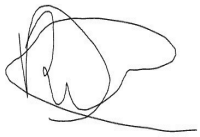
HMF-IVDD-275
OncoAct user manual

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 02:01 PM
2.1	Update related to first review round DEKRA: updated device label, aligned performance metrics, title of chapter 8 and 9; added reference to EUDAMED and description for Machine-readable report formats	All	SvdB, DP	20/09/2024 11:02
2.2	Update related to second review round DEKRA: updated device label, updated chapter 7.2 with Specific competences, added a warning about a lower sequencing coverage. Replaced reference to VAL-051 with VAL-055 and VAL-074 with Samsom et al. 2022.	2, 7.2, 8	SvdB	31/03/2025 06:02 PM
2.3	Update related to third review round DEKRA: Added information to IVD user description and information about used genes chapter 5. Updated sensitivity of TMB. Added extra warnings in chapter 8, updated appendix 11 to current state.	4.1; 5; 7.3; 7.4; 8; 11	SvdB	23/06/2025 15:53
2.4	Update related to fourth review round DEKRA: updated Hartwig logo on the label, analytical performance table for TMB and LoD; Added extra warnings in chapter 8. Corrected diagnosticssupport@hartwig.... In chapter 7.2.	2; 7.2 7.3; 8	SvdB	05/01/2026 17:29
2.5	Replaced label image with a new label image, see INCID26-321	2	XiZo	30/06/2026 15:00

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

Hartwig Medical OncoAct

SN V5.33-1.0

IVD In Vitro Diagnostic medical device

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

UDI (01)08720299486058(8012)v5.33-1.0

CE 0344

Instructions for use are supplied in electronic form instead of paper form.
URL: https://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 Hartwig 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).
Hartwig Medical Foundation is accredited for the ISO/IEC 27001:2022 information security management system (reference number ISMS-K-0216521/1). The analytical use of the device, which is related to running the software, is always done by a trained Hartwig employee following the ISO27001 norm. The software (including the data processed) is always run inside the cloud (Google Cloud Platform; in a Hartwig specific project internally managed under the ISO27001 norm.

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 specific genes and the type of genomic events that are reported for a gene can be found in HMF-IVDD-399 OncoAct tumor WGS specification sheet (https://www.oncoact.nl/specsheetOncoActWGS).

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.

Note: germline variants are also reported (in the same tables as the somatic variants), but are not actively indicated being germline.

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 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 4 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 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). Note: the minimal tumor-cell percentage as measured by OncoAct should be 8%.	*HMF-FOR-209*
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.
Specific competences for registered medical experts:

- Knowledge of the standard treatment options for specific genomic events
- Knowledge on how to contact medical oncologists in large (academic) cancer centers to discuss study treatment options

  Note: both are basic knowledge for certified medical oncologists in The Netherlands

Specific competences for Clinical molecular biologist in pathology:

- Expert knowledge on DNA sequencing technology including next generation sequencing
- Understanding of bioinformatic tools for analysis of DNA sequencing results.
- Expert knowledge on interpretation and classification of DNA results in the field of oncology/pathology

Note:
It is advised to discuss OncoAct results within a Molecular tumor board. Molecular tumor boards have the above knowledge available (these need to include at least the treating medical specialist; a pathologist; and a Clinical molecular biologist in pathology (see the Dutch quality guidelines for molecular diagnostics for oncology: https://www.zorginzicht.nl/binaries/content/assets/zorginzicht/kwaliteitsinstrumenten/kwaliteitsstandaard-organisatie-van-moleculaire-pathologie-diagnostiek-in-de-oncologie.pdf).
Important: if there is any doubt or unclarity about the OncoAct results, please always contact your hospitals specialist or Molecular Tumor Board; or contact the Clinical Molecular Biologists at Hartwig Medical Foundation trough diagnosticssupport@hartwigmedicalfoundation.nl.

7.2.1 Machine-readable report formats

Next to the OncoAct report in PDF format, two machine-readable formats (XML and JSON) are provided. The formats contain the same information as the OncoAct report. These formats enable automatic processing of the data using the hospital's own systems.

The XML format is specifically designed to be read in PALGA (https://www.palga.nl/), using the protocol module ‘Moleculaire Bepalingen’, see for more information:

https://www.palga.nl/moleculaire-diagnostiek. The responsibility for reading in this information is with the hospital. Please note: in the XML format not all the genomic events could be incorporated, and certain genomic events (such as gene disruptions) are not reported.

