Abstract #4832
Poster #1319

Repotrectinib, a new generation ROS1 inhibitor, is highly potent against fusion ROS1s and emerging resistance mutations

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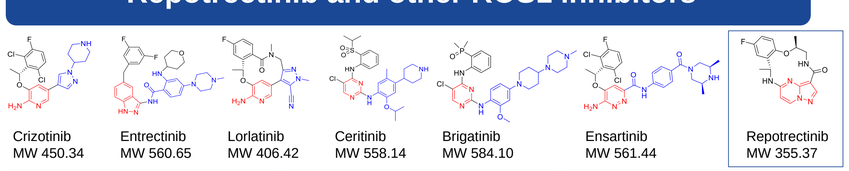
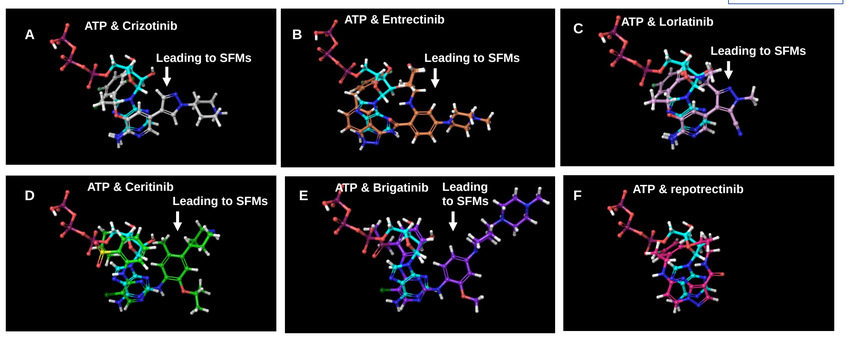
Introduction

ROS1 gene fusions (*ROS1*+) have been identified as oncogenic drivers in many malignancies, especially in non-small cell lung cancer (NSCLC). ALK/ROS1/MET inhibitor crizotinib is the only drug approved for the treatment of *ROS1*+ NSCLC.[1] The efficacy of crizotinib in *ROS1*+ NSCLC varied among different types of ROS1 fusion partners and patients with the most dominant fusion *CD74-ROS1* had a higher brain metastasis rate and shorter overall survival.[2] In addition, the emergence of drug resistance presents a major obstacle for targeted therapy. The most common resistance mutations to crizotinib treatment in *ROS1*+ NSCLC are the solvent front mutation (SFM) ROS1 G2032R and the gatekeeper mutation ROS1 L2026M. Repotrectinib (TPX-0005) was designed to overcome clinical resistance mutations systematically. The activity of repotrectinib against *ROS1* fusions with different partner genes and also with additional *ROS1* resistance mutations was investigated. Repotrectinib potently inhibited both wildtype (WT) and mutant *ROS1* fusions including SFMs and gatekeeper mutations. In cell growth assays using engineered Ba/F3 cells expressing ROS1 fusions with several partners, such as SDC4, CD74, TPM3 and EZR, repotrectinib demonstrated superior potency in comparison to other ROS1 inhibitors against multiple *ROS1* mutations, especially SFMs and gatekeeper mutations. In xenograft tumor model studies, repotrectinib treatment resulted in pronounced regression of the tumors harboring WT or SFM *ROS1* fusion genes. Overall, repotrectinib demonstrated a profound inhibition profile against WT and various mutated *ROS1* fusions with several partners. A Phase 1/2 clinical trial of repotrectinib is currently ongoing (TRIDENT-1, NCT03093116).

Repotrectinib and other ROS1 inhibitors



Crizotinib MW 450.34 | Entrectinib MW 560.65 | Lorlatinib MW 406.42 | Ceritinib MW 558.14 | Brigatinib MW 584.10 | Ensartinib MW 561.44 | Repotrectinib MW 355.37



- Crizotinib is the only ROS1 inhibitor approved for *ROS1*+ NSCLC with an objective response rate of 66% and a median duration of response of 18.3 months[1]
- Multiple ROS1 resistant mutations including G2032R, D2033N and L2026M are reported from crizotinib-resistant *ROS1*+ patients with up to 41% G2032R mutation[3]
- Crizotinib, entrectinib, lorlatinib, ceritinib, brigatinib and ensartinib have the common extended motifs (blue color) to solvent front exposure area that are susceptible for developing SFMs
- Repotrectinib (MW 355.37) was designed to bind completely inside the ATP pocket of the targeted kinase, without motifs extending to the back pocket or solvent front to systemically overcome resistance mutations, especially gatekeeper and solvent front mutations

Repotrectinib is a highly potent ROS1 inhibitor

- **Repotrectinib potently inhibited wildtype and mutant ROS1s at 10 μM ATP**

	ROS1 WT	ROS1 G2032R	ROS1 D2033N	ROS1 G2101A	ROS1 L1951R	ROS1 L2026M	ROS1 L2155S	ROS1 S1986F	ROS1 S1986Y	TPM3-ROS1
IC_{50} (nM)*	0.071	0.46	0.24	0.63	0.17	1.19	0.57	0.16	0.09	0.113

