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Entrectinib for the treatment of metastatic NSCLC: safety and efficacy

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Abstract

Introduction: Gene fusions are strong driver alterations in various cancers, increasingly diagnosed with multiple testing techniques. *ROS1* fusions can be found in 1-2% of non-small cell lung cancer (NSCLC) and several tyrosine kinase inhibitors (TKIs) have been tested in this oncogene-driven disease. *NTRK* fusions are characteristic of a few rare types of cancer, also infrequently seen in some common cancers including NSCLC. Entrectinib is a newer ROS1 and NTRK inhibitor developed across different tumour types harbouring rearrangements in these genes. Entrectinib was granted FDA accelerated approval in August 2019 for the treatment of ROS1+ NSCLC and NTRK-driven solid tumors.

Areas covered: This review covers the mechanism of action, safety, and efficacy of entrectinib in patients with metastatic NSCLC.

Expert opinion: Entrectinib is an orally bioavailable TKI of TrkA, TrkB, TrkC, and ROS1, with the ability to cross the blood-brain barrier. Entrectinib was effective and well-tolerated in patients harbouring *ROS1*- or *NTRK*-rearranged NSCLC treated within phase I and II studies. Entrectinib appears to be the most appropriate treatment choice for TKIs-*naïve* patients, especially in those presenting brain metastasis. Conversely, in case of systemic progression with the evidence of acquired resistance mutations in ROS1 or Trk proteins, a sequential therapy with entrectinib could not be successful.

Keywords: Entrectinib; Lung Cancer; NSCLC; NTRK; ROS1; Rozlytrek; Targeted Therapy

Article highlights:

- *ROS1* fusions can be found in 1-2% of non-small-cell lung cancers (NSCLCs), but they are enriched among younger and never-smokers patients.
- *NTRK* fusions interest less than 0.5% of all NSCLCs, and about 3% of cases with no other driver oncogenes.
- Entrectinib is a potent orally bioavailable tyrosine kinase inhibitor of TrkA, TrkB, TrkC, and ROS1, with the ability to cross the blood-brain barrier (BBB).
- Entrectinib encompasses higher intracranial activity than crizotinib, with similar duration of response and manageable toxicity profile and can be an alternative first-line treatment for patients with *ROS1*-rearranged NSCLC.
- Entrectinib demonstrated high response rate and durable response in patients affected by *NTRK*-rearranged tumours, including NSCLC.
- Because of the capability of entrectinib to cross the BBB and the neurophysiological function of Trk receptors, peculiar side-effects mediated by Trk inhibition should be monitored (including cognitive impairment, increased appetite or dizziness).
- Entrectinib is an appropriate treatment choice for TKIs-*naïve* patients with ROS1 or NTRK-positive NSCLC, while evidences of activity against the most frequent acquired mutations related to TKI-resistance are lacking.

1. Introduction

Lung cancer is the most commonly diagnosed cancer and the leading cause of cancer-related mortality worldwide [1], with approximately 180,000 expected deaths in the European Union in 2019 [2] and 150,000 in the United States, a quarter of all deaths from cancer [3] . Age-standardized incidence rate in developed countries is 19.1 per 100,000 among females and reaches 40.4 per 100,000 in males [1]. Tobacco control efforts have decreased and will continue to decrease smoking-related lung cancer rates, hence an increase in the proportion of people who have never smoked is expected among the population with lung cancer in the future [4].

On the other hand, treatment opportunities for patients with lung cancer have tremendously evolved in the last decade. The American Lung Association reported an increase of the 5-year survival rate from 17.2% in 2009 to 21.7% in 2019 in United States [5]. Both the detection of genetic drivers of oncogenesis suitable for pharmacological blockade and the discovery of immune-checkpoint inhibitors as new weapons against cancer have brought to reshape the treatment algorithms for non-small cell lung cancer (NSCLC), the most prevalent type of lung cancer.

