Hartwig Medical OncoAct

Technical Information

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A. Intended Use

Hartwig Medical OncoAct is a next generation sequencing (NGS) based in vitro cancer diagnostic test for genome-wide detection of genomic aberrations, including substitutions, insertion and deletion alterations (indels), copy number alterations (CNAs), gene fusions and other structural rearrangements (SVs), as well as genomic signatures including microsatellite instability (MSI), homologous recombination DNA-repair deficiency (HRD) and tumor mutational burden (TMB) using DNA isolated from fresh-frozen tumor tissue specimens and corresponding blood samples. The test is intended as a diagnostic tool to identify DNA mutations in patients who may benefit from specific treatments including targeted and immunotherapies, or patients who may be resistant/non-responsive to specific treatments. The Hartwig Medical OncoAct test is intended to provide tumor mutation profiling information to support treatment decision making by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms. The Hartwig Medical OncoAct is a single-site assay performed under ISO/ICE-17025:2005 accreditation at the Hartwig Medical Foundation (HMF) laboratory, Science Park 408 in Amsterdam, The Netherlands.

B. Warnings and Precautions

• Alterations reported only include alterations that are present in the tumor and that may be relevant for cancer treatment ('actionable' alterations). Detection and reporting of inherited germline variants that may confer cancer risk susceptibility is outside the scope of this test.

• This test requires fresh or fresh-frozen tumor tissue. Obtaining a tumor biopsy when fresh(-frozen) tissue is not available is an invasive procedure performed in a hospital which may pose a risk to the patient, dependent of the location of the tumor or metastatic lesion. The patient's physician should determine whether the patient can safely undergo the required biopsy procedure.

• Detection of tumor specific (somatic) aberrations is dependent on the amount of tumor cells in the biopsy and is less sensitive in samples with lower tumor cell purity. In case of a tumor purity below 20% the likelihood of failing to detect relevant variants increases and absence of specific variants should therefore be interpreted with caution.

C. Limitations

• For *in vitro* diagnostic use.

• For prescription use only. Hartwig Medical OncoAct must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.

• A negative result does not rule out the presence of an aberration below the limits of detection of the test.

• Concordance with other validated methods for Copy Number Alterations (CNA), with the exception of *ERBB2*, and gene rearrangements (fusions), with the exception of *ALK*, *ROS1* and *NRG1*, detection has not

been demonstrated. Confirmatory testing using a clinically validated assay for detection of CNA and gene rearrangements should be considered before clinical decision making.

• The MSI/MSS designation by Hartwig Medical OncoAct is based on genome-wide analysis of microsatellite loci. The threshold for MSI/MSS status was determined by analytical concordance with a MSI-PCR assay analyzing the 5 or 7 MSI loci described in current clinical practice guidelines. Homologous recombination DNA-repair deficiency (HRD) is determined by a classifier (CHORD) that uses genome-wide mutation characteristics and was trained on known BRCA1/2 deficient patient samples. The clinical validity of MSI and HRD as defined by Hartwig Medical OncoAct has not been established independently.

• The tumor mutational burden (TMB) is represented as the total number of single nucleotide variants (SNV) and small insertion/deletions (indels) per Mb based on the evaluable part of the genome. The mutational load (ML) is determined by the total number of somatic missense variants (SNV and short in-frame indels that affect the protein-coding sequence). The clinical validity of TMB and ML as defined by Hartwig Medical OncoAct has not been established independently.

• Decisions on patient care and treatment must be based on the independent medical judgment of the qualified treating physician, taking into consideration all applicable information concerning the patient's condition, including, but not limited to, patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given community.

• Hartwig Medical OncoAct is intended to be performed on specific instruments by Hartwig Medical Foundation, and is performed under ISO/ICE-17025:2005 accreditation.

D. Test Principle

Hartwig Medical OncoAct is performed as a laboratory test service using DNA extracted from fresh(-frozen) tumor tissue and blood samples. DNA extraction methods follow standard reagents and protocols from Qiagen, 50-200 ng gDNA is fragmented by sonication for NGS Truseq library preparation including PCR amplification. The Illumina® HiSeqX and NovaSeq6000 platforms are used for sequencing 90x and 30x average read coverage of tumor and normal genomes, respectively. Sequencing data is analyzed with an optimized in-house bioinformatic pipeline designed to detect all types of somatic alterations, including single and multiple nucleotide substitutions (SNV and MNV), insertions and deletions (indels), copy number alterations (amplifications and gene copy losses) and genomic rearrangements and structural variants (e.g. gene fusions). All variants that are potentially clinically relevant for treatment decision making are summarized in a patient report. Additionally, genome-wide mutational characteristics including microsatellite instability (MSI), tumor mutational burden (TMB) and Homologous Recombination DNA repair-deficiency are determined and reported.

