**Supplementary Table S1. Biopsy sites in patients with paired pre- and post-lorlatinib specimens**

|  |  |  |
| --- | --- | --- |
| Patient ID | Pre-lorlatinib | Post-lorlatinib |
| MGH952 | Abdominal lymph node | Liver metastasis |
| MGH098 | Left axillary lymph node | Malignant pleural effusion |
| MGH964 | Malignant pleural effusion | Brain metastasis |
| MGH9107 | Liver metastasis | Pancreas metastasis |
| MGH062 | Periportal lymph node | Liver metastasis |
| MGH953 | Malignant pleural effusion | Malignant pleural effusion |
| MGH087 | Right hilar mass | Right hilar mass |
| MGH086 | Left axillary lymph node | Subcutaneous metastasis |
| MGH065 | Right adrenal metastasis | Mediastinal lymph node |
| MGH9092 | Left lung apex mass | Left lower lung mass |
| MGH040 | Left lung mass | Right supraclavicular node |

**Supplementary Table S2. Clinical history of lorlatinib-resistant ALK-positive patients**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Patient ID | Prior first generation ALK TKI | Prior second generation ALK TKI | Prior second generation ALK TKI | Duration of lorlatinib treatment (days) |
| MGH947\* | crizotinib | brigatinib | alectinib | 58 |
| MGH048 | crizotinib | alectinib | -- | 82 |
| MGH962 | crizotinib | alectinib | -- | 257 |
| MGH952 | -- | brigatinib | -- | 43 |
| MGH098 | crizotinib | alectinib | -- | 91 |
| MGH964 | crizotinib | ceritinib | alectinib | 98 |
| MGH9107\* | crizotinib | ceritinib | alectinib | 220+ |
| MGH987\* | crizotinib | alectinib | ceritinib | 92 |
| MGH990 | crizotinib | alectinib | -- | 656+ |
| MGH9041 | crizotinib | alectinib | -- | 517+ |
| MGH062 | crizotinib | ceritinib | -- | 289 |
| MGH953 | crizotinib | alectinib | -- | 197 |
| MGH087 | crizotinib | alectinib | ceritinib | 527 |
| MGH086\*\* | crizotinib | brigatinib | -- | 480 |
| MGH065 | crizotinib | ceritinib | -- | 344 |
| MGH9092 | crizotinib | alectinib | -- | 194 |
| MGH040 | crizotinib | ceritinib | alectinib | 952+ |
| MGH9094 | crizotinib | ceritinib | alectinib | 347 |
| MGH9106\* | crizotinib | ceritinib | alectinib | 258 |
| MGH9108 | crizotinib | alectinib | ceritinib | 1070+ |

\*Lorlatinib obtained through single patient IND; \*\*Lorlatinib administered initially with a PDL1 inhibitor, then as single agent

Abbreviations: TKI, tyrosine kinase inhibitor; PD, progressive disease; SD, stable disease; PR, partial response; uPR, unconfirmed partial response

**Supplementary Table S3. NGS testing of lorlatinib-resistant biopsies**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Patient ID | NGS Platform | | *ALK* Mutation(s) | Other genetic alterations† |
| MGH947 | SNaPshot V2 | No *ALK* mutation | | NOTCH1 K2171N, BRCA2 R2651S |
| MGH048 | SNaPshot V1 | No *ALK* mutation | | APC L2342F, TP53 splice site donor variant |
| MGH962 | SNaPshot V2 | No *ALK* mutation | | None |
| MGH952 | SNaPshot V2 | No *ALK* mutation | | None |
| MGH098 | SNaPshot V2 | No *ALK* mutation | | TP53 E285Ter, MAP3K1 S939\_T942delinsCSS |
| MGH964 | SNaPshot V2 | No *ALK* mutation | | NRAS A155T |
| MGH9107 | FoundationOne | No *ALK* mutation | | **NRAS G12D**, PIK3CA E545K, CDKN2A/B loss, TP53 R213Ter |
| MGH987 | SNaPshot V2 | *ALK* I1171N + **L1198F** | | None |
| MGH990 | SNaPshot V2 | *ALK* I1171N + D1203N | | TP53 splice site donor variant, EGFR gain, CDKN2A loss |
| MGH9041 | FoundationOne | *ALK* G1202R + G1269A | | STK11 loss, ARID2 Q651Ter, CDKN2A/B loss, KEL M1T |
| MGH062 | SNaPshot V1 | *ALK* C1156Y + **L1198F** | | None |
| MGH953 | SNaPshot V2 | *ALK* G1202R + **L1196M** | | TP53 E17TfsTer23 |
| MGH087 | SNaPshot V2 | *ALK* G1202R + **L1204V** + **G1269A** | | DDR2 L610F, TP53 I195T, **NOTCH1 S1409G**, **PTCH1 N124S**, ARID1A A861T, VHL P2L |
| MGH086 | FoundationOne | *ALK* D1203N + E1210K + **G1269A** | | MTOR T1834\_T1837del, JAK3 R948C, CDKN2A/B loss, NFE2L2 E79Q |
| MGH065 | SNaPshot V1 | ***ALK* G1269A** | | TP53 Q192Ter |
| MGH9092 | SNaPshot V2 | No *ALK* mutation | | TP53 R280I, SMARCA4 L1126R, SMAD4 A532D, APC N389S |
| MGH040 | SNaPshot V2 | No *ALK* mutation | | ARID1A Q515Ter |
| MGH9094 | SNaPshot V2 | No *ALK* mutation | | TP53 QH192HY\* |
| MGH9106 | SNaPshot V2 | No *ALK* mutation | | TP53R306Ter |
| MGH9108 | SNaPshot V2 | No *ALK* mutation | | BRCA1 D1123G |

†Mutations in black were confirmed in both the pre- and post-lorlatinib biopsies. Mutations in red indicate new mutations in the lorlatinib-resistant specimen compared to the pre-lorlatinib specimen. Mutations in green cannot be confirmed as new due to: 1) there was no pre-lorlatinib biopsy, or 2) a different molecular testing platform was used on the pre-lorlatinib specimen.

\*The TP53 variant identified is a dinucleotide exchange, 576\_577delGCinsCT, affecting the last base of the Q encoding codon and the first base of the H encoding codon

Abbreviations: Ter indicates a translation stop codon

**Supplementary Table S4. Kinetic parameters of activated ALK (nonmutant and L1196M) kinase domains1**

| ALK variant | kcat (s-1) | KM,ATP (µM) | KM,YFF (µM) | Relative kcat/KM,ATP | Relative kcat/KM,YFF |
| --- | --- | --- | --- | --- | --- |
| Nonmutant ALK | 11.0 ± 1.7 | 116 ± 25 | 197 ± 27 | 1.0 | 1.0 |
| ALK L1196M | 22.8 ± 3.8 | 79 ± 8 | 79 ± 37 | 3.1 | 5.2 |

1Kinetic parameters were determined by a coupled spectrophotometric ATP-regenerating assay using a “YFF” analog of the ALK activation loop peptide, as described in Experimental Procedures. The values are best-fit values derived from the Michaelis-Menten equation for kinetic assays conducted at least in duplicate. Catalytic efficiencies were calculated relative to nonmutant enzyme.

Abbreviations: kcat (s-1), turnover rate; KM, Michaelis constant