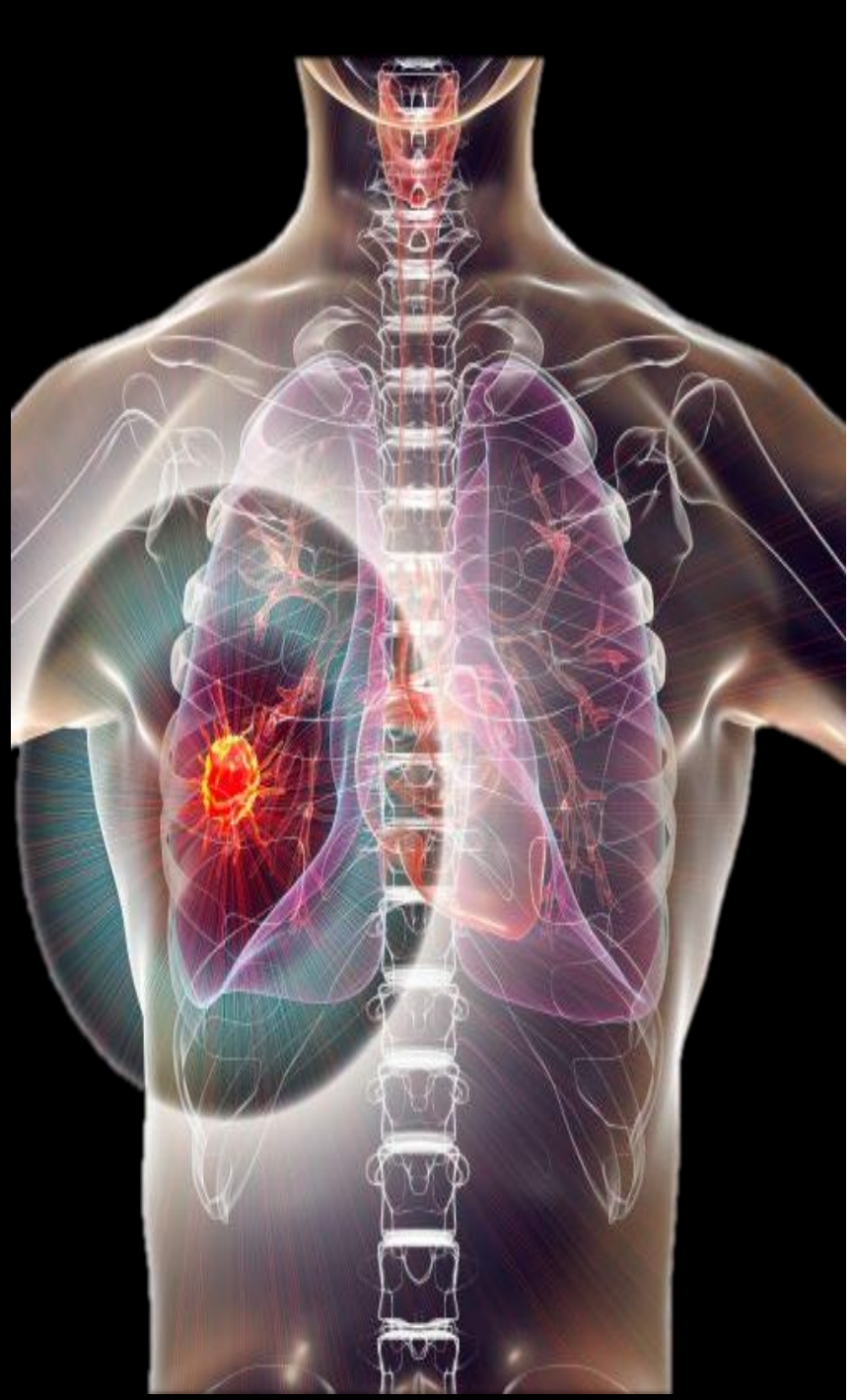

MECHANISM OF RESISTANCE TO ROS1 AND THERAPY POST RESISTANCE IN NSCLC

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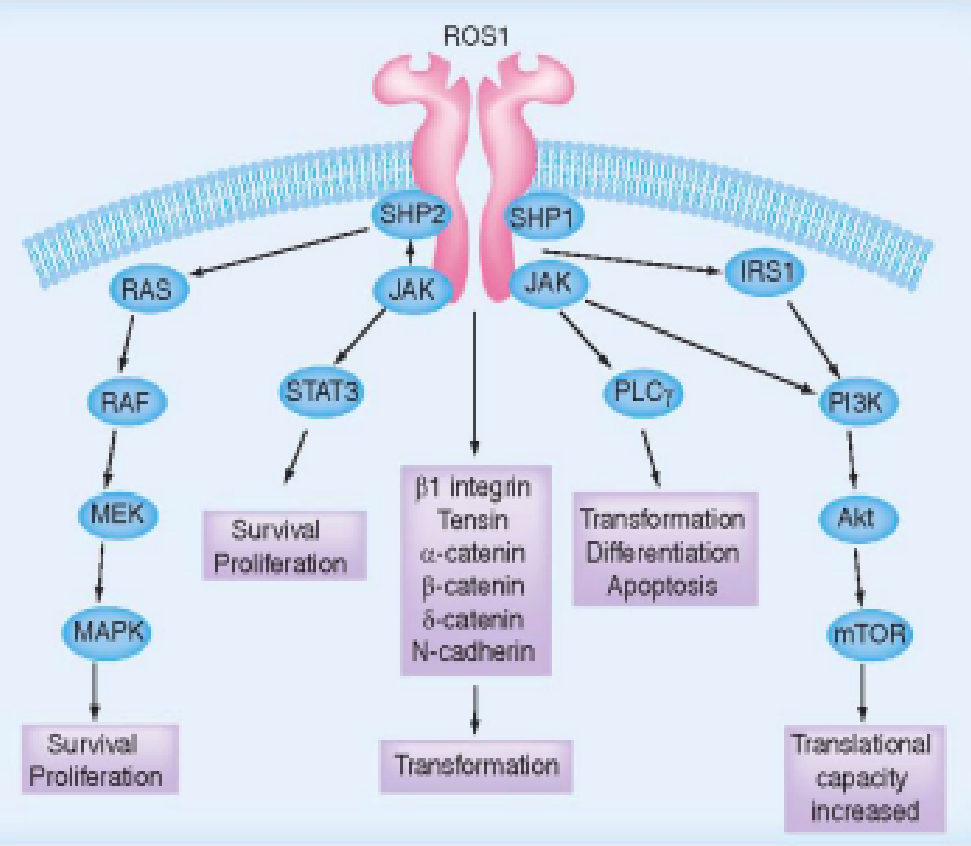
ROS1 Receptor Tyrosine Kinase Pathway



- ROS1 is a receptor tyrosine kinase that is phylogenetically related to ALK[1]
- ROS1 rearrangements are identified in approximately 1%-2% of NSCLC cases[2-4]
- ROS1 fusion kinases are constitutively activated and function as potent oncogenic drivers[4,5]
- ROS1 rearrangements rarely overlap with other oncogenic drivers, including ALK rearrangements, in NSCLC[5]

Reported Prevalence of ROS1-Rearranged NSCLCs[6,*]

