CeGaT

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

# CancerFusionRX report – Doe, Jane (*DD.MM.YYYY)

**Indication** **Colorectal cancer**

## Results

- **Detection of a therapeutically relevant fusion of the genes *TPM3* and *NTRK1*.**

**Fusions/structural variants with potential therapeutic relevance:**

| Gene | Functional category | Variant | Effect on protein function | Therapeutic option for discussion in the MTB | Approved by EMA/FDA | Approved for current entity |
|---|---|---|---|---|---|---|
| *TPM3-NTRK1* chr1:154142876-chr1:156844363 | fusion (inversion) | *TPM3* exon 8 (NM_152263.4) - *NTRK1* exon 10 (NM_001012331.2) | activating | NTRK inhibitor | EMA* & FDA* | EMA* & FDA* |

The influence of the detected structural variant on the function of the protein was classified into the following categories: activating/function altered, likely activating/function altered and unknown based on currently available data (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.**

## 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

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|---|---|
| **Order** | **Somatic molecular genetic analysis of a tumor tissue sample:**<br>RNA fusion-panel-analysis STR, evaluation of somatic variants of potential clinical relevance |
| **Sample material** | **Tumor tissue: Sample of the known colorectal carcinoma**<br>Sample collection DD.MM.YYYY<br>RNA isolation from tumor in FFPE (FFPE-ID: XXXX/YY) after macrodissection with estimated tumor content of 80% (HE staining) |
| **Sample receipt** | DD.MM.YYYY (Tumor-FFPE) |
| **Structural variants** | RNA fusion panel (STR) contains interpretation of translocations/fusions of the following cancer-relevant genes:<br><br>*ABL1*, *AFAP1*, *AGK*, *AKAP12*, *AKAP4*, *AKAP9*, *AKT2*, *AKT3*, *ALK*, *ASPSCR1*, *BAG4*, *BCL2*, *BCORL1*, *BCR*, *BICC1*, *BRAF*, *BRD3*, *BRD4*, *CCAR2*, *CCDC6*, *CD74*, *CIC*, *CLTC*, *CNTRL*, *COL1A1*, *CRTC1*, *DDIT3*, *EGFR*, *EML4*, *ERBB2*, *ERBB4*, *ERG*, *ESR1*, *ETV1*, *ETV4*, *ETV5*, *ETV6*, *EWSR1*, *EZR*, *FGFR1*, *FGFR2*, *FGFR3*, *FLI1*, *FN1*, *FUS*, *GOPC*, *JAZF1*, *KIAA1549*, *KIF5B*, *MAGI3*, *MAML1*, *MET*, *MGA*, *MYB*, *MYC*, *NAB2*, *NCOA4*, *NFIB*, *NOTCH2*, *NPM1*, *NRG1*, *NSD3*, *NTRK1*, *NTRK2*, *NTRK3*, *NUTM1*, *PAX3*, *PAX7*, *PAX8*, *PDGFB*, *PDGFRB*, *PIK3CA*, *PLAG1*, *PML*, *POU5F1*, *PRKAR1A*, *QKI*, *RAF1*, *RARA*, *RET*, *ROS1*, *SDC4*, *SHTN1*, *SLC34A2*, *SND1*, *SQSTM1*, *SS18*, *SSX1*, *STAT6*, *STRN*, *SUZ12*, *TACC1*, *TACC3*, *TAF15*, *TFE3*, *TFG*, *THADA*, *TMPRSS2*, *TPM3*, *TPR*, *TRIM24*, *TRIM33*, *WT1*, *YAP1*, *ZMYM2*, *ZNF703* (Structural Variants Panel version 6)<br><br>Selected break points within these fusion genes:<br><br>*TRIM24-BRAF, KIAA1549-BRAF, SND1-BRAF, EML4-ALK, CLTC-ALK, NPM1-ALK, TPM3-ALK, KIF5B-ALK, ETV6-NTRK3, EWSR1-ERG, EWSR1-FLI1, FGFR3-TACC3, FGFR2-BICC1, FGFR2-TACC3, FGFR1-TACC1, TMPRSS2-ERG, TPM3-NTRK1, TPR-NTRK1, TRIM24-NTRK2, AFAP1-NTRK2, QKI-NTKR2, ETV6-NTRK2, KIF5B-RET, CCDC6-RET, NCOA4-RET, PRKAR1A-RET, TRIM33-RET, CD74-ROS1, EZR-ROS1, SLC34A2-ROS1, TPM3-ROS1, SDC4-ROS1, BRD4-NUTM1, BRD3-NUTM1, MGA-NUTM1, NSD3-NUTM1, NAB2-STAT6*<br><br>Specific transcript variants:<br><br>*EGFR* del ex2-3, *EGFR* del ex2-4, *EGFR* del ex2-14, *EGFR* del ex2-22 (mLEEK), *EGFR* del ex5-6, *EGFR* del ex6-7, *EGFR* del ex9, *EGFR* del ex9-10, *EGFR* del ex10, *EGFR* del ex12, *EGFR* del ex25-26, *EGFR* del ex25-27, *EGFR* del ex26-27, *EGFR* VII, *EGFR* VIII, *MET* ex14 skipping |
| **Methods** | **RNA isolation:** The isolation of RNA was performed at CeGaT GmbH. Macrodissection prior to RNA isolation was performed, if necessary. The tumor material was assessed by a pathology specialist.<br><br>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.<br><br>**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). |

