practice	In the original validation only 1 case (out of 15), the WGS and FISH readouts were not aligned and could not be explained due to technical or interpretation issues which gives a concordance of 93.3%, in recent comparisons with standard of care tests a sensitivity of 97.6% is found	HMF-VAL-049 Validation of WGS based copy number_ERBB2, https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988
OncoAct analytical sensitivity for germline genomic events	8	For input data (tumor and reference) fulfilling all criteria as described under 6, the sensitivity for the detection of germline: SNVs, MNVs and indels, structural variants (with homozygous disruptions) should be 95% or higher	See claims 9 (SNVs, MNVs and indels) and 10 (structural variants, with homozygous disruptions)	See claims 9 (SNVs, MNVs and indels) and 10 (structural variants, with homozygous disruptions)	See claims 9 (SNVs, MNVs and indels) and 10 (structural variants, with homozygous disruptions)
Analytical sensitivity and positive predictive value/specificity for germline SNVs, MNVs and indels	9	Sensitivity and specificity for the detection of germline SNVs, MNVs and indels should be over 95% compared to previous version	Comparison to current 'standard-of-care' in clinical practice	The available analytical evidence demonstrates that this claim is met - sensitivity = 100%, specificity = 100%	HMF-VAL-072 Validation of germline analyses, HMF-VER-076 Verification of SAGE germline vs bachelor, HMF-VAL-077 Validation of PAVE
Analytical sensitivity for germline (homozygous) disruptions	10	Sensitivity for the detection of germline (homozygous) disruptions should be over 95% compared to previous version	Comparison to current 'standard-of-care' in clinical practice (but a bias towards a selection of more complex structural variants)	Recall 18 of 20 variants in the truth set and; which gives a sensitivity of 90%. However, there was a bias towards very complex variants making it justified to assume the general	HMF-VAL-072 Validation of germline analyses

				sensitivity is over 95%.	
Analytical sensitivity and concordance viral insertions and detected non-integrated viruses in the tumor	11	Sensitivity and concordance for viral insertions should be both over 95% compared to current standard of care tests	Comparison to current 'standard-of-care' in clinical practice	The available analytical evidence demonstrates that the claim is met - sensitivity = 100%, concordance = 97.8%	HMF-VAL-064 Validation of virus detection using WGS, HMF-VER-084 Verification virus interpreter v1.1
Analytical sensitivity and specificity tumor microsatellite score	12	Sensitivity and specificity for MSI should be over 95% compared to current standard of care tests	Comparison to current 'standard-of-care' in clinical practice	The available analytical evidence demonstrates that the claim is met - sensitivity = 100%, specificity = 97%	HMF-VAL-043 Validation of Microsatellite readout using WGS
Analytical exactness tumor homologous recombination deficiency score	13	Homologous recombination deficiency exactness should be over 95% compared to earlier homologous recombination deficiency classifications	Comparison to previous version/COLO829 that is scientifically validated + comparison with previous classifications, that have shown scientific/clinical validity	The available analytical evidence demonstrates that the claim is met - exactness = 99.1%	HMF-VAL-062 Validation of HR-deficiency classifier using WGS, HMF-VER-053 Verification of CHORD v2 (HR-deficiency classifier)
Analytical concordance tumor mutational burden/load	14	TMB correlation should be over 0.95 R2 compared to current standard of care tests (panel)	Comparison to current 'standard-of-care' in clinical practice	The available analytical evidence demonstrates that the claim is met - correlation R2 = 0.98	HMF-VAL-061 Validation of SNV-MNV-INDEL mutations using WGS
Analytical sensitivity and concordance pharmacogenetic calling (DPYD and UGT1A)	15	Sensitivity and concordance for DPYD and UGT1A pharmacogenetic calling should be both over 99% compared to current standard of care tests	Comparison to current 'standard-of-care' in clinical practice	The available analytical evidence demonstrates that the claim is met - sensitivity = 100%, concordance = 100%	HMF-VAL-069 Validation of DPYD genotype readout by WGS, HMF-VER-075 Verification of pharmacogenomics
Analytical sensitivity and specificity HLA status calling	16	Sensitivity and specificity for HLA calling should be over 99% compared to current clinically validated tests	Comparison to independent clinically validated orthogonal test	The available analytical evidence demonstrates that the claim is met - sensitivity = 100%, specificity = 100%	HMF-VAL-076 Validation of HLA typing by WGS