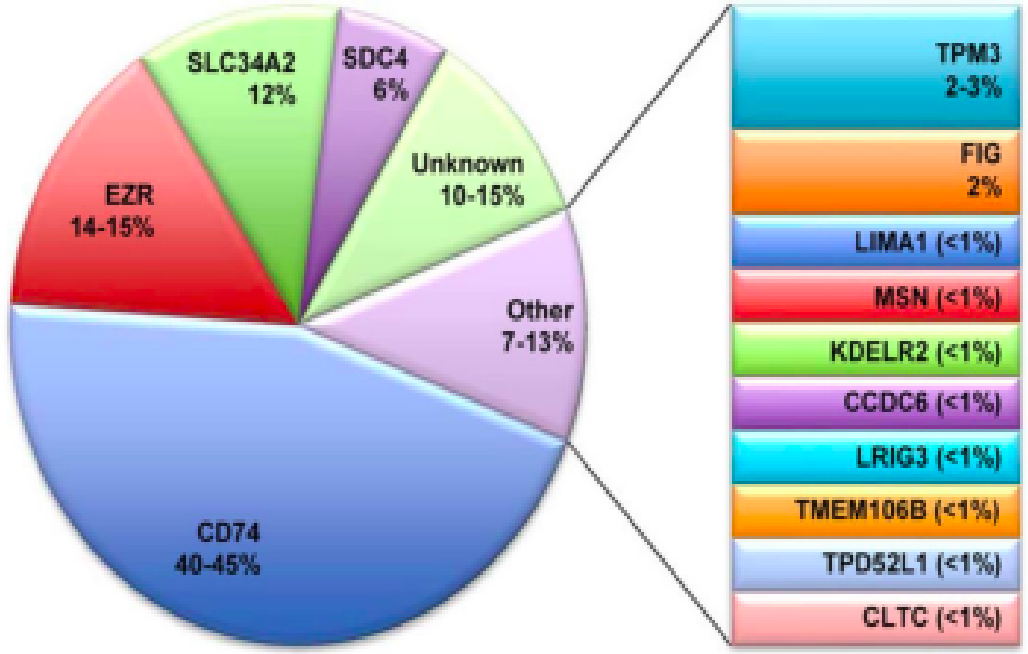
	No. of Patients Screened	No. of ROS1+ Tests (%)
North America	1,240	20 (**1.6**)
Europe	1,828	37 (**2.0**)
Asia	5,375	121 (**2.3**)

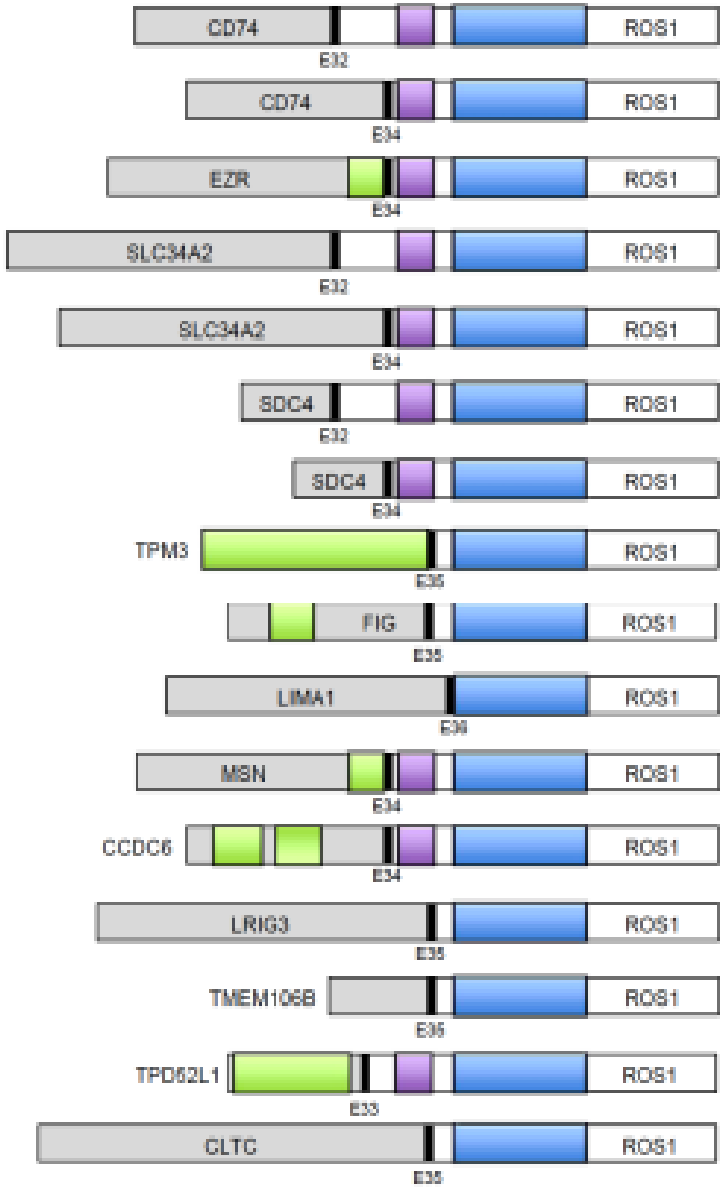
Once activated, ROS1 kinase activates the SHP-2 phosphatase and upregulates MAPK/ERK, PI3K/ AKT/mTOR and JAK/STAT3 signaling pathways to promote cell growth and survival

ROS1 Rearrangements

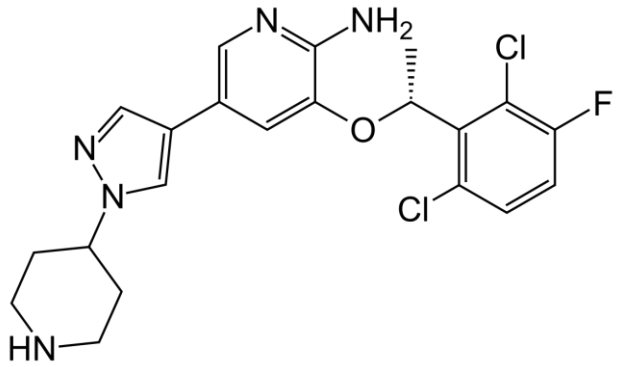
Prevalence of ROS1 Rearrangements in NSCLC

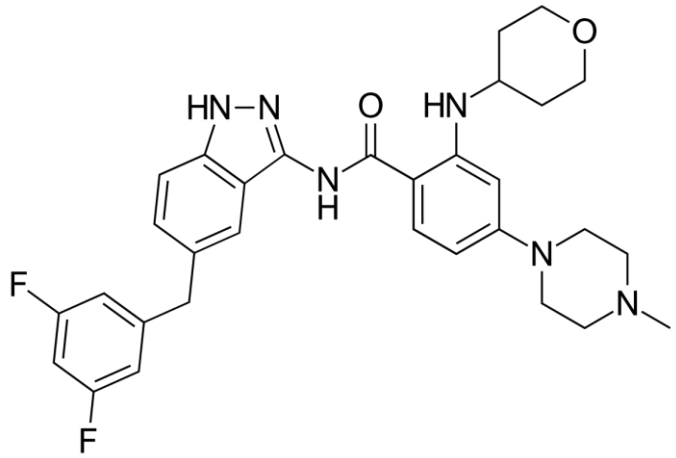
- In NSCLC, rearrangement of ROS1 gene results in several oncogenic ROS1 fusion proteins with constitutive kinase activity, the most common of which is CD74-ROS1, occurring in 40%-45% of ROS1-rearranged NSCLC





