CeGaT

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

CancerEssential® report – Doe, Jane (*DD.MM.YYYY)

Indication **Non-small cell lung cancer**

Results

- **We detected one alteration with potential therapeutic relevance in the current sample.**
- **No evidence for therapeutically relevant structural variants (fusions/translocations) on DNA level.**

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
EGFR	inframe	c.2235_2249del; p.Glu746_Ala750del	0.25	activating	EGFR inhibitor	EMA* & FDA*	EMA* & FDA*
					Resistance to Immune checkpoint inhibitor	N/A	N/A

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 Variants

The table below includes all variants (single nucleotide variants and small deletions/insertions (≤ 40bp)) detected automatically within the sequenced regions (Molecular Pathology version 3).

Gene	Functional category	Variant	Transcript-ID	NAF
EGFR	inframe	c.2235_2249del; p.Glu746_Ala750del	NM_005228.5	0.25

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	**Molecular genetic analysis of a tumor tissue sample** Molecular Pathology Module "Lung cancer"
Sample material	**Tumor tissue: Biopsy sample of the known non-small cell lung carcinoma** Sample collection MM/YYYY DNA isolation from tumor in FFPE (FFPE-ID: XXXX/YY) after macrodissection with estimated tumor content of 50% (HE staining) Diagnostically estimated tumor content 50%
Sample receipt	DD.MM.YYYY (Tumor-FFPE)
Requested Regions	*AKT1* (NM_005163.2), *ALK-EML4* translocations (NM_004304.5), *BRAF*, (including V600E) (NM_004333.6), *EGFR* (NM_005228.5), *ERBB2* (NM_004448.4), *FBXW7* (NM_033632.3), *FGFR1* (NM_023110.3), *FGFR2* (NM_000141.5), *FGFR3* (NM_001354810.2), *KRAS* (NM_004985.5), *MAP2K1* (NM_002755.4), *MET* (NM_001127500.3), *NOTCH1* (NM_017617.5), *NRAS* (NM_002524.5), *PIK3CA* (NM_006218.4), *PTEN* (NM_001304717.5), *RET* (NM_020630.6), *ROS1* translocations (NM_002944.3), *TP53* (NM_000546.6) (Lung cancer) (Molecular Pathology version 3)
Methods	**DNA isolation:** DNA from tumor tissue was isolated at CeGaT GmbH. Macrodissection prior to DNA isolation, and examination of tumor tissue by a pathologist were performed, if necessary. The pathological services (confirmation of the histological diagnosis and determination of the tumor content) were carried out on our behalf by a specialist in pathology. Pathology services are not within the scope of the ISO 15189 accreditation. **Sample quality:** The suitability of a sample for molecular genetic analysis depends on the tumor content as well as on the overall material quality (e.g. impairment of quality by chemical or physical stress due to fixation, Arreaza et al., 2016 PMID: 27657050; Einaga et al., 2017, PMID: 28498833; Jones et al., 2019, PMID: 31061401). In cases with low material quality the detection of variants and fusions/structural variations may be impaired or even impossible. **NGS-laboratory:** Protein-coding regions, as well as flanking intronic regions and additional disease-relevant non-coding regions, were enriched using in-solution hybridization technology, and were 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. Read duplicates that likely result from PCR amplification were removed. The remaining high-quality sequences 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 ≥ 5% in the tumor sample within the coding regions and their adjacent intronic regions (-/+ 8 base pairs) were evaluated. A list of all the variants with an allele frequency of 5% considered in the genetic data evaluation can be requested at any time. 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. In this case, > 99.9% of the targeted regions were covered by a minimum of 70 high-quality sequencing reads per base. The tumor content estimated by the pathologist was 50%. Therefore, somatic variants occur at a calculated NAF of around 25%. A theoretical sensitivity of >99% can be obtained for variants with a NAF ≥25% when a coverage of 43 reads per base is achieved. 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.

Prediction of structural variants: Genomic regions known to be involved in translocation, gene fusion or large insertion/deletion events are additionally enriched during the sequencing process. The alignment data is bioinformatically analysed for potential structural variants by identifying discordant read pairs and split reads (Chen et al., 2016, PMID: 26647377). Regions of interest are visually reviewed and possible structural variants are manually annotated. Please note that targets evaluated for the occurrence of relevant structural variants only represent a selection of hot spots frequently mutated. The absence of reported structural variants therefore does not ultimately guarantee the absence of structural variants.

