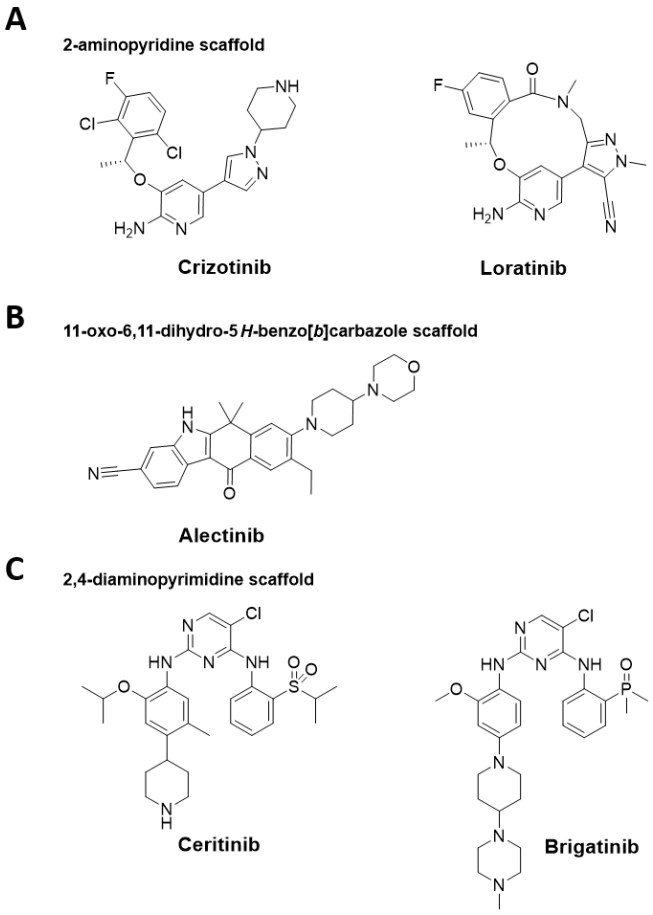
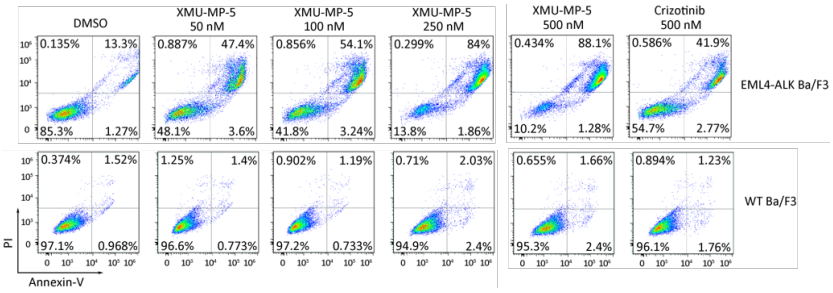
# *Appendix*

**A new ALK inhibitor overcomes resistance to first- and second-generation inhibitors in NSCLC** Yue Lu[1†], Zhenzhen Fan[2†], Su-Jie Zhu[3,4†], Xiaoxing Huang[1†], Zhongji Zhuang[1†], Yunzhan Li[1], Zhou Deng[1], Lei Gao[2], Xuehui Hong[5], Ting Zhang[1], Li Li[1], Xihuan Sun[1], Wei Huang[1], Jingfang Zhang[1], Yan Liu[1], Baoding Zhang[1], Jie Jiang[1], Fu Gui[1], Zheng Wang[1], Qiyuan Li[6], Siyang Song[1], Xin Huang[7], Qiao Wu[1], Lanfen Chen[1], Dawang Zhou[1], Jianming Zhang[8*], Cai-Hong Yun[3**], Liang Chen[2***], Xianming Deng[1****]

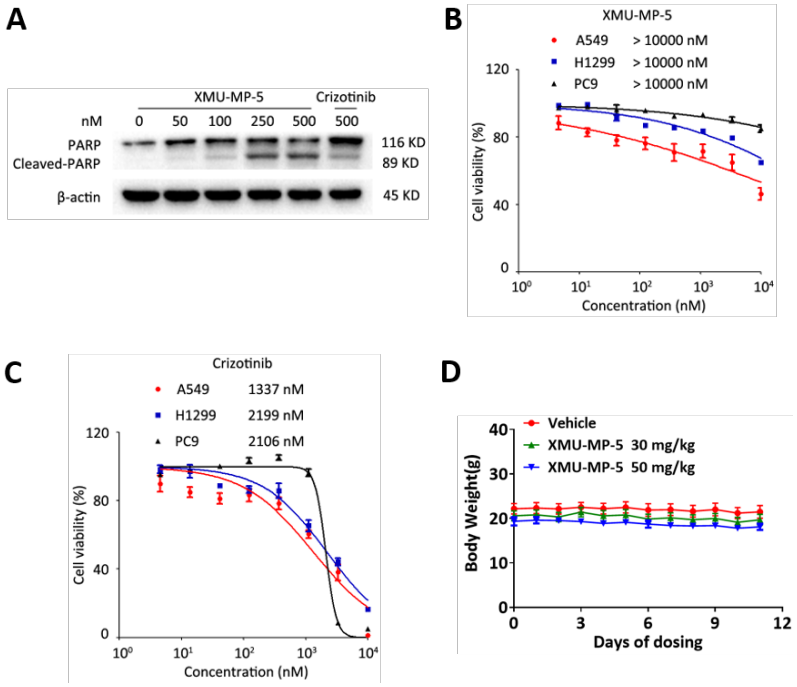
# Contents




**Appendix Figure S1. Chemical structures of FDA-approved ALK inhibitors with different scaffolds.** The core structures of FDA-approved ALK inhibitors were classified into 3 different scaffolds.




**Appendix Figure S2 XMU-MP-5 selectively induced cell apoptosis.**
EML4-ALK Ba/F3 and wild-type Ba/F3 cells were treated with XMU-MP-5 or crizotinib with the indicated concentrations for 24 hours. Apoptosis was detected by AnnexinV/PI staining.

