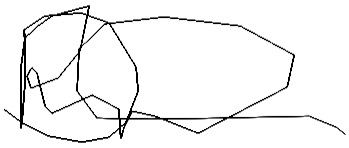
HMF-IVDD-275
OncoAct user manual

Approval Quality Manager:



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Version	**Description of the change**	**Section / Page nr.**	**Reviewed by**	**Issue date**	**Email send**
1.0	First Issue	All	PR	02/06/2021 04:21 PM	No

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Instructions for use IVDR device:
Hartwig Medical OncoAct

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

1 Identification

An OncoAct report can be identified by the following aspects:

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

2 Label

Device Hartwig Medical OncoAct

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

3 Intended purpose

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

4 Intended users

4.1 IVD users

Bioinformaticians and clinical molecular biologists working for Hartwig Medical Foundation are the intended users of OncoAct in terms of data analysis and reporting. The production process prior to the OncoAct analysis and reporting is also exclusively executed at the Hartwig Medical Foundation in Amsterdam by competent lab technicians.

4.2 Medical specialists

Medical specialists working in a hospital environment specialized in oncology are users of the results (the findings) that are listed in the OncoAct report. The medical specialist will use the results as diagnostic/treatment decision support, in dialogue with other specialists (e.g. in molecular tumor boards) and the patient.

5 Test principle

Whole Genome Sequencing can be performed to generate a complete picture of the genomic characteristics of a tumor. Besides performing Whole Genome Sequencing on the tumor (by sequencing DNA originating from a tumor tissue biopsy), Whole Genome Sequencing data is also generated for the normal (or 'reference', taken from blood). This results in a comprehensive analysis of the tumor material, including:

- Discovery of tumor specific (somatic) small variants (~<50 bp) in the tumor (somatic points to the mutations and events acquired besides the germline mutations and events: genetic information that is inherited), as well as information about the copy number, biallelic and if a variant is a hotspot or driver.
- Tumor characteristics: tumor purity and ploidy
- Tumor specific gains and losses of genes
- Tumor specific gene fusions
- Tumor specific homozygous disruptions
- Tumor specific gene disruptions
- Tumor specific viral insertions and detected non-integrated viruses
- Homologous recombination deficiency score
- Microsatellite status
- Pharmacogenetics for DPYD gene
- Molecular Tissue of Origin prediction
- Tumor mutational load and tumor mutational burden
- Tumor type specific therapy details: specific evidence and clinical trials
- Off-label therapy details: evidence for other tumor types
- Graphical overview of all events found within the tumor

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

6 Input biomaterial limitations

For somatic Whole Genome Sequencing analysis it is important to use biomaterials that are fresh or fresh frozen. The minimal tumor-cell percentage needed to ensure that sensitivity is high enough for diagnostic purposes, is 20%. Sample prep should be done efficiently and with sufficient input to result in a minimal sequencing data quality value of 85% Q30 and a read complexity that correlates with less than 30% total exclusion of raw data after mapping.

7 Calculations and interpretations of results

The software includes several different tools with different calculations to approximate the truth. Therefore, results should be interpreted with caution, and should be used solely as supporting evidence for decisions made by medical specialists.

7.1 Interpretation of reports

7.1.1 Types of reports

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

Type	**Purpose**	**Link to Hartwig documentation code:**
DNA analysis report	Samples that pass every quality check (so fulfill the requirements for limits of detection, see below under analytical performance)	HMF-FOR-080
Reports when below detection limits:		
DNA analysis report with 'low sensitivity' disclaimer	*Samples with low tumor cell percentage (10-20%) in deep sequencing*	*HMF-FOR-209*
Insufficient tumor cell percentage DNA analysis report	*Samples that do not meet the required tumor cell percentage (<20%) in shallow sequencing; Samples with insufficient tumor cell percentage (<20%) in deep sequencing*	*HMF-FOR-100*
Insufficient DNA report	*Samples with insufficient DNA after isolation.*	*HMF-FOR-082*
Sufficient tumor cell percentage quality check failure report	*Samples that do not pass bioinformatic quality checks*	*HMF-FOR-083*
Technical failure report	*Samples that fail during the lab process*	*HMF-FOR-102*

7.1.1.1 DNA analysis report

The OncoAct DNA analysis report is given out if a sample passed all quality control checks and reliable results were generated. At the end of this user manual an example OncoAct DNA analysis report is added with explanations about all the different sections, see 11 appendix: OncoAct DNA analysis report manual.

7.1.1.2 DNA analysis report with 'low sensitivity' disclaimer

Similar report as DNA analysis report (described above), but with additional low sensitivity disclaimer.

7.1.1.3 Insufficient tumor cell percentage DNA analysis report / Insufficient DNA report / Sufficient tumor cell percentage quality check failure report / Technical failure report

One page report without results of the Whole Genome Sequencing, but describing the features of the sample and the reason for failure.