Analytical accuracy molecular tumor of origin prediction	17	Molecular tumor of origin predictions should have an accuracy over 90% for conclusive results following the internal validation (note: this is the only performance that is lower and is specifically stated in the OncoAct report)	Internal validation using independent test set	The available analytical evidence demonstrates that the claim is met - 73.8% of the samples of the test set had conclusive results, among those there was an accuracy of 93.5%	HMF-VAL-071 Validation of CUPPA algorithm
OncoAct analytical reproducibility	18	Reproducibility is controlled using verifications after updates	All verifications	Verifications after every update control reproducibility	HMF-PRO-007 Validation and verification, HMF-VER-109 Verification of pipeline v5.33, HMF-VER-112 Verification of OncoAct reporting pipeline v1.0
Limits of detection OncoAct	19	When the input data provided does not fulfill the criteria as described under 6	All verifications and validations	NA	HMF-SOP-025

As

Also, the analytical performance has been described and published in scientific peer-reviewed journals, see https://www.jmdjournal.org/article/S1525-1578(21)00120-3/fulltext.

The conclusion was that whole genome sequencing has a >95% sensitivity and precision compared to routinely used DNA techniques in diagnostics and all relevant oncology related genomic events can be detected reliably in a single assay, as is also demonstrated by our verifications and/or validations.

7.4 Clinical performance

OncoAct is a diagnosis and treatment decision making support/advice tool. The registered medical expert uses it as support and advice, consequently, no sensitivity and specificity of effects for the patient can be defined. However, in a large clinical study (involving independent medical experts, the WIDE study (https://bmcmedgenomics.biomedcentral.com/articles/10.1186/s12920-020-00814-w)), the performance of OncoAct as compared to the 'standard-of-care' in clinical practice was evaluated. The below results are originating from that study:

Feature	***#***	***Performance claim***	***Method validation***	***Performance found***	***Evidence documentation available at Hartwig (can be viewed on request)***
Sensitivity OncoAct	1	Sensitivity is defined as the percentage of genomic events (biomarkers) that are present in the patient that are detected by OncoAct: the diagnostic sensitivity should be at least 95%	Clinical investigation (WIDE study)	The available clinical evidence demonstrates that the claim is met - the diagnostic sensitivity was 97.95%	HMF-VAL-074 Clinical Validation of OncoAct
Positive predictive value/specificity On coAct	2	Diagnostic positive predictive value/specificity is defined as: PPV = TP/(TP + FP): the diagnostic positive predictive value/specificity should be at least 95%	Clinical investigation (WIDE study)	The available clinical evidence demonstrates that the claim is met - the diagnostic positive predictive value/specificity was 99.7%	HMF-VAL-074 Clinical Validation of OncoAct
Likelihood ratio OncoAct	3	Likelihood ratio is defined as LR = Sensitivity / (1-Specificity): the likelihood ratio should be at least 300	Clinical investigation (WIDE study)	The available clinical evidence demonstrates that the claim is met - the diagnostic likelihood ratio was 326.25	HMF-VAL-074 Clinical Validation of OncoAct
Percentage extra patients (who initiated therapy) with treatment options - regular + early access - based on OncoAct	4	No performance claim	Clinical investigation (WIDE study)	The available clinical evidence demonstrates 10% extra patients	https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988
Percentage extra patients (who initiated therapy) with treatment options -clinical trials - based on OncoAct	5	No performance claim	Clinical investigation (WIDE study)	The available clinical evidence demonstrates 80% extra patients	https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988

To conclude, OncoAct has a high accuracy and added value compared to 'standard-of-care' in clinical practice with a sensitivity and specificity of over 95%. These results have also been published in a peer-reviewed journal: https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988.

