Abstract # B185

TPX-0005, a supreme ROS1 inhibitor, overcomes crizotinib-resistant ROS1 mutations including solvent front mutation G2032R and gatekeeper mutation L2026M

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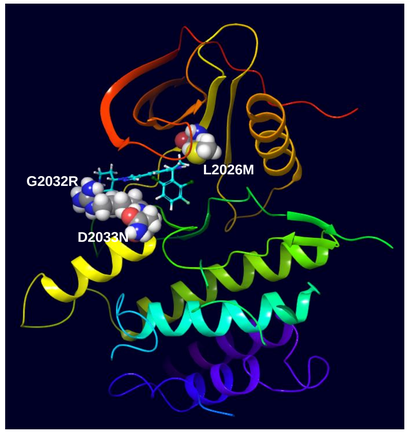
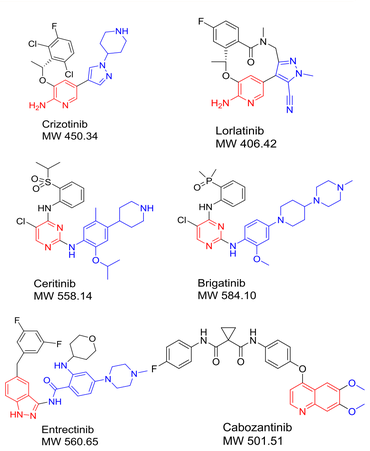
Introduction

The fusion ROS1 and ALK have been identified as oncogene drivers in small portions of many malignancies, especially in non-small cell lung cancer (NSCLC). ALK/ROS1/MET inhibitor crizotinib has been approved by the US Food and Drug Administration for the treatment of ALK or ROS1-positive non-small cell lung cancer in 2011 and 2016, respectively. The emergence of drug resistance presents a major issue for targeted therapy. Although ceritinib, alectinib and brigatinib have been approved for crizotinib-refractory ALK$^+$ patients with NSCLC, treatment options for patients with ROS1$^+$ NSCLC are limited, especially for crizotinib-refractory patients. Ceritinib and entrectinib demonstrated clinical efficacy only in crizotinib-naïve ROS1$^+$ patients. The most common resistance mechanisms to crizotinib treatment in ROS1$^+$ NSCLC are the solvent front mutation ROS1 G2032R and gatekeeper mutation ROS1 L2026M. TPX-0005, a novel three-dimensional macrocycle with a much smaller size than current ROS1 inhibitors in the clinic, was designed to overcome clinical resistance mutations systematically. TPX-0005 potently inhibited both wild type and mutant ROS1s including solvent front mutations and gatekeeper mutations. TPX-0005 showed pico-molar activity against ROS1 kinase (IC_{50} 0.0706 nM) in Reaction Biology kinase assay. The comparison of TPX-0005 with other ROS1 inhibitors in Ba/F3 cell proliferation assays is presented in the table. In the xenograft tumor model studies, TPX-0005 dramatically caused tumor regression in the tumors carrying WT or solvent-front mutated ROS1 fusion gene. Overall, TPX-0005 demonstrated desired drug-like properties, good safety profile, and is a supreme ROS1 inhibitor against WT and various mutated ROS1s. A Phase 1/2 clinical trial of TPX-0005 is actively pursued (NCT03093116).

ROS1 mutations and ROS1 inhibitor structures

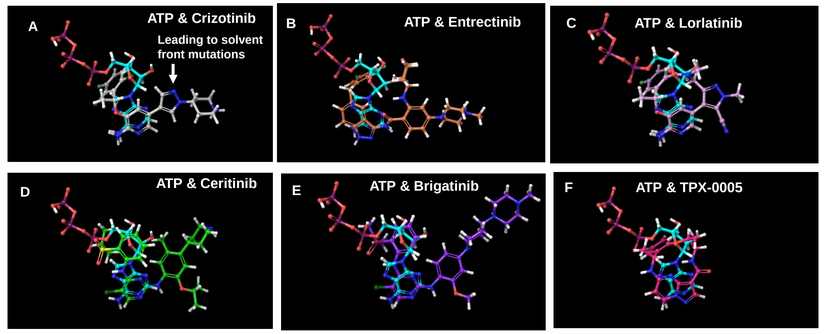


ROS1/crizotinib protein complex labeled with clinical mutations



- Crizotinib has demonstrated significant clinical activity with an ORR of 73% and a median PFS of 19.2 months as a single agent in *ROS1*+ NSCLC patients[1]
- G2032R[2], D2033N[3] and L2026M[4] are reported mutations from crizotinib refractory ROS1 patients
- Lorlatinib, ceritinib, entrectinib, brigatinib and cabozantinib have demonstrated activities against ROS1 kinase[4]
- Crizotinib, entrectinib, lorlatinib, ceritinib, and brigatinib are type I kinase inhibitors with extended motifs (blue color) to solvent exposure area
- Cabozantinib is a type II kinase inhibitor with a hydrophobic motif (black color) into the back pocket

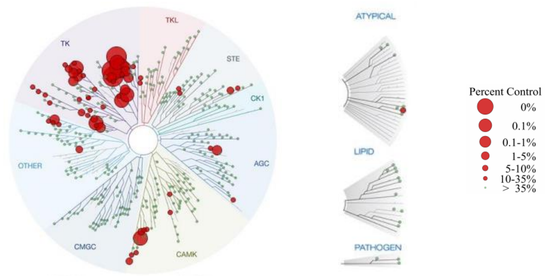
TPX-0005 is designed to overcome resistances systematically