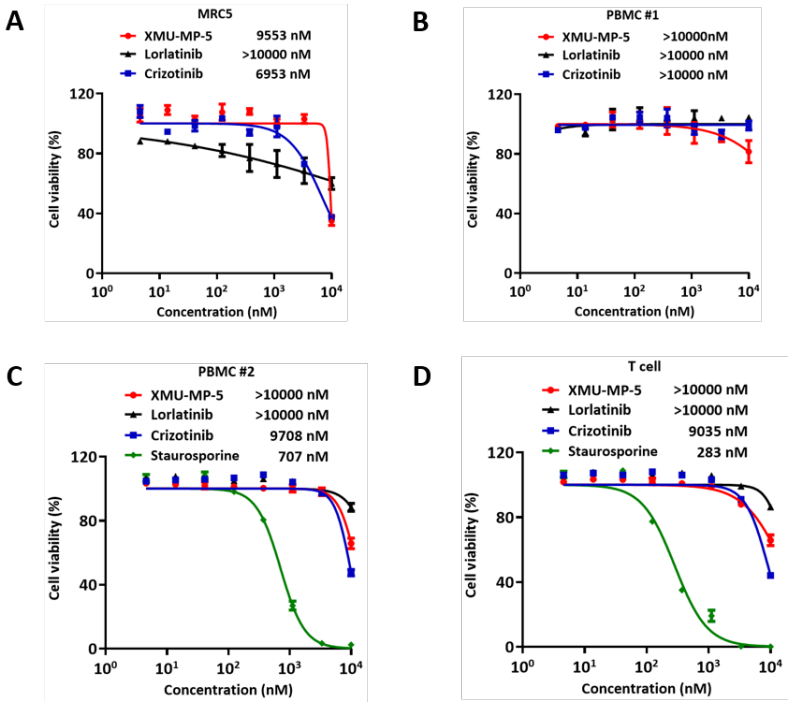



**Appendix Figure S3 Antiproliferative activity of XMU-MP-5 against non-small cell lung cancer cell lines in vitro and in vivo.**

A XMU-MP-5 induced cell apoptosis indicated by cleavage of PARP. H3122 cells were treated as indicated for 24 hours and then analyzed by immunobloting.

B, C XMU-MP-5 showed negligible antiproliferative activity against ALK negative lung cancer cell lines. Cells were treated with series concentrations of XMU-MP-5 for 48 h. Cell viability was detected by MTS assay. Crizotinib was used as reference.

D No Body weight change was observed after XMU-MP-5 treatment for 11 days. Data were shown as mean ± SD (n = 6).




**Appendix Figure S4 XMU-MP-5 has high selectivity over nontumorigenic cell lines.**

A Anti-proliferative activities of XMU-MP-5 and crizotinib against human lung fibroblast cell line MRC-5

B, C Effect of XMU-MP-5 and crizotinib on human peripheral blood mononuclear cells (PBMC) that freshly isolated from 2 healthy donors.

D Activity of XMU-MP-5 and crizotinib on human T cells.

All of the cells were treated with increasing concentration of each drug for 48 hours and then analyzed by CellTiter-Glo. Each concentration point was performed in triplicate. Data were presented as Mean ± SEM from three indepedent experiments (n = 3) .

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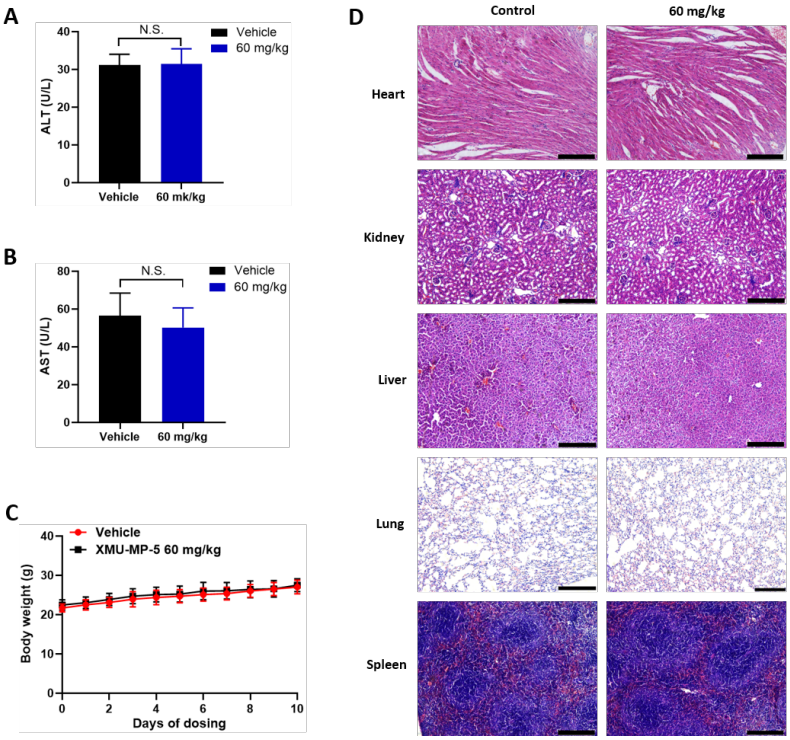
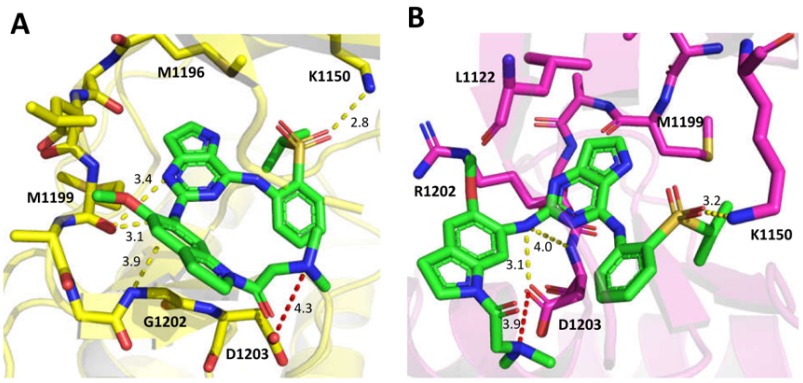



**Appendix Figure S5 Toxicity evaluation of XMU-MP-5 by ICR mice.**

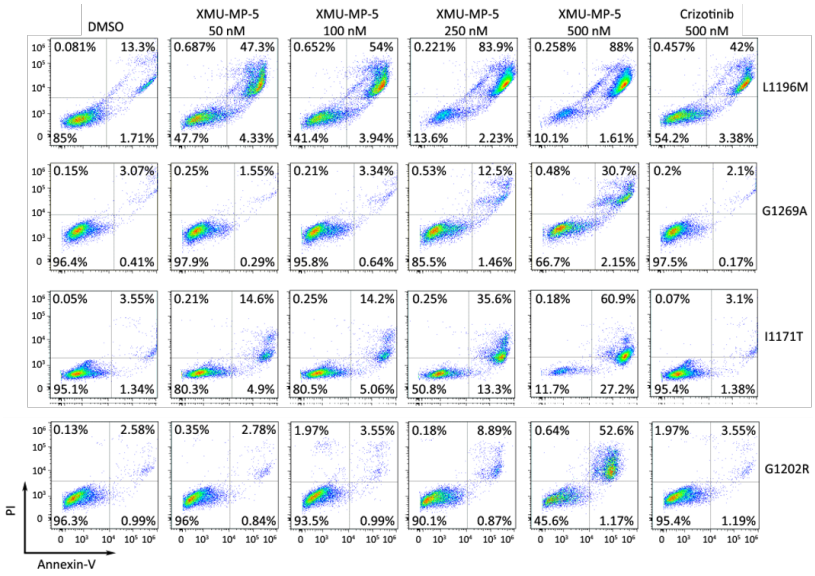
A, B The activity of ALT and AST after 10 days' dosing were measured. Mice were administrated with vehicle and 60 mg/kg XMU-MP-5 by tail vein injection. ALT and AST data were shown as Mean ± SD (n=7). Statistical comparasions were performed using an unpaired Student's *t* test for (A) and (B). (A) N.S., P = 0.9091; (B) N.S., P = 0.36.

