CeGaT

CeGaT GmbH | Paul-Ehrlich-Str. 23 | 72076 Tübingen | Germany
Dr. Richard Roe
Paul-Ehrlich-Straße 23
72076 Tübingen
Germany

Name	Doe, Jane (*DD.MM.YYYY)
Sex	Female
Patient-ID	123456
Report date	DD.MM.YYYY
Report-ID	R9999999999

CancerDetect® monitoring report (Cell-Free DNA analysis) Doe, Jane (*DD.MM.YYYY)

Indication **Breast Cancer (ED 10/2022)**

Results

- **In the current analysis, we detected the new variant c.1613A>G; p.Asp538Gly in gene *ESR1*.**
- **In the current analysis, the previously detected variants in genes *PIK3CA* and *TP53* were detected again.**

Monitored Alterations:

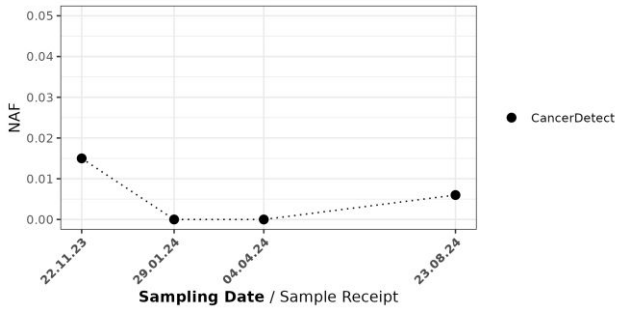
Gene	Functional category	Variant	Transcript-ID	NAF	Effect on protein function
PIK3CA	missense	c.3140A>G; p.His1047Arg chr3:178952085 A>G (hg19)	NM_006218.4	0.0060 (0.60%)	activating
TP53	essential_splice_site	c.994-2A>C; p.? chr17:7574035 T>G (hg19)	NM_000546.6	0.0054 (0.54%)	inactivating
ESR1	missense	c.1613A>G; p.Asp538Gly chr6:152419926 A>G (hg19)	NM_000125.4	0.0070 (0.70%)	activating

This table includes all alterations detected in all of the analyzed samples within the sequenced regions (CancerDetect® version 2). More details regarding the monitoring of these variants can be found in the supplement.
Information for the interpretation of this table can be found in section *Additional Information*.

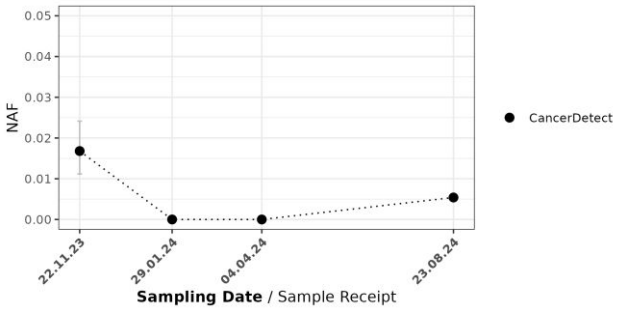
Frequency Change of Monitored Variants over Time:

For each monitored alteration, the figures below show the change in the allele frequency (NAF) within cell-free DNA as percentage over different sampling times. More details on these analyses are provided in the supplement, whereas further information for the interpretation of these figures can be found in section *Additional Information*.

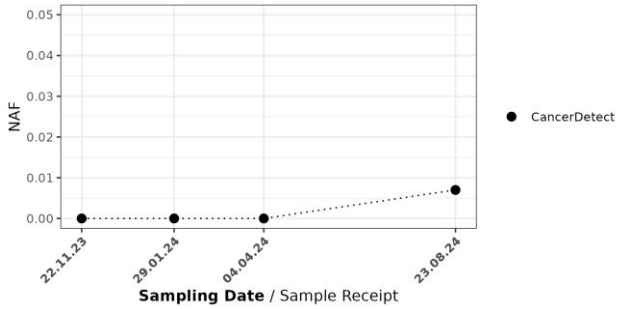
***PIK3CA*, c.3140A>G; p.His1047Arg**



***TP53*, c.994-2A>C; p.?**



***ESR1*, c.1613A>G; p.Asp538Gly**



Recommendation and Interpretation

Comparing the current analysis with the previous analysis, we have detected an increase in the occurrence of all detected alterations. This finding can be interpreted as evidence for a progressive disease course.

