CeGaT GmbH | Paul-Ehrlich-Str. 23 | D-72076 Tübingen | Germany

Dr. Physician
Doctorstreet 23
12345 Krankfurt

Patient	Doe, Jane (*01.01.1900)
Sex	Female
Patient-ID	205002
Sample receipt	03.01.2020
Material	Blood/liquor/ascites/pleural puncture for cfDNA
Report date	08.02.2021

Cell-free DNA analysis report – Doe, Jane (*01.01.1900)

Indication **Uveal melanoma (ID 09/2020)**

Material **Cell free DNA (cfDNA)**
Sample collection 01/2020
Isolation of cfDNA from blood/liquor/ascites/pleural puncture samples with an estimated tumor content of 6%

Order **UMI-based high sensitivity molecular genetic analysis of a liquid biopsy sample**

RESULTS

- **We detected one variant with potential therapeutic relevance in the current sample.**

Variant with potential therapeutic relevance:

Gene	Functional category	Variant	NAF	Effect on protein function	Related pathway	Therapeutic option	Predicted response	Level of evidence
GNAQ	missense	c.626A>C; p.Gln209Pro	0.03	activating	-	MEK/ERK inhibitor	sensitive	2B

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 and do not directly correlate with the variant's frequency in the tumor. The somatic alterations were classified with respect to their functional effect on protein levels in the following categories: inactivating/activating/function altered, likely inactivating/activating/function altered, unknown and benign (details in the methods section). **Predicted response:** represents the predicted response considering known interferences and pathway crosstalks. Please note that the predicted drug-response is made based on the identified biomarkers only and does not take clinical (or tumor entity specific) features into consideration. **Level of evidence:** for legend see supplement.

RECOMMENDATION

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.

With kind regards,

Dr. med. Dr. rer. nat. Saskia Biskup	Dr. rer nat. xxx	Dr. rer nat. xxx	Dr. rer nat. xxx
Consultant for Human Genetics	Diagnostics	Diagnostics	Diagnostics

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ADDITIONAL INFORMATION

Requested Regions

AKT1: Exon 2 (NM_005163), *ALK:* Exons 21-25 (NM_004304), *ARAF:* Exon 6 (NM_001654), *BRAF:* Exons 11 and 15 (NM_004333), *CTNNB1:* Exon 2 (NM_001904), *EGFR:* Exons 18-21 (NM_005228), *ERBB2:* Exon 8, 19-21 (NM_004448), *ERBB3:* Exons 3, 6-9, 23 (NM_001982), *ERBB4:* Exon 12 (NM_005235), *ESR1:* Exons 4-8 (NM_000125), *FGFR2:* Exons 6, 8, 11 (NM_000141), *FGFR3:* Exon 12 (NM_000142), *GNAQ:* Exon 5 (NM_002072), *GNAS:* Exon 8 and Exon 9 (NM_000516), *GNA11:* Exon 5 (NM_002067), *HRAS:* Exons 2-4 (NM_005343), *H3-3A:* Exon 1 (NM_002107), *H3-3B:* Exon 1 (NM_005324), *IDH1:* Exon 2 (NM_005896), *IDH2:* Exon 4 (NM_002168), *JAK2:* Exon 14 (NM_004972), *KIT:* Exons 9, 11, 13, 14, 17, 18 (NM_000222), *KRAS:* Exons 2-4 (NM_004985), *MAP2K1:* Exon 3 (NM_002755), *MET:* Exon 19 (NM_001127500), *MYCN:* Exon 1 (NM_005378), *NRAS:* Exons 2-4 (NM_002524), *PDGFRA:* Exons 4, 10-14, 18 (NM_006206), *PIK3CA:* Exons 4, 7, 9, 13, 20 (NM_006218), *PTEN:* Exons 5-7 (NM_000314), *RAC1:* Exon 2 (HS P29) (NM_018890), *RAF1:* Exon 6 (NM_002880), *RET:* Exon 10, 11, 13-16 (NM_020975), *STAT5B:* Exon 15 (NM_012448), *TERT:* Promotor (NM_198253), *TP53:* Entire coding region, all exons covered (NM_000546) (cfDNA focus panel version 1)

Methods

DNA isolation: Cell-free DNA was isolated at Praxis für Humangenetik/CeGaT, Tübingen.

