**Targeted Oncology**

**Supplemental Materials**

# Clinical Characteristics and Responses to Immune Checkpoint Inhibitors in *RET*-aberrant Digestive Tract Tumours

Chia-Jui Yen, MD., PhD.

Department of Oncology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan

yencj@mail.ncku.edu.tw

No. 138, Sheng-Li Road, Tainan 70403, Taiwan

Tel: +886-6-235-3535 ext. 6550

ORCID: 0000-0001-8744-6248

**Supplemental Note: introductory information about FoundationOne CDx**

## About the test

FoundationOne®CDx (F1CDx) is a next generation sequencing-based *in vitro* tumor assay from the FoundationOne panel, which is capable to detect genomic substitutions, insertions/deletions, copy number variations and selected gene rearrangements via targeted high throughput hybridization-based capture technology for clinical judgement, genomic characterization and therapeutic evaluations in oncological medicine. It also provides valid information on genomic signatures such as microsatellite instability (MSI) and tumor mutation burden (tMB), which may facilitate the decision and efficacy on antineoplastic immunotherapy. F1CDx is approved as a companion diagnostic by the US Food and Drug Administration in the year 2020 (medical device P170019/S017). The assayed DNA materials are derived from formalin-fixed paraffin-embedded (FFPE) tumor specimens, either as fresh or archival tissue samples, and can be integrated or compared with other blood or body fluid-based tumor genomic testing.

## Test principle

F1CDx employs a single DNA extraction method derived from surgical or biopsied FFPE tumor specimens. Briefly, the tumor DNA of 50 to 1,000 ng are processed with whole-genome shotgun library construction and hybridization-based capture of all coding exons from 309 cancer-related genes, one promoter region, one non-coding RNA and selective intronic regions from 34 commonly rearranged genes. In summary, the assay comprises of 324 targeted cancer-related genes and utilizes Illumina® HiSeq 4000 platform to conduct the hybrid capture–selected sequencing with a uniform read depth at least 500 times as a median coverage and at least 100 times in over 99% of the exons sequenced. Sequence data is then processed using a customized analysis pipeline designed to detect all classes of genomic alterations, including base substitutions, indels, copy number alterations (CNAs) (amplifications and homozygous gene deletions) and selective genomic rearrangements/fusions. Additionally, genomic signatures including MSI and tMB, and positive homologous recombination deficiency status are reported. The reference mapped genome library is human genome 19 (hg 19) in F1CDx.

## Test gene list

Genes sequenced with full coding exonic regions for the detection of substitutions, indels, and CNAs

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *ABL1* | *BRAF* | *CDKN1A* | *EPHA3* | *FGFR4* | *IKZF1* | *MCL1* | *NKX2-1* | *PMS2* | *RNF43* | *TET2* |
| *ACVR1B* | *BRCA1* | *CDKN1B* | *EPHB1* | *FH* | *INPP4B* | *MDM2* | *NOTCH1* | *POLD1* | *ROS1* | *TGFBR2* |
| *AKT1* | *BRCA2* | *CDKN2A* | *EPHB4* | *FLCN* | *IRF2* | *MDM4* | *NOTCH2* | *POLE* | *RPTOR* | *TIPARP* |
| *AKT2* | *BRD4* | *CDKN2B* | *ERBB2* | *FLT1* | *IRF4* | *MED12* | *NOTCH3* | *PPARG* | *SDHA* | *TNFAIP3* |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *AKT3* | *BRIP1* | *CDKN2C* | *ERBB3* | *FLT3* | *IRS2* | *MEF2B* | *NPM1* | *PPP2R1A* | *SDHB* | *TNFRSF14* |
| *ALK* | *BTG1* | *CEBPA* | *ERBB4* | *FOXL2* | *JAK1* | *MEN1* | *NRAS* | *PPP2R2A* | *SDHC* | *TP53* |
| *ALOX12B* | *BTG2* | *CHEK1* | *ERCC4* | *FUBP1* | *JAK2* | *MERTK* | *NT5C2* | *PRDM1* | *SDHD* | *TSC1* |
| *AMER1* | *BTK* | *CHEK2* | *ERG* | *GABRA6* | *JAK3* | *MET* | *NTRK1* | *PRKAR1A* | *SETD2* | *TSC2* |
| *APC* | *C11orf30* | *CIC* | *ERRFI1* | *GATA3* | *JUN* | *MITF* | *NTRK2* | *PRKCI* | *SF3B1* | *TYRO3* |
| *AR* | *CALR* | *CREBBP* | *ESR1* | *GATA4* | *KDM5A* | *MKNK1* | *NTRK3* | *PTCH1* | *SGK1* | *U2AF1* |
| *ARAF* | *CARD11* | *CRKL* | *EZH2* | *GATA6* | *KDM5C* | *MLH1* | *P2RY8* | *PTEN* | *SMAD2* | *VEGFA* |
| *ARFRP1* | *CASP8* | *CSF1R* | *FAM46C* | *GID4* | *KDM6A* | *MPL* | *PALB2* | *PTPN11* | *SMAD4* | *VHL* |
| *ARID1A* | *CBFB* | *CSF3R* | *FANCA* | *GNA11* | *KDR* | *MRE11A* | *PARK2* | *PTPRO* | *SMARCA4* | *WHSC1* |
| *ASXL1* | *CBL* | *CTCF* | *FANCC* | *GNA13* | *KEAP1* | *MSH2* | *PARP1* | *QKI* | *SMARCB1* | *WHSC1L1* |
| *ATM* | *CCND1* | *CTNNA1* | *FANCG* | *GNAQ* | *KEL* | *MSH3* | *PARP2* | *RAC1* | *SMO* | *WT1* |
| *ATR* | *CCND2* | *CTNNB1* | *FANCL* | *GNAS* | *KIT* | *MSH6* | *PARP3* | *RAD21* | *SNCAIP* | *XPO1* |
| *ATRX* | *CCND3* | *CUL3* | *FAS* | *GRM3* | *KLHL6* | *MST1R* | *PAX5* | *RAD51* | *SOCS1* | *XRCC2* |
| *AURKA* | *CCNE1* | *CUL4A* | *FBXW7* | *GSK3B* | *KMT2A* | *MTAP* | *PBRM1* | *RAD51B* | *SOX2* | *ZNF217* |
| *AURKB* | *CD22* | *CXCR4* | *FGF10* | *H3F3A* | *KMT2D* | *MTOR* | *PDCD1* | *RAD51C* | *SOX9* | *ZNF703* |
| *AXIN1* | *CD274* | *CYP17A1* | *FGF12* | *HDAC1* | *KRAS* | *MUTYH* | *PDCD1LG2* | *RAD51D* | *SPEN* | |
| *AXL* | *CD70* | *DAXX* | *FGF14* | *HGF* | *LTK* | *MYC* | *PDGFRA* | *RAD52* | *SPOP* | |
| *BAP1* | *CD79A* | *DDR1* | *FGF19* | *HNF1A* | *LYN* | *MYCL* | *PDGFRB* | *RAD54L* | *SRC* | |
| *BARD1* | *CD79B* | *DDR2* | *FGF23* | *HRAS* | *MAF* | *MYCN* | *PDK1* | *RAF1* | *STAG2* | |
| *BCL2* | *CDC73* | *DIS3* | *FGF3* | *HSD3B1* | *MAP2K1* | *MYD88* | *PIK3C2B* | *RARA* | *STAT3* | |
| *BCL2L1* | *CDH1* | *DNMT3A* | *FGF4* | *ID3* | *MAP2K2* | *NBN* | *PIK3C2G* | *RB1* | *STK11* | |
| *BCL2L2* | *CDK12* | *DOT1L* | *FGF6* | *IDH1* | *MAP2K4* | *NF1* | *PIK3CA* | *RBM10* | *SUFU* | |
| *BCL6* | *CDK4* | *EED* | *FGFR1* | *IDH2* | *MAP3K1* | *NF2* | *PIK3CB* | *REL* | *SYK* | |
| *BCOR* | *CDK6* | *EGFR* | *FGFR2* | *IGF1R* | *MAP3K13* | *NFE2L2* | *PIK3R1* | *RET* | *TBX3* | |
| *BCORL1* | *CDK8* | *EP300* | *FGFR3* | *IKBKE* | *MAPK1* | *NFKBIA* | *PIM1* | *RICTOR* | *TEK* | |

