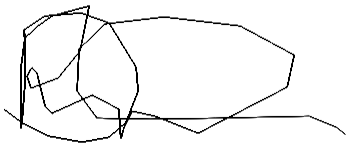
HMF-IVDD-275
OncoAct user manual

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 18:02

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

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 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 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.
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	*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). Note: the minimal tumor-cell percentage as measured by OncoAct should be 8%.	
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 diagnosticsupport@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 sensitivity of 99% is found	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
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	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

				recent comparisons with standard of care tests a sensitivity of 98% is found	
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 microsatellite score	12	Sensitivity and specificity for the detection of 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%; in recent comparisons with standard of care tests a sensitivity of 100% is found (although number that were compared were small)	HMF-VAL-043 Validation of Microsatellite readout using WGS, 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	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

				recombination deficiency within OncoAct is further supported	
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 - tumor mutational burden - sensitivity = 100%, specificity = 75%; tumor mutational load - sensitivity = 100%, specificity = 100%; (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
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	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%,	HMF-VAL-071 Validation of CUPPA algorithm, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9808446/

		lower and is specifically stated in the OncoAct report)		in a scientific publication the validity of the molecular tissue of origin prediction within OncoAct is further supported	
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

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 - the 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 there is truly no clinically relevant genomic event present in the tumor: the clinical specificity 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 clinical specificity (on patient level) was 100%	https://pathsocjournals.onlinelibrary.wiley.com/doi/10.1002/path.5988
Percentage extra patients (who initiated therapy) with treatment options - regular + early access - based on OncoAct	3	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 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	4	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 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 (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.

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.

- 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
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Tel: +31 (0) 20 – 235 2640
Website: https://www.hartwigmedicalfoundation.nl / https://www.oncoact.nl
Email: info@hartwigmedicalfoundation.nl / diagnosticssupport@hartwigmedicalfoundation.nl

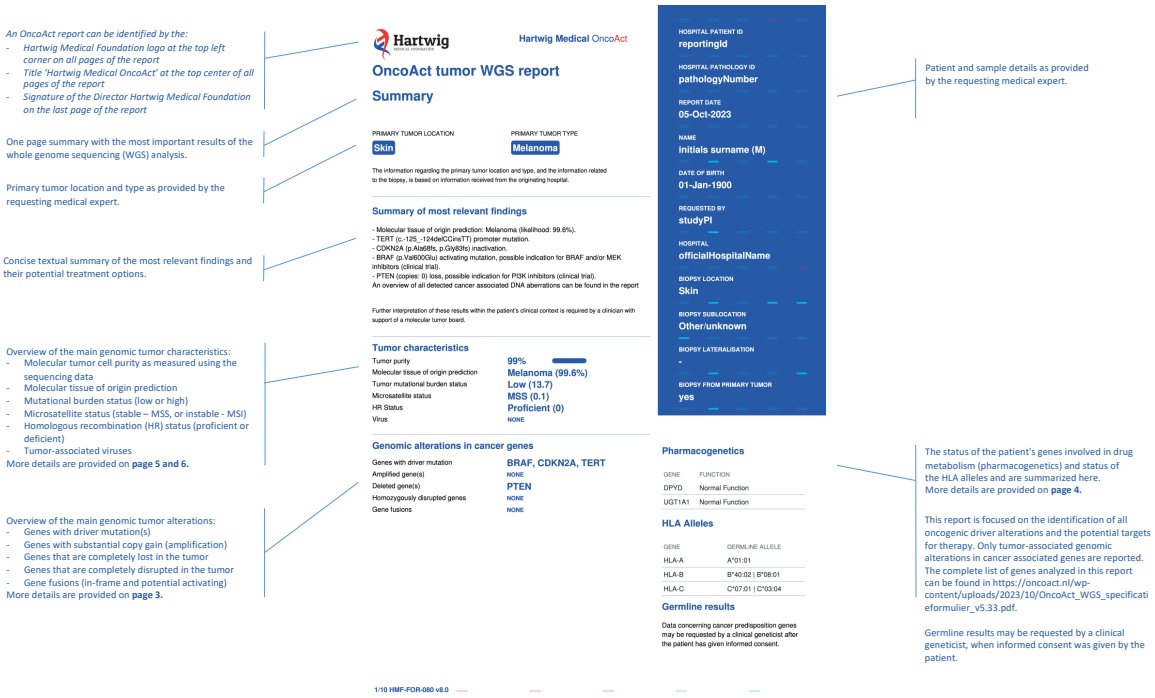
10 Final notices

These instructions for use have been issued on 31/03/2025 18:02 (version 2.2).