7.5 Mathematical approach upon which the calculation of the analytical result is made

The software includes several different software items (tools) with different calculations for a variety of problems. All the different tools are also available open-source and can be found for review of the mathematical approach under https://github.com/hartwigmedical/pipeline5.

8 Residual risks of use

- The OncoAct report is interpreted by someone who is not experienced in reviewing and interpreting results of molecular diagnostic tests (such as OncoAct).
- The clinical sensitivity of OncoAct is high, but there is always a risk of false negatives and false positives. The registered medical expert using the OncoAct report should always take this into account when reviewing and interpreting the results.

9 Manufacturer

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Email: info@hartwigmedicalfoundation.nl / diagnosticssupport@hartwigmedicalfoundation.nl

10 Final notices

These instructions for use have been issued on 15/11/2023 14:01 (version 2.0).

Please report any serious incident that has occurred in relation to the OncoAct device to the manufacturer and the competent authority of the Member State in which the (user) registered medical expert is established. Please use the contact details above.

11 Appendix: OncoAct DNA analysis report manual

Example report with explanations of all sections.

OncoAct user manual

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An OncoAct report can be identified by the:
- *Hartwig Medical Foundation logo at the top left corner on all pages of the report*
- *Title 'Hartwig Medical OncoAct' at the top center of all pages of the report*
- *Signature of the Director Hartwig Medical Foundation on the last page of the report*

One page summary with the most important results of the whole genome sequencing (WGS) analysis.

Primary tumor location and type as provided by the requesting medical expert.

Concise textual summary of the most relevant findings and their potential treatment options.

Overview of the main genomic tumor characteristics:
- Molecular tumor cell purity as measured using the sequencing data
- Molecular tissue of origin prediction
- Mutational burden status (low or high)
- Microsatellite status (stable – MSS, or instable - MSI)
- Homologous recombination (HR) status (proficient or deficient)
- Tumor-associated viruses

More details are provided on **page 5 and 6.**

Overview of the main genomic tumor alterations:
- Genes with driver mutation(s)
- Genes with substantial copy gain (amplification)
- Genes that are completely lost in the tumor
- Genes that are completely disrupted in the tumor
- Gene fusions (in-frame and potential activating)

More details are provided on **page 3.**

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.Gly63fs) 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
REPORT DATE: 05-Oct-2023
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

Patient and sample details as provided by the requesting medical expert.

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.

The status of the patient's genes involved in drug metabolism (pharmacogenetics) and status of the HLA alleles are summarized here. More details are provided on **page 4.**

This report is focused on the identification of all oncogenic driver alterations and the potential targets for therapy. Only tumor-associated genomic alterations in cancer associated genes are reported. The complete list of genes analyzed in this report can be found in https://oncoact.nl/wp-content/uploads/2023/10/OncoAct_WGS_specificatieformulier_v5.33.pdf.

Germline results may be requested by a clinical geneticist, when informed consent was given by the patient.

The "High level evidence" table shows the **tumor type specific and non-specific matches** of the identified biomarkers ("match" and "genomic event" columns) with available treatment ("drug type" column) possibilities. The match between the found genomic events with the treatment and predicted response are based on information collected in external knowledgebases.

Overview of the **clinical studies** in the Netherlands that have one (or more) of the observed genomic event(s) as study inclusion criteria, also including phase 1 clinical studies. Clinical study matching is performed using the iClusion database and is, as far as possible, tumor type specific.

Hartwig Medical OncoAct

HOSPITAL PATIENT ID: reportingId
REPORT DATE: 05-Oct-2023

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	D	▲	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 potential 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'.

Details of the evidence items:
- **LEVEL:** the level of evidence (LoE) of the biomarker-treatment association. Here, only the highest LoE items of the matched genomic events and treatments are shown, including validation associations (A, e.g. FDA/EMA approved, national guidelines, phase 3/4 clinical studies) and items with strong clinical evidence (B, e.g. phase 1/2 studies).
- **RESPONSE:** the predicted response to the treatment ("drug type" column) based on the matched genomic event. The tumor is predicted to be sensitive (blue triangle) or (innate or secondary) resistant (red triangle) to the drug.

