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Dr. Richard Roe
Paul-Ehrlich-Straße 23
72076 Tübingen
Germany

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

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

Indication **Melanoma**

Results

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

Variants with potential therapeutic relevance:

Gene	Functional category	Variant	NAF	Effect on protein function	Therapeutic option for discussion in the MTB	Approved by EMA/FDA	Approved for current entity
BRAF	missense	c.1799T>A; p.Val600Glu	0.0240 (2.40%)	activating	BRAF inhibitor	EMA* & FDA*	EMA* & FDA*
					MEK inhibitor	EMA* & FDA*	EMA* & FDA*
					Possible resistance to EGFR/HER inhibitor	N/A	N/A
TP53	missense	c.818G>A; p.Arg273His	0.0431 (4.31%)	function changed	CHK1 inhibitor	no	no
					Wee inhibitor	no	no

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. 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).
Approval: Only those organisations having approved the respective therapeutical option are listed here. An asterisk indicates approval restrictions (please refer to the appendix for details).

Please refer to the table in the appendix for more information regarding targeted approved drug therapies (EMA/FDA), including information on approval requirements and potential drug resistance.

Complete List of Automatically Detected Somatic Variants

The table below includes all somatic variants (single nucleotide variants and small deletions/insertions (≤ 40bp)) detected automatically within the sequenced regions (CFD panel V.1).

Gene	Functional category	Variant	Transcript-ID	NAF
BRAF	missense	c.1799T>A; p.Val600Glu	NM_004333.6	0.0240 (2.40%)
TP53	missense	c.818G>A; p.Arg273His	NM_000546.6	0.0431 (4.31%)

NAF: *Novel allele frequency*, the frequency with which the mutated allele was detected in the sequencing data (1 is 100%). The observed frequencies are influenced by the tumor content and do not correlate directly with the variant frequency in the tumor.

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.

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

Proofread by: Dr. rer. nat. Forename Surname

With kind regards,

Dr. med. Dr. rer. nat. Saskia Biskup
Dr. med. Friedmar Kreuz, M.A.

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 MM/YYYY Diagnostically estimated tumor content 5-10%
Sample receipt	DD.MM.YYYY (Streck-blood for cfDNA)
Requested Regions	*AKT1* Exon 2 (NM_005163.2), *ALK* Exons 21-25 (NM_004304.5), *ARAF* Exon 6 (NM_001654.5), *BRAF* Exons 11, 15 (NM_004333.6), *CTNNB1* Exon 2 (NM_001904.4), *EGFR* Exons 18-21 (NM_005228.5), *ERBB2* Exons 8, 19-21 (NM_004448.4), *ERBB3* Exons 3, 6-9, 23 (NM_001982.4), *ERBB4* Exon 12 (NM_005235.3), *ESR1* Exons 4-8 (NM_000125.4), *FGFR2* Exons 6, 8, 11 (NM_000141.5), *FGFR3* Exon 12 (NM_000142.5), *GNA11* Exon 5 (NM_002067.5), *GNAQ* Exon 5 (NM_002072.5), *GNAS* Exons 8+9 (NM_000516.7), *H3-3A* Exon 1 (NM_002107.7), *H3-3B* Exon 1 (NM_005324.5), *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+18 (NM_000222.3), *KRAS* Exons 1-3 (NM_004985.5), *MAP2K1* Exon 3 (NM_002755.4), *MET* Exon 18 (NM_000245.4), *MYCN* Exon 1 (NM_005378.6), *NRAS* Exons 1-3 (NM_002524.5), *PDGFRA* Exons 4, 9, 11, 13, 17 (NM_006206.6), *PIK3CA* Exons 4, 7, 9, 13, 20 (NM_006218.4), *PTEN* Exons 5-7 (NM_000314.8), *RAC1* Exon 2 (NM_018890.4), *RAF1* Exon 6 (NM_002880.4), *RET* Exons 10+11, 13-16 (NM_020975.6), *STAT5B* Exon 15 (NM_012448.4), *TERT* Promotor (NM_198253.3), *TP53* (NM_000546.6) (cfDNA diagnostics version 1, exon numbers referring to coding exons in a given transcript)
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 NovaSeq6000 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, 94.5% 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, IARC *TP53* database, 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.

Therapeutic options: The placement of drugs into different drug classes is done by cross referencing information from FDA, EMA, and PubChem. Approval status and limitations are taken from drugs.com (FDA) and ema.europa.eu (EMA).

In case of evidence (NCCN and/or ESMO guidelines) of a respective biomarker causing non-response, decreased response, or resistance to the specified medication class in the given entity, or in case of evidence in current literature suggesting non-response, decreased response, or resistance, the affected drugs will be marked with a warning sign in appendix.