Structural variants potentially affecting the following genes are being assessed:
NTRK1, *FGFR1*, *FGFR2*, *FGFR3*, *BRAF*, *ALK*, *RET*, *MET*, *ROS1*

Exon numbers referring to coding exons in a given transcript.

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 20% was taken as a basis.

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.

EGFR, c.2235_2249del; p.Glu746_Ala750del, NM_005228.5:

Relevant therapeutics for gene *EGFR*

Drug name	Tumor entity	Approval	Approval limited to biomarkers/others	Approval in combination with other drugs
Mobocertinib 3rd generation EGFR TKI EGFR inhibitor EGFR/HER inhibitor	**Non-small cell lung carcinoma**	**FDA**	**EGFR exon 20 insertion mutation** adult, locally advanced or metastatic, disease progression on or after platinum-based chemotherapy	
Osimertinib 3rd generation EGFR TKI EGFR inhibitor EGFR/HER inhibitor	**Non-small cell lung carcinoma**	**EMA**	**- for the adjuvant treatment after complete tumour resection in adult patients with stage IB-IIIA non-small cell lung cancer (NSCLC) whose tumours have EGFR exon 19 deletions or exon 21 (L858R) substitution mutations.** **- for the first-line treatment of adult patients NSCLC with activating EGFR mutations.** **- for the treatment of adult patients with locally advanced or metastatic EGFR T790M mutation-positive NSCLC.**	
		FDA	**- as adjuvant therapy after tumor resection in adult patients with non-small cell lung cancer (NSCLC) whose tumors have epidermal growth factor receptor (EGFR) exon 19 deletions or exon 21 L858R mutations, as detected by an FDA-approved test.** **- for the first-line treatment of adult patients with metastatic NSCLC whose tumors have EGFR exon 19 deletions or exon 21 L858R mutations, as detected by an FDA-approved test.** **- for the treatment of adult patients with metastatic EGFR T790M mutation positive NSCLC, as detected by an FDA-approved test, whose disease has progressed on or after EGFR TKI therapy.**	
Cetuximab EGFR inhibitor EGFR/HER inhibitor	Colon cancer	EMA	**EGFR expression, RAS wildtype** metastatic, - in combination with irinotecan-based chemotherapy or FOLFOX; - as a single agent in patients who have failed oxaliplatin- and irinotecan-based therapy and who are intolerant to irinotecan.	Irinotecan, 5-FU based chemotherapy
		FDA	**EGFR expression, KRAS wildtype** metastatic - in combination with FOLFIRI for first-line treatment, - in combination with irinotecan in patients who are refractory to irinotecan-based chemotherapy - as a single-agent in patients who have failed oxaliplatin- and irinotecan-based chemotherapy or who are intolerant to irinotecan.	Irinotecan, 5-FU based chemotherapy
	Neoplasm of head and neck	EMA	- in combination with radiation therapy for locally advanced disease. - in combination with platinum-based chemotherapy for recurrent and/or metastatic disease.	Carboplatin, Cisplatin, Oxaliplatin
		FDA	- Locally or regionally advanced squamous cell carcinoma of the head and neck in combination with radiation therapy. - Recurrent locoregional disease or metastatic squamous cell carcinoma of the head and neck in combination with platinum-based therapy with fluorouracil. - Recurrent or metastatic squamous cell carcinoma of the head and neck progressing after platinum-based therapy.	Carboplatin, Cisplatin, Oxaliplatin, Fluorouracil (5-FU)