Based on the results of this analysis, imaging methods (e.g. MRI/CT) to monitor a possible disease progression are strongly recommended.

Imaging methods (e.g. MRI/CT) to monitor the course of disease should be performed independently.

The subclonal composition of a tumor may change under therapy which may affect the occurrence of the targeted mutations. A lack of detection, a decrease in or no change to the occurrence of a somatic variant is not necessarily proof for a stable disease course or disease regression, while an increase in a variant's frequency is not proof for a progressive disease course.

The results of this report should be evaluated against this patient's current clinical status and should be reviewed by an interdisciplinary tumor board.

Please do not hesitate to contact us if you have any questions.

Medical report written by: Dr. rer. nat. Forename Surname

Proofread by: Dr. rer. nat. Forename Surname

Validated by: Dr. rer. nat. Forename Surname

With kind regards,

Dr. B. Kerner

Dr. med. Berit Kerner

Consultant for Human Genetics

Additional Information

Order	**UMI-based high sensitivity molecular genetic analysis of a liquid biopsy sample**
Sample material	**Tumor tissue: Cell free DNA (cfDNA)** Sample collection 23.08.2024 DNA isolation from STRECK blood with diagnostically estimated tumor content of 1.4%
Sample receipt	27.08.2024 (Tumor-DNA: Streck blood, ID P1234567_8)
Requested Regions	*AKT1* Exon 2 (NM_005163.2), *ALK* Exons 22-25 (NM_004304.5), *AR* Exons 4+5, 8 (NM_000044.6), *BRAF* Exons 11, 15 (NM_004333.6), *CDKN2A* complete gene (NM_000077.5), *CTNNB1* Exons 2, 6+7 (NM_001904.4), *EGFR* Exons 2+3, 6+7, 15, 18-21 (NM_005228.5), *ERBB2* Exons 8, 17, 19-21 (NM_004448.4), *ERBB3* Exons 3, 7-9, 23 (NM_001982.4), *ESR1* Exons 4+5, 7+8 (NM_000125.4), *FGFR1* Exons 11-13 (NM_023110.3), *FGFR2* Exons 6, 8, 11-13 (NM_000141.5), *FGFR3* Exons 6, 8, 13 (NM_000142.5), *GNA11* Exons 4+5 (NM_002067.5), *GNAQ* Exons 2, 4+5 (NM_002072.5), *GNAS* Exon 8 (NM_000516.7), *H3-3A* Exon 1 (NM_002107.7), *HRAS* Exons 1-3 (NM_005343.4), *IDH1* Exon 2 (NM_005896.4), *IDH2* Exon 4 (NM_002168.4), *JAK2* Exon 12 (NM_004972.4), *KIT* Exons 9, 11, 13+14, 17 (NM_000222.3), *KRAS* Exons 1-3 (NM_004985.5), *MET* Exons 13, 15, 18 (NM_001127500.3), *NRAS* Exons 1-3 (NM_002524.5), *PDGFRA* Exons 11, 13, 17 (NM_006206.6), *PIK3CA* Exons 1, 4, 7, 9, 13, 20 (NM_006218.4), *PTEN* complete gene (NM_000314.8), *RET* Exons 10+11, 13-16 (NM_020975.6), *TERT* Promotor (NM_198253.3), *TP53* complete gene (NM_000546.6) (cfDNA diagnostics version 2, exon numbers referring to coding exons in a given transcript)
Information for the interpretation of tables and figures	**NAF:** *Novel allele frequency*, the frequency with which the mutated allele occurs in the sequencing data (1 is 100%). The observed frequencies are influenced by the tumor content as well as copy number alterations and do not directly correlate with the variant's frequency in the tumor. A NAF value of zero or the abbreviation *n.d.* indicates that the variant has not been detected in the corresponding analysis. Please note that sequencing results based on different enrichment techniques can be used for monitoring. These may not be able to cover every variant suitable for monitoring due to technical reasons. In variant tables, for uncovered variants a "-" symbol is added to the NAF column whereas in the graphical progression diagrams for these variants no information on the frequency is provided. Moreover, different technological approaches have different sensitivities in the detection of somatic variants: in UMI-based CancerDetect® analyses variants with a NAF of ≥ 0.25% can be detected. For CancerPrecision® analyses (tumor panel) and CancerNeo® analyses (CeGaT ExomeXtra® based) variants with a NAF of ≥ 5% are detected (known hotspot variants may also be reported down to a NAF of ≥ 2%). **Protein function:** The somatic alterations were classified with respect to their effect on protein function with the following categories: inactivating/activating/function changed, likely inactivating/activating/function changed, unknown, and benign (details in the methods section).
Methods	**DNA isolation:** Cell-free DNA was isolated at CeGaT GmbH. **Sample quality:** The suitability of a sample for molecular genetic analysis depends on the tumor content as well as on the overall material quality. In case of low material quality, the detection of variants may be impaired or even impossible. **NGS-laboratory:** Extracted DNA molecules were labelled with dual unique molecular indices (UMI). The target region was enriched using in solution hybridization technology and was sequenced using the Illumina NovaSeq 6000/NovaSeq X Plus system. **Computational analysis:** Illumina bcl2fastq2 was used to demultiplex sequencing reads. Adapter removal was performed with Skewer. The trimmed reads were mapped to the human reference genome (hg19) using the Burrows-Wheeler Aligner. Reads mapping to more than one location with identical mapping score were discarded. UMI information was used to combine reads into single-molecule