Genes sequenced with intronic regions for the detection of gene rearrangements, one with 3'-UTR, one with a promoter region and one ncRNA gene

| | | | | | |
|---|---|---|---|---|---|
| *ALK introns 18, 19* | *CD74 introns 6- 8* | *EZR introns 9- 11* | *MSH2 intron 5* | *NUTM1 intron 1* | *RSPO2 intron 1* |
| *BCL2 3'UTR* | *EGFR introns 7, 15, 24-27* | *FGFR1 intron 1, 5, 17* | *MYB intron 14* | *PDGFRA introns 7, 9, 11* | *SDC4 intron 2* |
| *BCR introns 8, 13, 14* | *ETV4 intron 8* | *FGFR2 intron 1, 17* | *MYC intron 1* | *RAF1 introns 4-8* | *SLC34A2 intron 4* |
| *BRAF introns 7- 10* | *ETV5 introns 6, 7* | *FGFR3 intron 17* | *NOTCH2 intron 26* | *RARA intron 2* | *TERC ncRNA* |
| *BRCA1 introns 2, 7, 8, 12, 16, 19, 20* | *ETV6 introns 5, 6* | *KIT intron 16* | *NTRK1 introns 8-11* | *RET introns 7-11* | *TERT Promoter* |
| *BRCA2 intron 2* | *EWSR1 introns 7-13* | *KMT2A introns 6-11* | *NTRK2 Intron 12* | *ROS1 introns 31-35* | *TMPRSS2 introns 1- 3* |

**Limit of detections**

A subclonal change is noted when the alteration is present in a variant of allele frequency (VAF) less than 10% of the assayed tumor DNA. The copy number threshold for F1CDx is 5 times for *ERBB2* and 6 times all other genes. In addition, the MSI designation is based on genome-wide assay of 95 microsatellite loci. The threshold for a high instability is determined by analytical concordance to other comparator diagnostics, such as immunohistochemistry or qualitative polymerase chain reaction but the clinical confirmation has not been fully established by other orthogonal methods. In addition, the tMB is defined as the counting the total number of both synonymous and non-synonymous variants with at least 5% allele frequency and reported as mutations per megabase (mut/Mb). Conversely, the clinical validity has not been determined in conjunction with other orthogonal methods, such as whole genome-side sequencing of substitutions or variations.

**Verification of the test panel**

The referral laboratory and database unit of the tested panel were certified by Clinical Laboratory Improvement Amendments (Foundation Medicine) and approved as a diagnostic companion by the US Food and Drug Administration (FoundationOne CDx).

**Source of technical information**

Foundation Medicine, Inc.

150 Second Street, Cambridge, MA 02141

Phone: 617.418.2200

FOUNDATIONONE® CDx

## References


1. https://www.foundationmedicine.com/test/foundationone-cdx
2. Li M. Statistical Methods for Clinical Validation of Follow-On Companion Diagnostic Devices via an External Concordance Study. Statistics in Biopharmaceutical Research 8, 355-363 (2016).
3. FoundationOne®CDx Technical Information. US-FDX-2000037 V5.0 RAL-0081

**Supplemental Table 1: STROBE assessment of the study**

| | Item No. | Recommendation | Page No. | Relevant text from manuscript |
|---|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | p.1 | Title is provided. |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | p.3 | Abstract in provided. |
| **Introduction** | | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | p.6 | Line 97-109 (3rd paragraph), Introduction |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | p.6 | Line 97-109 (3rd paragraph), Introduction |
| **Methods** | | | | |
| Study design | 4 | Present key elements of study design early in the paper | p.6-7 | Line 112-129 (paragraph "Patient"), Materials and Methods |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | p.7 | Line 112-129 (paragraph "Patient"), Materials and Methods |
| Participants | 6 | (*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up<br>*Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls<br>*Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants | p.7 | Line 112-129 (paragraph "Patient"), Materials and Methods |
| | | (*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed<br>*Case-control study*—For matched studies, give matching criteria and the number of controls per case | Not applicable | |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | p.7-9 | Line 131-170 (paragraph "Clinical characteristics and treatment", |

| | | | | |
|---|---|---|---|---|
| | | | | "Tumour-based selective genome sequencing"), Materials and Methods |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | p.7-9 | Line 131-170 (paragraph "Clinical characteristics and treatment", "Tumour-based selective genome sequencing"), Materials and Methods |
| Bias | 9 | Describe any efforts to address potential sources of bias | p.9 | Line 172-188 (paragraph "Statistical consideration"), Materials and Methods |
| Study size | 10 | Explain how the study size was arrived at | Not applicable | |
| | | | | |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | p.7-9 | Line 131-170 (paragraph "Clinical characteristics and treatment", "Tumour-based selective genome sequencing"), Materials and Methods |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | p.9 | Line 172-188 (paragraph "Statistical consideration"), Materials and Methods |
| | | (*b*) Describe any methods used to examine subgroups and interactions | p.9 | Line 172-188 (paragraph "Statistical consideration"), Materials and Methods |
| | | (*c*) Explain how missing data were addressed | p.9 | Missing data were not imputed. Line 172-188 (paragraph "Statistical consideration"), Materials and Methods |
| | | (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed | Not applicable | Retrospective study |