More details are provided at the bottom of this page.

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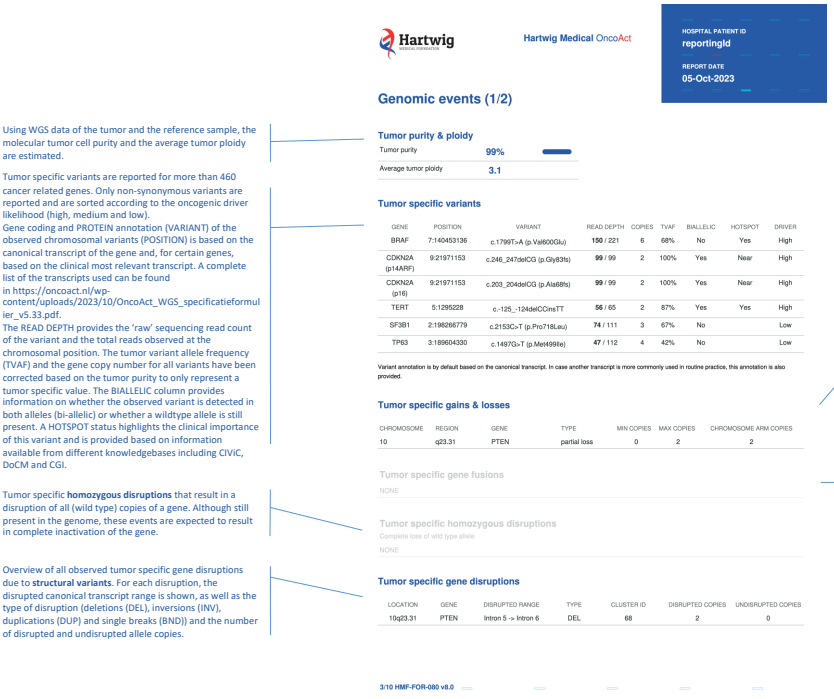


Using WGS data of the tumor and the reference sample, the molecular tumor cell purity and the average tumor ploidy are estimated.

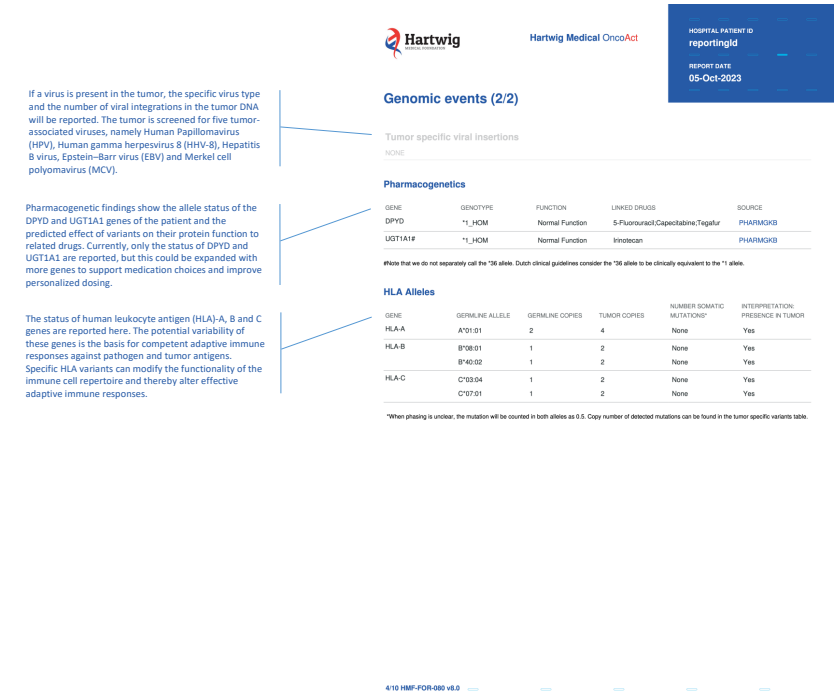
Tumor specific variants are reported for more than 460 cancer related genes. Only non-synonymous variants are reported and are sorted according to the oncogenic driver likelihood (high, medium and low).
Gene coding and PROTEIN annotation (VARIANT) of the observed chromosomal variants (POSITION) is based on the canonical transcript of the gene and, for certain genes, based on the clinical most relevant transcript. A complete list of the transcripts used can be found in https://oncoact.nl/wp-content/uploads/2023/10/OncoAct_WGS_specificatieformulier_v5.33.pdf.
The READ DEPTH provides the 'raw' sequencing read count of the variant and the total reads observed at the chromosomal position. The tumor variant allele frequency (TVAF) and the gene copy number for all variants have been corrected based on the tumor purity to only represent a tumor specific value. The BIALLELIC column provides information on whether the observed variant is detected in both alleles (bi-allelic) or whether a wildtype allele is still present. A HOTSPOT status highlights the clinical importance of this variant and is provided based on information available from different knowledgebases including CIViC, DoCM and CGI.