The identification of the *EGFR* gene mutation as a potential target in NSCLC has only been the first step toward the age of targeted therapy. Numerous additional driver mutations amenable to direct inhibition, especially in adenocarcinomas, have been recognized, making NSCLC a heterogeneous group of diseases and the most relevant example of precision oncology. Consequently, several effective molecular-targeted agents, acting as tyrosine kinase inhibitors (TKIs), have been developed for *EGFR, ALK*, *ROS1*, *BRAF*, *NTRK1,2,3* genetic alterations. Additionally, a quantity of new molecules are under clinical evaluation as inhibitors of other driver oncogenes in lung cancer, such as *RET*, *MET*, *HER2*, *NRG1*, or *KRAS*. Targeted treatments may produce higher response rates, higher quality of life, and longer survival compared with traditional cytotoxic chemotherapies [6–8].

The proto-oncogene *ROS1* (c-ros oncogene 1) is located on chromosome 6q22.1 and encodes a receptor tyrosine kinase (RTK) whose role has not been defined [9]. *ROS1* gene rearrangements are found in 1-2% of

NSCLC [10] and they can involve diverse fusion partners. The tyrosine kinase domain is always preserved and its constitutive activation is thought to stimulate the PI3K/AKT/mTOR, JAK/STAT, and MAPK/ERK pathways, resulting in proliferative growth and cell survival [11]. Consequently, pharmacological blockade of ROS1 signalling was demonstrated to induce growth inhibition in cell lines and durable clinical response in patients with advanced *ROS1*-rearranged NSCLC [12]. *ROS1* rearrangements are easier to be found among younger and never-smokers patients harbouring high grade adenocarcinomas of the lung [10]. Since March 2016, crizotinib has been the only TKI inhibitor approved by Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the treatment of advanced *ROS1*-rearranged NSCLC.

Trk A, Trk B and Trk C are transmembrane kinases encoded by *NTRK1* (1q21-q22), *NTRK2* (9q22.1), and *NTRK3* (15q25) genes, respectively. These receptors are the target of neurotrophins, which take part in development and function of the nervous system. Their activation lead to cellular proliferation and increasing survival driving a variety of downstream signalling pathways, including MAPK, PI3K and PKC [13]. A diversity of intrachromosomal or interchromosomal *NTRK* genes rearrangements has been described in several tumour types, and the originated chimeric oncoprotein presents a neurotrophin-independent constitutive activation of the Trk kinase [13, 14]. *NTRK1* gene fusions in NSCLC were firstly identified in 2012 by Vaishnavi et al. in a population of patients affected by lung adenocarcinomas with no other detectable oncogenes (3/91; 3.3%) [15]. Overall, *NTRK* gene fusions can be found in less than 0.5% of NSCLC, linked with no peculiar clinicopathologic features [15–17]. Upon its rarity, a mass screening approach with next generation sequencing technology or rational diagnostic algorithms (e.g. screening of driver-negative NSCLC by means of immunohistochemistry, fluorescence *in situ* hybridisation, or reverse transcriptase polymerase chain reaction) are important in terms of practical and economic feasibility [18]. Entrectinib and Larotrectinib have been the first TKIs studied with the aim to block the oncogenic driving function of Trk kinases.

Entrectinib is a novel ROS1 and NTRK inhibitor approved by FDA for the treatment of ROS1-positive NSCLC and all NTRK-driven solid tumors. Here we reviewed its mechanism of action, safety, and efficacy in patients with metastatic NSCLC.