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-055 Hartwig Medical OncoAct - technical and validation information; https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988
OncoAct analytical performance for somatic genomic events	2	For input data (tumor and reference) fulfilling all criteria as described under 6, the performance 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)	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 specificity for somatic SNVs, MNVs and indels	3	Sensitivity and 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 confirmation study showed: concordance = 98% the original validation showed: sensitivity = 99%, specificity = 95%; in recent comparisons with standard of care tests a	HMF-VAL-045 Validation of WGS based variants by smMIP, HMF-VAL-061 Validation of SNV-MNV-INDEL mutations using WGS, HMF-VAL-065 Validation of SAGE 2.2, https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988

				sensitivity of 99% is found	
Analytical sensitivity and specificity for 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 (although the tests look at different mechanisms/outputs so are not fully comparable)	The available analytical evidence demonstrates that this claim is met – the original validation showed: sensitivity = 93%, specificity = 100% (note: numbers in the validation are small but the results are supported by the data in the validation of the structural variant analysis); in recent comparisons with standard of care tests a sensitivity of 98% is found	HMF-VAL-066 Validation of structural variant analysis, https://genomebiology.biomedcentral.com/articles/10.1186/s13059-021-02423-x. HMF-VAL-060 Validation of fusion gene readout using WGS, https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988
Analytical sensitivity and specificity for somatic (homozygous) disruptions	5	Sensitivity and specificity for the detection of (homozygous) disruptions should be over 95% compared to current standard of care tests	Comparison to current 'standard-of-care' in clinical practice (although the tests look at different mechanisms/outputs so are not fully comparable)	The available analytical evidence demonstrates that this claim is met – the validation showed: sensitivity = 100%, specificity = 100% (note: numbers in the validation are small but the results are supported by the data in the validation of the structural variant analysis)	HMF-VAL-066 Validation of structural variant analysis, https://genomebiology.biomedcentral.com/articles/10.1186/s13059-021-02423-x. HMF-VAL-068 Validation of homozygous disruption readout
Analytical sensitivity and specificity for somatic gene copy number changes	7	Sensitivity and specificity 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 (although the tests look at different mechanisms/outputs so are not fully comparable)	The available analytical evidence demonstrates that this claim is met – the original validation showed: sensitivity = 100%, specificity = 83% (note: numbers in the validation are small); in recent comparisons with standard of care tests a sensitivity of 98% is found	HMF-VAL-049 Validation of WGS based copy number_ERBB2, https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988

OncoAct analytical performance for germline genomic events	8	For input data (tumor and reference) fulfilling all criteria as described under 6, the performance 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 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 current standard of care tests	Comparison to current 'standard-of-care' in clinical practice	The available analytical evidence demonstrates that this claim is met - sensitivity = 100%, specificity = 99%	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 current standard of care tests	Comparison to current 'standard-of-care' in clinical practice (in the selection for the validation there was a bias towards a selection of more complex structural variants)	The available analytical evidence demonstrates that this claim is met - sensitivity = 90%, however, there was a bias towards the selection of very complex variants in the validation making it justified to assume the general sensitivity is over 95%	HMF-VAL-072 Validation of germline analyses
Analytical sensitivity and specificity for viral insertions and detected non-integrated viruses in the tumor	11	Sensitivity and specificity for the detection of viral insertions should be both over 95% compared to current standard of care tests	Comparison to current 'standard-of-care' in clinical practice (although the tests look at different mechanisms/outputs so are not fully comparable)	The available analytical evidence demonstrates that the claim is met - the original validation showed: sensitivity = 100%, specificity = 100%; in recent comparisons with standard of care tests a sensitivity of 100% is found	HMF-VAL-064 Validation of virus detection using WGS, HMF-VER-084 Verification virus interpreter v1.1, https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988
Analytical sensitivity and specificity for tumor	12	Sensitivity and specificity for the detection of MSI should be over 95%	Comparison to current 'standard-of-care' in clinical practice	The available analytical evidence demonstrates that the claim is met -	HMF-VAL-043 Validation of Microsatellite readout using WGS,

microsatellite score		compared to current standard of care tests		sensitivity = 100%, specificity = 97%; in recent comparisons with standard of care tests a sensitivity of 100% is found (although number that were compared were small)	https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988
Analytical concordance of tumor homologous recombination deficiency score	13	Concordance of the homologous recombination deficiency results 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 - concordance = 99%; in recent comparisons with standard of care tests a sensitivity of 100% is found (although number that were compared were small); in a scientific publication the validity for detection of homologous recombination deficiency within OncoAct is further supported	HMF-VAL-062 Validation of HR-deficiency classifier using WGS, HMF-VER-053 Verification of CHORD v2 (HR-deficiency classifier), https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988, https://www.nature.com/articles/s41467-020-19406-4
Analytical sensitivity and specificity for tumor mutational burden/load	14	Sensitivity and specificity for the detection of tumor mutational burden/load 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 for tumor mutational load - sensitivity = 100%, specificity = 100%. For tumor mutational burden the claim is not met - sensitivity = 80%, specificity = 75%. This lower performance can be explained by the heterogeneity of tumor mutational burden calculations in different tests (note: numbers in the validations are small but the results are supported by the data in the validation of the somatic SNVs, MNVs and indels)	HMF-VAL-061 Validation of SNV-MNV-INDEL mutations using WGS, https://www.nature.com/articles/s41416-020-0762-5