| | | | | |
|---|---|---|---|---|
| | | *Case-control study*—If applicable, explain how matching of cases and controls was addressed<br><br>*Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy | | |
| | | (*e*) Describe any sensitivity analyses | Not applicable | |
| **Results** | | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | p.9-10 | Line 191-209 (paragraph "Patient and clinical characteristics"), Results<br><br>Figure 1 |
| | | (b) Give reasons for non-participation at each stage | p.9-10 | Line 191-209 (paragraph "Patient and clinical characteristics"), Results<br><br>Figure 1 |
| | | (c) Consider use of a flow diagram | Figure 1 | Flow diagram |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | p.9-10 | Line 191-209 (paragraph "Patient and clinical characteristics"), Results<br><br>Table 1 |
| | | (b) Indicate number of participants with missing data for each variable of interest | Not applicable | Missing data were not imputed nor analysed. |
| | | (c) *Cohort study*—Summarise follow-up time (eg, average and total amount) | p.12 | Median follow-up time |

| | | | | |
|---|---|---|---|---|
| Outcome data | 15* | *Cohort study*—Report numbers of outcome events or summary measures over time | p.11 | Line 211-226 (paragraph "RET and its co-occurring aberrations"), Results<br><br>Figure 2 |
| | | *Case-control study*—Report numbers in each exposure category, or summary measures of exposure | Not applicable | |
| | | *Cross-sectional study*—Report numbers of outcome events or summary measures | Not applicable | |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | p.11-12 | Line 241-252 (paragraph "Prognosis in patients with RET-aberrant versus non-aberrant tumours"), Results<br><br>Table 2 |
| | | (*b*) Report category boundaries when continuous variables were categorized | p.11-12 | Line 241-252(paragraph "Prognosis in patients with RET-aberrant versus non-aberrant tumours"), Results<br><br>Table 2 |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | Not applicable | Cox proportional hazard model; reporting hazard ratios |
| | | | | |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | p.11-12 | Line 241-252 (paragraph "Prognosis in patients with RET-aberrant versus non-aberrant tumours"), Results<br><br>Figure 3: stage subgroups |
| **Discussion** | | | | |

| | | | | |
|---|---|---|---|---|
| Key results | 18 | Summarise key results with reference to study objectives | p.14 | Line 281-298; (1st paragraph), Discussion |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | p.17 | Line 358-373; (5th paragraph), Discussion |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | p.14-17 | Line 300-356; 2nd-4th paragraph), Discussion |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | p.17 | Line 3258-373; (5th paragraph), Discussion<br><br>Real-world information |
| **Other information** | | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | p.19 | Line 385<br><br>Not funded |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

**Supplemental Table 2: information on passenger *RET* aberrations in digestive tract tumors**

**Patients with putative passenger *RET* aberrations (n=12)**

**Access and acquisition date: 10/Nov/2022**

| Patient | *RET* aberrations | Tumor type | cBioPortal: Oncogenicity (LOE) | ClinVar: Clinical significance (condition) | COSMIC: Pathogenicity (FATHMM score) | HGMD: Pathogenicity (disease) | Implicated therapeutics |
|---|---|---|---|---|---|---|---|
| 1 | *RET* p.R175H (c.524G>A) VAF 0.15 | COAD | Reported, unknown (NA) | US (MEN2) | Pathogenic[1] (0.75) | NA | NA |
| | *RET* p.W717C (c.2151G>T) VAF 0.23 | | Reported, unknown (NA) | NA | Pathogenic[2] (1.00) | NA | NA |
| 2 | *RET* p.V706M (c.2116G>A) VAF 0.19 | COAD | Reported, unknown (NA) | US (HCS, MEN2) | Pathogenic (0.89) | Likely disease causing (uterine corpus endometrial carcinoma)[3] | NA |
| 3 | *RET* p.V292M (c.874G>A) VAF 0.23 | STAD | Reported, unknown (NA) | NA | Pathogenic[4] (0.85) | Likely disease causing (PCC, MTC)[6] | NA |
| | *RET* p.T48M | | Reported, unknown (NA) | CIP (HCS, MEN2) | Neutral[5] (0.04) | NA | NA |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | (c.143C>T)<br>VAF 0.13 | | | | | | |
| 4 | *RET* p.V757M<br>(c.2269G>A)<br>VAF 0.11 | COAD | NA | NA | Pathogenic (0.99) | NA | NA |
| 5 | *RET* p.V292M<br>(c.874G>A)<br>VAF 0.17 | COAD | Reported, unknown (NA) | NA | Pathogenic[4] (0.85) | Likely disease causing (PCC, MTC)[6] | NA |
| 6. | *RET* p.R525W<br>(c.1573C>T)<br>VAF 0.13 | COAD | Reported, unknown (NA) | NA | Reported (NA) | NA | NA |
| 7. | *RET* p.W37L<br>(c.1573C>T)<br>VAF 0.27 | COAD | Reported, unknown (NA) | NA | NA | NA | NA |
| 8 | *RET* p.T1078R<br>(c.3233C>G)<br>VAF 0.10 | CHOL | NA | US (HCS) | Reported (NA) | NA | NA |
| 9 | *RET* p.F719L | CHOL | NA | US (HCS) | Reported (NA) | NA | NA |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | (c.2157C>A)<br>VAF 0.18 | | | | | | |
| 10 | *RET* p.Q681E<br>(c.2041C>G)<br>VAF 0.15 | ESCA | NA | US (HCS) | NA | NA | NA |
| 11 | *RET* p.G830R<br>(c.2488G>A)<br>VAF 0.14 | COAD | NA | CIP (HCS) | NA | NA | NA |
| 12 | *RET* p.P1103L<br>(c.3308C>T)<br>VAF 0.11 | CHOL | NA | US (HCS) | NA | NA | NA |

VAF, variant allele frequency; COAD, colorectal adenocarcinoma; STAD, gastric adenocarcinoma; CHOL, cholangiocarcinoma; ESCA, esophageal squamous cell carcinoma; FATHMM, Functional Analysis through Hidden Markov Models; NA, no available information; US, uncertain significance; CIP, conflicting interpretations of pathogenicity; MTC, medullary thyroid carcinoma; PCC, phaeochromocytoma; HCS, hereditary cancer-predisposing syndrome; MEN2, multiple endocrine neoplasia type 2.