Tumor specific copy number alterations are listed here, including gene copy-gains (amplification) and complete losses.
Gene **copy gains** are reported if the complete gene (full gain) or only part of the gene (partial gain) shows an increase in copy number, and the level of amplification is sufficiently high enough (defined as higher than 3x the tumor ploidy).
For **gene copy losses**, only tumor-specific complete losses are reported (0 copies). A distinction is made between a partial loss (only part of the gene has 0 copies) and a full loss (the complete gene has 0 copies) of the gene.

The detected gene fusions that are predicted to result in a viable fusion product are listed here. Information about the fusion partners include:
- The genetic breakpoints of the genes involved (exon level) and their position (5' or 3') in the fusion
- The phasing of the genes ('Inframe' or 'exon spiking', which is required for an inframe fusion product)
- The calculated copies of the fusion in the tumor
- The driver likelihood of the gene fusion, with a high-driver status for all known fusions.

Tumor specific **homozygous disruptions** that result in a disruption of all (wild type) copies of a gene. Although still present in the genome, these events are expected to result in complete inactivation of the gene.

Overview of all observed tumor specific gene disruptions due to **structural variants**. For each disruption, the disrupted canonical transcript range is shown, as well as the type of disruption (deletions (DEL), inversions (INV), duplications (DUP) and single breaks (BND)) and the number of disrupted and undisrupted allele copies.



If a virus is present in the tumor, the specific virus type and the number of viral integrations in the tumor DNA will be reported. The tumor is screened for five tumor-associated viruses, namely Human Papillomavirus (HPV), Human gamma herpesvirus 8 (HHV-8), Hepatitis B virus, Epstein–Barr virus (EBV) and Merkel cell polyomavirus (MCV).

Pharmacogenetic findings show the allele status of the DPYD and UGT1A1 genes of the patient and the predicted effect of variants on their protein function to related drugs. Currently, only the status of DPYD and UGT1A1 are reported, but this could be expanded with more genes to support medication choices and improve personalized dosing.

The status of human leukocyte antigen (HLA)-A, B and C genes are reported here. The potential variability of these genes is the basis for competent adaptive immune responses against pathogen and tumor antigens. Specific HLA variants can modify the functionality of the immune cell repertoire and thereby alter effective adaptive immune responses.

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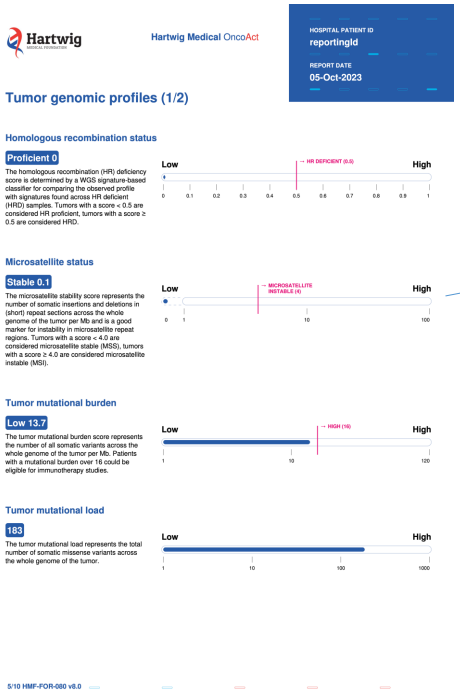