Analytical concordance of pharmacogenetic calling (DPYD and UGT1A)	15	Concordance of DPYD and UGT1A pharmacogenetic calling should be over 99% compared to current standard of care tests	Comparison to current 'standard-of-care' in clinical practice and population statistics from literature	The available analytical evidence demonstrates that the claim is met - concordance = 100% (note: numbers in the validation are small but the results are supported by the data in the validation of the germline SNVs, MNVs and indels; and a comparison with population statistics from literature)	HMF-VAL-069 Validation of DPYD genotype readout by WGS, HMF-VER-075 Verification of pharmacogenomics, HMF-VAL-072 Validation of germline analyses
Analytical concordance of HLA status calling	16	Concordance of HLA status calling should be over 99% compared to current clinically validated tests	Comparison to independent clinically validated test	The available analytical evidence demonstrates that the claim is met - concordance = 100%	HMF-VAL-076 Validation of HLA typing by WGS
Analytical concordance of molecular tissue of origin prediction	17	Concordance of molecular tissue of origin predictions should be 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 - 74% of the samples of the test set had conclusive results, among those concordance = 93%, in a scientific publication the validity of the molecular tissue of origin prediction within OncoAct is further supported	HMF-VAL-071 Validation of CUPPA algorithm, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9808446/
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	The input data provided should fulfill the criteria as described under 6	All verifications and validations	The data used in the verifications and validations fulfills the criteria as	HMF-PRO-007 Validation and verification, HMF-SOP-

		(input data limitations). These quality parameters ensure that a minimum amount of high-quality sequencing reads is achieved, thereby functioning as the practical determinants of the Limit of Detection		described under 6 (input data limitations)	025 Lab Test reporting and transfer of data

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 analysing whole genome sequencing data has a >95% sensitivity and precision compared to analysing data from 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 tool. The registered medical expert uses it as support in decision making, 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 (HMF-IVDD-268 Attachment 2 Protocol clinical performance study - WIDE; 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)***
Clinical sensitivity OncoAct	1	Clinical sensitivity is defined as the probability of finding a clinically relevant genomic event in a tumor conditioned that there truly is a clinically relevant genomic event present in the tumor: the clinical sensitivity should be at least 95%	Clinical investigation (WIDE study), by comparing the OncoAct report results to current 'Standard Of Care" results in clinical practice	The available clinical evidence demonstrates that the claim is met clinical sensitivity (on genomic event level) was 99% (and 3860 additional genomic events were found using OncoAct), the clinical sensitivity (on patient level) was 98%	https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988
Clinical specificity OncoAct	2	Clinical specificity is defined as the probability of not finding a clinically relevant genomic event in a tumor, conditioned that	Clinical investigation (WIDE study), by comparing	The available clinical evidence demonstrates that the claim is met - the clinical specificity	https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988

		there is truly no clinically relevant genomic event present in the tumor: the clinical specificity should be at least 95%	the OncoAct report results to current 'Standard Of Care" results in clinical practice	(on patient level) was 100%	
Positive predictive value	3	Positive predictive value is defined as the probability that a tumor truly harbors a clinically relevant genomic event given that the test has detected a clinically relevant genomic event in the tumor: the positive predictive value should be at least 95%	Clinical investigation (WIDE study), by comparing the OncoAct report results to current 'Standard Of Care" results in clinical practice	The available clinical evidence demonstrates that the claim is met - the positive predictive value (on patient level) was 100%	https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988
Negative predictive value	4	Negative predictive value is defined as the probability that a tumor does not harbor a clinically relevant genomic event given that the test has not detected a clinically relevant genomic event in the tumor: the negative predictive value should be at least 95%	Clinical investigation (WIDE study), by comparing the OncoAct report results to current 'Standard Of Care" results in clinical practice	The available clinical evidence demonstrates that the claim is met - the negative predictive value (on patient level) was 98%	https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988
Likelihood ratios	5	Positive likelihood ratio is defined as the ratio of the probability of a positive test result for a tumor with a clinically relevant genomic event and the probability of a positive test result in a tumor without such an event. Negative likelihood ratio is defined as the ratio of the probability of a negative test result for a tumor with a clinically relevant genomic event to the probability of a negative test result for a tumor without such an event. No performance claims	Clinical investigation (WIDE study), by comparing the OncoAct report results to current 'Standard Of Care" results in clinical practice	The available clinical evidence demonstrates (on patient level) a positive likelihood ratio of ∞ and a negative likelihood ratio of 0.02	https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988
Percentage extra patients (who initiated therapy) with treatment	6	No performance claim	Clinical investigation (WIDE study), by	The available clinical evidence demonstrates 10% extra patients	https://pathsocjournals.onlinelibrary.wiley.co

options - regular + early access - based on OncoAct			comparing the OncoAct report results to current 'Standard Of Care" results in clinical practice		m/doi/10.1002/path.5988
Percentage extra patients (who initiated therapy) with treatment options -clinical trials - based on OncoAct	7	No performance claim	Clinical investigation (WIDE study), by comparing the OncoAct report results to current 'Standard Of Care" results in clinical practice	The available clinical evidence demonstrates 80% extra patients	https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988

To conclude, OncoAct has a high performance and added value compared to 'standard-of-care' in clinical practice with a sensitivity, specificity, positive predictive value and negative predictive value 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 (warnings and safety precautions)

- The OncoAct report is interpreted by someone who is not experienced in reviewing and interpreting results of molecular diagnostic tests (such as OncoAct).
- Medical experts should always use OncoAct in addition to other standard diagnostic procedures and data considering the health condition and clinical background of the patient.
- The clinical sensitivity of OncoAct is high (>95%), 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.