**Supplemental Table 3: information on putatively oncogenic *RET* aberrations in digestive tract tumors**

**Patients with putatively oncogenic aberrations (n=20)**

**Access and acquisition date: 10/Nov/2022**

| Patient[a] | *RET* aberrations | Tumor type | cBioPortal: Oncogenicity (LOE)[b] | ClinVar: Clinical significance (condition) | COSMIC: Pathogenicity (FATHMM score) | HGMD: Pathogenicity (disease) | Implicated therapeutics[c] |
|---|---|---|---|---|---|---|---|
| 1 | *NCOA4::RET* | COAD | Oncogenic (I)[7] | NA | Reported (NA) | NA | Selpercatinib (I) |
| 2 | *KIF5B::RET* | LIHC | Oncogenic (NA) | NA | Reported (NA) | NA | Selpercatinib (I) |
| 3 | *RET::CCDC6* | STAD | Oncogenic (I)[8] | NA | Reported (NA) | NA | Selpercatinib (I) |
| 4 | *RET::CCDC6* | STAD | Oncogenic (I)[8] | NA | Reported (NA) | NA | Selpercatinib (I) |
| 5 | *RET::NCOA4* | PAAD | Oncogenic (I)[9] | NA | Reported (NA) | NA | Selpercatinib (I) |
| 6 | *RET* p.S671*<br>VAF 0.37 | LIHC | Reported, unknown (NA) | NA | NA | NA | NA |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 7 | *RET* p.V804M (c.2410G>A) VAF 0.41 | CHOL | Oncogenic (IIIB)[10] | Pathogenic (MTC)[11] | Reported (NA) | Likely disease causing (MTC) | Selpercatinib (IIIB) Pralsetinib (IIIB) |
| 8 | *RET* p.V804M (c.2410G>A) VAF 0.38 | COAD | Oncogenic (IIIB)[10] | Pathogenic (MTC)[11] | Reported (NA) | Likely disease causing (MTC) | Selpercatinib (IIIB) Pralsetinib (IIIB) |
| 9 | *RET* p.M848V (c.2542A>G) VAF 0.37 | COAD | Likely oncogenic (IIIB)[12] | US (HCS, MEN2) | Reported (NA) | NA | Selpercatinib (IIIB) Pralsetinib (IIIB) |
| 10 | *RET* p.R886Q (c.2657G>A) VAF 0.28 | COAD | Likely oncogenic (IIIB)[13] | CIP (HCS)[14] | Reported (NA) | Likely disease causing (PCC, MTC)[6] | Selpercatinib (IIIB) Pralsetinib (IIIB) |
| 11 | *RET* p.S904Y (c.2711C>A) VAF 0.35 | COAD | Likely oncogenic (IIIB)[15] | NA | Pathogenic[16] (0.97) | NA | Selpercatinib (IIIB) Pralsetinib (IIIB) |
| 12 | *RET* p.R833H (c.2498G>A) VAF 0.30 | STAD | Likely oncogenic (IIIB)[15] | US (HCS, MEN2) | Pathogenic[5] (1.00) | Likely disease causing (PCC, paraganglioma)[17] | Selpercatinib (IIIB) Pralsetinib (IIIB) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 13 | *RET* p.M848I<br>(c.2711C>A)<br>VAF 0.41 | COAD | Likely oncogenic (IIIB)[15] | US (HCS, MEN2) | Reported (NA) | Likely disease causing (MTC) | Selpercatinib (IIIB)<br>Pralsetinib (IIIB) |
| 14 | *RET* p.S891L<br>(c.2672C>T)<br>VAF 0.35 | COAD | Likely oncogenic (IIIB)[12] | NA | Reported (NA) | Likely disease causing (MTC) | Selpercatinib (IIIB)<br>Pralsetinib (IIIB) |
| 15 | *RET* p.V804M<br>(c.2410G>A)<br>VAF 0.19 | COAD | Oncogenic (IIIB)[10] | Pathogenic (MTC)[11] | Reported (NA) | Likely disease causing (MTC) | Selpercatinib (IIIB)<br>Pralsetinib (IIIB) |
| 16 | *RET* p.M848V<br>(c.2542A>G)<br>VAF 0.45 | COAD | Likely oncogenic (IIIB)[18] | US (HCS, MEN2) | Reported (NA) | NA | Selpercatinib (IIIB)<br>Pralsetinib (IIIB) |
| 17 | *RET* p.R833C<br>(c.2497C>T)<br>VAF 0.16 | STAD | Likely oncogenic (IIIB) | NA | Reported (NA) | NA | Selpercatinib (IIIB)<br>Pralsetinib (IIIB) |
| 18 | *RET* p.E511K<br>(c.1531G>A)<br>VAF 0.26 | ESCA | Likely oncogenic (IIIB)[13] | NA | Reported (NA) | Disease causing (MTC)[19] | Selpercatinib (IIIB)<br>Pralsetinib (IIIB) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 19 | *RET* p.S891L (c. 2672C>T) VAF 0.37 | CHOL | Likely oncogenic (IIIB)[20] | NA | Reported (NA) | NA | Selpercatinib (IIIB) Pralsetinib (IIIB) |
| 20 | *RET* p.E511K (c.1531G>A) VAF 0.25 | COAD | Likely oncogenic (IIIB)[13] | NA | Reported (NA) | Disease causing (MTC)[19] | Selpercatinib (IIIB) Pralsetinib (IIIB) |

a. the order of patients is corresponding to Figure2 (left to right) (n=20).

b. the implicated therapeutic levels of evidence are as the following: level I, II, IIIA, IIIB and IV. Only the highest level is shown.

c. Only the suggested therapeutics with the highest level of evidence are shown.

VAF, variant allele frequency; COAD, colorectal adenocarcinoma; LIHC, hepatocellular carcinoma; PAAD, pancreatic adenocarcinoma; STAD, gastric adenocarcinoma; ESCA, oesophageal squamous cell carcinoma; CHOL, cholangiocarcinoma; FATHMM, Functional Analysis through Hidden Markov Models; NA, no available information; US, uncertain significance; CIP, conflicting interpretations of pathogenicity;MTC, medullary thyroid carcinoma; PCC, phaeochromocytoma; HCS, hereditary cancer-predisposing syndrome; MEN2, multiple endocrine neoplasia type 2.