7.2 Recommendations for quality control procedures

No quality control procedures are needed to be performed by the user. However, medical specialists need the correct education and training and need to be 'competent' for the interpretation of DNA sequencing results in general and in interpretation of the report.

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 in 2017). Below on overview of all the analytical performance claims and the found 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 fresh-frozen tissue samples with a tumor cell purity of 20% or higher	Comparison to current 'standard-of-care' in clinical practice	The available analytical evidence demonstrates that this claim is met	HMF-VAL-051 Validation of molecular T% test; HMF-VAL-063 Validation of average tumor ploidy; HMF-VAL-074 Clinical Validation of OncoAct
OncoAct analytical sensitivity for events	2	For a tumor purity over 20%, the sensitivity for the detection of SNVs, MNVs and indels, structural variants, fusions and gene copy number changes should be 95% or higher	See claims 3 (SNVs, MNVs and indels), 4 (structural variants), 5 (fusions) and 7 (gene copy number changes)	See claims 3 (SNVs, MNVs and indels), 4 (structural variants), 5 (fusions) and 7 (gene copy number changes)	HMF-VAL-074 Clinical Validation of OncoAct; See claims 3 (SNVs, MNVs and indels), 4 (structural variants), 5 (fusions) and 7 (gene copy number changes)
Analytical sensitivity and positive predictive value/specificity for SNVs, MNVs and indels	3	Analytical sensitivity and positive predictive value/specificity for the detection of SNVs, MNVs and indels should both be over 95% compared to current standard of care tests	Comparison to current 'standard-of-care' in clinical practice	The available analytical evidence demonstrates that this claim is met - sensitivity = 99.2%, specificity = 95.8%. The smallest event that can be measured for SNVs, MNVs and indels: as of one nucleotide, and up to 50 nucleotides	HMF-VAL-061 Validation of SNV-MNV-INDEL mutations using WGS; HMF-VAL-045 Validation of WGS based variants by smMIP; HMF-VAL-065 Validation of SAGE 2.2

Analytical sensitivity for structural variants	4	Structural Variant detection from WGS is very new, and no analytical sensitivity for the detection of structural variants could be defined beforehand. Also, no overall structural variant standard of care test was available at the time.	Comparison to previous version/COLO829 that is scientifically validated	Recall 65 of 69 variants in the truth set and call one additional variant that is presumably a false positive; which gives a sensitivity of 94.2%. The smallest event that can be measured for structural variants: as of 10 nucleotides	HMF-VAL-066 Validation of structural variant analysis
Analytical sensitivity and specificity fusions from SVs	5	Analytical sensitivity and specificity for the detection of fusions from SVs should be over 90% and over 80% respectively 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 = 93%, specificity = 95%	HMF-VAL-060 Validation of fusion gene readout using WGS
Analytical concordance homozygous disruptions from SVs	6	Analytical concordance for the detection of homozygous disruptions from SVs should be over 99% compared to current standard of care tests, or another explanation should be found	Comparison to current 'standard-of-care' in clinical practice (although the current test looks at a different mechanism so is not fully comparable)	14 of the 16 samples were concordant; for 2 of the 16 samples discordant results were found but this was due to the difference in test type (and no mistakes).	HMF-VAL-066 Validation of structural variant analysis; HMF-VAL-068 Validation of homozygous disruption readout
Analytical concordance for gene copy number changes	7	Analytical concordance for the detection of gene copy number changes from SVs should be over 95% compared to current standard of care tests	Comparison to current 'standard-of-care' in clinical practice	For only 1 case (out of 15), the WGS and FISH readout were not aligned and could not be explained due to technical or interpretation issues which gives a concordance of 93.3%. The WGS ERBB2 copy numbers were very much in line with the HER2 FISH signals and showed a high correlation. Due to the small sample size and this high correlation, we still conclude the claim is met. The smallest event that can be measured for gene	HMF-VAL-049 Validation of WGS based copy number_ERBB2

				copy number changes: as of 10 nucleotides	
Analytical concordance germline variants	8	Analytical sensitivity and specificity for the detection of germline variants should be over 95% compared to previous version	Comparison to previous version that is scientifically validated (GATK, https://www.nature.com/articles/s41598-020-77218-4)	The available analytical evidence demonstrates that the claim is met - comparison to previous version of the germline caller showed a 100% concordance in true variants	HMF-VER-076 Verification of SAGE germline vs bachelor
Analytical sensitivity and concordance viral insertions	9	Sensitivity and concordance for viral insertions should be both over 95% compared to current standard of care tests	Comparison to current 'standard-of-care' in clinical practice	The available analytical evidence demonstrates that the claim is met - sensitivity = 100%, concordance = 97.8%	HMF-VAL-064 Validation of virus detection using WGS
Analytical sensitivity and concordance pharmacogeneti c calling	10	Sensitivity and concordance for DPYD pharmacogenetic calling should be both over 99% compared to current standard of care tests	Comparison to current 'standard-of-care' in clinical practice	The available analytical evidence demonstrates that the claim is met - sensitivity = 100%, concordance = 100%	HMF-VAL-069 Validation of DPYD genotype readout by WGS
Analytical sensitivity and specificity MSI	11	Sensitivity and specificity for MSI should be over 95% and 85% respectively compared to current standard of care tests	Comparison to current 'standard-of-care' in clinical practice	The available analytical evidence demonstrates that the claim is met - sensitivity = 100%, specificity = 97%	HMF-VAL-043 Validation of Microsatellite readout using WGS
Analytical exactness HRD	12	HRD exactness should be over 95% compared to earlier HRD and HRP classifications	Comparison to previous version/COLO829 that is scientifically validated > No external/other lab test is available for this purpose, so could not be used to set a threshold for sensitivity and specificity. Comparison with previous classifications, that have shown scientific/clinical	The available analytical evidence demonstrates that the claim is met - exactness = 99.1%	HMF-VAL-062 Validation of HR-deficiency classifier using WGS