For OncoAct low purity analysis reports the risk of false negatives is higher (sensitivity >85% instead of >95%), and the report should be interpreted with extra caution.

Important:
SPATA31A7, LINC01001, GTF2I, OR4F21, PMS2, RXRA, SLCO1B1 and BTK contain exon(s) for which the detection of genomic variants is less sensitive due to a lower sequencing coverage with sufficient mapping quality for these regions. Please interpret results for these genes with caution. When there is a suspicion that there might be relevant genomic events, please consider manual inspection or orthogonal validation.

Important:
There is an increased risk for a wrong molecular tissue of origin prediction (sensitivity >90% instead of standard >95%). Please interpret this prediction with caution, and only as support next to standard (histopathological) evaluation considering the full clinical context.

Important:
The homologous recombination deficiency score should always be interpreted in combination with the presence (or absence) of inactivation of homologous recombination deficiency-related genes (BRCA1, BRCA2, RAD50, RAD51B, RAD51C, RAD51D, RAD54L, PALB2, ATM, BARD1, BRIP1, CHEK1, CHEK2, CDK12, FANCL, PPP2R2A).

Important:
The cut-off (10 mut/Mb) used for determining the classification of the tumor mutational burden status (high/low) is based on current (scientific) insights and community consensus (https://pmc.ncbi.nlm.nih.gov/articles/PMC10248461/), but may change with new evidence and insights. The tumor mutational load is also provided but without any cut-off since there are currently no registered treatments based on. Interpretation of the tumor mutational load should therefore be done with caution.

Important:
The evidence for the detection of Epstein-Barr Virus (EBV) by OncoAct is still limited. When a positive EBV result is reported, please consider orthogonal validation of this result.

Important:
Extra annotations to genomic events (ploidy, tumor-adjusted variant allele frequency value) are provided but are not yet used in standard of care nor clinical guidelines. Interpretation of this information should be done with caution.

- The summary of safety and performance (HMF-IVDD-275 OncoAct summary of safety and performance) can be looked up in EUDAMED or can be requested at Hartwig Medical Foundation, see contact information below.

9 Manufacturer contact information

Hartwig Medical Foundation
Science Park 408
1098 XH Amsterdam
Tel: +31 (0) 20 – 235 2640
Website: https://www.hartwigmedicalfoundation.nl / https://www.oncoact.nl
Email: info@hartwigmedicalfoundation.nl / diagnosticssupport@hartwigmedicalfoundation.nl