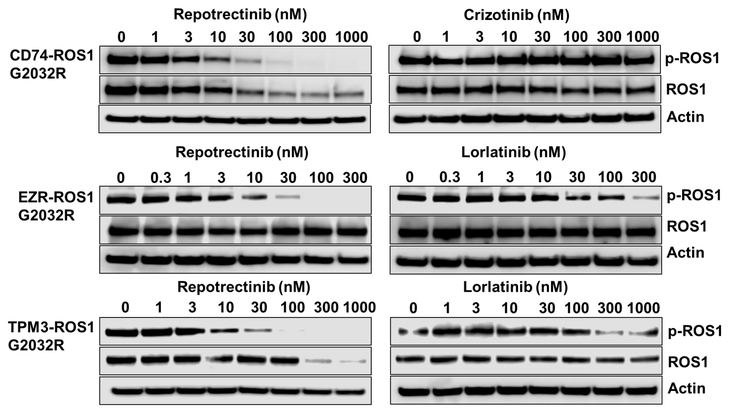
* Kinase activity was determined at Reaction Biology, Inc.

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

	Ba/F3 Cell Proliferation IC_{50} (nM)									
	No Kinase Domain Mutation				ROS1 G2032R				ROS1 L2026M	
Inhibitor	CD74-ROS1	SDC4-ROS1	EZR-ROS1	TPM3-ROS1	CD74-ROS1	SDC4-ROS1	EZR-ROS1	TPM3-ROS1	EZR-ROS1	TPM3-ROS1
Repotrectinib	<0.2	0.2	<0.1	<0.1	3.3	3	5	16.3	<0.2	<0.1
Crizotinib	14.6	19.6	19.4	31.1	266.2	4661	660	500.6	95.6	236.2
Lorlatinib	0.2	0.3	0.2	0.3	160.7	352.9	190.5	434.9	1.6	1.9
Entrectinib	10.5	ND	1.5	9.4	1813	ND	2947	1093	13.3	40.7
Ceritinib	42.8	59.8	33.1	105	1391	1883	885.8	543.7	12.6	66.5
Brigatinib	21	38.7	25.8	61	1172	1473	360.6	3000	24.4	41.3
Cabozantinib	0.5	3	0.4	4.5	11.3	169.4	39.5	60.7	3.4	12.6
Ensartinib	39.5	ND	118.6	433.1	371.8	ND	1757	4814	543.3	1463

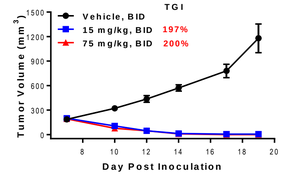
[a] Other than repotrectinib, data based on evaluation of comparable proxy chemical reagents purchased from commercial sources; ND: not determined

- **Repotrectinib more potently inhibited auto-phosphorylation of ROS1 G2032R in NIH3T3 or Ba/F3 cells engineered with different ROS1 fusion partners harboring G2032R mutation than crizotinib[a] or lorlatinib[a]**

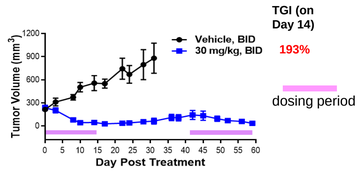


Efficacy of repotrectinib in xenograft tumor models

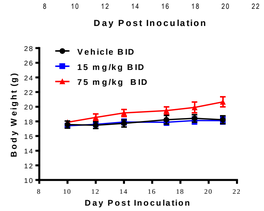
- **Antitumor effect of repotrectinib in a Ba/F3 cell-derived xenograft model with the *CD74-ROS1* fusion gene**

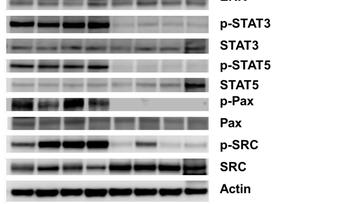


- **Antitumor effect of repotrectinib in a patient-derived xenograft model of lung cancer with the *CD74-ROS1* fusion gene**



- **Antitumor effect of repotrectinib in a Ba/F3 cell-derived xenograft model with the CD74-ROS1 G2032R mutation**





Conclusions

- The compact, 3-dimensional macrocycle structure of repotrectinib allows it to bind completely inside the ATP pocket of the target kinase with greater precision and affinity and be able to target both wildtype and mutant kinases with high potency
- Repotrectinib potently inhibited multiple WT and mutant ROS1 fusions, regardless of the fusion partners
- Repotrectinib is the most potent Type I ROS1 inhibitor against the SFM G2032R that is the major resistant mechanism developed after crizotinib treatment
- Repotrectinib has been well tolerated in the on-going Phase 1 portion of the TRIDENT-1 clinical trial and this preclinical data support that repotrectinib has great potential to address the unmet medical need for patients that are resistant to first generation ROS1 inhibitors
- A Phase 1/2 clinical trial of repotrectinib is on-going for patients with advanced solid tumors harboring *ROS1, NTRK1-3, or ALK* fusion gene (NCT03093116)

Reference
1. Kazandjian D, et al *Oncologist*. **2016**, 21:974-80. 2. Li Z, et al, *J Thorac Oncol.* **2018**,13:987-995. 3. Gainor JF, et al *JCO Precis Oncol.* **2017**, 2017.

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