C Body weights of each group were measured every day. Data were shown as mean ± SD (n = 7).

D Representative images of heart, kidney, liver, lung and spleen analyzed by H&E. Scale bars, 250 μm.

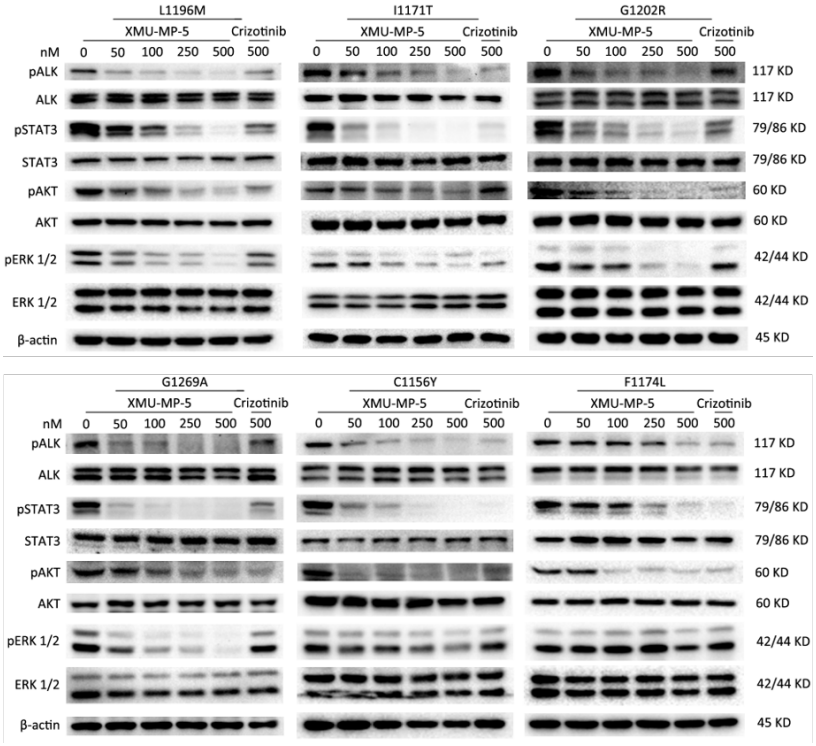



**Appendix Figure S6 Molecular docking of XMU-MP-5 with ALK mutant kinases.** A, B ALK (L1196M) mutant (PDB ID: 2YFX) was used in (A). Carbon chains of L1196M model were colored in yellow. ALK (G1202R) homology model (B, colored in purple) was developed based on the published X-ray structure of ALK(L1196M) (PDB ID: 2YFX).

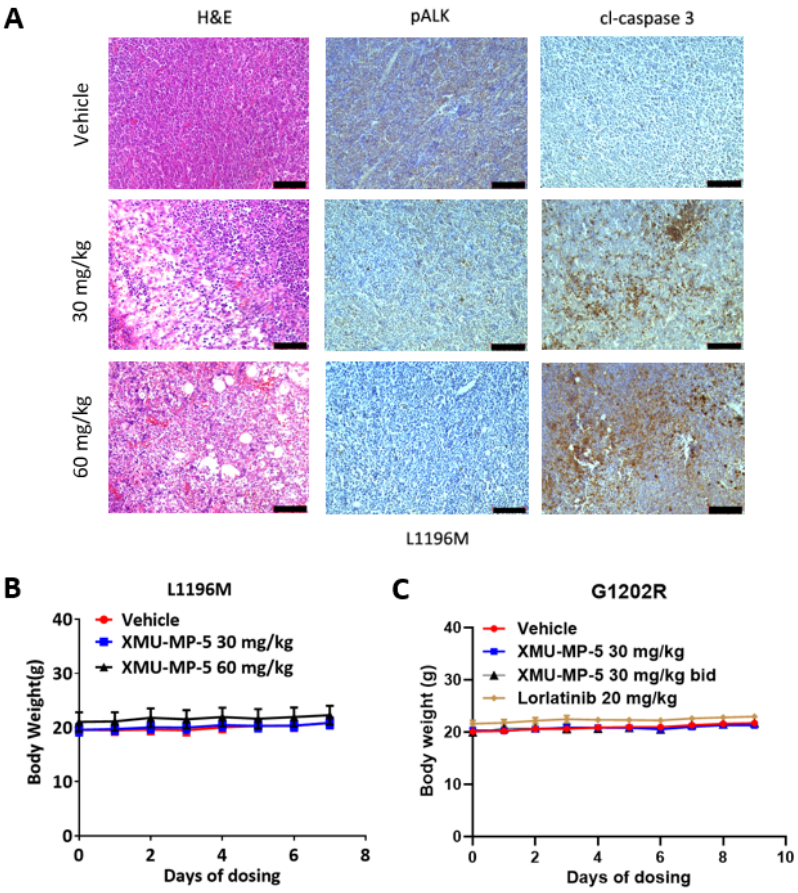



**Appendix Figure S7 Cellular apoptosis analysis of XMU-MP-5 against ALK mutant transformed Ba/F3 cell lines.**

Induction of cell apoptosis by XMU-MP-5 in ALK mutation Ba/F3 cell lines was assayed by Annexin-V-FLUOS/PI staining using flow cytometry. L1196M cells were treated for 24 hours, G1269A, I1171T and G1202R cell lines were treated for 48 hours.




**Appendix Figure S8 Effect of XMU-MP-5 on ALK signaling pathways in Ba/F3 cell lines harboring ALK mutations.** Cells were all treated with XMU-MP-5 or crizotinib for 4 h then analyzed by immunobloting.