References:


1. Zehir A, Benayed R, Shah RH, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med 2017;23:703-13.
2. Giannakis M, Hodis E, Jasmine Mu X, et al. RNF43 is frequently mutated in colorectal and endometrial cancers. Nature genetics 2014;46:1264-6.
3. Huang KL, Mashl RJ, Wu Y, et al. Pathogenic Germline Variants in 10,389 Adult Cancers. Cell 2018;173:355-70.e14.
4. Bertotti A, Papp E, Jones S, et al. The genomic landscape of response to EGFR blockade in colorectal cancer. Nature 2015;526:263-7.
5. Giannakis M, Mu XJ, Shukla SA, et al. Genomic Correlates of Immune-Cell Infiltrates in Colorectal Carcinoma. Cell reports 2016;15:857-65.
6. Castellone MD, Verrienti A, Magendra Rao D, et al. A novel de novo germ-line V292M mutation in the extracellular region of RET in a patient with phaeochromocytoma and medullary thyroid carcinoma: functional characterization. Clinical endocrinology 2010;73:529-34.
7. Sato K, Kawazu M, Yamamoto Y, et al. Fusion Kinases Identified by Genomic Analyses of Sporadic Microsatellite Instability-High Colorectal Cancers. Clinical cancer research : an official journal of the American Association for Cancer Research 2019;25:378-89.
8. Subbiah V, Shen T, Terzyan SS, et al. Structural basis of acquired resistance to selpercatinib and pralsetinib mediated by non-gatekeeper RET mutations. Annals

of oncology : official journal of the European Society for Medical Oncology 2021;32:261-8.
9. Subbiah V, Wolf J, Konda B, et al. Tumour-agnostic efficacy and safety of selpercatinib in patients with RET fusion-positive solid tumours other than lung or thyroid tumours (LIBRETTO-001): a phase 1/2, open-label, basket trial. The Lancet Oncology 2022;23:1261-73.
10. Cranston AN, Carniti C, Oakhill K, et al. RET is constitutively activated by novel tandem mutations that alter the active site resulting in multiple endocrine neoplasia type 2B. Cancer Res 2006;66:10179-87.
11. Innella G, Rossi C, Romagnoli M, et al. Results and Clinical Interpretation of Germline RET Analysis in a Series of Patients with Medullary Thyroid Carcinoma: The Challenge of the Variants of Uncertain Significance. Cancers 2020;12.
12. Wirth L, Sherman E, Drilon A, et al. LBA93 - Registrational results of LOXO-292 in patients with RET-altered thyroid cancers. Annals of Oncology 2019;30:v933.
13. Prazeres H, Couto JP, Rodrigues F, et al. In vitro transforming potential, intracellular signaling properties, and sensitivity to a kinase inhibitor (sorafenib) of RET proto-oncogene variants Glu511Lys, Ser649Leu, and Arg886Trp. Endocrine-related cancer 2011;18:401-12.
14. Tsaousis GN, Papadopoulou E, Apessos A, et al. Analysis of hereditary cancer syndromes by using a panel of genes: novel and multiple pathogenic mutations. BMC Cancer 2019;19:535.
15. Cosci B, Vivaldi A, Romei C, et al. In silico and in vitro analysis of rare germline allelic variants of RET oncogene associated with medullary thyroid cancer. Endocrine-related cancer 2011;18:603-12.
16. Abou Alaiwi S, Nassar AH, Xie W, et al. Mammalian SWI/SNF Complex Genomic Alterations and Immune Checkpoint Blockade in Solid Tumors. Cancer immunology research 2020;8:1075-84.
17. Ben Aim L, Pigny P, Castro-Vega LJ, et al. Targeted next-generation sequencing detects rare genetic events in pheochromocytoma and paraganglioma. J Med Genet 2019;56:513-20.
18. Subbiah V, Gainor JF, Rahal R, et al. Precision Targeted Therapy with BLU-667 for RET-Driven Cancers. Cancer discovery 2018;8:836-49.
19. Muzza M, Cordella D, Bombled J, et al. Four novel RET germline variants in exons 8 and 11 display an oncogenic potential in vitro. European journal of endocrinology 2010;162:771.
20. Schulte KM, Machens A, Fugazzola L, et al. The clinical spectrum of multiple endocrine neoplasia type 2a caused by the rare intracellular RET mutation S891A. The Journal of clinical endocrinology and metabolism 2010;95:E92-7.

**Supplemental Table 4: Frequently altered genes in *RET*-aberrant versus non-aberrant tumours (n=453)**

| Gene | *RET*-aberrant tumours | | | | *RET* non-aberrant tumours | | | | FDR[b] |
|---|---|---|---|---|---|---|---|---|---|
| | Total alteration frequencies[a] | Type | | | Total alteration frequencies[a] | Type | | | |
| | | NSM[c] | indels | Others[d] | | NSM[c] | indels | Others[d] | |
| *APC* | 50.0 | 20.0 | 30.0 | | 70.3 | 39.4 | 26.9 | 4.0 | 3.82E-06** |
| *KRAS* | 20.0 | 20.0 | | | 44.2 | 42.2 | | 2.0 | 6.36E-06** |
| *TP53* | 50.0 | 30.0 | 20.0 | | 81.1 | 62.2 | 12.0 | 6.9 | 1.87E-04** |
| *MSH6* | 15.0 | 15.0 | | | 0.8 | | 0.8 | | 0.0474* |
| *STK11* | 15.0 | 5.0 | 10.0 | | 0.8 | 0.2 | 0.6 | | 0.0474* |
| *TERT* | 20.0 | | | 20.0 | 4.4 | | | 4.4 | 0.2239 |
| *SMAD4* | 10.0 | 5.0 | | 5.0 | 17.7 | 10.0 | 7.2 | 0.5 | 0.2345 |
| *FGF12* | 10.0 | | | 10.0 | 1.2 | 0.4 | | 0.8 | 0.2916 |
| *KDM6A* | 10.0 | | 5.0 | 5.0 | 1.2 | 0.4 | 0.8 | | 0.2916 |
| *PTCH1* | 10.0 | 5.0 | 5.0 | | 1.2 | 0.8 | 0.4 | | 0.2916 |
| *MLL2* | 20.0 | 5.0 | 10.0 | 5.0 | 6.0 | 0.4 | 4.8 | 0.8 | 0.3464 |
| *TSC2* | 10.0 | | 10.0 | | 1.6 | 0.4 | 0.8 | 0.4 | 0.3464 |
| *BRCA2* | 15.0 | 5.0 | 10.0 | | 4.0 | 1.2 | 2.8 | | 0.3882 |
| *ASXL1* | 10.0 | 5.0 | 5.0 | | 2.0 | | 2.0 | | 0.3882 |
| *ARID1A* | 5.0 | | 5.0 | | 1.6 | 0.8 | 0.4 | | 0.3882 |

a. per 100 tumours.
b. pairwise multiple comparisons were adjusted by Benjamini-Hochberg method and presented as false discovery rates. The top fifteen frequently altered genes were reported.
c. including missense and nonsense mutations.
d. including splice site variations, promoter sequence variations, amplifications and fusions/translocations.
NSM, nonsynonymous mutations; FDR, false discovery rate. * FDR<0.05 **<0.001.