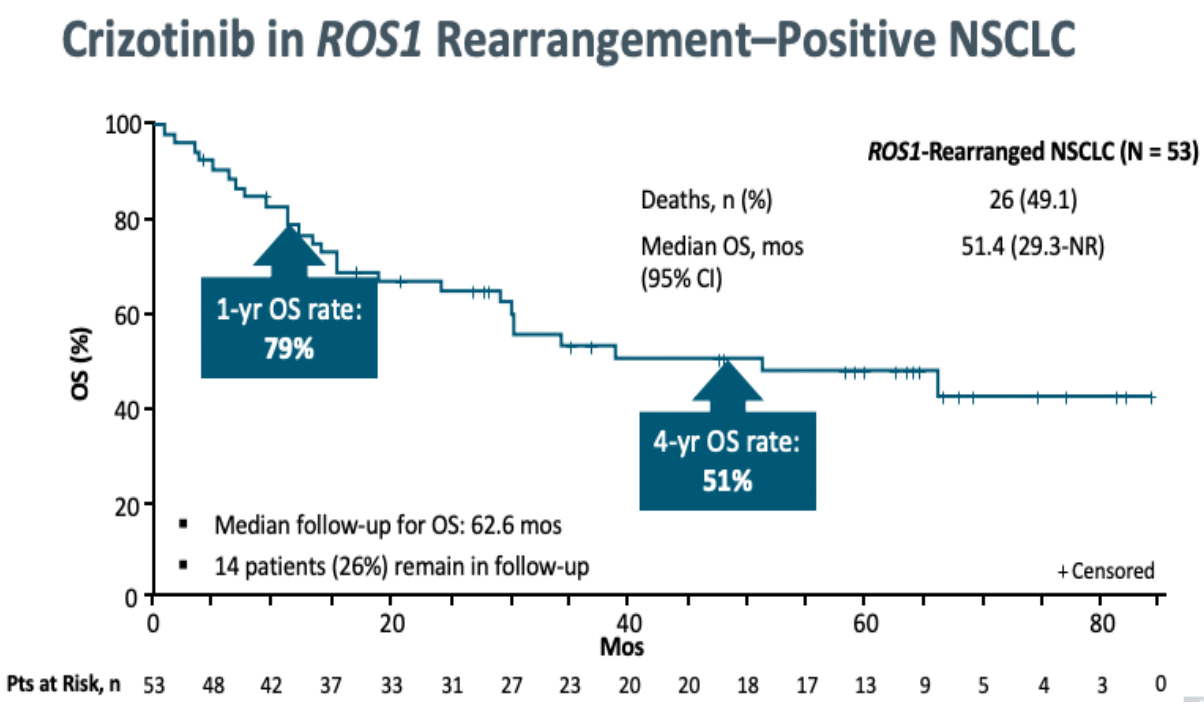
ROS1 inhibitors: Approved*

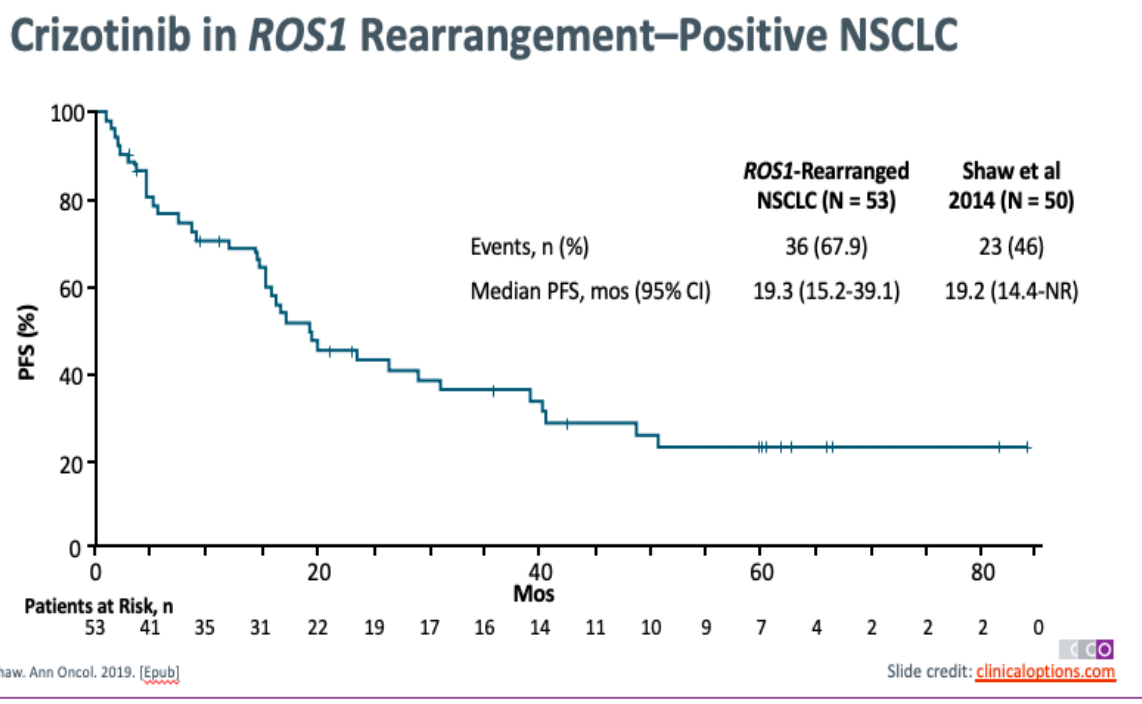




	XALKORI (crizotinib)[1]	ROZLYTREK (entrectinib)[2,3]
Mechanism of action	• First-Generation ALK Inhibitor | ROS1-TKI • Crizotinib is an inhibitor of receptor tyrosine kinases, including ALK, HGFR/c-Met, ROS1, and RON • Crizotinib demonstrated concentration-dependent inhibition of ALK, ROS1, and c-Met phosphorylation in cell-based assays • Using tumor cell lines, crizotinib demonstrated antitumor activity in preclinical studies in mouse xenografts that expressed EML4-ALK or NPM-ALK fusion proteins or c-Met	• ROS1-TKI | Pan-TRK TKI • Entrectinib inhibits all 3 types of TRK (TRKA, TRKB, TRKC), ALK, and ROS1[2,a] • Entrectinib demonstrated in vitro and in vivo inhibition of cancer cell lines derived from multiple tumor types harboring NTRK, ROS1, and ALK fusion genes[2] • Entrectinib demonstrated steady-state brain-to-plasma concentration ratios of 0.4 to 2.2 in multiple animal species (mice, rats, and dogs) and demonstrated in vivo antitumor activity in mice with intracranial implantation of TRKA-driven and ALK-driven tumor cell lines[2] ○ Entrectinib is not a substrate of P-gp or BCRP, but M5 is a substrate of P-gp and BCRP[3]

Crizotinib in *ROS1* Rearrangement–Positive NSCLC

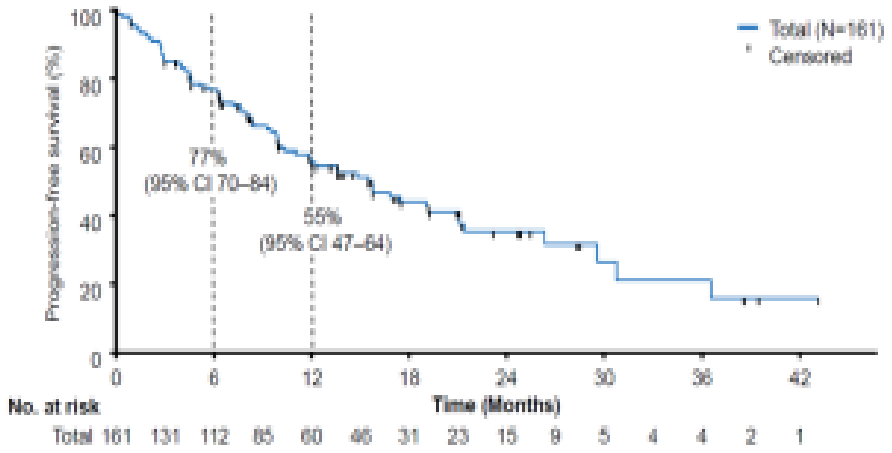
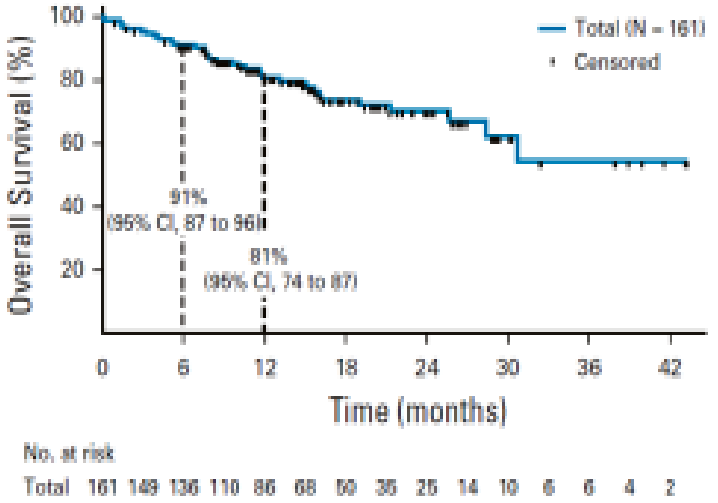




Shaw. Ann Oncol. 2019. [Epub]

Slide credit: clinicaloptions.com

Entrectinib | Key Clinical Trials Data: PFS and OS | Available KM curves

	ROZLYTREK (entrectinib)[1]	
Key study	**Phase 1:** ALKA-372-001 **Phase 2:** STARTRK-1, STARTRK-2	
Dosing	**ROZLYTREK (N=161)** 600 mg QD	
Efficacy Data	**Median PFS, months**	**Median OS, months**
		
	15.7 (95% CI, 11.0, 21.1)‡	NE (95% CI, 28.3, NE)