			validity, is the best we can do.		
Analytical concordance TMB/TML	13	TMB correlation should be over 0.95 R2 compared to to current standard of care tests (panel)	Comparison to current 'standard-of-care' in clinical practice	The available analytical evidence demonstrates that the claim is met - correlation R2 = 0.98	HMF-VAL-061 Validation of SNV-MNV-INDEL mutations using WGS
Analytical accuracy molecular tumor of origin prediction	14	Molecular tumor of origin predictions should have an accuracy over 95% for conclusive results following the internal validation	Internal validation using independent test set	The available analytical evidence demonstrates that the claim is met - 75.6% of the samples of the test set had conclusive results, among those there was an accuracy of 96.1%	HMF-VAL-071 Validation of CUPPA algorithm
OncoAct analytical reproducibility	15	Reproducibility is controlled using verifications after updates	All verifications	Verifications after every update control reproducibility	HMF-PRO-007 Validation and verification; HMF-VER-077 IVDR production pipeline verification
Limits of detection OncoAct	16	We consider a few limits of detection : -Samples that fail during the lab process -Samples with insufficient DNA after isolation (HMF-SOP-017, HMF-SOP-027) - Samples that don't meet the required molecular tumor percentage (20% mTCP) in shallow sequencing (HMF-SOP-030)	All verifications and validations	NA	HMF-SOP-025

Also, the analytical performance has been described and published in a scientific peer-reviewed journal, see https://www.jmdjournal.org/article/S1525-1578(21)00120-3/fulltext. **The conclusion was that whole genome sequencing has a >95% sensitivity and precision compared to routinely used DNA techniques in diagnostics and all relevant mutation types 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 support advice tool. The medical specialist uses it as support and advice, consequently, no diagnostic sensitivity and specificity can be defined. However, in a large clinical investigation (involving independent medical specialists) 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)***
Diagnostic sensitivity OncoAct	1	Diagnostic sensitivity is defined as the percentage of biomarkers that are present in the patient that are detected by OncoAct: the diagnostic sensitivity for samples with TCP > 20% should be at least 95%	Clinical investigation (WIDE study)	The available clinical evidence demonstrates that the claim is met - the diagnostic sensitivity was 97.95% for samples with >20% TCP	HMF-VAL-074 Clinical Validation of OncoAct
Diagnostic positive predictive value/specificity OncoAct	2	Diagnostic positive predictive value/specificity is defined as: PPV = TPTP + FP : the diagnostic positive predictive value/specificity for samples with TCP > 20% should be at least 95%	Clinical investigation (WIDE study)	The available clinical evidence demonstrates that the claim is met - the diagnostic positive predictive value/specificity was 99.7% for samples with >20% TCP	HMF-VAL-074 Clinical Validation of OncoAct
Diagnostic likelihood ratio OncoAct	3	Likelihood ratio is defined as LR+ = Sensitivity1-Specificity: the diagnostic likelihood ratio for samples with TCP > 20% should be at least 300	Clinical investigation (WIDE study)	The available clinical evidence demonstrates that the claim is met - the diagnostic likelihood ratio was 326.25 for samples with >20% TCP	HMF-VAL-074 Clinical Validation of OncoAct

To conclude, OncoAct has a high clinical accuracy compared to 'standard-of-care' in clinical practice with a sensitivity and specificity of over 95%.

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

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

8 Residual risks of use

- The OncoAct DNA analysis report is interpreted by a non-medical specialist and/or by someone that is not experienced in reviewing molecular diagnostic biomarkers in tumors.
- The clinical sensitivity of OncoAct is high, but there is always a risk of false negatives and false positives. The interpreting medical specialist should always take this into account when reviewing the information.

9 Manufacturer

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10 Final notices

These instructions for use have been issued on 02/06/2021 04:21 PM (version 1.0).

Please report any serious incident that has occurred in relation to the OncoAct device to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established. Please use the contact details above.

11 Appendix: OncoAct DNA analysis report manual

Example report with explanations of all sections.