Drug name	Tumor entity	Approval	Approval limited to biomarkers/others	Approval in combination with other drugs
Erlotinib EGFR inhibitor EGFR/HER inhibitor	**Non-small cell lung carcinoma**	**EMA**	**- first-line treatment of patients with locally advanced or metastatic non-small cell lung cancer (NSCLC) with EGFR activating mutations** **- switch maintenance treatment in patients with locally advanced or metastatic non-small cell lung cancer with EGFR activating mutations and stable disease after first-line chemotherapy.** **- treatment of patients with locally advanced or metastatic NSCLC after failure of at least one prior chemotherapy regimen.** **- tumors without EGFR activating mutations, indicated when other treatment options are not considered suitable.**	
		FDA	**EGFR exon 19 deletion or L858R** locally advanced or metastatic NSCLC, treatment after progression following at least one prior chemotherapy regimen	
Gefitinib EGFR inhibitor EGFR/HER inhibitor	**Non-small cell lung carcinoma**	**EMA**	**EGFR activating mutation** adult patients, locally advanced or metastatic NSCLC	
		FDA	**EGFR exon 19 deletion or L858R** first-line treatment, metastatic	
Necitumumab EGFR inhibitor EGFR/HER inhibitor	**Non-small cell lung carcinoma**	**FDA**	**metastatic squamous non-small cell lung cancer**	**Cisplatin, Gemcitabin**
Panitumumab EGFR inhibitor EGFR/HER inhibitor	Colon cancer	EMA	**RAS wildtype** adult patients, metastatic disease - in first-line in combination with FOLFOX or FOLFIRI - in second-line in combination with FOLFIRI for patients who have received first-line fluoropyrimidine-based chemotherapy (excluding irinotecan).	FOLFOX, FOLFIRI
		EMA	**RAS wildtype** adult patients, metastatic disease, monotherapy after failure of fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy regimens	
		FDA	**RAS wildtype** metastatic	
Afatinib EGFR inhibitor EGFR/HER inhibitor HER2 inhibitor HER4 inhibitor	**Non-small cell lung carcinoma**	**EMA**	**EGFR activating mutation** -adult, EGFR TKI-naïve, locally advanced or metastatic NSCLC -adult, locally advanced or metastatic NSCLC of squamous histology progressing on or after platinum-based chemotherapy	
		FDA	**metastatic squamous NSCLC, progressed after treatment with platinum-based chemotherapy**	
		FDA	**EGFR exon 19 deletions, exon 21 L858R, S768I, L861Q, G719X** metastatic	
Dacomitinib EGFR inhibitor EGFR/HER inhibitor HER2 inhibitor HER4 inhibitor	**Non-small cell lung carcinoma**	**EMA**	**EGFR activating mutation** first-line, adult patients, locally advanced or metastatic NSCLC	
		FDA	**EGFR exon 19 deletion or L858R** metastatic NSCLC	
Lapatinib EGFR inhibitor EGFR/HER inhibitor HER2 inhibitor HER4 inhibitor	Breast cancer	EMA	**HER2 overexpression** adult patients, HR-negative metastatic disease, progressed on prior trastuzumab therapy(ies)	Trastuzumab
		EMA	**HER2 overexpression** adult patients, advanced or metastatic, progression under prior treatment which must have included anthracyclines and taxanes and therapy with trastuzumab in the metastatic setting	Capecitabine
		EMA	**HER2 positive** postmenopausal women with hormone receptor positive metastatic disease, not currently intended for chemotherapy	Exemestane, Letrozole, Anastrozole
		FDA	**HER2 overexpression** advanced or metastatic disease, following prior therapy including an anthracycline, a taxane, and trastuzumab; disease progression on trastuzumab	Capecitabine

Drug name	Tumor entity	Approval	Approval limited to biomarkers/others	Approval in combination with other drugs
Neratinib EGFR inhibitor EGFR/HER inhibitor HER2 inhibitor HER4 inhibitor	Breast cancer	FDA	**HER2 overexpression** metastatic disease, postmenopausal women, hormonal therapy indicated	Letrozole
		EMA	**HER2 positive, HR positive** adjuvant treatment, adult patients with early breast cancer, following treatment with trastuzumab	
		FDA	**HER2 positive** adult patients, extended adjuvant treatment, early stage, to follow adjuvant trastuzumab-based therapy	
		FDA	**HER2 positive** adult, advanced or metastatic disease, two or more prior anti-HER2 based regimens in the metastatic setting	Capecitabine
Amivantamab EGFR inhibitor EGFR/HER inhibitor HGF/MET inhibitor	**Non-small cell lung carcinoma**	**EMA**	**EGFR Exon 20 insertion mutation** adult, locally advanced or metastatic, after failure of platinum-based chemotherapy	
		FDA	**EGFR Exon 20 insertion mutation** adult, locally advanced or metastatic, progress after platinum-based chemotherapy	
Vandetanib EGFR inhibitor EGFR/HER inhibitor RET inhibitor VEGF/VEGFR inhibitor	Thyroid carcinoma	EMA	aggressive and symptomatic medullary thyroid cancer (MTC) in patients with unresectable locally advanced or metastatic disease, age ≥5 years, RET mutation not known or is negative, a possible lower benefit should be taken into account before individual treatment decision	
		FDA	symptomatic or progressive medullary thyroid cancer, unresectable (non-operable) locally advanced or metastatic disease	