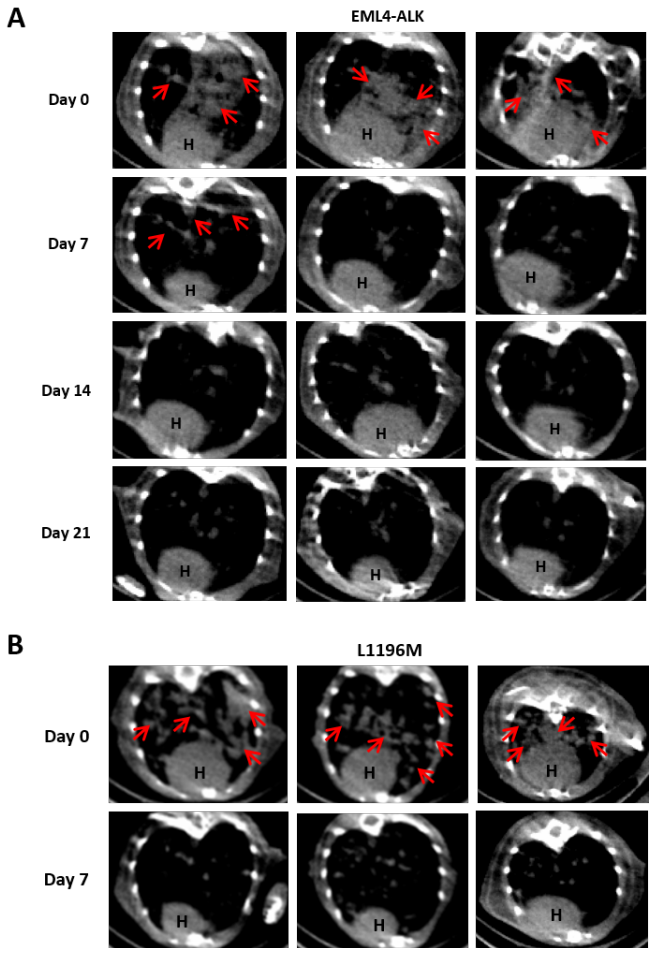



**Appendix Figure S9 In vivo efficacy of XMU-MP-5 in xenograft mouse models with ALK mutations.**

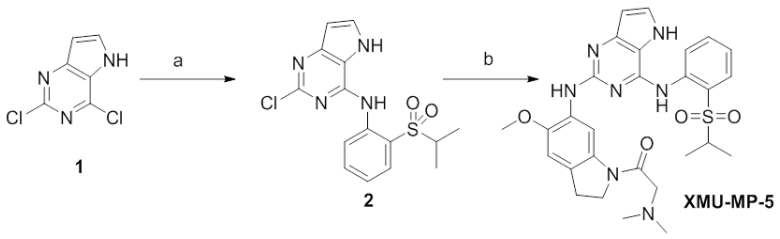
A H&E and IHC analysis of xenograft tumors from L1196M mice. Scale bar: 100 μm.

B Body weight change of mice bearing L1196M xenograft tumors during XMU-MP-5 treatment. Data were shown as mean ± SD (n = 6).

C Body weights of each group bearing G1202R xenograft tumors were measured every day. Data were shown as mean ± SD (n = 6).




**Appendix Figure S10 In vivo efficacy of XMU-MP-5 in different GEM models.**
A EML4-ALK transgenic mice were treated with 30 mg/kg XMU-MP-5 twice daily for 3 weeks. Tumor burdens were recorded by CTscan. H: heart; arrow indicated tumor. (n = 3).
B L1196M transgenic mice were treated with 40 mg/kg XMUMP-5 twice daily for 1 week. H: heart; arrow indicated tumor (n = 3).




**Appendix Figure S11 Synthesis of XMU-MP-5.**
Reagents and conditions: a) 2-(isopropylsulfonyl)aniline, TFA, 2-BuOH, 110 ℃; b) 1-(6-amino-5-methoxyindolin-1-yl)-2-(dimethylamino)ethan-1-one, TFA, 2-BuOH, 130 ℃.

**Appendix Table S1 The full list of KINOMEscan profiling data of XMU-MP-5.**

XMU-MP-5 was profiled against a panel of 468 kinases using KINOMEscan technology, an active site–dependent competition binding assay at 1000 nM. The results were reported as percent of control DMSO (ctrl%), in which lower numbers represent higher affinity binding; ctrl% = (test compound signal − positive control signal)/(negative control signal − positive control signal) × 100; where the negative control was set at 100%, and the positive control compound was set at 0%. The kinase hits with ctrl% less than 1 were highlighted, the lower numbers were marked with the more intense color.

| Cmpd ID | | XMU-MP-5 |
|---|---|---|
| Concentration (μM) | | 1 |
| DiscoveRx Gene Symbol | Entrez Gene Symbol | |
| AAK1 | AAK1 | 99 |
| ABL1(E255K)-phosphorylated | ABL1 | 71 |
| ABL1(F317I)-nonphosphorylated | ABL1 | 77 |
| ABL1(F317I)-phosphorylated | ABL1 | 69 |
| ABL1(F317L)-nonphosphorylated | ABL1 | 61 |
| ABL1(F317L)-phosphorylated | ABL1 | 88 |
| ABL1(H396P)-nonphosphorylated | ABL1 | 66 |
| ABL1(H396P)-phosphorylated | ABL1 | 77 |
| ABL1(M351T)-phosphorylated | ABL1 | 89 |
| ABL1(Q252H)-nonphosphorylated | ABL1 | 56 |
| ABL1(Q252H)-phosphorylated | ABL1 | 91 |
| ABL1(T315I)-nonphosphorylated | ABL1 | 63 |
| ABL1(T315I)-phosphorylated | ABL1 | 84 |
| ABL1(Y253F)-phosphorylated | ABL1 | 81 |
| ABL1-nonphosphorylated | ABL1 | 53 |
| ABL1-phosphorylated | ABL1 | 70 |
| ABL2 | ABL2 | 86 |
| ACVR1 | ACVR1 | 98 |
| ACVR1B | ACVR1B | 99 |
| ACVR2A | ACVR2A | 100 |
| ACVR2B | ACVR2B | 98 |
| ACVRL1 | ACVRL1 | 100 |
| ADCK3 | CABC1 | 100 |