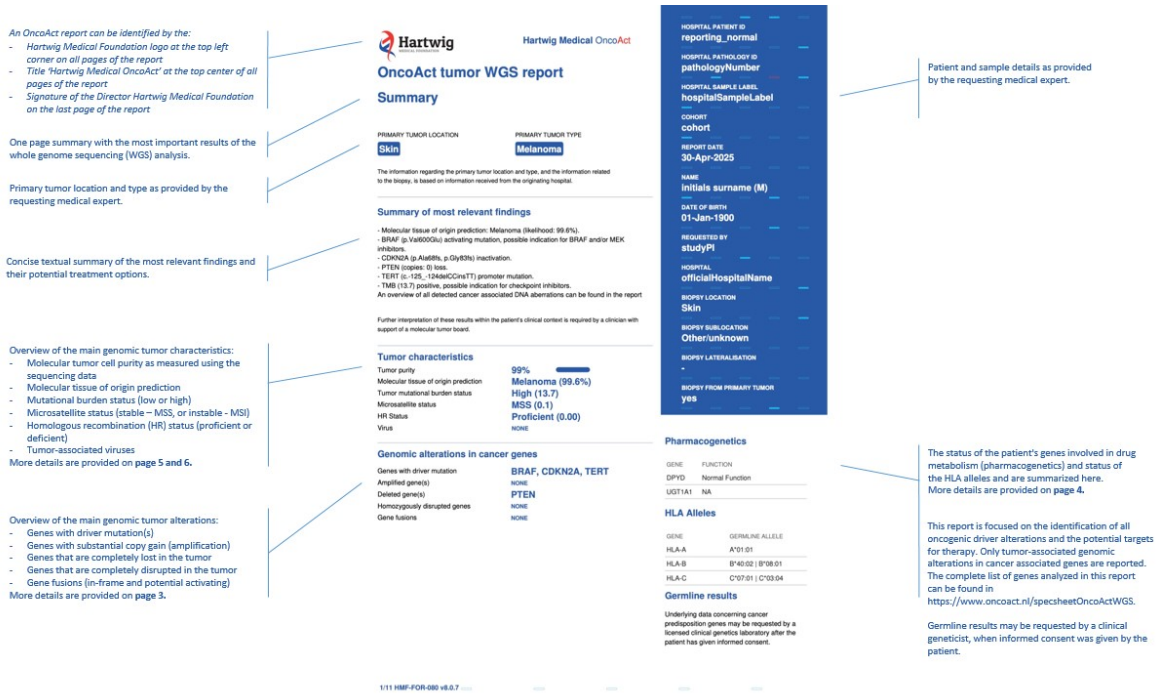
10 Final notices

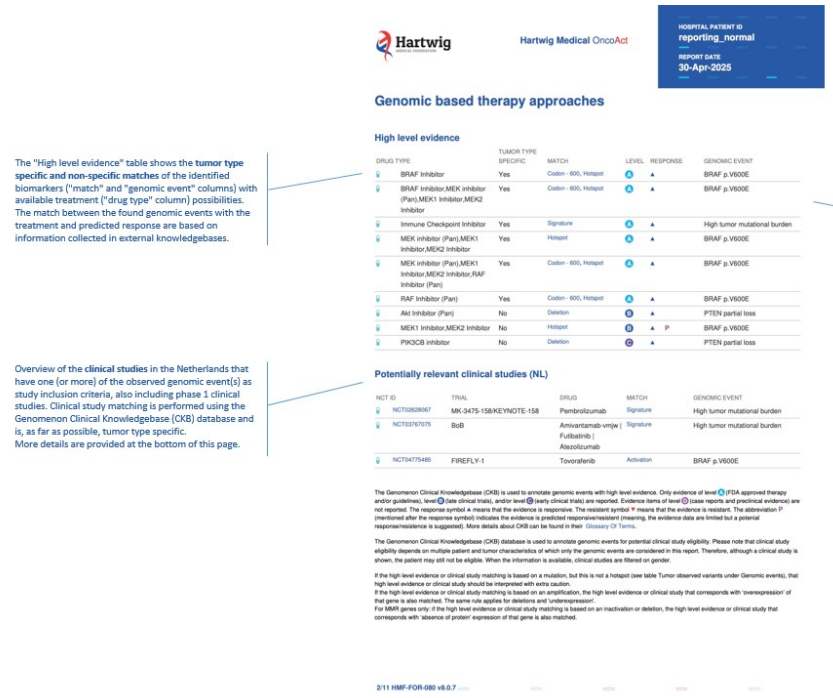
These instructions for use have been issued on 30/06/2026 15:00 (version 2.5).

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 tumor WGS report manual

Example report with explanations of all sections.





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Genomic based therapy approaches

High level evidence

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

Potentially relevant clinical studies (NL)

NCT ID	TRIAL	DRUG	MATCH	GENOMIC EVENT
NCT02629067	MK-3475-158/KEYNOTE-158	Pembrolizumab	Signature	High tumor mutational burden
NCT03767075	BoB	Amivantamab-vmjw | Futibatinib | Atezolizumab	Signature	High tumor mutational burden
NCT04775485	FIREFLY-1	Tovorafenib	Activation	BRAF p.V600E

The Genomenon Clinical Knowledgebase (CKB) is used to annotate genomic events with high level 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 resistant symbol ▼ means that the evidence is resistant. The abbreviation P (mentioned after the response symbol) indicates the evidence is predicted responsive/resistant (meaning, the evidence data are limited but a potential response/resistance is suggested). More details about CKB can be found in their Glossary Of Terms.

The Genomenon Clinical Knowledgebase (CKB) 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. Therefore, although a clinical study is shown, the patient may still not be eligible. When the information is available, clinical studies are filtered on gender.

If the high level evidence or clinical study matching is based on a mutation, but this is not a hotspot (see table Tumor observed variants under Genomic events), that high level evidence or clinical study should be interpreted with extra caution.
If the high level evidence or clinical study matching is based on an amplification, the high level evidence or clinical study that corresponds with 'overexpression' of that gene is also matched. The same rule applies for deletions and 'underexpression'.
For MMR genes only: if the high level evidence or clinical study matching is based on an inactivation or deletion, the high level evidence or clinical study that corresponds with 'absence of protein' expression of that gene is also matched.

