**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 next generation ALK inhibitors to overcome multi-resistance mechanisms simultaneously.

SRC kinase is involved in contributing to cancer progression and metastasis. SRC/FAK signaling plays important roles in regulating antimurmur 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 significantly gain-of-function ALK and solvency from mutations. At clinically relevant concentrations, TPX-0005 also inhibits JAK2, SRC and FAK that are important in modulating multiple resistance mechanisms.

Upregulation of the bypassing kinase EGFR, EMT marker vimentin, and cancer stem 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.

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

![Image](https://example.com/image1)

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

**TPX-0005 Potently Inhibited ALK in intracranial xenograft tumor models**

![Image](https://example.com/image2)

**Modulation of SRC/FAK Signaling**

**Inhibition of Clinical Resistance ALK Mutants**

![Image](https://example.com/image3)

**Cell Proliferation of Ba/F3 Cells**

<table>
<thead>
<tr>
<th>Compound</th>
<th>EML-ALK/WT</th>
<th>EML-ALK/L1196M</th>
<th>EML-ALK/G1202R</th>
<th>L1199F/G1202R</th>
<th>L1199F/L1198M</th>
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<td>TPX-0005</td>
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<td>20.5</td>
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<td>NA</td>
<td>78</td>
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**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**

**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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