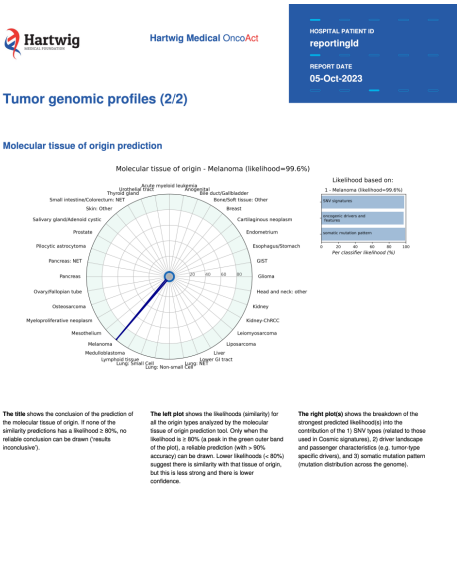
Using WGS data, the HR status of the tumor can be accurately predicted by the CHORD classifier tool based on specific single nucleotide variants (SNV), insertions and deletions (indels), and structural variant (SV) types. A score higher than 0.5 indicates HR deficiency caused by complete (bi-allelic) inactivation of BRCA1/2 or possibly other genes in the HR pathway (e.g. RAD51C, PALB2). More details are described in Nguyen *et al.* Nature Communications, 2020.

Tumors with a microsatellite stability score lower than 4 are considered microsatellite stable (MSS) and tumors with a score larger than 4 are considered microsatellite instable (MSI). The WGS-based MSI readout has been validated against the routine MSI-PCR assay and immunohistochemistry status of proteins involved in the mismatch repair (MMR) pathway.

The tumor mutational burden is reported as:

- The mutational load (ML), which is defined by the total number of somatic missense variants across the whole genome of the tumor.
- Tumor mutational burden (TMB) score, which is calculated by the number of all somatic variants per genome Mb.

Although closely related, differences between both metrics exist. For TMB, tumors with a score >16 are considered to have a high mutational burden, which has clinical significance for possible treatment with immunotherapy.



The molecular tissue of origin prediction tool shows the predicted tissue of tumor origin based on three different read-outs of the WGS data (right plot). A visual representation of the prediction distributed over the different origins is shown in the left plot. The likelihood (similarity) for a specific origin prediction must be over 80%, otherwise no reliable conclusion can be drawn ('results inconclusive').

Details on how to interpret the molecular tissue of origin prediction plots are described at the bottom of this page.

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CIRCOS plot

A CIRCOS plot visualizes the position, size and orientation of all tumor-specific genomic alterations. These alterations include single nucleotide variants, insertions/deletions, copy numbers changes, translocations and other structural variants.

Details on how to interpret a CIRCOS plot are described on the bottom of this page.

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.

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Report explanation (1/2)

At the end of each OncoAct report a comprehensive explanation is provided for reference (**page 8 and 9**).

Details on the report in general

This report is created using NovaSeq 6000 (Illumina) WGS analysis, which data is processed using Hartwig MedicalOncoActB 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, L833).

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'.

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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).

Each OncoAct report ends with more **Sample details** (left side), **Disclaimers** (right side) and the signature of the Director Hartwig Medical Foundation (below).

The **Sample details** include additional information about the sample processing and report generation.
The **Disclaimers** include the version of the report, the UDI-DI of the OncoAct product and general aspects of the performance of OncoAct (sensitivity).

For feedback and complaints, please contact: qualitysystem@hartwigmedicalfoundation.nl.

For questions regarding the contents of a report, please contact: diagnosticssupport@hartwigmedicalfoundation.nl.

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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 **05-Oct-2023**.

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

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-SNP22B v5.0-PREP041 v4.0**

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

Comments: this is a test report and based on COLO829.

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)87202994869258(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 —

Edwin Cuppen,
Director Hartwig Medical Foundation

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