E. Summary and Explanation

Hartwig Medical OncoAct is a broad cancer diagnostics test for genome-wide detection of DNA aberrations in all solid malignancies (including lymphoma). Information generated by this test is an aid in the identification of patients who are most likely to benefit (or not) from associated therapies (typically targeted therapies and immunotherapies) in routine as well as experimental clinical settings. Hartwig Medical OncoAct employs total DNA (gDNA) isolation from tumor and control blood samples, gDNA is sonicated for NGS Truseq library preparation including PCR amplification and whole genome sequencing on the Illumina® HiSeqX or NovaSeq600 platforms. Following sequencing, optimized algorithms and software is used to identify tumor-specific genomic variants including substitutions, insertion and deletion alterations (indels), copy number alterations (CNAs), gene fusions and other structural rearrangements (SVs), as well as genomic signatures including microsatellite instability (MSI), homologous recombination DNA-repair deficiency status (HRD) and tumor mutational burden (TMB).

F. Test Kit Contents

Hartwig Medical OncoAct includes a sample shipping kit, which is sent to ordering laboratories. The shipping kit contains the following components:

- Specimen Preparation Instructions
- Sample collection tube
- Barcode labels
- Registration form
- Return shipping materials

All other reagents, materials and equipment needed to perform the test are used exclusively in the HMF laboratory. Hartwig Medical OncoAct is intended to be performed with serial number-controlled instruments.

G. Sample Collection and Test Ordering

To order Hartwig Medical OncoAct, a test request needs to be sent to Hartwig Medical Foundation (forms can be obtained by email to info@hartwigmedicalfoundation.nl) or, in case of an ongoing study, a study registration form must be fully completed and signed by the ordering physician or other authorized medical professional. Please refer to the Specimen Preparation Instructions and mailing instructions included in the sample collection kit for details regarding the sample collection and shipping.

H. Instruments and Reagents

Hartwig Medical OncoAct is intended to be performed with the following instruments:

- Qiasymphony isolation robot
- Covaris LE220 Focused ultrasonicator
- Beckman Coulter Biomek4000 and i7 automated liquid handler
- Illumina® cBot System
- Illumina® HiSeqX ten System and NovaSeq6000

And using the following reagents:

- Qiasymphony DSP DNA Midi Kit
- Qiasymphony DSP DNA Mini Kit
- Illumina® TruSeq Nano kit
- Illumina® KAPA Library Quantification Kit
- Illumina® TruSeq DNA Index Set
- Illumina® HiSeq X™ Ten Reagent Kit v2.5
- Illumina® NovaSeq 6000 S4 Reagent Kit

Hartwig Medical Foundation OncoAct is a single-site assay performed under ISO-17025 accreditation at the Hartwig Medical Foundation (HMF) laboratory, Science Park 408 in Amsterdam, The Netherlands.

I. Performance Characteristics and clinical concordance studies

Performance characteristics were established using DNA derived from a wide range of fresh-frozen tumor tissue types and cell line-based DNA. The **table** below provides a summary of the performance studies, including the type and number of samples used for the specific performance study together with an overview of the clinical concordance studies. Details of performance (validation) studies and clinical performance studies are available upon request. Please contact info@hartwigmedicalfoundation.nl. In addition to the information provided in this technical validation document, details regarding the technology, data, analysis and software/algorithms underlying Hartwig Medical OncoAct can be found on Github (https://github.com/hartwigmedical/hmftools) and in the scientific manuscript by Priestley *et al*. with an

associated extensive methods section.[1]

Table. Summary overview of the performance and clinical concordance studies.

Performance and clinical concordance studies	Nr samples	Sample type
Detection of substitutions and insertion/deletions	2	GIAB
Detection and quantification of chromosomal copy numbers	1	COLO829
Comparison against smMIP based NGS panel sequencing	30	Tumor biopsies
Validation of gene fusion detection with RNA-seq	60	Tumor biopsies
OncoAct test tissue characteristics and compatibility	2921	Tumor biopsies
DNA damage estimation (G>T/C>A transversions)	2520	Tumor biopsies
Performance of OncoAct at cancer hotspot positions	25	Tumor biopsies
Instrument-to-instrument reproducibility and stability	23	GIAB
Reproducibility of OncoAct somatic analysis pipeline	18	Tumor biopsies
Reproducibility on duplicate diagnostic samples	2	Tumor biopsies
Comparison and reproducibility between sequencing platforms	15	GIAB
Impact of sequencing coverage on variant calling sensitivity	10	Tumor biopsies
Tumor purity and ploidy assessment and sensitivity	7	COLO829
Sensitivity on mix-in samples with decreasing tumor DNA content	5	COLO829
Clinical concordance study for microsatellite instability readout	48	Tumor biopsies
Clinical concordance study for ERBB2 copy-number variation	16	Tumor biopsies
In silico comparison of mutational load measurements	18	Tumor biopsies
OncoAct TMB versus panel based TMB assessment	10	Tumor biopsies
Clinical concordance study for gene fusions detection	24	Tumor biopsies
Clinical concordance study for BRAF mutations and other oncogenes	48	Tumor biopsies

[1] *Pan-cancer whole genome analyses of metastatic solid tumors.* Priestley P, Baber J, Lolkema MP, Steeghs N, de Bruijn E, Duyvesteyn K, Haidari S, van Hoeck A, Onstenk W, Roepman P, Shale C, Voda M, Bloemendal HJ, Tjan-Heijnen VCG, van Herpen CML, Labots M, Witteveen PO, Smit EF, Sleijfer S, Voest EE, and Cuppen E, available at https://www.biorxiv.org/content/early/2019/01/16/415133