consensus sequences. Only patient DNA molecules sequenced in both directions with matching consensus were used to determine sequence variants (single nucleotide changes and small insertions/deletions). The variants were annotated based on several internal as well as external databases.

Genetic data evaluation: Only variants (SNVs/small indels) with a novel allele frequency (NAF) of ≥ 0.25% in the tumor sample were reported. The clinical interpretation of variants is based on different external and internal databases and on information from scientific literature. The sensitivity of the test is dependent on the tumor content of the analyzed material, the sample quality, and the sequencing depth. A coverage of 1000 reads per base achieves a sensitivity of > 91% for the detection of variants with a NAF ≥0.25%. In this case, 78.61% of the targeted regions were covered by a minimum of 1000 high-quality sequencing reads per base. Variants are named according to the HGVS recommendations without any information regarding the cis or trans configuration. Please be aware that a germline origin of reported variants cannot be excluded.

Variant classification: The somatic alterations were assessed with respect to their possible impact on protein function based upon the available data (i.e. cBioPortal, My Cancer Genome, Clinical Interpretations of Variants in Cancer (CIVIC), MD Anderson Personalized Medicine Center Database, *TP53* database (ISB-CGC), CKB, OncoKB, PubMed research) and/or using *in silico* predictions (MetaLR, PrimateAI, and SpliceAI). The functional categories assigned are: inactivating, activating, function altered, likely inactivating/activating/function altered, unknown or benign. "Inactivating": known inactivating variants as well as frameshift, nonsense and essential splice site variants, unless they are described as activating or benign. "Activating" and "function altered": known activating/function changing variants. The functional evidence of variants classified as inactivating, activating and function altered is highly reliable (i.e. ClinVar/ClinGen data with a review status of at least two stars, databases of specific consortia and/or *in vivo/in vitro* analyses). "Likely inactivating/activating/function altered": an impact of the variant on protein function is considered as likely with respect to the affected amino acid position (e.g. known hot spot, pathogenic variant in the same codon, high conservation, *in silico* predictions), but there are insufficient functional data available. "Unknown": based upon the available data, we are not able to conclusively confirm or exclude a possible functional relevance of the variant. "Benign": the variant is described as benign and does not impair protein function.