Entrectinib | Key Clinical Trials Data: DoR and ORR

	ROZLYTREK (entrectinib)[1]	
Key study	**Phase 1:** ALKA-372-001 **Phase 2:** STARTRK-1, STARTRK-2	
Dosing	**ROZLYTREK (N=161)** 600 mg QD	
Efficacy Data	**BICR**	
	Data cutoff: May 31, 2018[1]	
Baseline CNS Status	No	Yes
ORR (%) (95% CI)	80.0 (61.4, 92.3)	73.9 (51.6, 89.8)
Complete response (%)	3 (10.0)	0
Partial response (%)	21 (70.0)	17 (73.9)
DoR, median, months (95% CI)	24.6 (11.4, 34.8)	12.6 (6.5, NE)
	Median follow-up, 15.8 mo[2]	
ORR (%) (95% CI)	67.1 (59.3, 74.3)	
Complete response (%)	8.7	
Partial response (%)	58.4	
DoR, median, months (95% CI)	15.7 (13.9 to 28.6)	

Entrectinib | Key Clinical Trials Data: CNS Data

	ROZLYTREK (entrectinib)[1,2]
Key study	**Phase 1:** ALKA-372-001 **Phase 2:** STARTRK-1, STARTRK-2
Dosing	**ROZLYTREK (N=161)** 600 mg QD
Efficacy Data	**BICR**
	Median follow-up, 15.5 mo‡[1]
Intracranial PFS, median, months (95% CI)	7.7 (3.8, 19.3)
CNS ORR (%)	55.0 (31.5, 76.9)
CR (%)	NA
Median DoR, months (95% CI)	12.9 (5.6, NE)
	Median follow-up, 15.8 mo*[2]
Intracranial PFS, median, months (95% CI)	12.0 (6.2, 19.3)
CNS ORR (%)	79.2 (57.9 to 92.9)
CR (%)	12.5
Median DoR, months (95% CI)	12.9 (6.8, 22.1)

ENTRECTINIB

Entrectinib can effectively cross the blood-brain barrier acting on pre-existing CNS lesions and preventing or delaying the onset of metastases to the brain.

NO activity against the ROS1 resistance mutations L2026M, G2032R, and D2033N and seems to be ineffective in treating crizotinib resistant ROS1- rearranged tumours

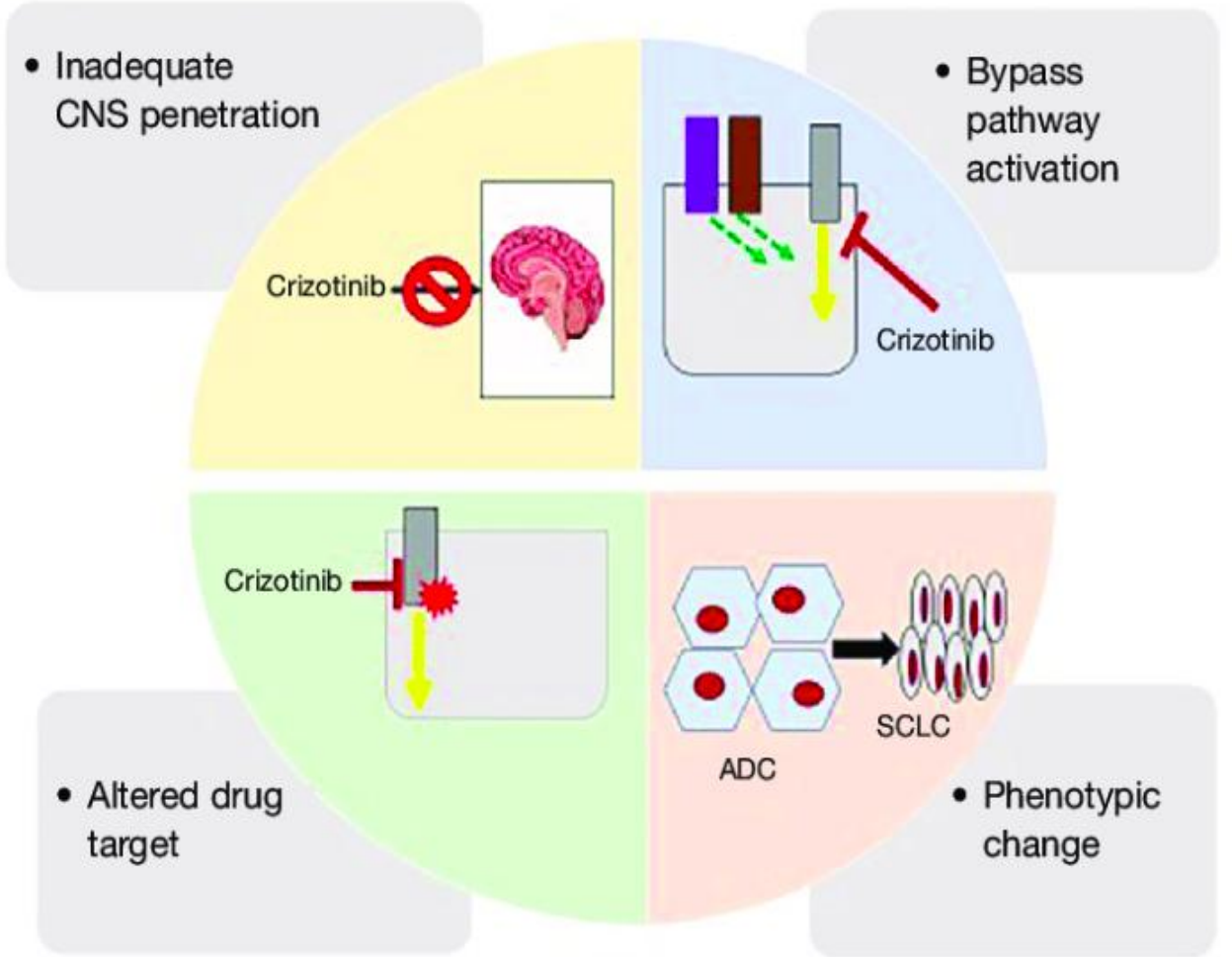
MECHANISMS OF RESISTANCE

On Target/Intrinsic Resistance

Activation of bypass signaling pathways/Extrinsic Resistance

Inadequate CNS Penetration

Phenotypic Change



Mechanism of acquired resistance to ROS1 TKIs. Acquired resistance can occur through either pharmacological or biological mechanism.

ON-TARGET RESISTANCE / INTRINSIC RESISTANCE

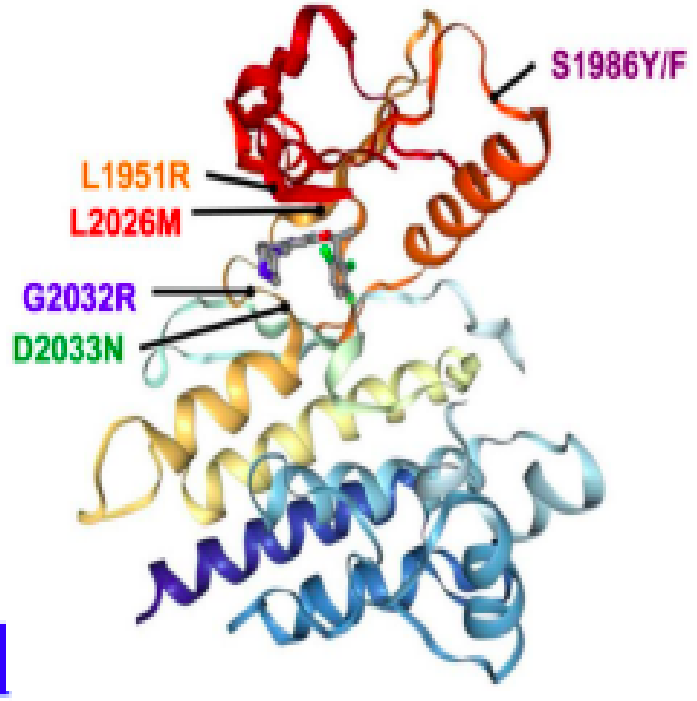
- Secondary point mutations within the ROS1 kinase domain occur in 50−60% of crizotinib resistant tumors .
- First documented and most reported ROS1 secondary mutation - ROS1 G2032R mutation (41%).
- The G2032R mutation, a glycine-to-arginine substitution at codon 2032 in the solvent-front, causes resistance to crizotinib through steric interference with the drug binding at ROS1-kinase residues exposed to solvent
- Other secondary resistance mutations on the ROS1 tyrosine kinase domain includes D2033 (6%), L1951 and the gatekeeper mutation L2026M, and other secondary mutations include L1982F, E1990G, F1994L, S1986Y/F and L2086F.
- ROS1-independent mechanisms, like mutations in KIT Proto-Oncogene (KIT) and in β-catenin have also been detected.

