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Taletrectinib, a Next-Generation Selective ROS1 Inhibitor, Exhibits a Differentiated Profile in ROS1 Fusion Models

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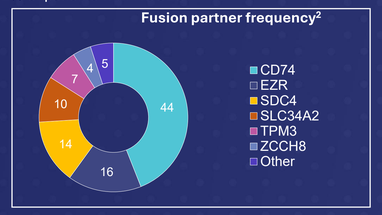
Background

- *ROS1* gene fusions occur in approximately 2% of patients with NSCLC[1]



  - *ROS1* gene fusions result in increased ROS1 autophosphorylation and constitutive activation[3]
- While crizotinib, entrectinib, and repotrectinib are approved by the US FDA for the treatment of *ROS1+* NSCLC, an unmet need remains for effective and tolerable treatment options[1]
  - Crizotinib and entrectinib are not active against many resistance mutations, including ROS1^{G2032R}, the most common mutation[1]
  - Repotrectinib, while active in the CNS, is associated with a high rate of neurologic AEs such as dizziness (65%), ataxia (28%), and cognitive impairment (25%), which are attributed to the drug's inhibition of TRKB[4]
- Taletrectinib is currently approved in China and the United States for the treatment of adult patients with locally advanced or metastatic *ROS1+* NSCLC[5]
  - Taletrectinib is an oral next-generation, CNS-active, ROS1 TKI with selectivity over TRKB[6]
  - Pooled results from the TRUST-I (NCT04395677) and TRUST-II (NCT04919811) studies of taletrectinib demonstrated a cORR of 89%, an intracranial cORR of 77%, a mDOR of 44.2 months, and mPFS of 45.6 months in patients with advanced *ROS1+* NSCLC who had not previously received a ROS1 TKI[6]

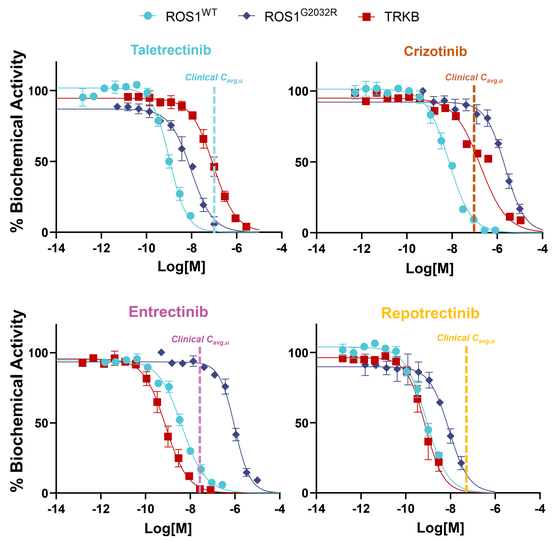
Methods

Study Design

- **Biochemical inhibition:** In vitro kinase activity was detected via Reaction Biology HotSpot kinase assay and measured using the P81 filter-binding method[7]
- **In vitro cell viability:** Ba/F3 cells harboring the respective *ROS1* wild type or mutant fusions were plated at a density of 500–2500 cells/well and treated the next day with respective treatments; viability was assessed after 5 or 6 days of treatment, and data are presented as IC_{50} values, where 50% of growth inhibition relative to control was observed
- **Western blotting:** Cells were harvested 2 hours postdosing, and protein expression was analyzed using the antibodies Phospho-ROS1 Tyr 2274 (CST-3028), ROS1 (CST-3287, OTI1A1-Invitrogen), and GAPDH (Proteintech_60004); protein expression was normalized to GAPDH expression
- **Mutagenesis resistance screen:** CD74-ROS1 WT cells were treated with ENU for 16 h. Cells were then seeded into 96 well plates (2 plates per treatment group) and treated with vehicle or a TKI for 4 weeks. Resistant clones were counted and sent for sequencing
- **In vivo CDX/PDX:** CDX or PDX studies were run as per standard practice
  - Briefly, cells or tumor fragments were implanted in mice, and mice were housed in pathogen-free housing with access to sterilized food and water ad libitum
  - Taletrectinib, crizotinib, entrectinib, or repotrectinib was administered orally
  - For subcutaneous models, tumors were measured twice/week and tumor volume was calculated using the formula (L*W^2)*0.52; for intracranial models, survival of mice was evaluated[8]

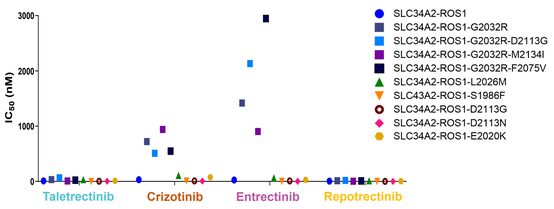
Results

Taletrectinib Demonstrates Effective Target Coverage for ROS1^{WT} and ROS1^{G2023R} at Clinically Relevant Concentrations