The sample fulfilled our quality criteria upon arrival and during/after each processing step in the laboratory.

The procedure described above was developed and validated in-house (Laboratory developed test; LDT). A minimal tumor content of 0.5% was taken as a basis.

Communication, dissemination and usage of this report for scientific purposes is only permitted in accordance with the German Genetic Diagnostics Legislation.

Supplement - Frequency Change of Monitored Alterations

The tables below include all alterations detected in all of the analyzed samples within the sequenced regions (CancerDetect® version 2).

PIK3CA, c.3140A>G; p.His1047Arg, NM_006218.4

Report	Sampling Date / *Sample Receipt*	Isolate Type	Sample Material	Analysis	Tumor Content	NAF	cell-free DNA Amount
R1234567	23.08.2024	cell-free DNA	STRECK blood	CancerDetect® version 2	1.4%	0.0060 (0.60%)	0.24 µg / 18.00 ng/µl
R1234567	04.04.2024	cell-free DNA	STRECK blood	CancerDetect® version 1	n.d.	n.d.	0.09 µg / 1.80 ng/µl
R1234567	29.01.2024	cell-free DNA	STRECK blood	CancerDetect® version 1	n.d.	n.d.	0.07 µg / 2.50 ng/µl
R1234567	22.11.2023	cell-free DNA	STRECK blood	CancerDetect® version 1	3%	0.0150 (1.50%)	0.18 µg / 12.90 ng/µl

Information for the interpretation of this table can be found in section *Additional Information*.

TP53, c.994-2A>C; p.?, NM_000546.6

Report	Sampling Date / *Sample Receipt*	Isolate Type	Sample Material	Analysis	Tumor Content	NAF	cell-free DNA Amount
R1234567	23.08.2024	cell-free DNA	STRECK blood	CancerDetect® version 2	1.4%	0.0054 (0.54%)	0.24 µg / 18.00 ng/µl
R1234567	04.04.2024	cell-free DNA	STRECK blood	CancerDetect® version 1	n.d.	n.d.	0.09 µg / 1.80 ng/µl
R1234567	29.01.2024	cell-free DNA	STRECK blood	CancerDetect® version 1	n.d.	n.d.	0.07 µg / 2.50 ng/µl
R1234567	22.11.2023	cell-free DNA	STRECK blood	CancerDetect® version 1	3%	0.0168 (1.68%)	0.18 µg / 12.90 ng/µl

Information for the interpretation of this table can be found in section *Additional Information*.

ESR1, c.1613A>G; p.Asp538Gly, NM_000125.4

Report	Sampling Date / *Sample Receipt*	Isolate Type	Sample Material	Analysis	Tumor Content	NAF	cell-free DNA Amount
R1234567	23.08.2024	cell-free DNA	STRECK blood	CancerDetect® version 2	1.4%	0.0070 (0.70%)	0.24 µg / 18.00 ng/µl
R1234567	04.04.2024	cell-free DNA	STRECK blood	CancerDetect® version 1	n.d.	n.d.	0.09 µg / 1.80 ng/µl
R1234567	29.01.2024	cell-free DNA	STRECK blood	CancerDetect® version 1	n.d.	n.d.	0.07 µg / 2.50 ng/µl
R1234567	22.11.2023	cell-free DNA	STRECK blood	CancerDetect® version 1	3%	n.d.	0.18 µg / 12.90 ng/µl

Information for the interpretation of this table can be found in section *Additional Information*.

CeGaT GmbH | Paul-Ehrlich-Str. 23 | 72076 Tübingen | Germany
Tel: + 49 7071 565 44 55 | Fax: + 49 7071 565 44 56 | info@cegat.de | www.cegat.de
Court District Stuttgart - HRB 729958 | VAT No: DE265504070
Volksbank in der Region eG | IBAN: DE73 6039 1310 0543 4480 02 | SWIFT / BIC: GENODES1VBH
Managing Directors: Dr. Dr. Saskia Biskup, Dr. Dirk Biskup