**Supplemental Table 5: Frequently altered genes in *RET*-aberrant versus non-aberrant colorectal cancer (n=231)**

| Gene | *RET*-aberrant colorectal tumours | | | | *RET* non-aberrant colorectal tumours | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total alteration frequencies[a] | Type | | | Total alteration frequencies[a] | Type | | | FDR[b] |
| | | NSM[c] | indels | Others[d] | | NSM[c] | indels | Others[d] | |
| *TP53* | 50.0 | 20.0 | 30.0 | | 74.8 | 58.8 | 9.6 | 6.4 | 7.12E-03* |
| *APC* | 40.0 | 20.0 | 20.0 | | 75.9 | 41.7 | 28.7 | 5.5 | 8.96E-03* |
| *KRAS* | 20.0 | 20.0 | | | 35.6 | 31.9 | | 3.7 | 0.0239* |
| *STK11* | 10.0 | 10.0 | | | 0.5 | | 0.5 | | 0.1104 |
| *PTCH1* | 10.0 | 10.0 | | | 0.5 | 0.5 | | | 0.1104 |
| *TERT* | 10.0 | | | 10.0 | 1.8 | | | 1.8 | 0.2401 |
| *FGF12* | 10.0 | | | 10.0 | 2.1 | 2.1 | | | 0.2388 |
| *KDM6A* | 10.0 | | 10.0 | | 2.1 | | 2.1 | | 0.2388 |
| *ATM* | 20.0 | 10.0 | 10.0 | | 13.2 | 5.3 | 5.3 | 2.6 | 0.3849 |
| *ERBB2* | 10.0 | | | 20.0 | 4.6 | | | 4.6 | 0.4764 |
| *SMAD4* | 10.0 | 10.0 | | | 16.2 | 7.8 | 6.6 | 1.8 | 0.5337 |
| *MLL2* | 20.0 | 10.0 | 10.0 | | 4.8 | | 4.8 | | 0.5630 |
| *MSH6* | 10.0 | 10.0 | | | 2.9 | | 1.9 | | 0.7051 |
| *ASXL1* | 10.0 | 10.0 | | | 13.9 | | 13.9 | | 0.8326 |
| *BRAF* | 10.0 | 10.0 | | | 6.0 | 5.5 | | 0.5 | 0.8910 |

a. per 100 tumours.
b. pairwise multiple comparisons were adjusted by Benjamini-Hochberg method and presented as false discovery rates. The top fifteen frequently altered genes were reported.
c. including missense and nonsense mutations.
d. including splice site variations, promoter sequence variations, amplifications and fusions/translocations.
NSM, nonsynonymous mutations; FDR, false discovery rate. * FDR<0.05.

**Supplemental Table 6: Details for patients with *RET*-aberrant tumours treated with ICPi**

| Patient[a] | Age/Sex | Tumour type | *RET* aberrations | Frontline treatment | Timing of ICPi | ICPi treatment | TTD, ms | Best evaluable response | tMB | PD-L1[b] | MSI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 47 M | COAD | NSM | systemic C/T<br>surgery | R/R | nivolumab | 13.6 | SD | 6.5 | CPS 15 | S |
| 2 | 55 F | CHOL | NSM | systemic C/T | R/R | nivolumab | 9.2 | PD | 2.5 | CPS 2 | S |
| 3 | 62 M | STAD | NSM | systemic C/T<br>surgery | R/R | nivolumab | 5.4 | PD | 6.1 | CPS<1 | S |
| 4 | 67 M | STAD | Fusion | systemic C/T<br>+ nivolumab | Frontline | nivolumab<br>+ XELOX | 5.2 | SD | 1.3 | CPS 8 | S |
| 5 | 74 F | COAD | NSM | systemic C/T<br>surgery | R/R | nivolumab | 3.4 | PD | 5.0 | NA | S |
| 6 | 34 M | LIHC | Fusion | surgery | R/R | nivolumab | 2.8 | PD | 2.5 | TPS 8%,<br>CPS<1 | S |
| 7 | 61 M | COAD | NSM | ICPi + mTKI | Frontline | pembrolizumab<br>+ lenvatinib | 2.8 | SD | 1.3 | TPS 15% | S |
| 8 | 50 M | LIHC | indel | local ablation | R/R | pembrolizumab<br>+ lenvatinib | 2.6 | PD | 6.3 | CPS<1 | S |
| 9 | 46 F | CHOL | NSM | surgery | R/R | pembrolizumab | 2.1 | PD | 1.3 | CPS<1 | S |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 67 M | PAAD | Fusion | systemic C/T<br>surgery<br>PARPi | R/R | pembrolizumab | 2.0 | PD | 12.6 | CPS 3 | S |
| 11 | 65 F | COAD | Fusion | systemic C/T<br>selpercatinib | R/R | nivolumab<br>+ irinotecan | 1.9 | PD | 1.3 | CPS<1 | S |

a. patients who received ICPi and were evaluable for the therapeutic response (n=11). The order of patients is corresponded with the lower panel of Figure 4 (downward).

b. Both PD-L1 TPS and CPS were determined by PD-L1 immunohistochemical staining (Dako, 22C3 pharmDx).

ICPi, immune checkpoint inhibitor; tMB, tumour mutation burden; MSI, microsatellite instability; C/T, chemotherapy; R/R, recurrence or refractory; mTKI, multitargeted tyrosine kinase inhibitor; PARPi, poly ADP-ribose polymerase inhibitor; COAD, colorectal adenocarcinoma, LIHC, hepatocellular carcinoma; PAAD, pancreatic adenocarcinoma; STAD, gastric adenocarcinoma; CHOL, cholangiocarcinoma; NSM, nonsynonymous mutation; SD, stable disease; PD, progressive disease; TTD, time to treatment discontinuation; S, stable; TPS, tumor proportion score; CPS, combined proportion score; NA, not available.

**Supplemental Table 7: Treatment responses with ICPi according to tumour type**

| | ***RET*-aberrant tumours** | ***RET* non-aberrant tumours** | ***p*** |
|---|---|---|---|
| COAD, n (%)[a] | (n=4) | (n=25) | |
| ORR | 0 | 5 (20.0) | 0.3340 |
| DCR | 2 (50.0) | 17 (68.0) | 0.5926 |
| STAD, n (%) | (n=2) | (n=12) | |
| ORR | 0 | 4 (30.0) | 0.3865 |
| DCR | 1 (50.0) | 11 (91.7) | 0.2747 |
| CHOL, n (%) | (n=2) | (n=6) | |
| ORR | 0 | 3 (50.0) | 0.4643 |
| DCR | 0 | 4 (66.7) | 0.4286 |
| LIHC, n (%) | (n=2) | (n=2) | |
| ORR | 0 | 1 (50.0) | 0.3173 |
| DCR | 0 | 2 (100.0) | 0.0833 |
| PAAD, n (%) | (n=1) | (n=2) | |
| ORR | 0 | 0 | - |
| DCR | 0 | 2 (100.0) | 0.3333 |

a. evaluated as the best tumour responses per RECIST 1.1.