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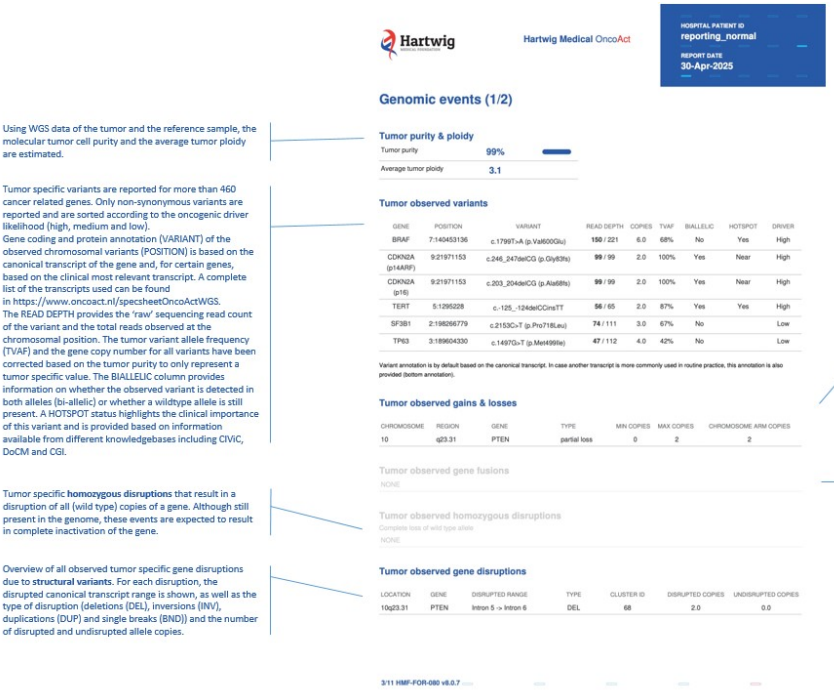
The "High level evidence" table shows the **tumor type specific and non-specific matches** of the identified biomarkers ("match" and "genomic event" columns) with available treatment ("drug type" column) possibilities.
The match between the found genomic events with the treatment and predicted response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

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.

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 Genomenon Clinical Knowledgebase (CKB) database and is, as far as possible, tumor type specific.
More details are provided at the bottom of this page.



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Genomic events (1/2)

Tumor purity & ploidy

Tumor purity	99%
Average tumor ploidy	3.1

Tumor observed variants

GENE	POSITION	VARIANT	READ DEPTH	COPIES	TVAF	BIALLELIC	HOTSPOT	DRIVER
BRAF	7:140453136	c.1799T>A (p.Val600Glu)	150 / 221	6.0	68%	No	Yes	High
CDKN2A (p14ARF)	9:21971153	c.246_247delCG (p.Gly83fs)	99 / 99	2.0	100%	Yes	Near	High
CDKN2A (p16)	9:21971153	c.203_204delCG (p.Ala68fs)	99 / 99	2.0	100%	Yes	Near	High
TERT	5:1295228	c.-125_-124delCCinsTT	56 / 65	2.0	87%	Yes	Yes	High
SF3B1	2:198266779	c.2153C>T (p.Pro718Leu)	74 / 111	3.0	67%	No		Low
TP63	3:189604330	c.1497G>T (p.Met499Ile)	47 / 112	4.0	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 (bottom annotation).

Tumor observed gains & losses

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

Tumor observed gene fusions

NONE

Tumor observed homozygous disruptions

Complete loss of wild type allele

NONE

Tumor observed gene disruptions

LOCATION	GENE	DISRUPTED RANGE	TYPE	CLUSTER ID	DISRUPTED COPIES	UNDISRUPTED COPIES
10q23.31	PTEN	Intron 5 -> Intron 6	DEL	68	2.0	0.0

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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.

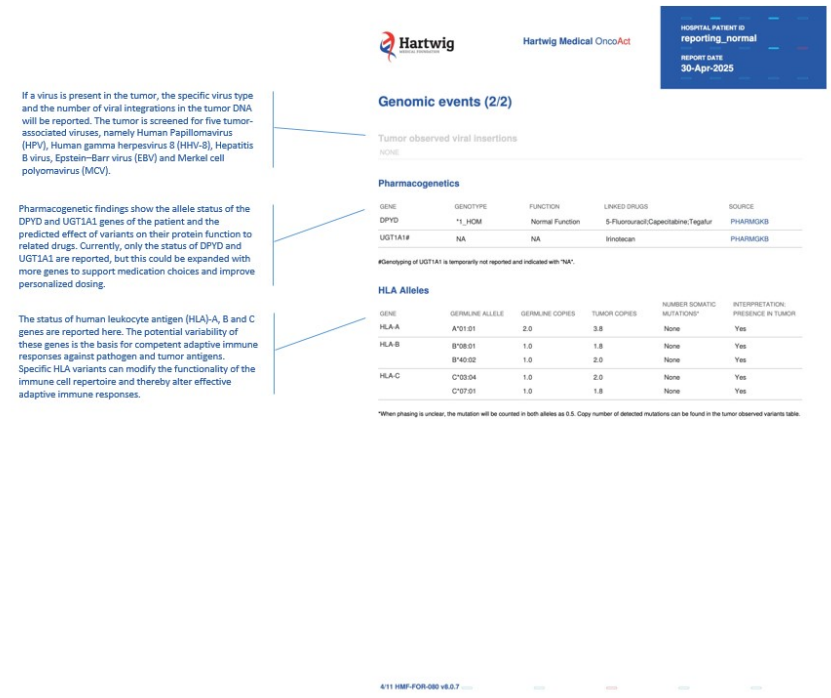
Tumor specific variants are reported for more than 460 cancer related genes. Only non-synonymous variants are reported and are sorted according to the oncogenic driver likelihood (high, medium and low).
Gene coding and protein annotation (VARIANT) of the observed chromosomal variants (POSITION) is based 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://www.oncoact.nl/specsheetOncoActWGS.
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 **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.

