Order no.: documented by:

Order received: DD/MM/YYYY

Sample type: FFPE tissue, block

Sample collection date: DD/MM/YYYY

Report date: DD/MM/YYYY

Report type: Final Report

Patient no.: , First Name: , Last Name:
DOB: **DD/MM/YYYY**, Sex: , Your ref.:

Additional report recipient(s):

Test(s) requested: Solid tumor panel

CLINICAL INFORMATION

Lung adenocarcinoma; Non-small cell lung carcinoma
(Clinical information indicated above follows HPO nomenclature.)

Family history: Unknown.
Consanguineous parents: No.

➕ ACTIONABLE VARIANT IDENTIFIED (TIER 1)

INTERPRETATION

A likely pathogenic variant was identified in the *EGFR* gene at high allele frequency. **The identified variant is a predictive biomarker for primary sensitivity of non-small cell lung carcinoma to treatment with afatinib, crizotinib, erlotinib, gefitinib, osimertinib, dacomitinib, cetuximab, and pembrolizumab (FDA and/or NCCN approved guidelines).**

Furthermore, likely pathogenic variants were identified in the *TP53* and *RB1* genes at low allele frequencies. These variants are considered driver mutations of cancer development. However, these variants are not a known FDA or NCCN-approved biomarker for a targeted therapy of the present type of cancer.

In the remainder of the panel genes (see methods), no other actionable, or otherwise clinically relevant variant was identified.

RECOMMENDATIONS

- Oncology counseling is recommended for targeted therapies.
- Genetic counseling is recommended to explain the given results.

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RESULT SUMMARY

GENE	VARIANT	FREQUENCY	CLASSIFICATION
EGFR	**Chr7(GRCh37):g.55242483_55242506del NM_005228.3:c.2253_2276del p.(Ser752_Ile759del)**	**82.3% of 5535 NGS reads**	**Likely pathogenic Tier 1 COSM13556**
RB1	Chr13(GRCh37):g.49039339A>T NM_000321.2:c.2326-2A>T	21.6% of 1060 NGS reads	Likely pathogenic Tier 2
TP53	Chr17(GRCh37):g.7576855del NM_000546.4:c.991del p.(Gln331Argfs*14)	36.9% of 923 NGS reads	Likely pathogenic Tier 2

VARIANT INTERPRETATION

EGFR, c.2253_2276del p.(Ser752_Ile759del)

The *EGFR* variant c.2253_2276del p.(Ser752_Ile759del) is an in-frame deletion of 24 bps in exon 19, which causes the loss of 8 residues. Exon 19 deletions are known drivers of lung cancer development. In the COSMIC database this variant is listed for tumor samples derived from primary lung tissue (COSM13556) It is classified as likely pathogenic (class 2) according to the recommendations of CENTOGENE and ACMG (please, see additional information below). **The identified variant is a predictive biomarker for primary sensitivity of non-small cell lung carcinoma to treatment with afatinib, crizotinib, erlotinib, gefitinib, osimertinib, dacomitinib, cetuximab, and pembrolizumab (FDA and/or NCCN approved guidelines).**

Afatinib, may be used as single agent (FDA, NCCN), or may be considered in combination with cetuximab after progression on afatinib, erlotinib, gefitinib, or dacomitinib, and chemotherapy (NCCN).

Erlotinib may be used as single agent (FDA, NCCN), or in combination with bevacizumab (non-squamous only) or ramucirumab (NCCN).

Osimertinib is preferred first-line therapy, per NCCN, Pembrolizumab, for PD-L1 expressing tumors (Tumor Proportion Score >=1%, clone 22C3) with an *EGFR* or *ALK* alteration: FDA-approved as subsequent line of therapy following targeted therapy. However, per NCCN, data in the second-line setting suggest that subsequent pembrolizumab monotherapy is less effective in tumors with an *EGFR* mutation or *ALK* rearrangement (FDA guidelines).

For the *EGFR* sensitizing mutations afatinib + cetuximab combination, per NCCN, may be considered after progression on afatinib, erlotinib, gefitinib, or dacomitinib, and chemotherapy (NCCN guidelines).