ORR, objective response rate; DCR, disease control rate; COAD, colorectal adenocarcinoma, LIHC, hepatocellular carcinoma; PAAD, pancreatic adenocarcinoma; STAD, gastric adenocarcinoma; CHOL, cholangiocarcinoma.

**Supplemental Table 8: Cross-organ analysis of *RET* aberrations in digestive tract tumours**

| Tumour type | Tumour tested at primary site | n | Tested tumour at metastatic site | n | *RET* aberrations, (n) | mtMB, mut/$10^6$ bp | Best response of ICPi, (n)[a] | mTTD, ms | p[b] |
|---|---|---|---|---|---|---|---|---|---|
| COAD | Colorectum | 3 | | - | NSM (3) | 5.1 | SD (1/2) | 11.4 | 0.0634 |
| | | - | Lung | 4 | NSM (4) | 2.5 | PD (1) | 3.4 | |
| | | - | Liver | 2 | Fusion (1), NSM (1) | 1.3 | SD (1/2) | 2.3 | |
| | | - | Peritoneum | 1 | NSM (1) | 1.3 | - | - | |
| STAD | Stomach/GEJ | 3 | | - | Fusion (1), NSM (2) | 6.1 | SD (1) | 5.4 | 0.3173 |
| | | - | Lung | 1 | Fusion (1) | 1.3 | PD (1) | 5.2 | |
| LIHC | Liver | 2 | | - | Fusion (1), Indel (1) | 4.4 | PD (2/2) | 2.7 | |
| CHOL | Biliary tract | 2 | | - | NSM (2) | 1.9 | PD (1) | 2.1 | |
| ESCA | oesophagus | 1 | | - | NSM (1) | NA | - | - | |
| PAAD | Pancreas | 1 | | - | Fusion (1) | 12.6 | PD (1) | 2.0 | |

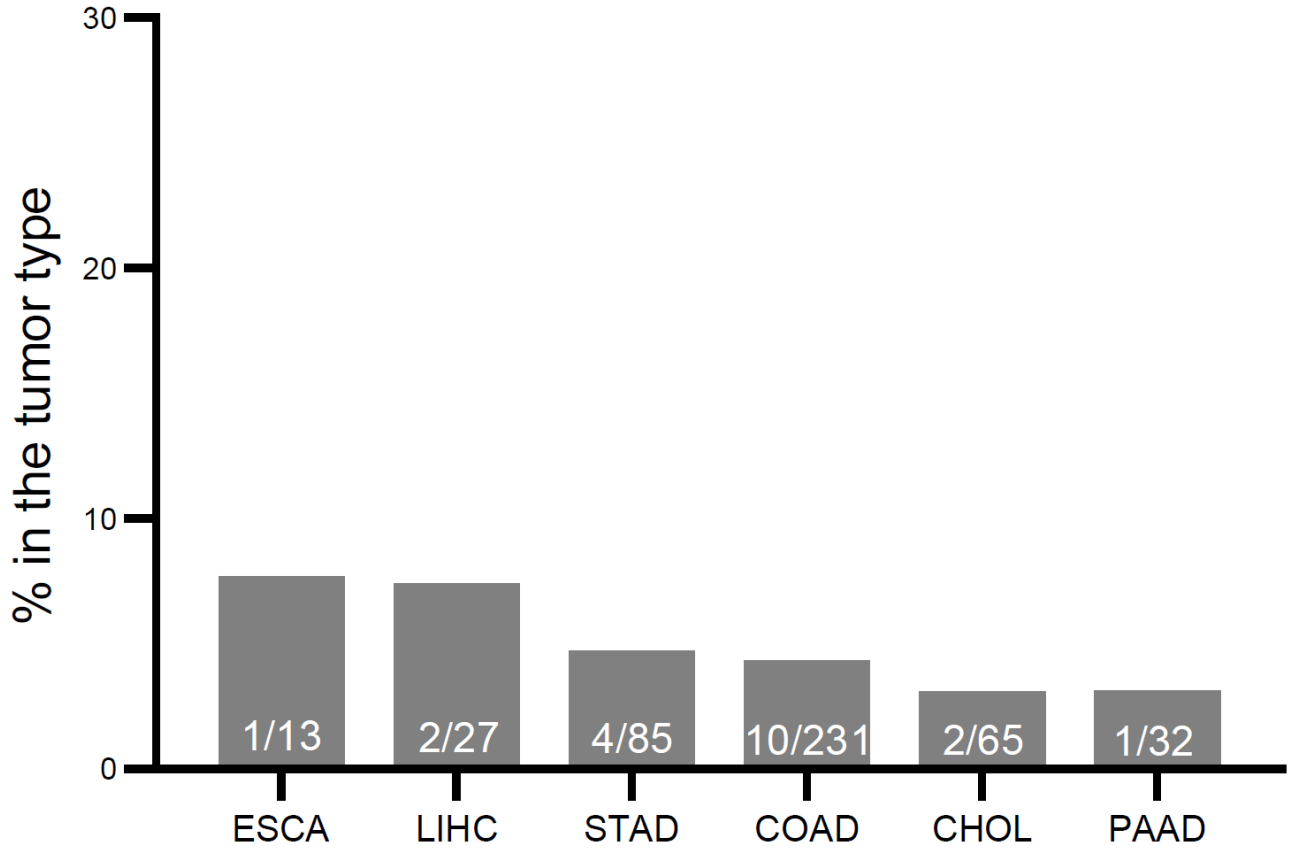
a. patients who received ICI and were evaluable for the therapeutic response (n=11).

b. comparisons between tumours tested at primary vs. metastatic site by log-rank test.

$p<0.05$ as statistically significant and shown in *.