| | | |
|---|---|---|
| ADCK4 | ADCK4 | 95 |
| AKT1 | AKT1 | 100 |
| AKT2 | AKT2 | 100 |
| AKT3 | AKT3 | 100 |
| ALK | ALK | 0.45 |
| ALK(C1156Y) | ALK | 0 |
| ALK(L1196M) | ALK | 0 |
| AMPK-alpha1 | PRKAA1 | 81 |
| AMPK-alpha2 | PRKAA2 | 88 |
| ANKK1 | ANKK1 | 58 |
| ARK5 | NUAK1 | 100 |
| ASK1 | MAP3K5 | 100 |
| ASK2 | MAP3K6 | 100 |
| AURKA | AURKA | 98 |
| AURKB | AURKB | 73 |
| AURKC | AURKC | 95 |
| AXL | AXL | 88 |
| BIKE | BMP2K | 98 |
| BLK | BLK | 94 |
| BMPR1A | BMPR1A | 96 |
| BMPR1B | BMPR1B | 77 |
| BMPR2 | BMPR2 | 72 |
| BMX | BMX | 100 |
| BRAF | BRAF | 100 |
| BRAF(V600E) | BRAF | 68 |
| BRK | PTK6 | 95 |
| BRSK1 | BRSK1 | 100 |
| BRSK2 | BRSK2 | 97 |
| BTK | BTK | 83 |
| BUB1 | BUB1 | 71 |
| CAMK1 | CAMK1 | 54 |
| CAMK1B | PNCK | 75 |
| CAMK1D | CAMK1D | 69 |
| CAMK1G | CAMK1G | 92 |
| CAMK2A | CAMK2A | 75 |
| CAMK2B | CAMK2B | 75 |
| CAMK2D | CAMK2D | 87 |
| CAMK2G | CAMK2G | 90 |
| CAMK4 | CAMK4 | 100 |
| CAMKK1 | CAMKK1 | 93 |
| CAMKK2 | CAMKK2 | 83 |
| CASK | CASK | 100 |
| CDC2L1 | CDK11B | 94 |
| CDC2L2 | CDC2L2 | 100 |
| CDC2L5 | CDK13 | 0.9 |
| CDK11 | CDK19 | 100 |
| CDK2 | CDK2 | 90 |
| CDK3 | CDK3 | 99 |

| | | |
|---|---|---|
| CDK4 | CDK4 | 100 |
| CDK4-cyclinD1 | CDK4 | 77 |
| CDK4-cyclinD3 | CDK4 | 88 |
| CDK5 | CDK5 | 94 |
| CDK7 | CDK7 | 75 |
| CDK8 | CDK8 | 91 |
| CDK9 | CDK9 | 82 |
| CDKL1 | CDKL1 | 64 |
| CDKL2 | CDKL2 | 94 |
| CDKL3 | CDKL3 | 100 |
| CDKL5 | CDKL5 | 67 |
| CHEK1 | CHEK1 | 90 |
| CHEK2 | CHEK2 | 100 |
| CIT | CIT | 89 |
| CLK1 | CLK1 | 80 |
| CLK2 | CLK2 | 69 |
| CLK3 | CLK3 | 89 |
| CLK4 | CLK4 | 81 |
| CSF1R | CSF1R | 98 |
| CSF1R-autoinhibited | CSF1R | 98 |
| CSK | CSK | 79 |
| CSNK1A1 | CSNK1A1 | 75 |
| CSNK1A1L | CSNK1A1L | 91 |
| CSNK1D | CSNK1D | 89 |
| CSNK1E | CSNK1E | 94 |
| CSNK1G1 | CSNK1G1 | 98 |
| CSNK1G2 | CSNK1G2 | 85 |
| CSNK1G3 | CSNK1G3 | 76 |
| CSNK2A1 | CSNK2A1 | 72 |
| CSNK2A2 | CSNK2A2 | 91 |
| CTK | MATK | 94 |
| DAPK1 | DAPK1 | 100 |
| DAPK2 | DAPK2 | 72 |
| DAPK3 | DAPK3 | 72 |
| DCAMKL1 | DCLK1 | 51 |
| DCAMKL2 | DCLK2 | 100 |
| DCAMKL3 | DCLK3 | 99 |
| DDR1 | DDR1 | 93 |
| DDR2 | DDR2 | 82 |
| DLK | MAP3K12 | 100 |
| DMPK | DMPK | 100 |
| DMPK2 | CDC42BPG | 82 |
| DRAK1 | STK17A | 95 |
| DRAK2 | STK17B | 89 |
| DYRK1A | DYRK1A | 72 |
| DYRK1B | DYRK1B | 100 |
| DYRK2 | DYRK2 | 50 |
| EGFR | EGFR | 98 |

| | | |
|---|---|---|
| EGFR(E746-A750del) | EGFR | 53 |
| EGFR(G719C) | EGFR | 96 |
| EGFR(G719S) | EGFR | 86 |
| EGFR(L747-E749del, A750P) | EGFR | 59 |
| EGFR(L747-S752del, P753S) | EGFR | 68 |
| EGFR(L747-T751del,Sins) | EGFR | 84 |
| EGFR(L858R) | EGFR | 78 |
| EGFR(L858R,T790M) | EGFR | 13 |
| EGFR(L861Q) | EGFR | 63 |
| EGFR(S752-I759del) | EGFR | 33 |
| EGFR(T790M) | EGFR | 9.1 |
| EIF2AK1 | EIF2AK1 | 77 |
| EPHA1 | EPHA1 | 96 |
| EPHA2 | EPHA2 | 91 |
| EPHA3 | EPHA3 | 95 |
| EPHA4 | EPHA4 | 100 |
| EPHA5 | EPHA5 | 89 |
| EPHA6 | EPHA6 | 100 |
| EPHA7 | EPHA7 | 93 |
| EPHA8 | EPHA8 | 91 |
| EPHB1 | EPHB1 | 82 |
| EPHB2 | EPHB2 | 88 |
| EPHB3 | EPHB3 | 99 |
| EPHB4 | EPHB4 | 87 |
| EPHB6 | EPHB6 | 95 |
| ERBB2 | ERBB2 | 73 |
| ERBB3 | ERBB3 | 94 |
| ERBB4 | ERBB4 | 20 |
| ERK1 | MAPK3 | 84 |
| ERK2 | MAPK1 | 78 |
| ERK3 | MAPK6 | 85 |
| ERK4 | MAPK4 | 75 |
| ERK5 | MAPK7 | 82 |
| ERK8 | MAPK15 | 76 |
| ERN1 | ERN1 | 96 |
| FAK | PTK2 | 15 |
| FER | FER | 4.9 |
| FES | FES | 12 |
| FGFR1 | FGFR1 | 94 |
| FGFR2 | FGFR2 | 75 |
| FGFR3 | FGFR3 | 96 |
| FGFR3(G697C) | FGFR3 | 94 |
| FGFR4 | FGFR4 | 94 |
| FGR | FGR | 97 |
| FLT1 | FLT1 | 97 |
| FLT3 | FLT3 | 100 |
| FLT3(D835H) | FLT3 | 88 |
| FLT3(D835V) | FLT3 | 88 |