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.

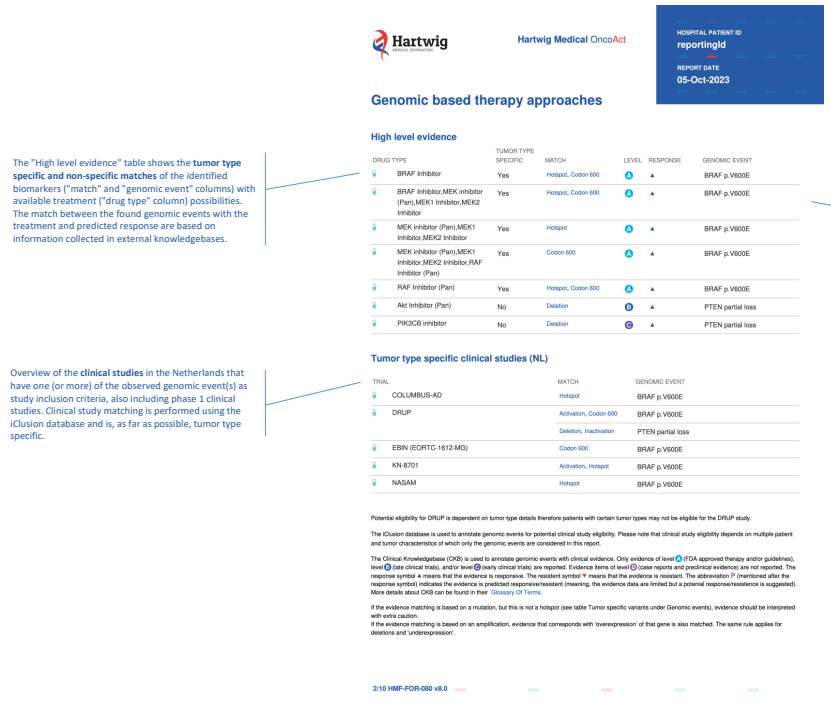
11 Appendix: OncoAct DNA analysis report manual

Example report with explanations of all sections.



OncoAct user manual

HMF-IVDD-275 V2.2

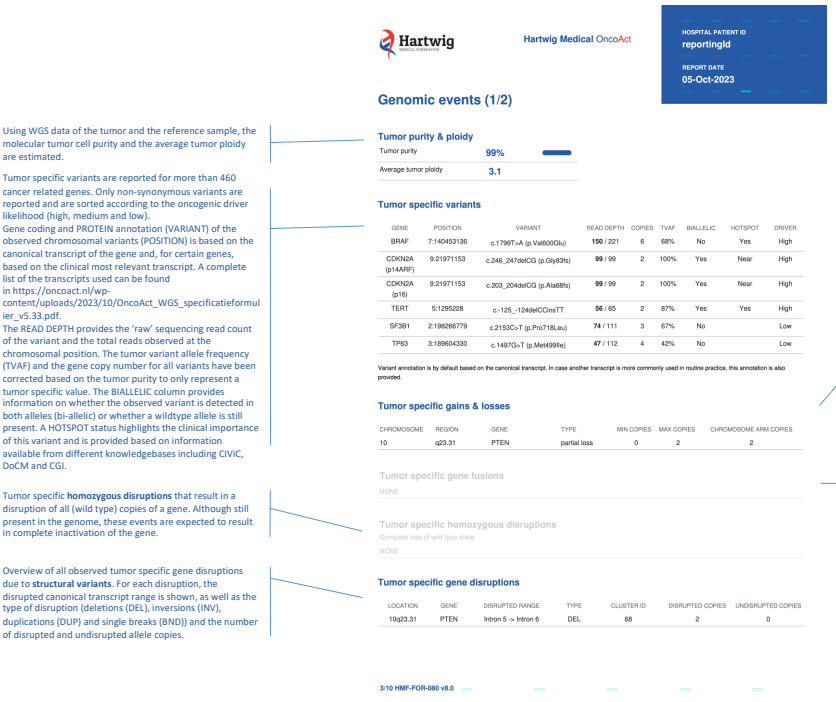


The "High level evidence" table shows the **tumor type specific and non-specific matches** of the identified biomarkers ("match" and "genomic event" columns) with available treatment ("drug type" column) possibilities. The match between the found genomic events with the treatment and predicted response 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 iClusion database and is, as far as possible, tumor type specific.