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.



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Genomic events (2/2)

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).

Tumor observed viral insertions

NONE

Pharmacogenetics

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.

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

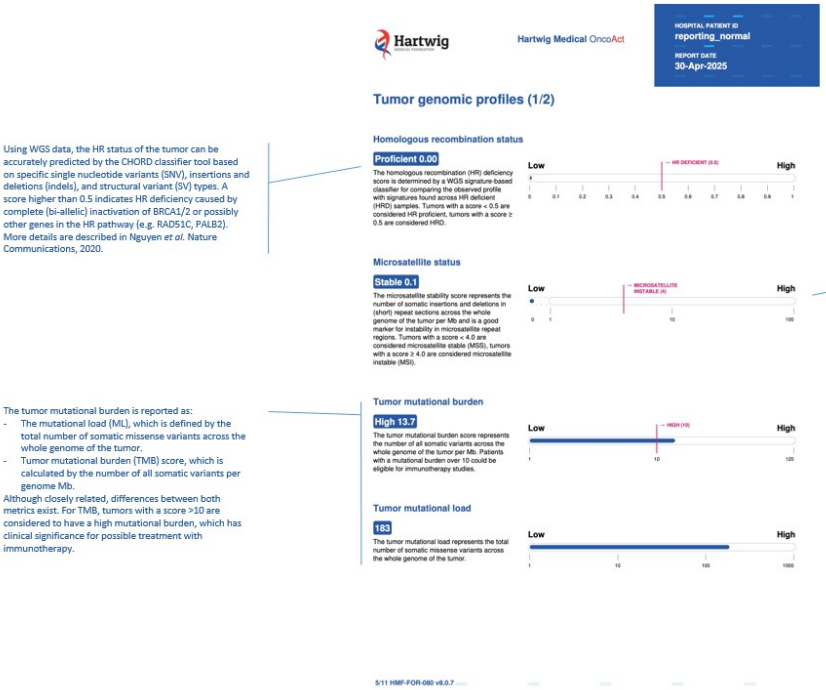
#Genotyping of UGT1A1 is temporarily not reported and indicated with "NA".

HLA Alleles

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.

GENE	GERMLINE ALLELE	GERMLINE COPIES	TUMOR COPIES	NUMBER SOMATIC MUTATIONS*	INTERPRETATION: PRESENCE IN TUMOR
HLA-A	A*01:01	2.0	3.8	None	Yes
HLA-B	B*08:01	1.0	1.8	None	Yes
	B*40:02	1.0	2.0	None	Yes
HLA-C	C*03:04	1.0	2.0	None	Yes
	C*07:01	1.0	1.8	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 observed variants table.



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Tumor genomic profiles (1/2)

Homologous recombination status

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.

Proficient 0.00

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.

Low — High; HR DEFICIENT (0.5); 0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1

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).

Low — High; MICROSATELLITE INSTABLE (4); 0 1 10 100

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.

Tumor mutational burden

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 >10 are considered to have a high mutational burden, which has clinical significance for possible treatment with immunotherapy.

High 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 10 could be eligible for immunotherapy studies.

Low — High; HIGH (10); 1 10 125

Tumor mutational load

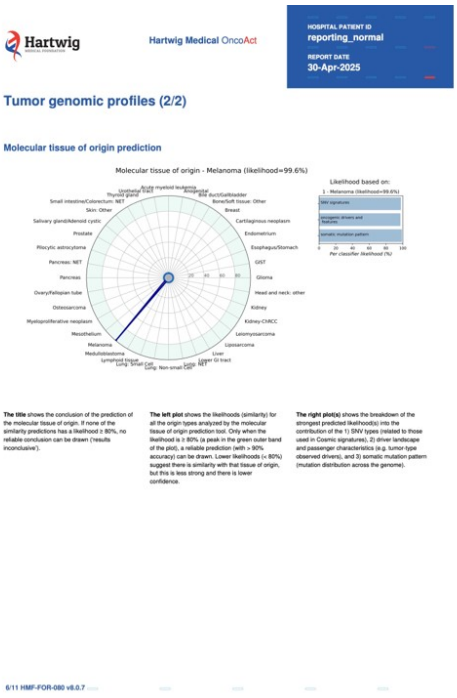
183

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

Low — High; 1 10 100 1000

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').

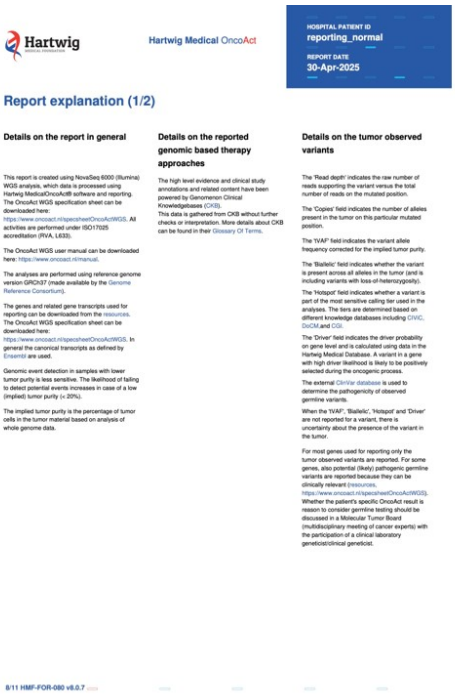
Details on how to interpret the molecular tissue of origin prediction plots are described at the bottom of this page.