| | | |
|---|---|---|
| FLT3(D835Y) | FLT3 | 89 |
| FLT3(ITD) | FLT3 | 79 |
| FLT3(ITD,D835V) | FLT3 | 75 |
| FLT3(ITD,F691L) | FLT3 | 92 |
| FLT3(K663Q) | FLT3 | 96 |
| FLT3(N841I) | FLT3 | 97 |
| FLT3(R834Q) | FLT3 | 100 |
| FLT3-autoinhibited | FLT3 | 88 |
| FLT4 | FLT4 | 100 |
| FRK | FRK | 77 |
| FYN | FYN | 94 |
| GAK | GAK | 83 |
| GCN2(Kin.Dom.2,S808G) | EIF2AK4 | 100 |
| GRK1 | GRK1 | 78 |
| GRK2 | ADRBK1 | 100 |
| GRK3 | ADRBK2 | 100 |
| GRK4 | GRK4 | 97 |
| GRK7 | GRK7 | 77 |
| GSK3A | GSK3A | 100 |
| GSK3B | GSK3B | 90 |
| HASPIN | GSG2 | 69 |
| HCK | HCK | 69 |
| HIPK1 | HIPK1 | 67 |
| HIPK2 | HIPK2 | 78 |
| HIPK3 | HIPK3 | 85 |
| HIPK4 | HIPK4 | 85 |
| HPK1 | MAP4K1 | 100 |
| HUNK | HUNK | 97 |
| ICK | ICK | 89 |
| IGF1R | IGF1R | 1.1 |
| IKK-alpha | CHUK | 63 |
| IKK-beta | IKBKB | 74 |
| IKK-epsilon | IKBKE | 100 |
| INSR | INSR | 0.1 |
| INSRR | INSRR | 0.8 |
| IRAK1 | IRAK1 | 63 |
| IRAK3 | IRAK3 | 92 |
| IRAK4 | IRAK4 | 68 |
| ITK | ITK | 88 |
| JAK1(JH1domain-catalytic) | JAK1 | 100 |
| JAK1(JH2domain-pseudokinase) | JAK1 | 97 |
| JAK2(JH1domain-catalytic) | JAK2 | 71 |
| JAK3(JH1domain-catalytic) | JAK3 | 77 |
| JNK1 | MAPK8 | 66 |
| JNK2 | MAPK9 | 88 |
| JNK3 | MAPK10 | 81 |
| KIT | KIT | 100 |
| KIT(A829P) | KIT | 74 |

| | | |
|---|---|---|
| KIT(D816H) | KIT | 70 |
| KIT(D816V) | KIT | 84 |
| KIT(L576P) | KIT | 90 |
| KIT(V559D) | KIT | 96 |
| KIT(V559D,T670I) | KIT | 100 |
| KIT(V559D,V654A) | KIT | 100 |
| KIT-autoinhibited | KIT | 74 |
| LATS1 | LATS1 | 100 |
| LATS2 | LATS2 | 100 |
| LCK | LCK | 90 |
| LIMK1 | LIMK1 | 100 |
| LIMK2 | LIMK2 | 88 |
| LKB1 | STK11 | 100 |
| LOK | STK10 | 80 |
| LRRK2 | LRRK2 | 92 |
| LRRK2(G2019S) | LRRK2 | 78 |
| LTK | LTK | 5.1 |
| LYN | LYN | 91 |
| LZK | MAP3K13 | 86 |
| MAK | MAK | 96 |
| MAP3K1 | MAP3K1 | 87 |
| MAP3K15 | MAP3K15 | 76 |
| MAP3K2 | MAP3K2 | 97 |
| MAP3K3 | MAP3K3 | 68 |
| MAP3K4 | MAP3K4 | 89 |
| MAP4K2 | MAP4K2 | 86 |
| MAP4K3 | MAP4K3 | 100 |
| MAP4K4 | MAP4K4 | 100 |
| MAP4K5 | MAP4K5 | 100 |
| MAPKAPK2 | MAPKAPK2 | 100 |
| MAPKAPK5 | MAPKAPK5 | 78 |
| MARK1 | MARK1 | 100 |
| MARK2 | MARK2 | 93 |
| MARK3 | MARK3 | 91 |
| MARK4 | MARK4 | 100 |
| MAST1 | MAST1 | 83 |
| MEK1 | MAP2K1 | 68 |
| MEK2 | MAP2K2 | 60 |
| MEK3 | MAP2K3 | 69 |
| MEK4 | MAP2K4 | 85 |
| MEK5 | MAP2K5 | 74 |
| MEK6 | MAP2K6 | 92 |
| MELK | MELK | 100 |
| MERTK | MERTK | 60 |
| MET | MET | 84 |
| MET(M1250T) | MET | 82 |
| MET(Y1235D) | MET | 77 |
| MINK | MINK1 | 64 |

| | | |
|---|---|---|
| MKK7 | MAP2K7 | 42 |
| MKNK1 | MKNK1 | 81 |
| MKNK2 | MKNK2 | 82 |
| MLCK | MYLK3 | 100 |
| MLK1 | MAP3K9 | 90 |
| MLK2 | MAP3K10 | 69 |
| MLK3 | MAP3K11 | 100 |
| MRCKA | CDC42BPA | 97 |
| MRCKB | CDC42BPB | 100 |
| MST1 | STK4 | 93 |
| MST1R | MST1R | 99 |
| MST2 | STK3 | 86 |
| MST3 | STK24 | 100 |
| MST4 | MST4 | 98 |
| MTOR | MTOR | 81 |
| MUSK | MUSK | 100 |
| MYLK | MYLK | 75 |
| MYLK2 | MYLK2 | 67 |
| MYLK4 | MYLK4 | 91 |
| MYO3A | MYO3A | 93 |
| MYO3B | MYO3B | 100 |
| NDR1 | STK38 | 73 |
| NDR2 | STK38L | 100 |
| NEK1 | NEK1 | 89 |
| NEK10 | NEK10 | 83 |
| NEK11 | NEK11 | 97 |
| NEK2 | NEK2 | 99 |
| NEK3 | NEK3 | 77 |
| NEK4 | NEK4 | 93 |
| NEK5 | NEK5 | 91 |
| NEK6 | NEK6 | 96 |
| NEK7 | NEK7 | 100 |
| NEK9 | NEK9 | 100 |
| NIK | MAP3K14 | 93 |
| NIM1 | MGC42105 | 72 |
| NLK | NLK | 97 |
| OSR1 | OXSR1 | 74 |
| p38-alpha | MAPK14 | 96 |
| p38-beta | MAPK11 | 92 |
| p38-delta | MAPK13 | 75 |
| p38-gamma | MAPK12 | 88 |
| PAK1 | PAK1 | 77 |
| PAK2 | PAK2 | 79 |
| PAK3 | PAK3 | 87 |
| PAK4 | PAK4 | 76 |
| PAK6 | PAK6 | 79 |
| PAK7 | PAK7 | 90 |
| PCTK1 | CDK16 | 74 |

