Abstract # B186

TPX-0005, a polypharmacology inhibitor, overcomes ALK treatment resistances from acquired mutations, bypass signaling and EMT

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Introduction

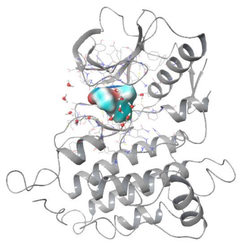
ALK kinase inhibitors have achieved tremendous success in the treatment of lung cancer patients with abnormal ALK gene. However, the emergence of drug resistance limits their long term clinical applications. The mechanisms of resistance often include target gene amplification, acquired resistance mutations, bypass signaling, epithelial-mesenchymal transition (EMT) and metastasis. The bypass and EMT-based resistance mechanisms represent a major challenging in primary and acquired resistances, especially after multiple kinase inhibitor treatment. None of the current ALK inhibitors can overcome bypass or EMT-based resistance when applied as a single agent therapy. Therefore, a new strategy needs to be deployed for the design of new generation ALK inhibitors to overcome multi-resistance mechanisms simultaneously.

SRC kinase has been identified to contribute broadly to cancer progression and matastasis. SRC/FAK signaling plays important roles in regulating antitumor immunity, cancer stem-like properties, and EMT. TPX-0005 is a potent kinase inhibitor against WT and mutated ALK, ROS1 and TRK family kinases, especially the clinically significant gatekeeper and solvent front mutations. At clinically relevant concentrations, TPX-0005 also inhibits JAK2, SRC and FAK that are important in modulating multiple resistance mechanisms.

Upregulation of the bypass signaling kinase EGFR, EMT marker vimentin, and cancer stem-like marker CD44 was reported in H2228 cells, likely leading to intrinsic resistance to ALK inhibitors. TPX-0005 suppressed the phosphorylation of YB-1, leading to the downregulation of EGFR, CD44 and vimentin, and eventually to anti-proliferation effect on H2228 cells. This unprecedented polypharmacology profile of TPX-0005 provides opportunities in clinic to combat multiple resistance mechanisms including a broad spectrum of acquired mutations, bypass signaling, cancer stemness, and metastasis.

Polypharmacology Profile of TPX-0005

Target	Kinase IC_{50} (nM) at 10 μM ATP
ALK	1.04
ROS1	0.0706
TRKA	0.826
TRKB	0.0517
TRKC	0.0956
SRC	5.29
FAK	6.96
JAK2	1.04



The model of TPX-0005 complex with ALK Kinase

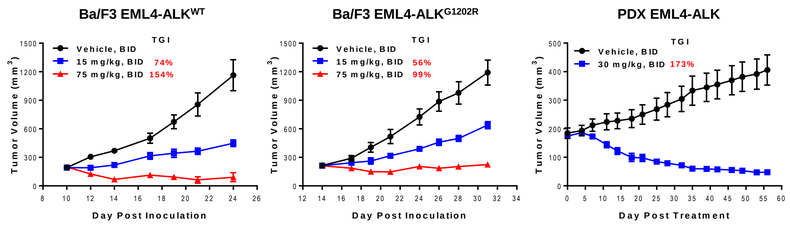
Inhibition of Clinical Resistance ALK Mutants

Cell Proliferation of Ba/F3 Cells IC_{50} (nM)

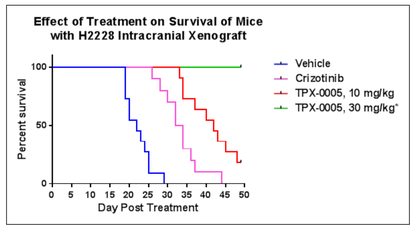
Compound	EML4-ALK V1 WT	EML4-ALK L1196M	EML4-ALK G1202R	L1198F/ C1156Y	L1198F/ G1202R	L1198F/ L1196M
TPX-0005	17.8	50	20.5	1.1	0.2	34.8
Crizotinib	74.8	713	359.4	22	43.7	350.7
Ceritinib	8.7	6	388	1123	476.8	1794
Alectinib	18.9	131	607	158	1369	1249
Brigatinib	11.8	13	399	118	1187	341
Lorlatinib	0.7	18	NA	89	131.6	1169