Lin JJ, Choudhary NJ et al. Spectrum of Mechanism of Resistance to Crizotinib and Lorlatinib in ROS1 Fusion Positive Lung Cancer. Clin Cancer Res 2021 May 1;27(10)

Mechanisms of Resistance

ROS1 Point Mutations

- The most frequently observed ROS1 resistance mutation is G2032R, a mutation that causes steric hindrance to crizotinib binding but does not alter the oncogenic kinase activity of ROS1[1]
- Resistance to crizotinib is a key driver for development of next-generation ROS1 inhibitors[1]
- The next-generation ROS1 inhibitor repotrectinib has demonstrated activity against G2032R ROS1 resistance mutations in the TRIDENT-1 trial[2]



CD74-ROS1 Ba/F3 Cell Proliferation IC_{50} (nM)[3]

ROS1	Crizotinib	Ceritinib	Cabozantinib	Entrectinib	Lorlatinib	Repotrectinib
WT	14.6	42.8	0.5	10.5	0.2	<0.2
G2032R	266.2	1391	11.3	1813	160.7	3.3
D2033N	200.9	535.4	0.2	169.2	3.3	1.3
L2026M	606.4	ND	29.1	2026	930.6	10.0
S1986F	63.7	68.0	5.5	3.4	0.4	<0.2
L1951R	157.6	785.5	91.8	35.4	2.8	<0.2

Lorlatinib is not indicated for use in ROS1 NSCLC.

ACTIVATION OF BYPASS PATHWAY/OFF TARGET RESISTANCE

- Less common than on-target resistance mutations
- Retrospective studies have reported resistance to crizotinib driven by one of these bypass-signaling pathways in 42–44% of crizotinib-resistant ROS1-rearranged NSCLC tumors
- Tumor cells under the pressure of the targeted TKI can become resistant through upregulation of either downstream or parallel cell signaling pathways like EGFR, MET, HER2, KRAS, KIT, BRAF and MEK.
- Neurofibromatosis 1 (NF1) alterations can be present in 7% of crizotinib-resistant cases
- Combination therapies to overcome this kind of off-target resistance is a promising strategy.

INTRACRANIAL FAILURE

Crizotinib has limited blood–brain barrier (BBB) penetration as it is a substrate of P-glycoprotein and human ATP-binding cassette subfamily efflux transporters

CNS progression to crizotinib reflect a pharmacokinetic failure of therapy rather than true biological resistance.

CSF concentrations of crizotinib are usually low, and the intracranial ORR with this agent was only 33%.

Incidence of brain metastases in advanced NSCLC is between 20% and 40% prior to start treatment and can increase to up to 30−50% in patients pre-treated with TKIs in ROS1+ lung cancer patients.

Novel ROS1 inhibitors have higher efficacy for patients with brain metastasis. In metastatic patients in first-line setting, entrectinib (intracranial ORR 55%), lorlatinib (iORR 64%) and repotrectinib (iORR 100%) showed substantial intracranial activity in patients with treatment naïve ROS1

PHENOTYPICAL CHANGES

Epithelial to mesenchymal transition (EMT)- can emerge as a resistance mechanism to crizotinib (reduced E-cadherin and increased vimentin)	SCLC- The most extreme phenotypic change is the histologic transformation from adenocarcinoma to small-cell lung cancer (SCLC).	Currently, these patients should receive standard platinum-etoposide based chemotherapy and be treated like a SCLC

OVERCOMING ROS-1 RESISTANCE

- Liquid biopsies have been shown to be non-inferior to up-front tumor tissue genotyping in NSCLC (Second and subsequent lines of therapy in ROS1- rearranged NSCLC
- The non-invasive nature of liquid biopsy allows serial monitoring for acquired resistance mechanisms.

Depending on the nature of the resistance mechanisms and type of progression and involvement of CNS

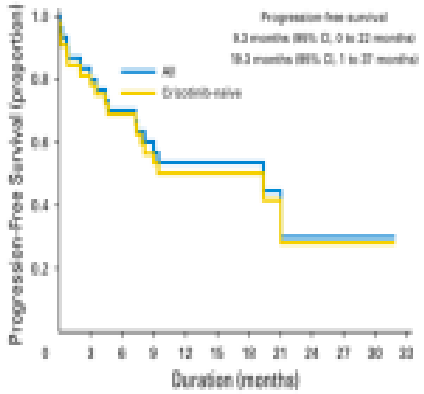
- ROS1 inhibitor with better CNS efficacy
- Combination of ROS1-targeted TKIs and/or other standard-of-care therapies (for example, chemotherapy)
- Chemoimmunotherapy
- According to bypass activation pathway or phenotype change

Loss of G2032R resistance mutation upon chemotherapy treatment enables successful crizotinib rechallenge in patient with ROS1-rearranged NSCLC

- The 62-year-old patient developed disease progression while on long-term crizotinib .
- Molecular analysis of tumor cells confirmed the rearrangement and revealed a G2032R substitution in ROS1.
- He received, six cycles of carboplatin and pemetrexed followed by pemetrexed maintenance were administered for approximately 9 months, resulting in a good clinical and radiologic response.
- Tissue re-biopsy at progression on chemotherapy revealed wild-type sequences in exon 38 of ROS1.
- Consequently, crizotinib treatment was re-initiated and resulted in a good confirmed partial response (-41.2%).

Michels et al.

Not Approved: ROS1 Inhibitor Key Clinical Trials Data: PFS (1 of 3)

	ZYKADIA (ceritinib)[1]		Repotrectinib (TPX-0005)[2]	
Key study	**Phase 2:** NCT01964157		**Phase 1/2:** TRIDENT-1[†]	
Dosing	**ZYKADIA (all patients, N=32; crizotinib-naïve, N=30)** 750 mg QD		**Repotrectinib (N=11, TKI-naïve; N=60, 1 prior TKI)** 40 mg QD to 200 mg BID	
Efficacy Data				
	All patients by independent review	**Crizotinib-naïve by independent review**	**TKI-naïve by BICR (N=11)**	**Pretreated with 1 TKI by BICR (N=60)**
Median PFS, months			NE	NE
	9.3 (95% CI, 0, 22)*	19.3 (95% CI, 1, 37)*	24.6 (95% CI, 7.2, NR)	NE

Repotrectinib (TPX-0005) and ZYKADIA (ceritinib) are currently not indicated for patients with ROS1-rearranged NSCLC.