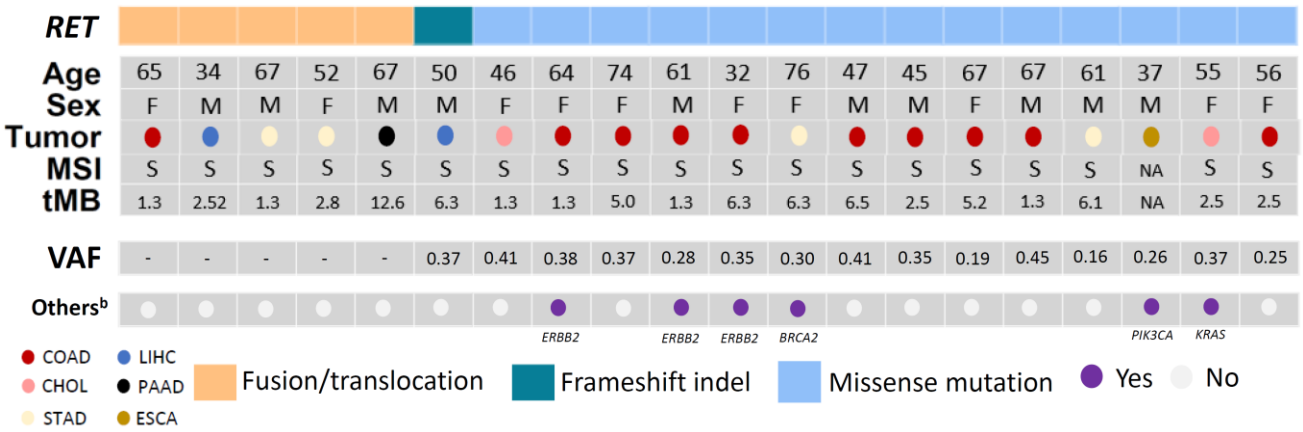
COAD, colorectal adenocarcinoma, LIHC, hepatocellular carcinoma; PAAD, pancreatic adenocarcinoma; STAD, gastric adenocarcinoma; ESCA, oesophageal squamous cell carcinoma; CHOL, cholangiocarcinoma; GEJ, gastrooesophageal junction; NSM, nonsynonymous mutation; NA, not available; SD, stable disease; PD, progressive disease; mTTD, median time to treatment discontinuation.

**Supplemental Figure 1: *RET*-aberrant tumours by type**




COAD, colorectal adenocarcinoma, LIHC, hepatocellular carcinoma; PAAD, pancreatic adenocarcinoma; STAD, gastric adenocarcinoma; ESCA, oesophageal squamous cell carcinoma; CHOL, cholangiocarcinoma

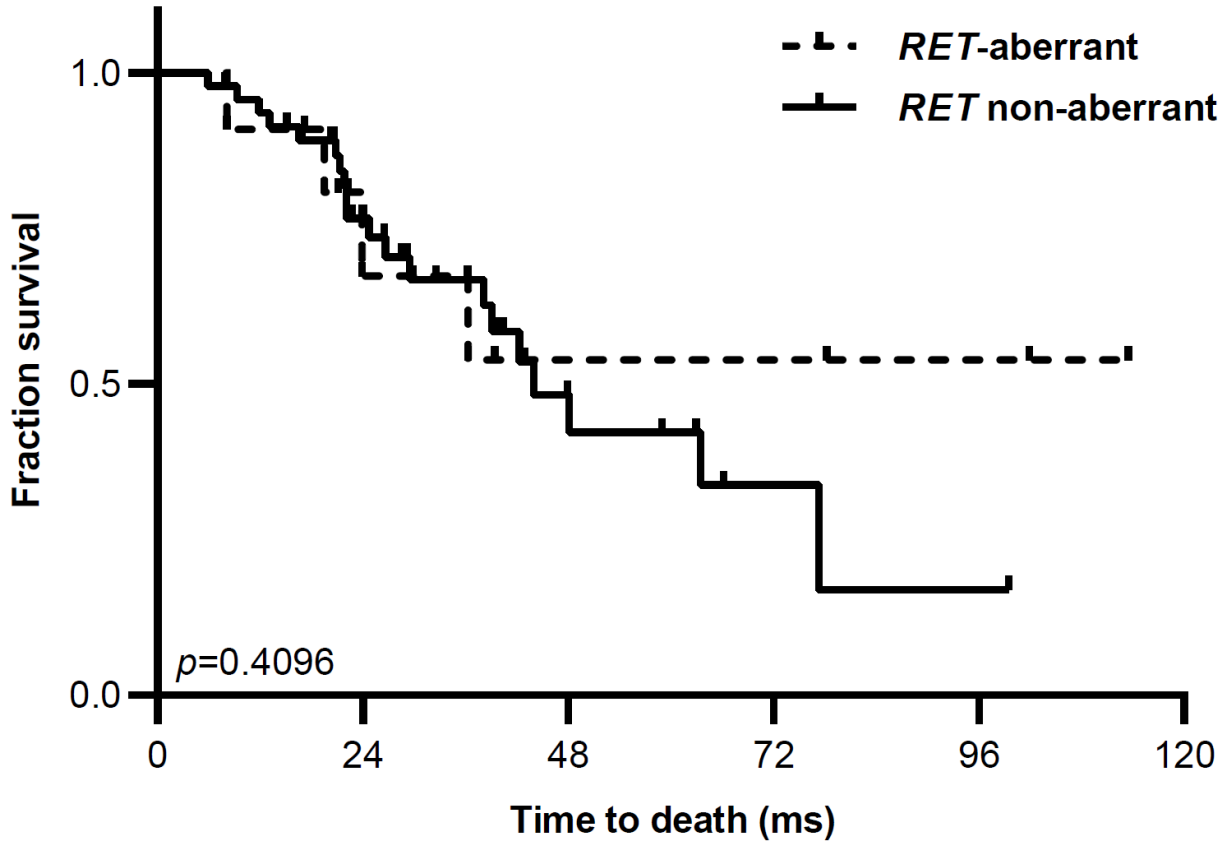
**Supplemental Figure 2: Co-occurring actionable aberrations in addition to *RET***




b. other putatively actionable co-occurring aberrations are determined by COSMIC, ClinVar and cBioPortal databases with a level of evidence I, II, IIIA, IIIB and IV (*ERBB2* amplifications (level 1), n=3; *KRAS* p.G12V, n=1 (level 4); *PIK3CA* p.H1047R, n=1 (level 3B); *BRCA2* p.E2981Kfs*7, n=1 (level 3B))

COAD, colorectal adenocarcinoma, LIHC, hepatocellular carcinoma; PAAD, pancreatic adenocarcinoma; STAD, gastric adenocarcinoma; ESCA, oesophageal squamous cell carcinoma; CHOL, cholangiocarcinoma; M, male; F, female; tMB, tumor mutation burden; MSI, microsatellite instability; NA, not available or known. S, stable; H, high.

**Supplemental Figure 3: Overall survival in patients who received ICPi**




Compared by log-rank test