For the *EGFR* sensitizing mutations afatinib + crizotinib; crizotinib + dacomitinib; crizotinib + erlotinib; crizotinib + gefitinib; crizotinib + osimertinib combinations are used to overcome acquired resistance. To overcome resistance by secondary mutational events like MET amplification, *EGFR* must still be inhibited and additionally, a MET inhibitor must be added to the treatment (NCCN guidelines).

RB1, c.2326-2A>T

The *RB1* variant c.2326-2A>T is predicted to disrupt the highly conserved acceptor splice site of intron 22. It is classified as likely pathogenic (class 2) according to the recommendations of CENTOGENE and ACMG (please, see additional information below). This variant is considered driver mutation of cancer development. However, the variant is not a known FDA or NCCN-approved biomarker for a targeted therapy of the present type of cancer.

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TP53, c.991del p.(Gln331Argfs*14)

The *TP53* variant c.991del p.(Gln331Argfs*14) creates a shift in the reading frame starting at codon 331. The new reading frame ends in a stop codon 13 positions downstream. It is classified as likely pathogenic (class 2) according to the recommendations of CENTOGENE and ACMG (please, see additional information below). However, the variant is not a known FDA or NCCN-approved biomarker for a targeted therapy of the present type of cancer.

Tumor protein p53 (*TP53*) is a gene that codes for a tumor suppressor protein, cellular tumor antigen p53. The protein regulates expression of genes involved in cell cycle arrest, apoptosis, senescence, DNA repair, and changes in metabolism. *TP53* is the most frequently mutated gene in cancer; it is mutated in about half of all cancers. *TP53* is most frequently mutated in ovarian, colon, and esophageal cancers, although it is significantly mutated in many other cancer types.

CENTOGENE VARIANT CLASSIFICATION (BASED ON ACMG RECOMMENDATIONS)

Class 1 – Pathogenic

Class 2 – Likely pathogenic

Class 3 – Variant of uncertain significance (VUS)

Class 4 – Likely benign

Class 5 – Benign

Additionally, other types of clinically relevant variants can be identified (e.g. risk factors, modifiers).

ADDITIONAL TIERING OF SOMATIC VARIANTS IN TUMORS (BASED ON AMP RECOMMENDATIONS)

Somatic variants can be additionally categorized according to actionability specific for primary tumor site or histology. The level of current clinical and/or experimental evidence for the detected variant regarding therapeutic options, prognosis and/or diagnosis allows categorization (based on AMP recommendations):

Tier 1 – strong clinical significance
FDA-approved studies included in professional guidelines or well-powered studies with consensus from experts in the field

Tier 2 – potential clinical significance
multiple small studies with some consensus, or proved strong clinical significance/actionability for different tumor types or only preclinical trials of few case reports without consensus

Tier 3 – unknown clinical significance

Tier 4 – (likely) no clinical significance

METHODS

Genomic DNA is enzymatically fragmented and DNA capture probes targeting the coding regions of the panel genes and known relevant hotspot regions for solid tumors (gene list is described below). The libraries are subsequently sequenced on an Illumina platform to achieve at least 200x depth of coverage for 97% of the target region.

Unless otherwise stated, the coding regions and +/- 2 bp of flanking intronic sequences of the Solid tumor panel genes (*ABL1, AKT1, AKT2, AKT3, APC, AR, ARID1A, ATM, ATR, ATRX, BAP1, BRAF, BRCA1, BRCA2, CDH1, CDK12, CDK4, CDKN1B, CDKN2A, CDKN2B, CHEK1, CHEK2, CREBBP, CSF1R, CTNNB1, DDR2, EGFR, ERBB2, ERBB3, ERBB4, EZH2, FANCA, FANCD2, FANCI, FBXW7, FGFR1, FGFR2, FGFR3, FGFR4, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, KDR, KEAP1, KIT, KMT2A, KMT2C, KMT2D, KRAS, MAP2K1, MAP2K2, MEN1, MET, MLH1, MPL, MRE11, MSH2, MSH6, MTOR, NBN, NF1, NF2, NFE2L2, NOTCH1, NOTCH2, NOTCH3, NRAS, NTRK3, PALB2, PDGFRA, PIK3CA, PIK3R1, PMS2, POLE, PTCH1, PTEN, PTPN11, RAD50, RAD51, RAD51B, RAD51C, RAD51D, RB1, RBM10, RET, RIT1, RNF43, SETD2, SLX4, SMAD4, SMARCA4, SMARCB1, SMO, SPOP, SRC, STK11, TP53, TSC1, TSC2, TSHR, VHL*) are targeted as well as the hotspot regions for the following genes (*ALK, ARAF, AXL, BTK, CBL, CCND1, CDK6, ERCC2, ESR1, FLT3, FOXL2, GATA2, H3F3A, HIST1H3B, JAK1, JAK2, JAK3, KNSTRN, MAGOH, MAP2K4, MAPK1, MAX, MDM4, MED12, MYC, MYCN, MYD88, NTRK1, NTRK2, PDGFRB, PIK3CB, PPP2R1A, RAC1, RAF1, RHEB, RHOA, ROS1, SF3B1, STAT3, TERT, TOP1, U2AF1, XPO1*) are targeted.

