

SUPPLEMENTAL METHODS

Next Generation Sequencing Platforms and MET Detection Assays

Three different platforms were utilized for solid tumor NGS at each institution. MSKCC: Briefly, normalized coverage values for each exon in the panel from the tumor are divided by normalized coverage values from a sample type matched normal (i.e. FFPE normal for FFPE tumor). Logarithmic fold change values are used for segmentation and parametrizing a null background model from which p-values are generated. Genes which fall onto segments with fold change ≥ 2 and p values < 0.05 are reported as amplifications²². Dana Farber: RobustCNV performs a robust linear regression against the panel of normal samples to calculate copy ratios. Circular binary segmentation is again used to segment the data, and event identification is performed based on the observed variance of the data points. Estimated copy number was calculated using the following published formula: $CR = (TC/2) \times P + (2/2)(1 - P)$, where CR is the median copy ratio for all intervals in a gene, TC is the number of copies in the tumor sample, and P is purity^{23,24}. Sarah Cannon: The Foundation One platform was used for solid tumor NGS and MET detection²⁵.

HBEC-RET cell lines

LC-2/ad cells were obtained from Riken Bioresource Center (Japan). The HBEC-RET cell line expressing a *CCDC6/RET* fusion cDNA was generated as previously described²⁶. HBEC-RET cells were infected with retroviruses generated from empty pMSCV-PIG retroviral vector (HBEC-RET-EV) or pMSCV-PIG vector containing a *MET* cDNA (HBEC-RET+MET).

Cell viability assays: Cells were plated directly into selpercatinib and grown for 96 hours, and AlamarBlue was added during the last 6 hours and quantified using a fluorescence plate

reader (Ex 530 nm, Em 585 nm). All conditions were tested in triplicate, and nonlinear regression was used to plot growth curves and estimate IC₅₀ values.

Polymerase chain reaction (PCR): CCDC6-RET was detected by RT-PCR using CCDC6 exon 1 forward (5'-ATGGCGGACAGCGCCAGCGAGA-3') RET exon 12 reverse (5'-CTTTCAGCATCTTCACGGCCA-3') primers. Amplified products were separated by gel electrophoresis, and quantitative PCR to detect MET utilized a Taqman MET gene expression assay kit (Hs04329783).

Patient-derived organoids

A pleural effusion specimen was obtained from the patient described in Case 1. After RBC lysis and CD45 depletion, the remaining cells resuspended in Matrigel (Corning) and were allowed to solidify and organoids were usable after passage 3 in Renaissance Essential Tumor Medium (Cellaria) supplemented with B-27 (Thermo Fisher Scientific).

Fluorescence In Situ Hybridization (FISH) of MET amplification: FISH analysis was performed on paraffin embedded organoids. Two-color probes against MET (MET-20-OR) and Centromere 7 (CHR07-10-GR) were purchased from Empire Genomics. Baked slides were prepared, antigen retrieval performed, and treated with 0.004% pepsin in 0.01N hydrogen chloride. Slide denaturation with probe mixture that was followed by overnight hybridization, which were then washed, dehydrated, and mounted.

Cell growth assay: TrypLE Express (Invitrogen) was applied to the cultured organoids to dissociate them into single cells and seeded on ultra-low attachment microplates (Corning). After overnight incubation, cells were treated with DMSO, selpercatinib (Chemgood), crizotinib (Thermo Fisher Scientific), or with combination, with representative images of organoids captured after six days. CellTiter-Glo 3D (Promega) was used to measure cell viability.

Statistical significance was assessed using a two-tailed t-test.

Western blots and antibodies: The dissociated organoids were treated with drug overnight, lysed with RIPA buffer (Boston BioProducts) and protease and phosphatase inhibitor cocktails (Sigma Aldrich). BCA assay (Pierce) was performed for equivalent protein concentration of each sample. Denatured samples were immunoblotted using anti-phospho-MET (Tyr1234/1235; #3126), anti-phospho-RET (Tyr905; #3221), anti-MET (#8198), anti-RET (#3223), Akt (#9272), pS473 Akt (#4060), ERK1/2 (#9102), pT202/Y204 ERK1/2 (#4370), all purchased from Cell Signaling Technology, and anti-HSP90 (#sc-7947) purchased from Santa Cruz. All Western blots shown were run using the same protein lysate, but AKT, pAKT, ERK, and pERK were blotted together, and the HSP90 loading control shown is from that set of blots.

Single patient protocols of selpercatinib and crizotinib

Each SPP was sponsored by LOXO and drafted in collaboration between LOXO and the site primary investigator. Each protocol enrolled a single patient after review by the FDA and approval by the site IRB.