TPX-0005 Potently Inhibited ALK in *in vivo* models

- **Efficacy of TPX-0005 against WT and mutant ALK in xenograft tumor models**



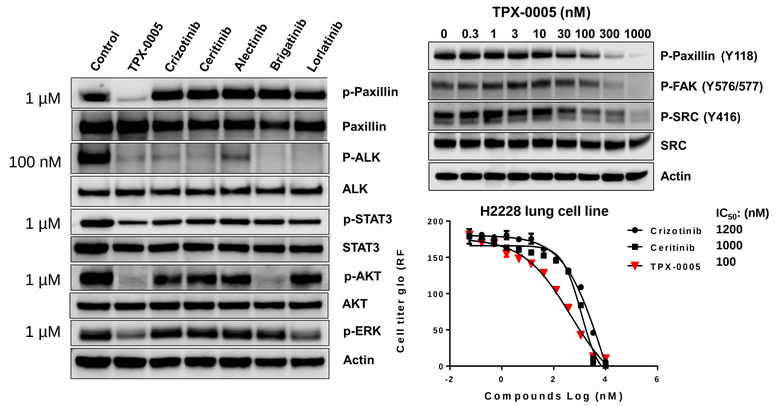
- **Efficacy of TPX-0005 against ALK in intracranial xenograft tumor models**



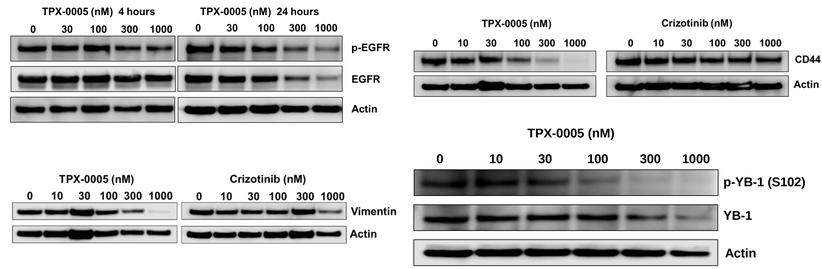
Group	Median Survival (days)
Vehicle, BID	22
Crizotinib, 50 mg/kg, BID	33
TPX-0005, 10 mg/kg, BID	42
TPX-0005, 30 mg/kg, BID	Not reached

Modulation of SRC/FAK Signaling

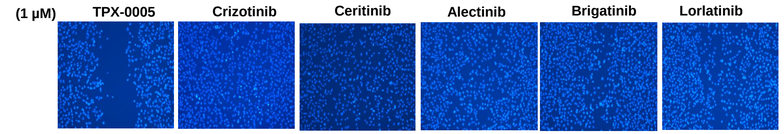
- H2228 lung cancer cell line endogenously expressed *EML4-ALK* fusion gene. The activation of EGFR, SRC, and FAK confers primary resistance to ALK inhibitors, such as crizotinib and ceritinib.
- TPX-0005 effectively inhibited SRC/FAK signaling in addition to ALK leading to the inhibition of H2228 cell proliferation, and overcame the primary resistance.



- **TPX-0005 down-regulated the expression of EGFR, CD44, vimentin, and YB-1 in H2228 cells**

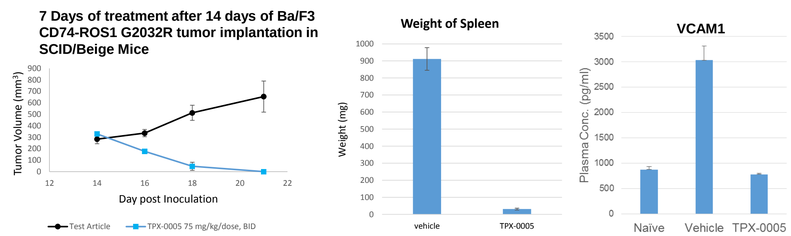


- **TPX-0005 effectively inhibited HT-1080 tumor cell migration in wound healing assay**



- **TPX-0005 effectively eliminated metastasis and restored cytokines to normal levels in metastasis tumor model**

7 Days of treatment after 14 days of Ba/F3 CD74-ROS1 G2032R tumor implantation in SCID/Beige Mice



Conclusions

- TPX-0005 was designed to target the center of ATP binding pocket with a compact three dimensional structure, and potently inhibited WT and mutant ALK, including the solvent front mutations ALK^{G1202R}, the compound mutations L1198F/C1156Y, L1198F/G1202R, L1198F/L1196M and other acquired resistant mutations.
- TPX-0005 potently inhibited the WT and mutant ALKs, ROS1s and TRKs in vitro and in vivo, especially the solvent front mutations which render common resistances to ALK.
- TPX-0005 significantly extended survival time in mouse H2228 orthotopic brain tumor model.
- TPX-0005 also effectively suppressed SRC/FAK signaling, leading to down-regulation of bypass signaling kinase EGFR, cancer stem cell marker CD44 and EMT marker vimentin, restoration of the sensitivity to ALK inhibition, and reduction of the potential for metastasis.
- The multi-faceted kinase inhibitor TPX-0005 has the potential in clinic to address the primary and acquired resistances to ALK inhibitor treatment caused by multiple mechanisms that include a broad spectrum of acquired resistance mutations, bypass signaling, cancer stemness, and EMT.
- A phase 1/2 clinical trial (NCT03093116) of TPX-0005 is actively pursued.

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