**NGS-laboratory:** RNA from tumor tissue was sequenced. Fusion transcripts were enriched using in-solution hybridization technology. For fusion transcripts with known breakpoints, breakpoint spanning probes were used. For genes with unknown breakpoints or a large number of possible fusion partners, the coding sequence was used for enrichment. Sequencing was performed on Illumina NovaSeq6000 systems.

**Computational analysis:** Sequencing data was demultiplexed using bcl2fastq2. Adapter sequences were removed using Skewer and the resulting reads were mapped to the human reference genome hg19 using STAR aligner. Fusions were detected using the software STAR-Fusion (Haas et al., 2017). Additional intragene structural events in genes *EGFR* and *MET* were extracted from STAR output.

**Genetic data evaluation:** The sensitivity of the test is dependent on the tumor content of the analyzed material, the sample quality, and the amount of transcripts sequenced. In this case, an amount of 14.66 gigabases RNA was sequenced. Therefore, this analysis is appropriate to detect structural variants on RNA level.

**Variant classification:** The structural variants were assessed with respect to their possible impact on function of the fusion protein based upon the available data (i.e. FASMIC, PubMed research). The functional categories assigned are: activating, function altered, likely activating/function altered, or unknown. "Activating" and "function altered": known activating/function changing structural variants. The functional evidence of structural variants classified as activating and function altered is highly reliable, indicated by *in vivo/in vitro* analyses). "Likely activating/function altered": an impact of the structural variant on protein function is considered to be likely with respect to the genes/breakpoints within the described regions, the currently available literature, and frequency in tumor samples. But there are insufficient functional data available. "Unknown": based upon the available data, we are not able to conclusively confirm nor exclude the possible functional relevance of the structural variant.

**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.



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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.

### *TPM3-NTRK1* fusion (inversion), exon 8 (NM_152263.4), exon 10 (NM_001012331.2):

**Relevant therapeutics for fusion gene *TPM3-NTRK1***

| Drug name | Tumor entity | Approval | Approval limited to biomarkers/others | Approval in combination with other drugs |
|---|---|---|---|---|
| **Larotrectinib**<br>NTRK inhibitor | **Neoplasm** | **EMA** | **NTRK gene fusion**<br>adult and pediatric patients, solid tumors, locally advanced, metastatic or inoperable, no other satisfactory treatment options | |
| | | **FDA** | **NTRK gene fusion**<br>adult and pediatric patients, solid tumors, metastatic or inoperable, no satisfactory alternative treatments or progress following treatment | |
| **Entrectinib**<br>NTRK inhibitor<br>ROS1 inhibitor<br>ROS1/ALK inhibitor | **Neoplasm** | **EMA** | **NTRK gene fusion**<br>adult and pediatric patients 12 years of age and older with solid tumors, locally advanced or metastatic disease or where surgical resection is likely to result in severe morbidity, no prior treatment with a NTRK inhibitor, no satisfactory treatment options | |
| | | **FDA** | **NTRK gene fusion**<br>adult and pediatric patients 12 years of age and older with solid tumors, metastatic disease or surgical resection is likely to result in severe morbidity, progression following treatment or no satisfactory alternative therapy | |