| | | |
|---|---|---|
| PCTK2 | CDK17 | 84 |
| PCTK3 | CDK18 | 100 |
| PDGFRA | PDGFRA | 74 |
| PDGFRB | PDGFRB | 100 |
| PDPK1 | PDPK1 | 97 |
| PFCDPK1(P.falciparum) | CDPK1 | 85 |
| PFPK5(P.falciparum) | MAL13P1.279 | 98 |
| PFTAIRE2 | CDK15 | 98 |
| PFTK1 | CDK14 | 97 |
| PHKG1 | PHKG1 | 98 |
| PHKG2 | PHKG2 | 60 |
| PIK3C2B | PIK3C2B | 87 |
| PIK3C2G | PIK3C2G | 85 |
| PIK3CA | PIK3CA | 100 |
| PIK3CA(C420R) | PIK3CA | 72 |
| PIK3CA(E542K) | PIK3CA | 69 |
| PIK3CA(E545A) | PIK3CA | 68 |
| PIK3CA(E545K) | PIK3CA | 62 |
| PIK3CA(H1047L) | PIK3CA | 89 |
| PIK3CA(H1047Y) | PIK3CA | 86 |
| PIK3CA(I800L) | PIK3CA | 89 |
| PIK3CA(M1043I) | PIK3CA | 84 |
| PIK3CA(Q546K) | PIK3CA | 82 |
| PIK3CB | PIK3CB | 72 |
| PIK3CD | PIK3CD | 94 |
| PIK3CG | PIK3CG | 100 |
| PIK4CB | PI4KB | 45 |
| PIKFYVE | PIKFYVE | 92 |
| PIM1 | PIM1 | 75 |
| PIM2 | PIM2 | 98 |
| PIM3 | PIM3 | 84 |
| PIP5K1A | PIP5K1A | 92 |
| PIP5K1C | PIP5K1C | 100 |
| PIP5K2B | PIP4K2B | 100 |
| PIP5K2C | PIP4K2C | 76 |
| PKAC-alpha | PRKACA | 95 |
| PKAC-beta | PRKACB | 100 |
| PKMYT1 | PKMYT1 | 100 |
| PKN1 | PKN1 | 76 |
| PKN2 | PKN2 | 99 |
| PKNB(M.tuberculosis) | pknB | 100 |
| PLK1 | PLK1 | 82 |
| PLK2 | PLK2 | 68 |
| PLK3 | PLK3 | 65 |
| PLK4 | PLK4 | 93 |
| PRKCD | PRKCD | 93 |
| PRKCE | PRKCE | 83 |
| PRKCH | PRKCH | 87 |

| | | |
|---|---|---|
| PRKCI | PRKCI | 77 |
| PRKCQ | PRKCQ | 100 |
| PRKD1 | PRKD1 | 85 |
| PRKD2 | PRKD2 | 100 |
| PRKD3 | PRKD3 | 84 |
| PRKG1 | PRKG1 | 90 |
| PRKG2 | PRKG2 | 72 |
| PRKR | EIF2AK2 | 85 |
| PRKX | PRKX | 99 |
| PRP4 | PRPF4B | 100 |
| PYK2 | PTK2B | 74 |
| QSK | KIAA0999 | 100 |
| RAF1 | RAF1 | 100 |
| RET | RET | 91 |
| RET(M918T) | RET | 92 |
| RET(V804L) | RET | 100 |
| RET(V804M) | RET | 100 |
| RIOK1 | RIOK1 | 85 |
| RIOK2 | RIOK2 | 84 |
| RIOK3 | RIOK3 | 95 |
| RIPK1 | RIPK1 | 91 |
| RIPK2 | RIPK2 | 100 |
| RIPK4 | RIPK4 | 81 |
| RIPK5 | DSTYK | 65 |
| ROCK1 | ROCK1 | 92 |
| ROCK2 | ROCK2 | 73 |
| ROS1 | ROS1 | 18 |
| RPS6KA4(Kin.Dom.1-N-terminal) | RPS6KA4 | 100 |
| RPS6KA4(Kin.Dom.2-C-terminal) | RPS6KA4 | 85 |
| RPS6KA5(Kin.Dom.1-N-terminal) | RPS6KA5 | 96 |
| RPS6KA5(Kin.Dom.2-C-terminal) | RPS6KA5 | 100 |
| RSK1(Kin.Dom.1-N-terminal) | RPS6KA1 | 95 |
| RSK1(Kin.Dom.2-C-terminal) | RPS6KA1 | 100 |
| RSK2(Kin.Dom.1-N-terminal) | RPS6KA3 | 86 |
| RSK2(Kin.Dom.2-C-terminal) | RPS6KA3 | 81 |
| RSK3(Kin.Dom.1-N-terminal) | RPS6KA2 | 91 |
| RSK3(Kin.Dom.2-C-terminal) | RPS6KA2 | 100 |
| RSK4(Kin.Dom.1-N-terminal) | RPS6KA6 | 85 |
| RSK4(Kin.Dom.2-C-terminal) | RPS6KA6 | 91 |
| S6K1 | RPS6KB1 | 87 |
| SBK1 | SBK1 | 73 |
| SGK | SGK1 | 72 |
| SgK110 | SgK110 | 96 |
| SGK2 | SGK2 | 72 |
| SGK3 | SGK3 | 63 |
| SIK | SIK1 | 95 |
| SIK2 | SIK2 | 100 |
| SLK | SLK | 95 |

| | | |
|---|---|---|
| SNARK | NUAK2 | 57 |
| SNRK | SNRK | 88 |
| SRC | SRC | 94 |
| SRMS | SRMS | 76 |
| SRPK1 | SRPK1 | 100 |
| SRPK2 | SRPK2 | 100 |
| SRPK3 | SRPK3 | 84 |
| STK16 | STK16 | 82 |
| STK33 | STK33 | 81 |
| STK35 | STK35 | 100 |
| STK36 | STK36 | 90 |
| STK39 | STK39 | 72 |
| SYK | SYK | 90 |
| TAK1 | MAP3K7 | 74 |
| TAOK1 | TAOK1 | 97 |
| TAOK2 | TAOK2 | 80 |
| TAOK3 | TAOK3 | 86 |
| TBK1 | TBK1 | 88 |
| TEC | TEC | 94 |
| TESK1 | TESK1 | 100 |
| TGFBR1 | TGFBR1 | 98 |
| TGFBR2 | TGFBR2 | 100 |
| TIE1 | TIE1 | 91 |
| TIE2 | TEK | 92 |
| TLK1 | TLK1 | 100 |
| TLK2 | TLK2 | 89 |
| TNIK | TNIK | 90 |
| TNK1 | TNK1 | 100 |
| TNK2 | TNK2 | 55 |
| TNNI3K | TNNI3K | 100 |
| TRKA | NTRK1 | 94 |
| TRKB | NTRK2 | 63 |
| TRKC | NTRK3 | 67 |
| TRPM6 | TRPM6 | 85 |
| TSSK1B | TSSK1B | 100 |
| TSSK3 | TSSK3 | 92 |
| TTK | TTK | 71 |
| TXK | TXK | 100 |
| TYK2(JH1domain-catalytic) | TYK2 | 64 |
| TYK2(JH2domain-pseudokinase) | TYK2 | 59 |
| TYRO3 | TYRO3 | 88 |
| ULK1 | ULK1 | 79 |
| ULK2 | ULK2 | 71 |
| ULK3 | ULK3 | 72 |
| VEGFR2 | KDR | 74 |
| VPS34 | PIK3C3 | 57 |
| VRK2 | VRK2 | 84 |
| WEE1 | WEE1 | 98 |

| | | |
|---|---|---|
| WEE2 | WEE2 | 93 |
| WNK1 | WNK1 | 64 |
| WNK2 | WNK2 | 65 |
| WNK3 | WNK3 | 72 |
| WNK4 | WNK4 | 92 |
| YANK1 | STK32A | 84 |
| YANK2 | STK32B | 87 |
| YANK3 | STK32C | 100 |
| YES | YES1 | 89 |
| YSK1 | STK25 | 86 |
| YSK4 | MAP3K19 | 63 |
| ZAK | ZAK | 100 |
| ZAP70 | ZAP70 | 49 |