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.

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



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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. The OncoAct WGS specification sheet can be downloaded here: https://www.oncoact.nl/specsheetOncoActWGS. 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 high level evidence and clinical study annotations and related content have been powered by Genomenon Clinical Knowledgebases (CKB).
This data is gathered from CKB without further checks or interpretation. More details about CKB can be found in their Glossary Of Terms.

Details on the tumor observed 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 'VAF' 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 COSMIC.

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.

When the 'VAF', 'Biallelic', 'Hotspot' and 'Driver' are not reported for a variant, there is uncertainty about the presence of the variant in the tumor.

For most genes used for reporting only the tumor observed variants are reported. For some genes, also potential (likely) pathogenic germline variants are reported because they can be clinically relevant (resources, https://www.oncoact.nl/specsheetOncoActWGS). Whether the patient's specific OncoAct result is reason to consider germline testing should be discussed in a Molecular Tumor Board (multidisciplinary meeting of cancer experts) with the participation of a clinical laboratory geneticist/clinical geneticist.

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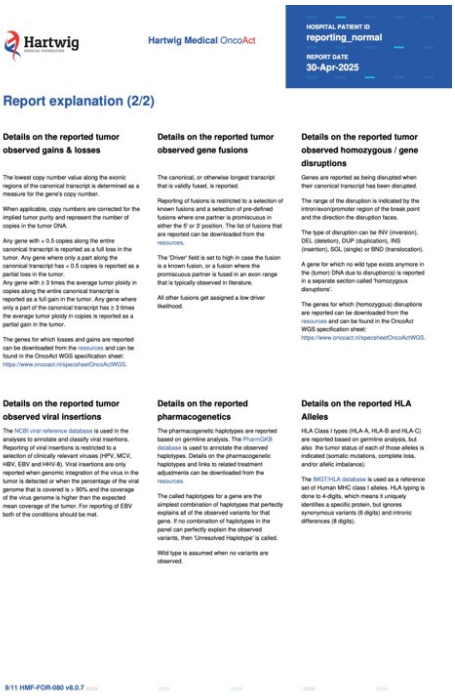

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Report explanation (2/2)

Details on the reported tumor observed 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.

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

The genes for which losses and gains are reported can be downloaded from the resources and can be found in the OncoAct WGS specification sheet: https://www.oncoact.nl/specsheetOncoActWGS.

Details on the reported tumor observed 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 list of fusions that are reported 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 observed 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'.

The genes for which (homozygous) disruptions are reported can be downloaded from the resources and can be found in the OncoAct WGS specification sheet: https://www.oncoact.nl/specsheetOncoActWGS.

Details on the reported tumor observed 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).

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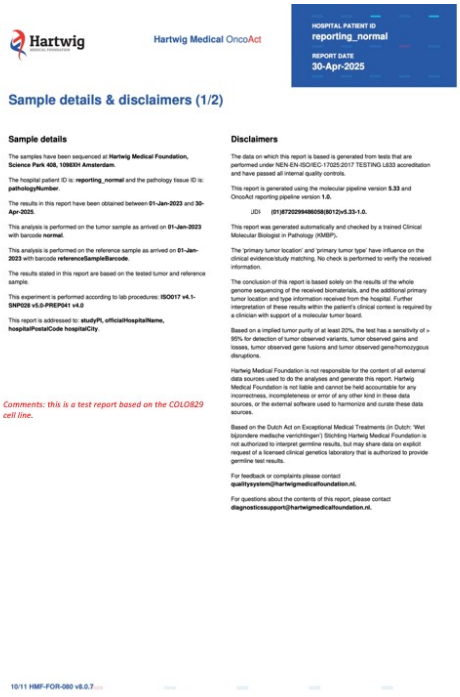
Each OncoAct report ends with more Sample details and Disclaimers (**page 10**) and the signature of the Director Hartwig Medical Foundation (**page 11**).

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 (1/2)

Sample details

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

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

The results in this report have been obtained between **01-Jan-2023** and **30-Apr-2025.**

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.**

Comments: this is a test report based on the COLO829 cell line.

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.**

UDI (01)8720299480058(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 observed variants, tumor observed gains and losses, tumor observed gene fusions and tumor observed 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 bijzondere medische verrichtingen') Stichting Hartwig Medical Foundation is not authorized to interpret germline results, but may share data on explicit request of a licensed clinical genetics laboratory that is authorized to provide germline test results.

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

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

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Hartwig Medical OncoAct

HOSPITAL PATIENT ID
reporting_normal
REPORT DATE
30-Apr-2025

Sample details & disclaimers (2/2)

— End of report —

Edwin Cuppen,
Director Hartwig Medical Foundation

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