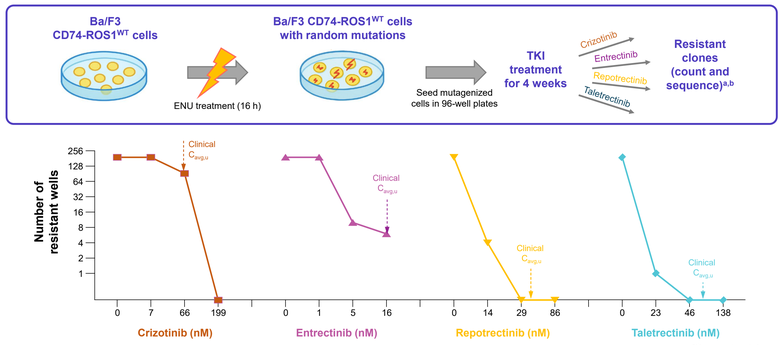
Kinase	Percent coverage at clinically relevant concentrations (Clinical $C_{avg,u}$)			
	Taletrectinib	Crizotinib	Entrectinib	Repotrectinib
ROS1^{WT}	99%	87%	79%	97%
ROS1^{G2032R}	88%	11%	7%	83%
TRKB	47%	36%	95%	99%

Taletrectinib Demonstrates Activity Against Single and Double ROS1^{G2032R} Mutants Resistant to Crizotinib and Entrectinib



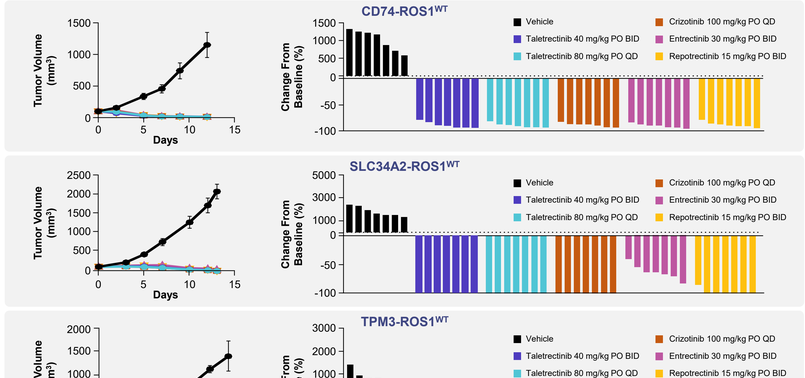
	IC_{50} (nM)									
	SLC34A2-ROS1	SLC34A2-ROS1-G2032R	SLC34A2-ROS1-D2113G	SLC34A2-ROS1-G2032R-D2113G	SLC34A2-ROS1-G2032R-M2134I	SLC34A2-ROS1-G2032R-F2075V	SLC34A2-ROS1-L2026M	SLC43A2-ROS1-S1986F	SLC34A2-ROS1-D2113G	SLC34A2-ROS1-D2113N	SLC34A2-ROS1-E2020K
Taletrectinib	10	33	65	8	23	31	1	3	3	10
Crizotinib	32	721	509	940	548	107	6	10	6	76
Entrectinib	26	1419	2132	903	2947	63	2	10	3	29
Repotrectinib	5	12	19	4	10	12	<1	1	1	5

Taletrectinib Prevents Emergence of Resistance Clones at Clinically Relevant Concentrations

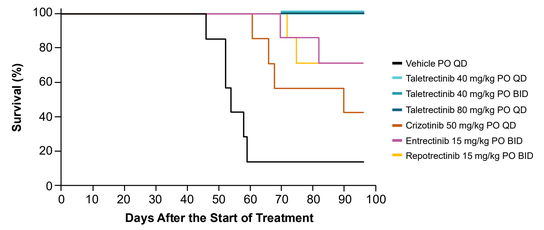


[a]Sequencing data pending.
[b]Taletrectinib treatment group was followed for an additional 2 weeks; no resistance clones emerged.