**Appendix Table S2 S score of XMU-MP-5 and approved ALK TKIs.**

The S score of XMU-MP-5 was determined according to KINOMEscan profile data, data for crizotinib, ceritinib, alectinib and brigatinib were acquired from published reference. ND = Not Determined.

| TKI | S(10) | S(35) |
|---|---|---|
| **XMU-MP-5** | 0.02 | 0.03 |
| **Crizotinib** | ND | 0.21 |
| **Ceritinib** | 0.19 | ND |
| **Alectinib** | 0.07 | ND |
| **Brigatinib** | 0.34 | ND |

**Appendix Table S3 Pharmacokinetic properties of XMU-MP-5 in mice.**

The pharmacokinetics of XMU-MP-5 were determined following single intravenous and oral administration in ICR mice (N = 3/each point) at the dose indicated. Blood samples were collected at 0.08, 0.25, 0.5, 1, 2, 4, 8 & 24 hr (IV) & 0.08, 0.25, 0.5, 1, 2, 4, 6, 8 hr (PO) post dose. Then the samples were quantified by LC-MS/MS, and data analysis was conducted using WinNonlin V6.3. intravenous injection, PO = oral delivery, $T_{max}$ = time of maximum plasma concentration, $C_{max}$ = maximum plasma concentration, = area under the curve (measure of exposure), $T_{1/2}$ = half life, CL = plasma clearance, Vz = volume of distribution, F = oral bioavailal

| Route | Dose (mg/kg) | $t_{1/2}$ hr | $T_{max}$ hr | $C_{max}$ ng/mL | $C_0$ ng/mL | $AUC_{(0-t)}$ ng/mL*hr | $AUC_{(0-\infty)}$ ng/mL*hr | Vz L/kg | Cl L/hr/kg | $MRT_{(0-\infty)}$ hr | F % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| IV | 5 | 3.99 | 0.08 | 4303.44 | 5718.82 | 3299.95 | 3311.99 | 8.70 | 1.51 | 1.74 | - |
| IV | 30 | 4.93 | - | - | 10200 | 13100 | 13200 | 6.70 | 2.27 | 2.95 | - |
| IV | 60 | 4.01 | - | - | 16300 | 26200 | 26400 | 7.71 | 2.27 | 3.39 | - |
| PO | 10 | 1.53 | 0.50 | 474.50 | - | 1205.37 | 1274.20 | - | - | 3.08 | 18.26 |

**Appendix Table S4 X-ray data collection and refinement statistics.**

| | ALK S1281G/XMU-MP-5 |
|---|---|
| **PDB ID** | 7BTT |
| **Data collection** | |
| Space group | $P2_12_12_1$ |
| Cell dimensions□□ | |
| *a*, *b*, *c* (Å) | 51.5, 57.4, 105.4 |
| □□□□□□□□□□□□□□□°□□ | 90.0, 90.0, 90.0 |
| Resolution (Å) | 50.0-1.86 (1.90-1.86) * |
| $R_{pim}$ | 0.066 (0.426) |
| □□□□□□ | 10.8 (1.9) |
| Completeness (%) | 99.4 (99.8) |
| Redundancy | 4.1 (4.3) |
| | |
| **Refinement** | |
| Resolution (Å) | 46.3-1.86 |
| No. reflections | 26858 |
| $R_{work}$ / $R_{free}$ | 0.187/0.209 |
| No. atoms | |
| Protein | 2391 |
| Ligand/ion | 40 |
| Water | 157 |
| *B*-factors | |
| Protein | 36.7 |
| Ligand/ion | 34.5 |
| Water | 39.6 |
| R.m.s. deviations | |
| Bond lengths (Å) | 0.007 |
| Bond angles (°) | 0.938 |
| Ramachandran Plot | |
| Favored regions (%) | 98.33 |
| Allowed regions (%) | 1.67 |
| Outliers (%) | 0.00 |

*Values in parentheses are for highest-resolution shell.

**Appendix Table S5 $IC_{50}$ values of XMU-MP-5 against wild type and ALK mutant Ba/F3 cell lines[a].**

| Compound ($IC_{50}$, nM) | WT | EML4-ALK | L1196M | G1269A | I1171T | G1202R | S1206Y | C1156Y | F1174L |
|---|---|---|---|---|---|---|---|---|---|
| XMU-MP-5 | 4098 | 4.15 | 12 | 30.9 | 16.3 | 49.4 | 27.4 | 22.9 | 123.2 |
| Crizotinib | 1116 | 45.86 | 467.3 | 305.1 | 145.4 | 556.3 | 71.4 | 73.2 | 81 |
| Alectinib | ND | 16.9 | 167.3 | 165.1 | 158 | 478.9 | ND | 103.6 | 47.5 |
| Ceritinib | ND | 1.2 | 6.84 | 1.8 | 1.5 | 206.1 | ND | 6.1 | 53.6 |
| Brigatinib | ND | 17.3 | 38.5 | 14 | 86.7 | 432.8 | ND | 17.2 | 47.5 |
| Lorlatinib | ND | 1.5 | 56.9 | 31.5 | 20.8 | 89.5 | ND | 5.9 | 2.4 |

[a] Antiproliferative activity was determined by the MTS assay. ND means not determined.

**Appendix Table S6 Fold change $IC_{50}$s of ALK mutant Ba/F3 cell lines to WT Ba/F3 cell lines.**

| Fold change $IC_{50}$ | EML4-ALK | L1196M | G1269A | I1171T | G1202R | S1206Y | C1156Y | F1174L |
|---|---|---|---|---|---|---|---|---|
| XMU-MP-5 | 987 | 341.5 | 132.6 | 251.4 | 83 | 149.6 | 179 | 33.3 |
| Crizotinib | 24.3 | 2.4 | 3.7 | 7.7 | 2 | 15.6 | 13.2 | 13.8 |