2. Overview of the market

2.1. *ROS1 inhibition*

Crizotinib represents the standard treatment choice for patients affected by *ROS1*-rearranged NSCLC, since it is the first ROS1 inhibitor approved by both FDA and EMA and it is supported by the widest amount of evidences so far. Crizotinib demonstrated activity at nanomolar concentrations as inhibitor of MET, ALK and ROS1 in biochemical assays [19] and the Profile 1001 trial firstly evaluated its safety and clinical activity among patients with advanced *ROS1*-rearranged NSCLC [12]. The most updated data of Profile 1001 derive from results on 53 patients (87% pre-treated) who received crizotinib, 250 mg twice daily, with a median of follow-up period of 62.6 months in total. The overall response rate (ORR) was 72%, with 24.7 months of median duration of response (mDOR), and the median progression free survival (mPFS) was 19.3 months (95% CI, 15.2–39.1). The safety profile was favourable, even with long-term treatment, with most of treatment-related adverse events (TRAE) being grade 1 or 2 (mainly vision disorders, emesis and oedema) [8]. Similar results were obtained in other Asian, European and Italian phase II studies examining crizotinib in *ROS1*-rearranged NSCLC, where ORR was 72%, 70% and 65%, and mPFS was 15.9, 20 and 22.8 months, respectively [20–22]. A fourth French study, with 37 patients, reported an ORR of 69.4% with a mPFS of 5.5 months. By the way, this study-population was more heavily pretreated, with ECOG PS 2 in 25% of patients versus 2% in Profile 1001 [23]. A new "benchmark" of 51.4 months for median overall survival (mOS) was achieved by Profile 1001 study for patients with advanced *ROS1*-rearranged NSCLC [8]. An enhanced efficacy of pemetrexed-based chemotherapy in patients harbouring *ROS1* fusions as driver oncogene [24, 25] could have contributed to this outcome. Otherwise, the activity of anti-PD1/PD-L1 directed immune-checkpoint-inhibitors seems to be poor in the subset of oncogene-addicted NSCLC, but limited data are available for patients harbouring *ROS1*-rearranged cancers [26]. On the basis of this amount of data, crizotinib is approved by regulatory agencies worldwide and both NCCN [27] and ESMO guidelines [28] recommend it as first treatment choice in *ROS1*-rearranged NSCLC.

Crizotinib clinical efficacy is limited by two main issues. First, as it happens for other oncogene-driven

NSCLC, *ROS1*-rearranged lung cancers ultimately acquire mechanisms of resistance under the therapeutic pressure of crizotinib, leading to disease progression [29]. Such acquired resistance mechanisms can arise in the ROS1 kinase domain, where the ROS1 G2032R mutation is the most frequently observed, or they can involve "off-target" pathways [9]. Second, crizotinib has a reduced penetrance across the blood-brain barrier (BBB) [30], that could be an issue since brain metastases are a key reason of morbidity and mortality in patients with NSCLC [31]. No data on brain activity was reported in Profile 1001 trial. In a retrospective analysis of two trials evaluating crizotinib in the setting of *ALK*-rearranged NSCLC, Costa et al. reported a systemic ORR of 53% but an intracranial response rate of 18% and intracranial mDOR of 6.6 months among patient with untreated brain metastasis [32]. Taking into consideration these unmet needs, a number of next-generation TKIs with anti-ROS1 activity are under clinical development: cabozantinib, ceritinib, DS-6051b, entrectinib, lorlatinib, and repotrectinib **(Table 1)** [33–39]. Most of these new molecules have already demonstrated high capability to cross the BBB in humans. In a phase II study, ceritinib exhibited similar ORR and PFS as crizotinib among TKI-naïve patients, with 69% of patients requiring at least one dose reduction due to toxicity and high rate of serious adverse events [35]. In preclinical studies, ceritinib has been able to overcome the gatekeeper L2026M mutation, but no other ROS1 mutations [40]. In a phase I-II study, lorlatinib showed systemic and intracranial activity in patients with ROS1-positive NSCLC after crizotinib failure [38]. Lorlatinib already presented preclinical activity against different crizotinib-resistant mutations, including L2026M, S1986Y/F, D2033N [9, 41, 42]. On the other hand, lorlatinib exhibited limited clinical activity against G2032R [41], providing stable disease as best response [43]. $ROS1^{G2032R}$ is the most common acquired mutation after crizotinib, preventing the drug access to the kinase domain. Both DS-6051b, a selective ROS1/NTRK inhibitor, and cabozantinib, a multikinase inhibitor, have shown activity against $ROS1^{G2032R}$ in preclinical models [44, 40]. Repotrectinib, a new ROS1/TRK/ALK inhibitor, has been designed to overcome G2032R and has proven both preclinical and clinical activity [39, 45]. Among 5 patients with NSCLC harbouring $ROS1^{G2032R}$ treated in a dose-finding trial (TRIDENT-1), a tumor shrinkage was obtained in all cases using different doses of repotrectinib, with 40% of confirmed ORR [45].

Entrectinib enters as a new TKI in the crowded context of ROS1-driven NSCLC [46]. The NCCN guidelines indicate entrectinib as an alternative to crizotinib as first-line treatment option for *ROS1*-rearranged NSCLC, and ceritinib as second choice. Lorlatinib is recommended after progression on first-line TKI [27].