Taletrectinib Demonstrates Growth Inhibition and Tumor Regression in Models Harboring Prevalent ROS1 Fusions

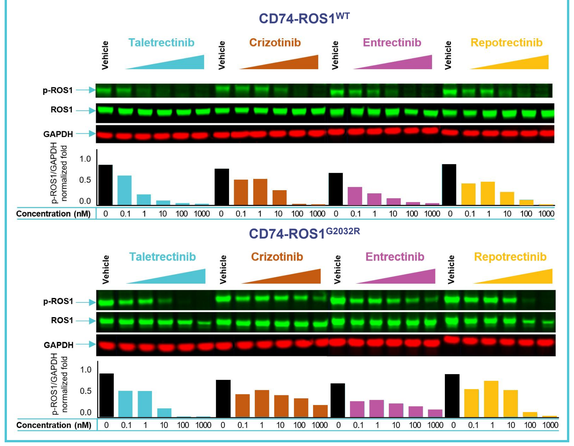


Taletrectinib Prolongs Survival in LU-01-0414 Intracranial Mouse PDX Model Harboring an SDC4-ROS1 Fusion



N=7 per treatment.

Taletrectinib Inhibits ROS1 Phosphorylation in Both ROS1^{WT} and ROS1^{G2032R} Resistance Mutation Settings



Conclusions

- Taletrectinib demonstrates complete inhibition of ROS1^{WT} and selectivity over TRKB at clinically relevant concentrations, whereas repotrectinib and entrectinib did not demonstrate any selectivity over TRKB, a kinase whose inhibition is associated with CNS toxicity
- Taletrectinib demonstrates complete inhibition of ROS1^{G2032R} at clinically relevant concentrations, while crizotinib and entrectinib show little activity against this mutation
- Taletrectinib demonstrated in vitro and in vivo activity against ROS1 regardless of fusion partner or resistance mutation, including the ROS1^{G2032R} mutation, which is resistant to crizotinib and entrectinib
- Taletrectinib treatment at clinically relevant concentrations prevented resistance in CD74-ROS1^{WT} fusion-positive cells, whereas crizotinib and entrectinib treatment both lead to emergence of resistant clones
- Taletrectinib demonstrated tumor regression in models harboring prevalent ROS1^{WT} and ROS1^{G2032R} fusions
- Taletrectinib inhibits ROS1 phosphorylation in ROS1^{WT} and ROS1^{G2032R} models
- Taletrectinib treatment prolonged survival (>100 days) in mice implanted intracranially with a PDX model harboring a SDC4-ROS1 fusion

Abbreviations

AE, adverse event; ATP, adenosine triphosphate; BID, twice daily; $C_{avg,u}$, average unbound concentration; CDX, cell line–derived xenograft; CNS, central nervous system; cORR, confirmed objective response rate; ENU, *N*-ethyl-*N*-nitrosourea; FDA, Food and Drug Administration; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IC_{50}, half-maximal inhibitory concentration; mDOR; median duration of response; mPFS, median progression-free survival; NSCLC, non-small cell lung cancer; PDX, patient-derived xenograft; PO, orally; p-ROS1, phosphorylated ROS1; QD, once daily; ROS1, proto-oncogene tyrosine-protein kinase 1; ROS1+, ROS1 positive; SLC34A2, solute carrier family 34 member 2; TKI, tyrosine kinase inhibitor; TRKB, tropomyosin receptor kinase B; WT, wild type.