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.

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.

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.

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.

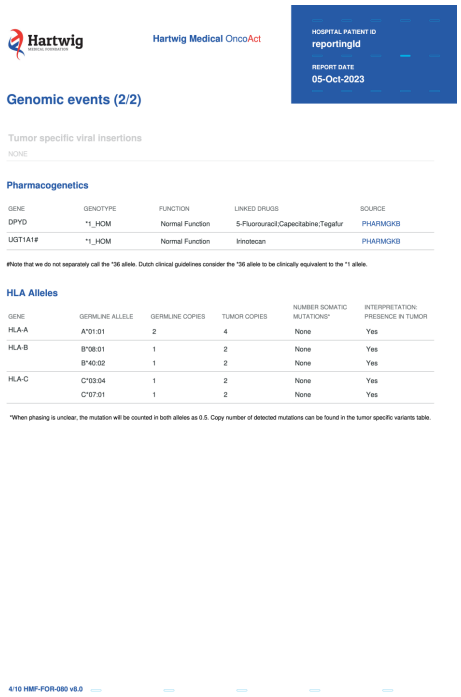
OncoAct user manual

HMF-IVDD-275 V2.2

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).

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.



Hartwig Medical OncoAct

HOSPITAL PATIENT ID
reportingId
REPORT DATE
05-Oct-2023

Genomic events (2/2)

Tumor specific viral insertions

NONE

Pharmacogenetics

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

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

HLA Alleles

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

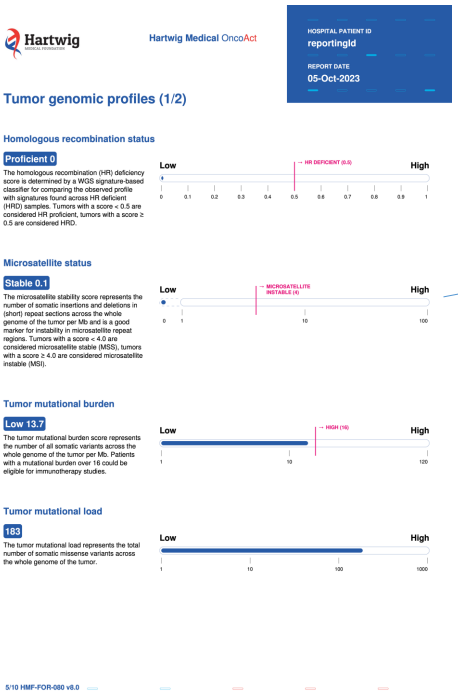
*When phasing is unclear, the mutation will be counted in both alleles as 0.5. Copy number of detected mutations can be found in the tumor specific variants table.

4/10 HMF-FOR-080 v8.0

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.

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.



Hartwig Medical OncoAct

HOSPITAL PATIENT ID
reportingId
REPORT DATE
05-Oct-2023

Tumor genomic profiles (1/2)

Homologous recombination status

Proficient 0

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

Low — → HR DEFICIENT (0.5) — High

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 — → MICROSATELLITE INSTABLE (4) — High

0 1 10 100

Tumor mutational burden

Low 13.7

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

Low — → HIGH (16) — High

1 10 120

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

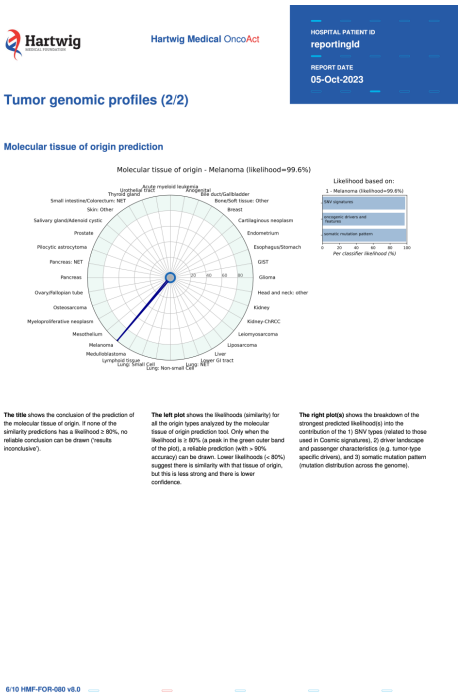
1 10 100 1000

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.

5/10 HMF-FOR-080 v8.0

OncoAct user manual

HMF-IVDD-275 V2.2



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.