2.2. ***NTRK inhibition***

Regarding *NTRK*-fusion positive cancers, larotrectinib and entrectinib have been the first drugs to be approved as inhibitors of Trk proteins, with tissue-agnostic criteria (**Table 1**). Larotrectinib is a highly selective pan-Trk inhibitor and has been tested in different tumour types of adult and paediatric patients, showing an ORR of 73% among adults. Toxicity were low, mostly grade 1-2, with 9% of dose reduction and < 1% of discontinuation [47, 48]. Data from 12 patients with *TRK* fusion-positive NSCLC were recently reported by Hong and colleagues: after a median follow-up of 12.9 months, ORR was 75% overall and 67% in 6 patients with brain metastases, with durable responses [48]. Larotrectinib is approved in US, Canada and Brazil. The European Medicines Agency (EMA) also recently recommended approval of larotrectinib for *NTRK* fusion-positive solid tumors [11].

Next generation Trk inhibitors are already under development with the aim to overcome the on-target resistance mechanisms developing under treatment with entrectinib or larotrectinib. As it happens for EGFR, ALK or ROS1 kinases, on-target acquired resistant mutations result in amino acid substitutions which sterically prevent the binding of the inhibitor drug. The analogous $TrkA^{G595R}$, $TrkB^{G639R}$ and $TrkC^{G623R}$ are the most frequent mutations [49, 50]. Two main agents specifically designed to best accommodate the Trk binding pocket in presence of these clinically observed resistance mutations are currently in development: selitrectinib (LOXO-195) and repotrectinib (TPX-0005) [39, 51]. Preliminary data, presented at the AACR 2019 meeting, showed an ORR of 50% with selitrectinib in patients with acquired $TrkA^{G595R}$, $TrkB^{G639R}$ or $TrkC^{G623R}$ mutations in different cancers. Tumor shrinkage was observed in 2/2 evaluable patients with acquired mutations in the “gatekeeper” domain and in 1 out of 2 patients with mutations in DFG (Asp-Phe-Gly) motif of Trk protein, as well [52]. Repotrectinib led to partial responses in two patients with different tumors after progression to first-generation TKIs: a

cholangiocarcinoma with acquisition of G595R and *in trans*-F589L mutations in *NTRK1* after larotrectinib and a salivary gland tumor with G623R in *NTRK3* after entrectinib [53].

3. Introduction to the drug

3.1. *Chemistry*

Entrectinib, previously known as NMS-E628 and then as RXDX-101, is a small molecule with a chemical structure of N-[5-(3,5-difluoro-benzyl)-1 H-indazol-3-yl]-4-(4-methyl-piperazin-1-yl)-2-(tetrahydropyran-4-ylamino)-benzamide ($C_{31}H_{34}F_2N_6O_2$) in a specific cristalline form (WO2009/013126). The compound was synthesized and optimized at Nerviano Medical Sciences srl (NMS), Nerviano, Italy in 2008, firstly evaluated as an inhibitor of ALK kinase activity [54, 55].

3.2. *Pharmacodynamics*

Entrectinib is active as a multi-kinase inhibitor, more particularly of TrkA, TrkB, TrkC, ROS1 and ALK kinases, which share analogous amino-acidic sequences in their tyrosine kinase domain. Entrectinib behaves as a pure ATP competitor. The half maximal inhibitory concentration (IC_{50}) values for TrkA, TrkB, TrkC, ROS1 and ALK purified kinases are 1, 3, 5, 7 and 12 nM/L, respectively [55, 56]. It has shown a higher potency than crizotinib against ALK [57]. In the setting of ROS1-driven cell lines, entrectinib results 40-fold more potent than crizotinib [56]. Blocking of these deregulated receptor tirosine kinases (RTKs) results in inhibition of downstream oncogenic pathways [9, 13].