References

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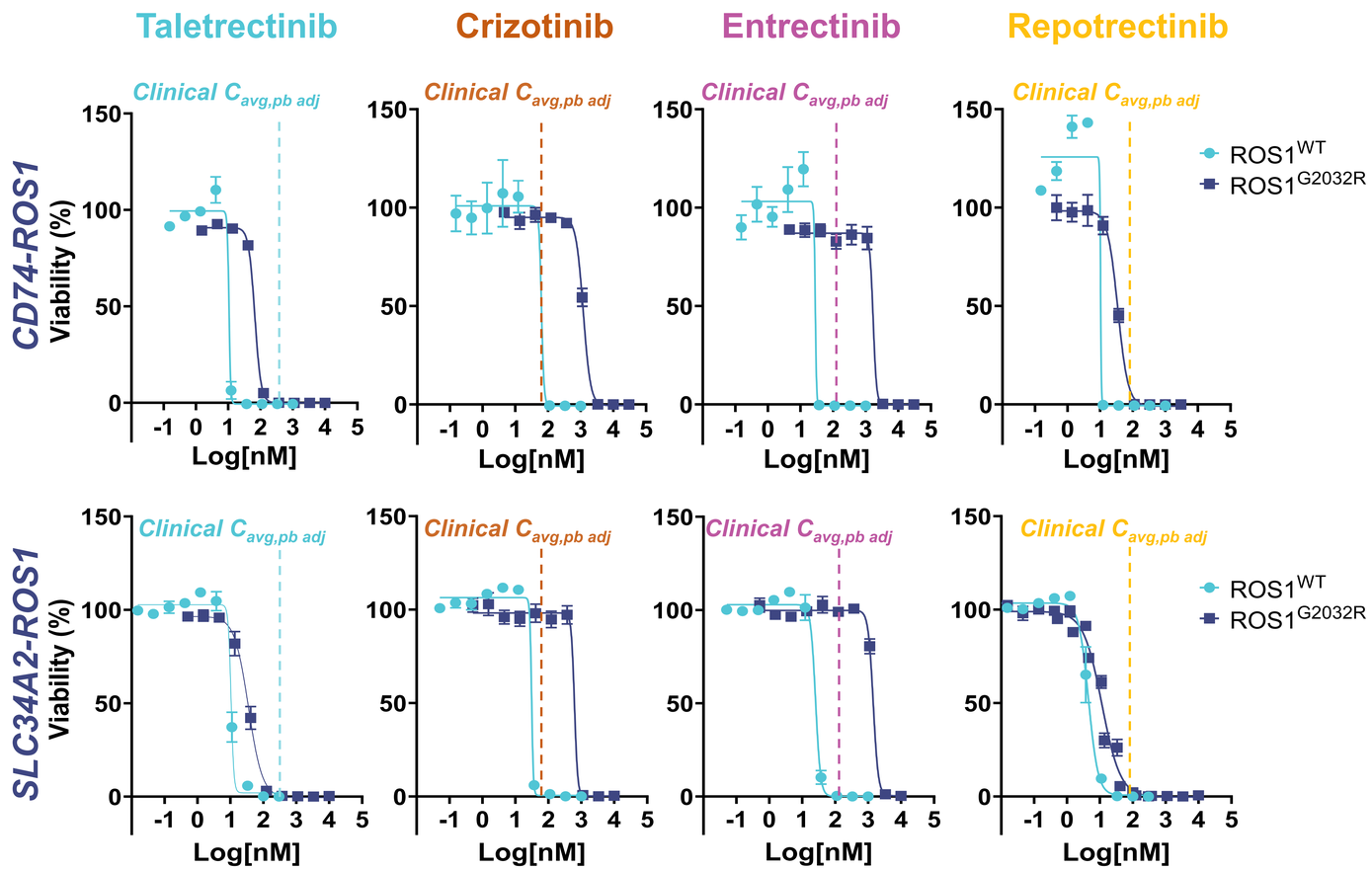
Supplemental Materials

Supplemental

	AUC (nM*h)	C_{avg} (nM) (AUC/24h)	Fu (10% FBS)	Clinical $C_{avg,unbound}$ (nM)	Fu (Human plasma)	$C_{avg,pb\ adj}$ (nM)[d]
Taletrectinib, 600 mg	23800[a]	992	0.07[a]	69	0.23[b]	302
Repotrectinib, 160 mg QD/BID-ss	20300[a]	846	0.046[a]	39	0.41[b]	95
Entrectinib, 600 mg QD	48000[a]	2000	<0.01[a]	<20	0.16[c]	<125
Crizotinib, 250 mg BID	17200[a]	796	0.09[a]	65	0.83[c]	78

[a] US FDA label as of July 15, 2025, and Crizotinib NDA 202570 Clinical Pharmacology Review (steady state).
[b] Internal data protein binding determination using ultracentrifugation at 1 μM.
[c] External data from AACR 2024 https://doi.org/10.1158/1538-7445.AM2024-LB182.
[d] In vivo average plasma concentration over the dosing interval (C_{avg}) after correcting for the difference in protein binding between the in vivo system (human plasma) and the in vitro assay conditions (10% FBS).
AUC, area under the curve; BID, twice daily; C_{avg}, average concentration (AUC/24hr); $C_{avg,unbound}$, average unbound drug concentration; $C_{avg,pb\ adj}$, protein binding adjusted average drug concentration; FBS, fetal bovine serum; Fu, fraction unbound; QD, once daily.

Taletrectinib Inhibits Cell Growth in Models Harboring ROS1^{WT} and ROS1^{G2032R} Fusions



	Cell Line	IC_{50} (nM)			
		Taletrectinib	Crizotinib	Entrectinib	Repotrectinib
CD74	ROS1^{WT}	11	63	28	10
	ROS1^{G2032R}	67	1198	1695	35
SLC34A2	ROS1^{WT}	10	31	25	4
	ROS1^{G2032R}	35	620	1428	12

$C_{avg,pb\ adj}$, protein binding adjusted average drug concentration; IC_{50}, half-maximal inhibitory concentration; ROS1, proto-oncogene tyrosine-protein kinase 1; SLC34A2, solute carrier family 34 member 2; WT, wild type.