- The ROS1 inhibitors including crizotinib (A, MW 450. 34), entrectinib (B, MW 560. 65), lorlatinib (C, MW 406.42), ceritinib (D, MW 558.14), and brigatinib (E, MW 584.10) are oversized, and develop various resistance mutations outside ATP binding boundary. The solvent front mutation ROS1 G2032R conferred resistance to all of the type I ROS1 inhibitors because of the structure motifs in the common area leading to the solvent exposure as shown above.
- TPX-0005 (F, MW 355.37) was designed to locate completely inside the ATP adenine binding pocket, without motifs extending to the back pocket or solvent front, and have higher binding affinity with the active kinase conformation.
- The macrocyclic structure of TPX-0005 anchors the three dimensional interactions with the protein with higher binding efficiency, and allows to target wild type (WT) and mutant kinases equally.

TPX-0005 is a polyphamacology kinase inhibitor

- **TPX-0005 kinase selectivity profile over 395 non-mutant kinases (screened at DiscoveRx)**



Selectivity score*	S (1)	S (10)	S (35)
TPX-0005	0.025	0.071	0.157

* S score = the number of hits / the number of the non-mutant kinases screened

- **Kinase activity of the S (10) hits at 10 μM ATP concentration**

	TRKB	ROS1	TRKC	TRKA	ALK	JAK2	SRC	DDR1	FAK
IC_{50} (nM)*	0.052	0.071	0.10	0.83	1.04	1.04	5.30	5.70	6.96

* Kinase activity was determined at Reaction Biology, Inc.

TPX-0005 Potently Inhibited WT and Mutant ROS1

- **Kinase activity against wildtype, fusion and mutant ROS1s IC_{50} (nM) by TPX-0005**

ROS1 WT	0.0706	ROS1 G2032R	0.456	TPM3-ROS1	0.113

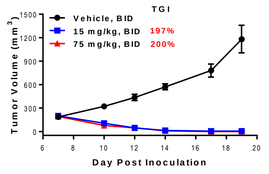
* Kinase activity was determined at Reaction Biology, Inc.

- **Inhibition of cell proliferation in Ba/F3 cell lines by ROS1 inhibitors**

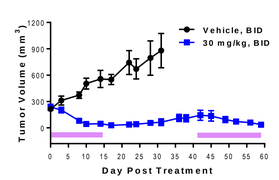
	CD74-ROS1 Ba/F3 Cell Proliferation IC_{50} (nM)							
Inhibitor	WT	G2032R	D2033N	L2026M	S1986F	S1986Y	L1951R	G2101A
TPX-0005	**<0.2**	**8.4**	**0.2**	**10**	**<0.2**	**<0.2**	**<0.2**	**0.2**
Crizotinib	18.4	1402	139	606.4	20.9	19	157.6	184.9
Lorlatinib	0.2	262.4	2.4	930.6	0.3	0.3	2.8	4.7
Entrectinib	11.3	2404	ND	2026	ND	ND	35.4	175.2
Ceritinib	70.9	2000	ND	ND	14.2	26.9	785.5	156.2
Brigatinib	34.5	1385	167.1	2115	27.7	24.6	2919	74.7
Cabozantinib	0.5	60.7	0.1	29.1	ND	ND	91.8	4.4

- **Efficacy of TPX-0005 against WT and mutant ROS1 in xenograft tumor models**

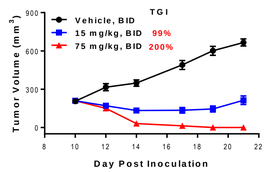
Ba/F3 CD74-ROS1^{WT}



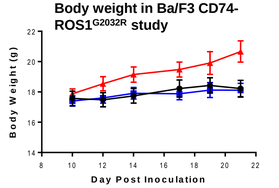
PDX CD74-ROS1



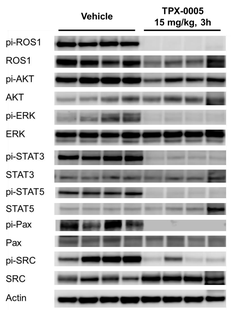
Ba/F3 CD74-ROS1^{G2032R}



Body weight in Ba/F3 CD74-ROS1^{G2032R} study



❑ **TPX-0005 inhibited oncogenic ROS1 signaling in Ba/F3 CD74-ROS1^{G2032R} tumors.**



Conclusions

- The compact three dimensional structure of TPX-0005 allows it to bind **WITHIN** the ATP binding boundary with high affinity and be able to target both wild type and mutant kinases.
- TPX-0005 potently inhibited WT and mutant ROS1s *in vitro* and *in vivo*, especially the solvent front mutations which render common resistances to ROS1 inhibitors that are currently in clinic.
- TPX-0005 has good drug-like properties, and is able to address the unmet medical needs for patients that are resistant to ROS1 inhibitors.
- A Phase 1/2 clinical trial is on-going for TPX-0005 for cancer patients with solid tumors harboring ALK, ROS1 or NTRK fusion gene (NCT03093116).

Reference
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3. Drilon A, et al Clin Cancer Res. 2016, 22:2351-8.
4. Lin JJ and Shaw AT, J Thorac Oncol. 2017 Aug 14. pii: S1556-0864(17)30668-8.

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