Not Approved: ROS1 Inhibitor Key Clinical Trials Data: OS

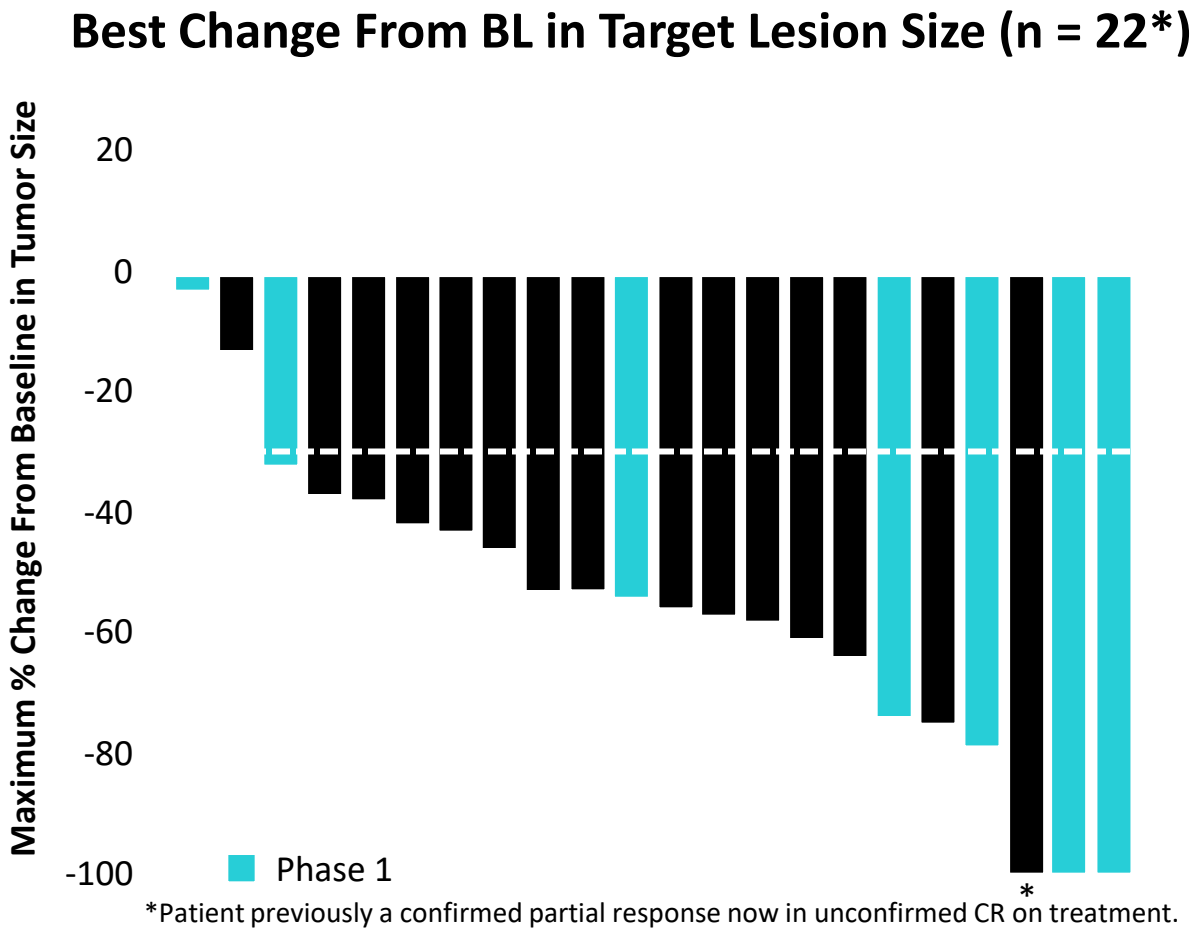
	ZYKADIA (ceritinib)[1]	Repotrectinib (TPX-0005)[2]	
Key study	**Phase 2:** NCT01964157	**Phase 1/2:** TRIDENT-1	
Dosing	**ZYKADIA (N=32)** 750 mg QD	**Repotrectinib** 40 mg QD to 200 mg BID	
Efficacy Data			
Median OS, months	**≥ 2 previous treatments (N=32)**	**TKI-naïve by BICR (N=55)**	**Pretreated with 1 TKI by BICR (N=60)**
	24 (95% CI, 5, 43)	NE	NE

Repotrectinib (TPX-0005) and ZYKADIA (ceritinib) are currently not indicated for patients with ROS1-rearranged NSCLC.

No responses in the two crizotinib-resistant patients were reported. Ceritinib shows no activity against most ROS1 resistant mutations, including ROS1 G2032R, so its use is limited to crizotinib-naïve patients.

Repotrectinib is a potent ALK/ROS1/TRK inhibitor and is active in CNS and against several ROS1- point resistance mechanisms, including ROS1 G2032R
The phase I/II trial (TRIDENT-1, NCT03093116), showing an ORR of 82%. In 18 TKI pre-treated patients, ORR was 57% The intracranial ORR was 75%.

TRIDENT-1: Phase I/II Study of Repotrectinib, a 2nd-Gen ROS1/ALK/TRK TKI, for TKI-Naive *ROS1*+ Adv NSCLC



*Patient previously a confirmed partial response now in unconfirmed CR on treatment.

Responses	Phase II (n = 15)	Phase I†/II (n = 22*)
Confirmed ORR, % (95% CI)	93 (68-100)	91 (71-99)
	Phase I (n = 11)‡	
ORR, % (95% CI)	91 (59-100)	
▪ At ≥ RP2D	86	
Median DoR, mo (95% CI)	23.1 (5.6-NR)	
Median PFS, mo (95% CI)	24.6 (7.2-NR)	

Any Grade TEAE in ≥20%, %	All Treated Patients§ (n = 301)
Dizziness	60.1
Dysgeusia	43.9
Constipation	33.6
Paresthesia	28.9
Dyspnea	27.9
Anemia	27.2
Fatigue	24.3
Nausea	20.6

*Includes patients with BL and ≥2 post-BL scans.
†Phase I patients treated at or above RP2D. ‡Previously reported.
§Includes all solid tumors with *ALK, ROS1,* or *NTRK1-3* rearrangements.

Cho. WCLC 2020. Abstr 3255.
Besse. AACR 2021. Abstr P02-01.

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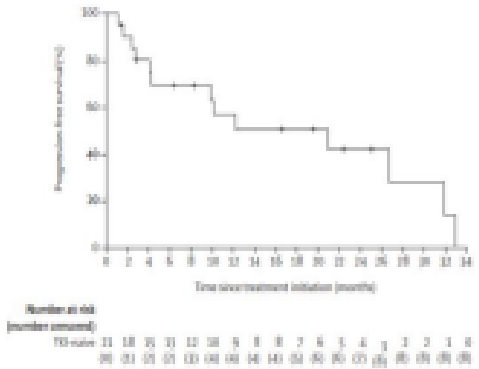
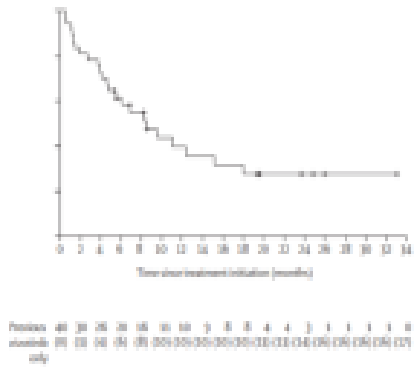
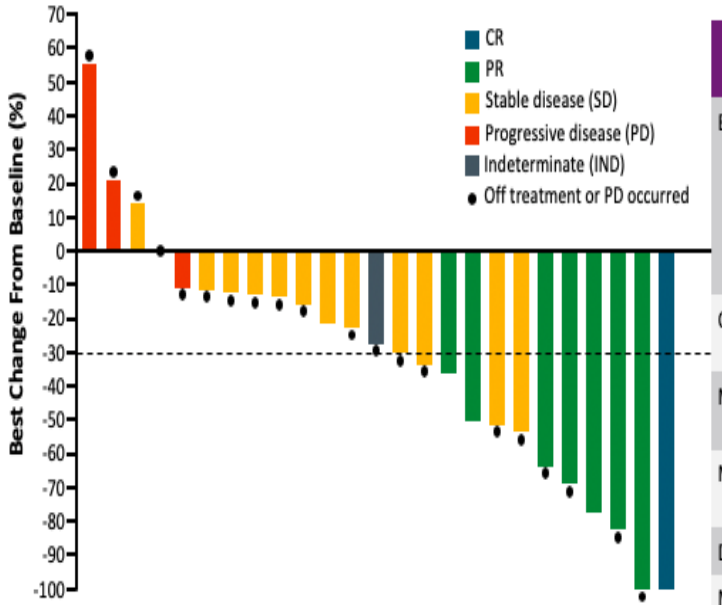
Not Approved: ROS1 Inhibitor Key Clinical Trials Data: PFS (2 of 3)

	LORBRENA/LORVIQUA (lorlatinib)	
Key study	**Phase 1/2:** B7461001 (NCT01970865)	
Dosing	**Lorlatinib (N=69 ROS+ [EXP6])** **Escalating doses (10 mg QD to 100 mg BID)**	
Efficacy Data		
	TKI-naïve patients, N=21	**Prior crizotinib only, N=40**
Median PFS, months		
	21.0 (95% CI, 4.2, 31.9)	8.5 (95% CI, 4.7, 15.2)

Lorlatinib is not indicated for use in ROS1 NSCLC.

