**EGFR ligands induce resistance to ALK inhibitors in a dose dependent manner in H2228 cells**

Tumor cells were incubated with crizotinib (100 nmol/L) and TAE684 (100 nmol/L), and increasing concentrations of EGF, TGF-α, or HB-EGF. Cell growth was determined as described in the legend to Figure 1A. Each experiment included triplicate determinations, and each experiment was repeated at least three times independently.
H2228 cells were highly sensitive to siRNAs specific for ALK, whereas HGF and/or EGFR ligands (EGF, TGF-α, and HB-EGF) reduced the sensitivity of siRNAs specific for ALK.

(A) Control or ALK-specific siRNAs (#1, #2) were introduced into H2228 cells, with cell growth measured by the MTT assay. Cell extracts were prepared and immunoblotted with the indicated antibodies. Each experiment included triplicate determinations, and each experiment was repeated at least three times independently.

(B) Control or ALK-specific siRNA#1 were introduced into H2228 cells. After 24 hours, the cells were incubated with or without EGF, TGF-α, IGF-1, or PDGF-AA (100 ng/mL each), HB-EGF (10 ng/mL), or HGF (50 ng/mL), with cell growth determined after 72 hours. The percentage of growth is shown relative to untreated controls. Each experiment included triplicate determinations, and each experiment was repeated at least three times independently.
**Induction of apoptosis in H2228 and H3122 cells by crizotinib.**

The cells (3x10^3 cells) were seeded in 96-well, white walled plates and incubated overnight, and then treated with 1μM crizotinib or vehicle (DMSO) for 48 h. Caspase-3/7 activity was measured by using Caspase-Glo3/7 assay kit. Cellular apoptosis was expressed relative to DMSO treated control cells shown as mean ± SD, *P<0.05 (one-way ANOVA).
Supplementary Figure 4

MANA2

In MANA2 cells, HGF and EGFR ligands trigger ALK inhibitor resistance via Met and EGFR, respectively.

(A) Crizotinib inhibited the phosphorylation of ALK and STAT-3 but did not that of EGFR, Akt and Erk1/2 in the presence of EGF, TGF-α, or HB-EGF. MANA2 cells were treated with or without crizotinib (100 nmol/L) for 1 h and/or EGF (100 ng/mL), TGF-α (100 ng/mL), or HB-EGF (10 ng/mL) for 15 min. The cells were lysed and the indicated proteins were detected by immunoblotting. The results shown are representative of 3 independent experiments.

(B) TAE684 inhibited the phosphorylation of ALK and STAT-3, but not of Met, Gab1, Akt and Erk1/2 in the presence of HGF. MANA2 cells were treated with or without TAE684 (100 nmol/L) for 1 h and/or HGF (50 ng/mL) for 15 min. The cells were lysed and the indicated proteins were detected by immunoblotting. The results shown are representative of 3 independent experiments.