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.

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Supplement - Possible Therapeutic Strategies

Please note that the provided information on potential drugs is only a specific selection and makes no claim of completeness. Furthermore, the listing is limited to targeted therapies and does not include common chemotherapies.

Approvals affecting your patient's tumor entity are highlighted in blue.

BRAF, c.1799T>A; p.Val600Glu, NM_004333.6:

Relevant therapeutics for gene *BRAF*

Drug name	Tumor entity	Approval	Approval limited to biomarkers/others	Approval in combination with other drugs
Dabrafenib BRAF inhibitor	**Melanoma**	**EMA**	**BRAF V600 mutation** adult patients, unresectable or metastatic melanoma or stage III melanoma after surgery	**Trametinib**
		EMA	**BRAF V600 mutation** adult patients, unresectable or metastatic melanoma	
		FDA	**BRAF V600E or V600K** unresectable or metastatic melanoma; adjuvant treatment and involvement of lymph node(s), following complete resection	**Trametinib**
		FDA	**BRAF V600E** unresectable or metastatic	
	Neoplasm	**FDA**	**BRAF V600E** patients 6 years and older, unresectable or metastatic solid tumor, progress following prior treatment, no satisfactory alternative treatment options not indicated for colorectal cancer!	**Trametinib**
Encorafenib BRAF inhibitor	**Melanoma**	**EMA**	**BRAF V600 mutation** adult patients, unresectable or metastatic melanoma	**Binimetinib**
		FDA	**BRAF V600E or BRAF V600K** unresectable or metastatic melanoma	**Binimetinib**
Vemurafenib BRAF inhibitor	**Melanoma**	**EMA**	**BRAF V600 mutation** adult patients, metastatic or unresectable melanoma	
		FDA	**BRAF V600E** metastatic or unresectable melanoma	
Sorafenib BRAF inhibitor FLT3 inhibitor KIT inhibitor PDGFR inhibitor VEGF/VEGFR inhibitor pan-RAF(dimer) inhibitor	Hepatocellular carcinoma	EMA		
		FDA	unresectable hepatocellular carcinoma (HCC)	
	Renal cell carcinoma	EMA	advanced renal cell carcinoma who have failed prior interferon-alpha or interleukin-2 based therapy or are considered unsuitable for such therapy	
		FDA	locally advanced / metastatic Renal Cell (clear cell) Carcinoma (RCC), failure or intolerance to prior systemic therapy	
	Thyroid carcinoma	EMA	progressive, locally advanced or metastatic, differentiated (papillary/follicular/Hürthle cell) thyroid carcinoma, refractory to radioactive iodine	
		FDA	late-stage (metastatic) differentiated thyroid cancer, radioactive iodine-refractory	
Binimetinib MEK inhibitor	**Melanoma**	**EMA**	**BRAF V600** adult patients with unresectable or metastatic melanoma	**Encorafenib**
		FDA	**BRAF V600E or V600K** unresectable or metastatic melanoma	**Encorafenib**
Cobimetinib MEK inhibitor	**Melanoma**	**EMA**	**BRAF V600** unresectable or metastatic melanoma	**Vemurafenib**
		FDA	**BRAF V600E or V600K** unresectable or metastatic melanoma	**Vemurafenib**
Selumetinib MEK inhibitor	Neurofibromas	EMA	pediatric patients 3 years of age and older with neurofibromatosis type 1 (NF1) who have symptomatic, inoperable plexiform neurofibromas	
		FDA	pediatric patients 2 years of age and older with neurofibromatosis type 1 (NF1) who have symptomatic, inoperable plexiform neurofibromas	

Drug name	Tumor entity	Approval	Approval limited to biomarkers/others	Approval in combination with other drugs
Trametinib MEK inhibitor	**Melanoma**	**EMA**	**BRAF V600** adult patients with unresectable or metastatic melanoma; adjuvant treatment of adult patients with Stage III melanoma following complete resection	**Dabrafenib**
		EMA	**BRAF V600** adult patients with unresectable or metastatic melanoma; monotherapy has not demonstrated clinical activity in patients who have progressed on a prior BRAF inhibitor therapy	
		FDA	**BRAF V600E or V600K** -unresectable or metastatic melanoma -adjuvant treatment and involvement of lymph node(s), following complete resection	**Dabrafenib**
		FDA	**BRAF V600E or V600K** BRAF-inhibitor treatment-naïve patients with unresectable or metastatic melanoma	
	Neoplasm	**FDA**	**BRAF V600E** patients 6 years and older, unresectable or metastatic solid tumor, progress following prior treatment, no satisfactory alternative treatment options; not indicated for colorectal cancer	**Dabrafenib**