Lorlatinib: Overall Efficacy in Crizotinib-Pretreated *ROS1*-Positive NSCLC Patients



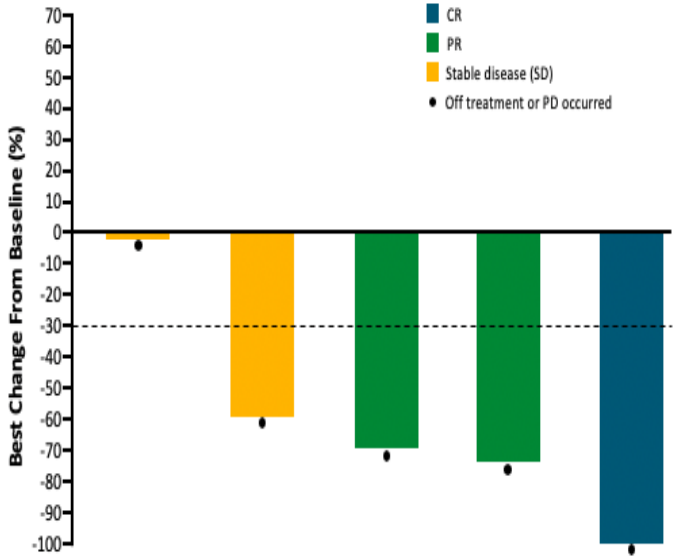
	Crizotinib Pretreated (n = 34)
BOR, n (%)	
CR	2 (5.9)
PR	7 (20.6)
SD	16 (47.1)
PD	3 (8.8)
IND	6 (17.6)
ORR, n (%)	9 (26.5)
95% CI	12.9-44.4
Median TTR, mos	2.5
Range	1.4-4.2
Median DoR, mos	NR
95% CI	7.1-NR
DoR ≥ 12 mos, n/N (%)	5/9 (55.6)
Median PFS, mos	8.5
95% CI	4.4-18.0

Patients with at least 1 on-study target lesion assessment as per independent central review were included. If any procedure was different and not interchangeable from the procedure at screening, the percent change from baseline could not be calculated and is not displayed.

Shaw. Lancet Oncol. 2017;18:1590.

Slide credit: clinicaloptions.com

Lorlatinib: Intracranial Efficacy in Crizotinib-Pretreated *ROS1*-Positive NSCLC



	Crizotinib Pretreated (n = 19)*
IC-BOR, n (%)	
CR	8 (42.1)
PR	2 (10.5)
SD	5 (26.3)
PD	0
IND	4 (21.1)
IC-ORR, n (%)	10 (52.6)
95% CI	28.9-75.6
Median IC-DoR, mos	NR
95% CI	5.0-NR
IC-DoR ≥ 6 mos, n/N (%)	6/10 (60)

*Based on all intracranial lesions.

Patients with at least 1 on-study target lesion assessment as per independent central review were included. If any procedure was different and not interchangeable from the procedure at screening, the percent change from baseline could not be calculated and is not displayed.

Shaw. Lancet Oncol. 2017;18:1590.

Slide credit: clinicaloptions.com

lorlatinib has in vitro activity against several crizotinib-resistant mutations, including L2026M, S1986Y/F, and D2033N. lorlatinib has limited efficacy against the ROS1 G2032R in preclinical models

Lorlatinib in pretreated ALK- or ROS1-positive lung cancer and impact of TP53 co-mutations: results from the German early access program

Nikolaj Frost, Petros Christopoulos, Diego Kauffmann-Guerrero, Jan Stratmann, Richard Riedel, Monica Schaefer, Jürgen Alt, Sylvia Gütz, Daniel C. Christoph, Eckart Laack, Martin Faehling, Richard Fischer, Klaus Fenchel, Sebastian Haen, Lukas Heukamp, Christian Schulz and Frank Griesinger

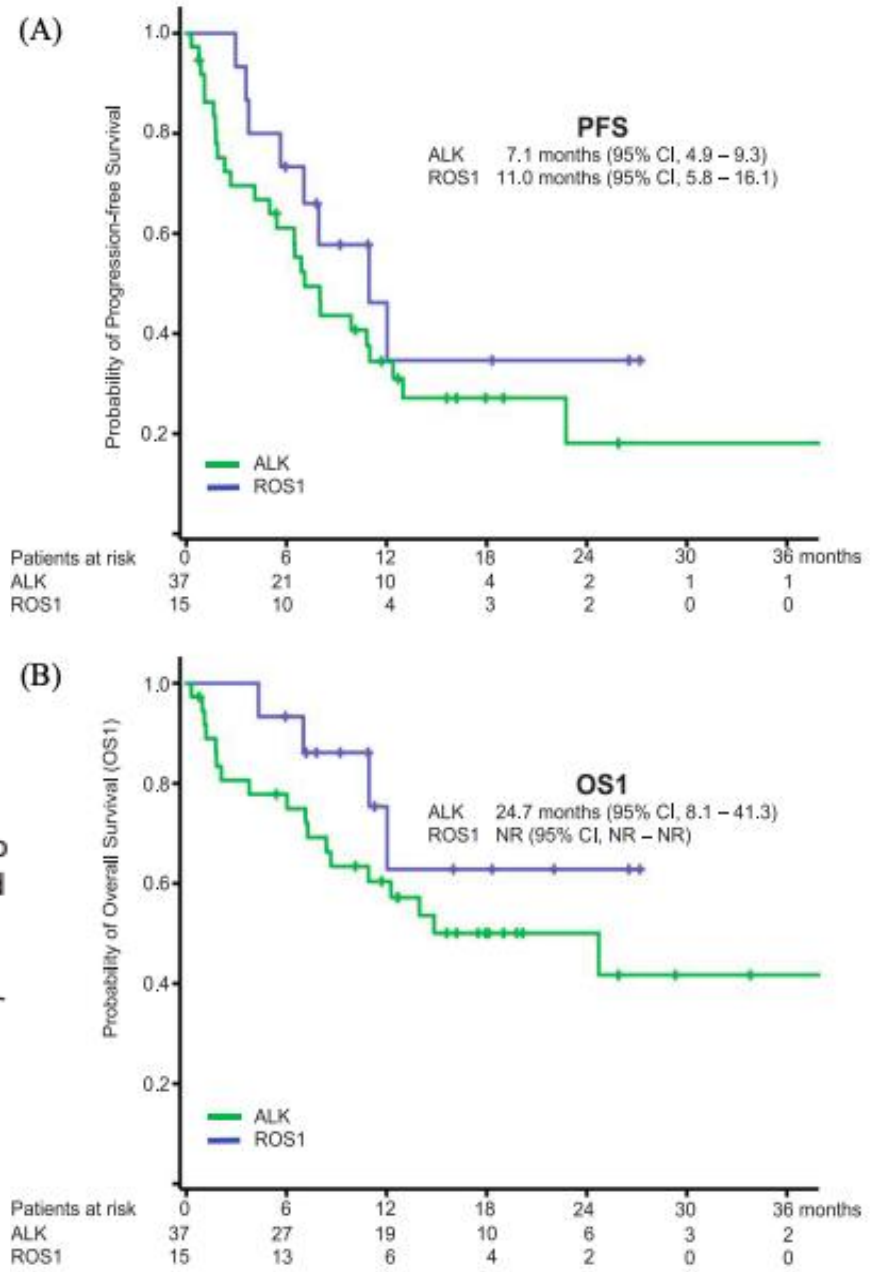
Abstract

Introduction: We report on the results of the German early access program (EAP) with the third-generation ALK- and ROS1-inhibitor lorlatinib.

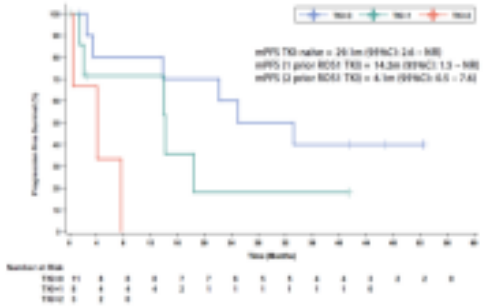
Patients and Methods: Patients with documented treatment failure of all approved ALK/ROS1-specific therapies or with resistance mutations not covered by approved inhibitors or leptomeningeal carcinomatosis were enrolled and analyzed.