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 were sequenced using the Illumina HiSeq/NovaSeq 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, 95.6% 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. Catalogue of somatic mutations in cancer (COSMIC), cBioPortal, My Cancer Genome, Clinical Interpretations of Variants in Cancer (CIVIC), MD Anderson Personalized Medicine Center Datenbank, IARC *TP53* database, CKB, OncoKB, PubMed research) and/or using *in silico* predictions (Mutation Taster, fathmm, Mutation Assessor, SIFT, fathmm-MKL coding, LRT and PROVEAN). 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.

Classification criteria for theoretical response:

sensitive: We expect a favorable response to the specified medication class, in the presence of this biomarker. This prediction is based on the affected signal transduction pathway and is made considering all therapeutically relevant biomarkers. However, the potential interference of other mutated genes with the effectiveness of this class of medication cannot be ruled out. A level of evidence (LoE) of 1-4 must be indicated to assign a sensitive theoretical response.

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unclear: An unclear theoretical response is given if there is another mutated biomarker in the pathway of this biomarker, or downstream of the medication target, that could reduce the efficacy of the specified drug class. Also, where evidence of non-response (see resistance) has been described in the scientific literature. Moreover, an LoE of 5 is always given an "unclear" theoretical response, unless the corresponding drug class has already been provided with an LoE of 1-4 with a "sensitive" theoretical response due to another biomarker. In this case, a "sensitive" theoretical response is also assigned for the biomarker with an LoE of 5.

resistant: If there is evidence that the current biomarker (NCCN and/or ESMO) will have a non-response, decreased response, or resistance to the specified medication class in the given entity, then it will be given an LoE of R1 with a "resistant" theoretical response.

However, if the resistance of the biomarker towards the medication class is not indicated in the NCCN- or ESMO-guidelines, but there is data in the current literature suggesting non-response, decreased response, or resistance, then an LoE of R2 is given with an "unclear" theoretical response.

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.

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SUPPLEMENT - LEVEL OF THERAPEUTIC EVIDENCE

LoE	
1A	**Approved drug, specific to the biomarker and entity** Drug is approved for the biomarker within the same entity (FDA and/or EMA)
1B	**Approved drug, specific to entity but not specific to the biomarker OR specific to biomarker, but only in organ related entities** Drug is approved independently of the biomarker within the same entity OR drug is approved for the biomarker in an organ related entity, e. g. benign tumor (FDA and/or EMA). The reported biomarker must have significant clinical relevance, despite biomarker-independent approval of the indicated drug.
2A	**Approved drug, specific to the biomarker for a different entity** Drug is approved for the biomarker in a different entity (FDA and/or EMA)
2B	**Approved drug, not specific to the biomarker for a different entity** Drug is approved independently of the biomarker in a different entity (FDA and/or EMA). The reported biomarker must have significant clinical relevance, despite biomarker-independent approval of the indicated drug.
3	**Efficacy of the drug is currently being/was analyzed in clinical trials**
4	**Efficacy of the drug is based on preclinical analyses and/or case reports**
5	**Hypothetical response** The biomarker could hypothetically induce response to the drug
R1	**The variant and/or biomarker is associated with a non-response, decreased response, or resistance to a specific drug or drug class in the same entity. The information is based on high impact guidelines (NCCN and/or ESMO)** The variant and/or biomarker is associated with a non-response, decreased response, or resistance to a specific drug or drug class in the same entity. The information is based on high impact guidelines (NCCN and/or ESMO)
R2	**Biomarker might be associated with a non-response or a resistance** The biomarker might be associated with a non-response, reduced response, or resistance to the stated drug class in this or another tumor entity (based on current literature)

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