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

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

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

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.

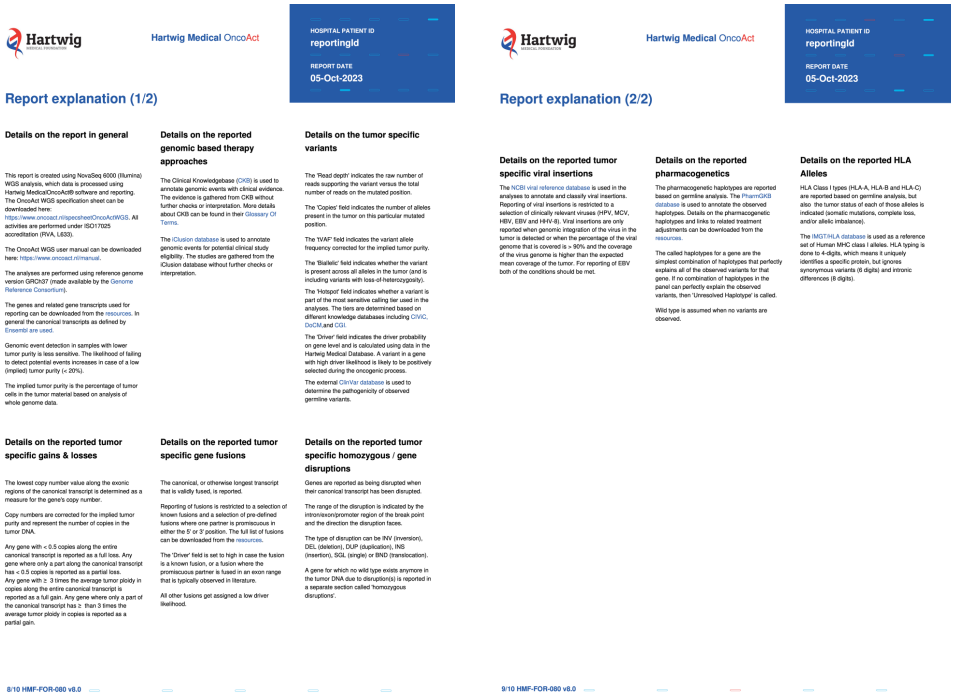
The fourth circle represents the observed 'minor allele copy numbers' across the chromosome. The range of the chart is from 0 to 3. The expected normal minor allele copy number is 1, and anything below 1 is shown as a loss and represents a LOH event (orange). Minor allele copy numbers above 1 indicate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

OncoAct user manual

HMF-IVDD-275 V2.2

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



Hartwig Medical OncoAct

HOSPITAL PATIENT ID
reportingId
REPORT DATE
05-Oct-2023

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/sitecheetOncoActWGS. All activities are performed under ISO17025 accreditation (RVA. L.633).

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'.

Hartwig Medical OncoAct

HOSPITAL PATIENT ID
reportingId
REPORT DATE
05-Oct-2023

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).

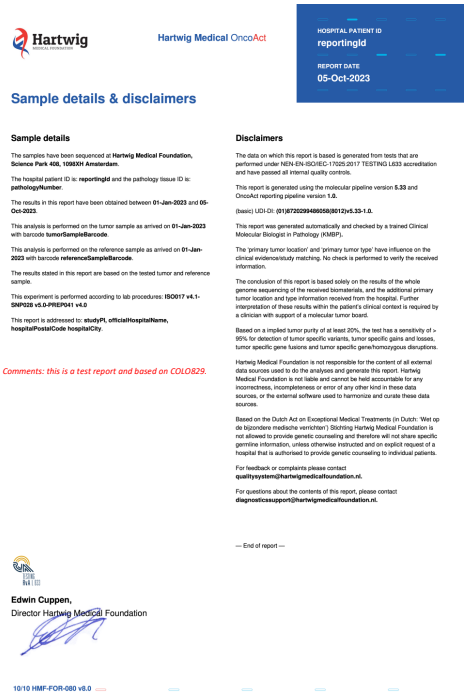
The IMGT/HLA database is used as a reference set of Human MHC class I alleles. HLA typing is done to 4-digits, which means it uniquely identifies a specific protein, but ignores synonymous variants (6 digits) and intronic differences (8 digits).

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.



Hartwig Medical OncoAct

HOSPITAL PATIENT ID
reportingId
REPORT DATE
05-Oct-2023

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-SNP025 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)87320294860058(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