Results: In total, 52 patients were included [median age 57 years (range 32–81), 54% female, 62% never smokers, 98% adenocarcinoma]; 71% and 29% were ALK- and ROS1-positive, respectively. G1202R and G2032R resistance mutations prior to treatment with lorlatinib were observed in 10 of 26 evaluable patients (39%), 11 of 39 patients showed TP53 mutations (28%). Thirty-six patients (69%) had active brain metastases (BM) and nine (17%) leptomeningeal carcinomatosis when entering the EAP. Median number of prior specific TKIs was 3 (range 1–4). Median duration of treatment, progression-free survival (PFS), response rate and time to treatment failure were 10.4 months, 8.0 months, 54% and 13.0 months. Calculated 12-, 18- and 24-months survival rates were 65, 54 and 47%, overall survival since primary diagnosis (OS2) reached 79.6 months. TP53 mutations were associated with a substantially reduced PFS (3.7 *versus* 10.8 month, HR 3.3, $p = 0.003$) and were also identified as a strong prognostic biomarker (HR for OS2 3.0 $p = 0.02$). Neither prior treatments with second-generation TKIs nor BM had a significant influence on PFS and OS.

Conclusions: Our data from real-life practice demonstrate the efficacy of lorlatinib in mostly heavily pretreated patients, providing a clinically meaningful option for patients with resistance mutations not covered by other targeted therapies and those with BM or leptomeningeal carcinomatosis.



Not Approved: ROS1 Inhibitor Key Clinical Trials Data: PFS (3 of 3)

	Taletrectinib (AB-106/DS-6051b)[1-3]	
Key study	**Phase 1:** United States (U101, NCT02279433); Japan (J102, NCT02675491) **Phase 2:** People's Republic of China TRUST (NCT04395677)	
Dosing	**Taletrectinib (Estimated enrollment: 106 (As of June 16, 2021, 21 crizotinib treatment-naïve patients and 16 crizotinib pre-treated patients were confirmed to be ROS1 fusion-positive) Doses = 400 or 600 mg QD**	
Efficacy Data		
	Pooled Analysis (US—Japan) = 22* Treatment naïve = 11; Crizotinib refractory Only = 8; two previous ROS1 TKIs = 3	**21 crizotinib treatment-naïve patients 16 crizotinib pre-treated patients[†2, 3]**
Median PFS, months		NA
	TKI-naïve = 29.1m (95%CI: 2.6 – NR); 1 prior ROS1 TKI = 14.2m (95%CI: 1.5 – NR); 2 prior ROS1 TKIs = 4.1m (95%CI:: 0.5 – 7.6)[1]	NA

Taletrectinib is not indicated for use in ROS1 NSCLC.

In preclinical models, taletrectinib overcomes resistance to ROS1 G2032R mutation. Taletrectinib showed an ORR of 33% in crizotinib-resistant patients.

BRIGATINIB

A phase 1/2 study of brigatinib included 3 patients with ROS1-rearranged NSCLC. One patient was crizotinib- naïve and had a partial response.

In vitro, brigatinib inhibits the L2026M mutation, but not other common resistance mutations as G2032R, L1951R, D2033N.

P1.14-50 A Phase 2 Trial of Cabozantinib in ROS1-Rearranged Lung Adenocarcinoma

R. Guo • I. Preeshagul • A. Schoenfeld • ... M. Ladanyi • M. Kris • A. Drilon • Show all authors

- Cabozantinib is a multi-kinase inhibitor with activity against RET, MET, VEGFR2, ALX, TIE2, KIT, and ROS1.
- Cabozantinib has also demonstrated clinical activity against ROS1 fusions, particularly against solvent front resistance mutations in ROS1 including G2032R and D2033N.
- PFS durations ranged from 4.9 to 13.8 months in previously treated patients.

Chemo-Immunotherapy

After the failure of targeted therapies, conventional cytotoxic chemotherapy remains a standard treatment.

Pemetrexed-based chemotherapy for ROS1 fusion-positive tumors is associated with better responses rates and longer PFS compared with patients with NSCLC harbouring other driver mutations.

Potential role of ICIs either alone or in synergistic combination in ROS1-rearranged NSCLC

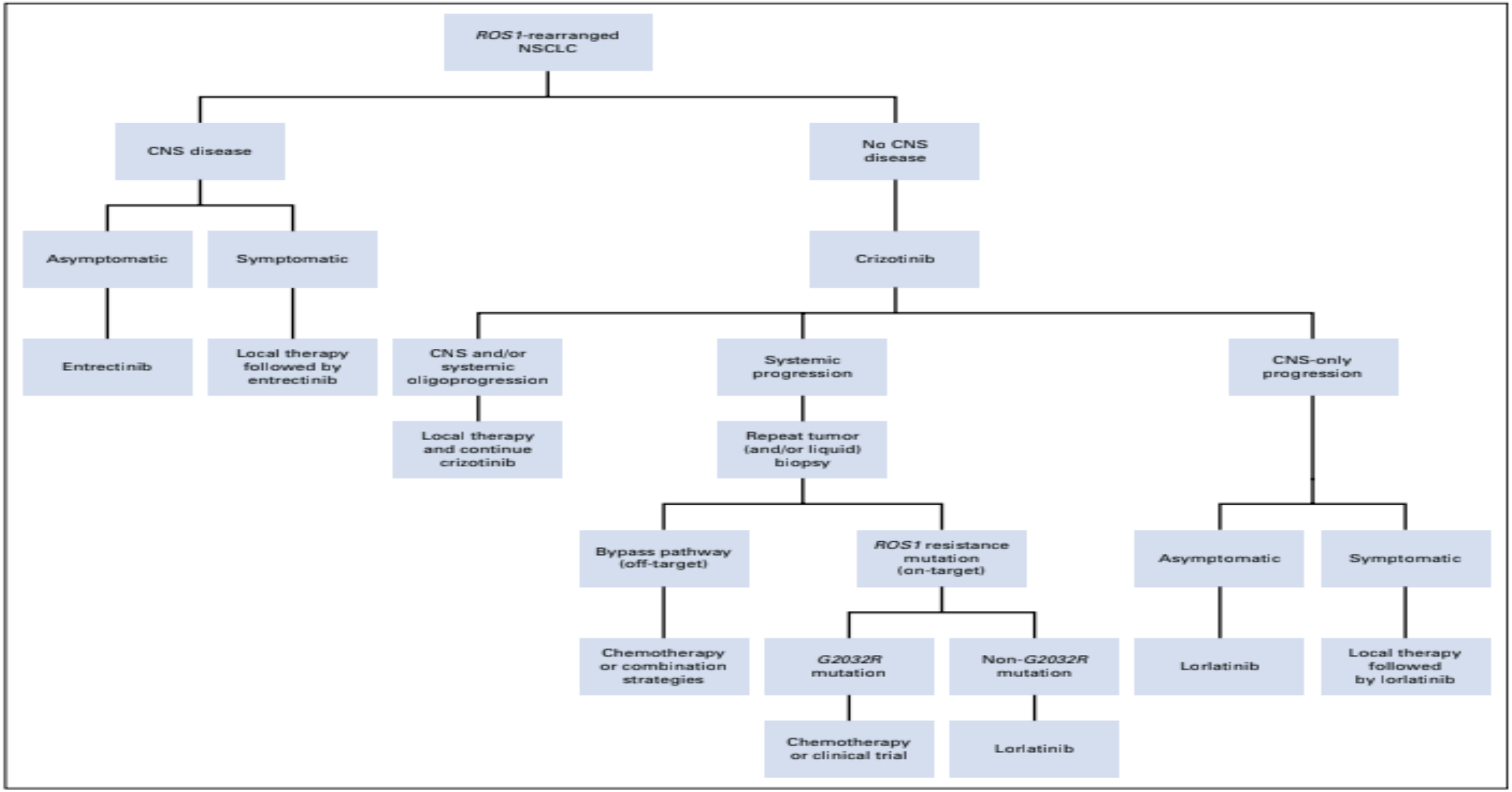


FIG 1. Treatment algorithm. NSCLC, non–small-cell lung cancer.

SUMMARY

- Several major resistance mechanisms have arisen to oral TKI that lead to treatment failure and next generation TKIs have been developed in order to overcome resistances.
- Intracranial activity, efficacy against resistant ROS1- mutant kinases (particularly G2032R), and testing combination therapies for bypass-signaling resistance mechanisms may be keys for prolonging disease control and improving survival.
- Tissue or blood-based NGS can help to identify the resistance mechanism to ROS1 TKI and may be considered after tumor progression in order to better select further lines of targeted therapy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