Raw sequence data analysis, including base calling, demultiplexing, alignment to the hg19 human reference genome (Genome Reference Consortium GRCh37) and variant calling (single nucleotide variants and InDels) is performed using validated in-house software. All identified variants are evaluated with respect to their pathogenicity and causality and categorized into classes (see above). Tumor-specific (potentially) actionable and clinically relevant variants are reported. Analysis includes but is not restricted to mycancergenome.org and cancergenomeinterpreter.org. CENTOGENE has established stringent quality criteria and validation processes for variants detected by NGS. Relevant variants with low quality are confirmed by Sanger sequencing. Consequently, we warrant a specificity of >99.9% for all reported variants.

99.53% of the target base pairs of the analyzed genes were covered ≥200x, with an overall average coverage of 1103.24x.

Clinically relevant variants are reported at a read depth of ≥ 50 reads and variant frequency of ≥ 5%. Please note that we cannot exclude allele drop off, which can be caused by normal genomic variation in the patient sample may interfere with mutation detection.

LIMITATIONS

The genetic results are interpreted in the context of the provided clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the provided information is inaccurate and/or incomplete. The used method is not designed to, and therefore cannot, detect complex genetic events such as inversions, translocations, and repeat expansions. The sensitivity is decreased for repetitive and homologous regions, such as pseudogenes. Due to technology limitations, certain regions may be either not or poorly covered. In these regions variants cannot be confidently detected. Extremely low coverage calls (homo/hemizygous or heterozygous calls with less than three or four reads, respectively) are expected to be artifacts based on our extensive validations and are consequently not considered during the analysis.

Genomic DNA is enzymatically fragmented and DNA capture probes targeting the coding regions of the panel genes and known relevant hotspot regions for solid tumors (gene list is described below). The libraries are subsequently sequenced on an Illumina platform to achieve at least 200x depth of coverage for 97% of the target region.

ADDITIONAL INFORMATION

This test was developed, and its performance validated by CENTOGENE GmbH. The US Food and Drug Administration (FDA) has determined that clearance or approval of this method is not necessary and thus neither have been obtained. This test has been developed for clinical purposes. All test results are reviewed, interpreted and reported by our scientific and medical experts.

The classification of variants of uncertain clinical significance can change over the time. Please feel free to contact CENTOGENE (customer.support@centogene.com) in the future to determine if there have been any changes in classification of these variants. CENTOGENE performs confirmatory testing by an independent DNA aliquot in all cases with a mutation (class 1), in all cases with a likely pathogenic variant (class 2) and in most cases with variants of uncertain significance (class 3). We will only contact you if the results are inconsistent. To also exclude mistaken identity in your clinic, several guidelines recommend testing a second sample that is independently obtained from the proband. Please note that any further analysis will result in additional costs. If you would like to enquire about additional analyses, please do not hesitate to contact us (customer.support@centogene.com).

DISCLAIMER

Any preparation and processing of a sample from patient material provided to CENTOGENE by a physician, clinical institute, or a laboratory (by a "Partner") and the requested genetic and/or biochemical testing itself is based on the highest and most current scientific and analytical standards. However, in very few cases genetic or biochemical tests may not show the correct result, e.g., because of the quality of the material provided by a Partner to CENTOGENE or in cases where any test provided by CENTOGENE fails for unforeseeable or unknown reasons that cannot be influenced by CENTOGENE in advance. In such cases, CENTOGENE shall not be responsible and/or liable for the incomplete, potentially misleading or even wrong result of any testing if such issue could not be recognized by CENTOGENE in advance.

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