In the condition of crizotinib-resistant *ROS1*-rearranged NSCLC, the ROS1 kinase can acquire mutations which alter its conformation and the capability to bind the drug: $ROS1^{G2032R}$ (the most frequent), $ROS1^{D2033R}$, $ROS1^{S1986Y/F}$ (in the alfaC-helix of the kinase domain), $ROS1^{L2026M}$ ("gatekeeper"), $ROS1^{L1951R}$ [9]. Entrectinib has failed to demonstrate activity in preclinical setting against $ROS1^{G2032R}$, conflicting data exist for $ROS1^{L2026M}$ and $ROS1^{D2033N}$, while entrectinib showed IC50 of 3.0 nM in Ba/F3-CD74-$ROS1^{L1951R}$ cell lines [44, 56, 58].

$ROS1^{G2032R}$ mutation is analogous to $TrkA^{G595R}$, $TrkB^{G639R}$ and $TrkC^{G623R}$, that have been identified in patients with entrectinib-resistant *NTRK*-rearranged cancers [49, 50]. The gatekeeper $ROS1^{L2026M}$

mutation is analogous to TrkA^{F589L}, TrkB^{F633L} and TrkC^{F617L}, also described after treatment with first-generation TKI in *NTRK*-rearranged cancers. Finally, acquired mutations in the DFG motif of Trk (TrkA^{G667C}, TrkB^{G709C} and TrkC^{G696A}), which plays a role in regulation of kinase activity, have been reported after entrectinib or larotrectinib [52].

3.3. *Pharmacokinetics and metabolism*

Entrectinib is orally bioavailable (hard capsules) and has a systemic distribution, with the ability to cross the blood-brain barrier (BBB). Brain levels reach nearly 50% of plasma levels in mice [55]. The pharmacokinetic profile of entrectinib has been valued in two phase I studies [37, 59, 60]. Here we report the main findings:

- the identified recommended phase 2 dose (RP2D) is 600 mg daily, with or without food;
- the estimated plasma half-life is 20–22 hours, compatible with a once daily, continuous dosing regimen;
- the peak plasma concentration is reached from 2 to 4 h after administration (in the fed condition);
- entrectinib is highly bound by plasma proteins (~99.5%), with free-drug concentration 4-fold higher than that of trough concentrations observed in animal models with complete tumour inhibition;
- entrectinib is mainly metabolized by CYP3A4 (minor contribution from CYP2C9 and CYP1C19) to its active metabolite (M5) [61];
- steady state is achieved within one week for entrectinib and two weeks for M5 [61];
- entrectinib and M5 are mainly excreted in feces, with minimal excretion in urine (3%) [61].

4. **Clinical efficacy**

4.1. Phase I studies

Entrectinib has been tested in two phase I trials, ALKA-372-001 (EudraCT 2012-000148-88; 2 sites, Italy) and STARTRK-1 (NCT02097810; 10 sites, United States, Korea, and Spain) [37]. Overall, 119

patients with a median age of 55 years (18–80 years) were enrolled between 2012 and 2016. The cohort of patients in studies harboured several solid tumour types (60% NSCLC, 15% gastro-intestinal cancers, 4% central nervous system cancers, and others), mostly heavily pretreated (in 25 cases even with ROS1 or ALK inhibitors). A half of them (60 patients) had a gene fusion involving *NTRK*1/2/3, *ROS1*, or *ALK*. The other 59 patients had point mutations, amplifications, copy number variants, insertions/deletions, or no alterations (in 6 cases) of *NTRK1/2/3*, *ROS1*, or *ALK* genes. Responses by RECIST were observed exclusively in the sub-population of patients harbouring gene fusions with no history of prior TKI-treatment. Among 25 evaluable patients, one case of NSCLC with *SQSTM1-NTRK1* fusion, 13 cases of NSCLC with *ROS1* fusions and 4 cases of NSCLC with *ALK* fusions were included. 15 of these 18 patients (83%) achieved a partial or complete response by RECIST. Entrectinib showed also intracranial activity, consistently with preclinical data, with one patient with *NTRK1*-rearranged NSCLC achieving intracranial complete response.

4.2. ***Phase II studies***

The phase II STARTRK-2 basket trial (NCT02568267) is evaluating the activity of entrectinib in patients with advanced tumours harbouring *NTRK1/2/3*, *ROS1*, or *ALK* rearrangements. Integrated data from phase I and phase II trials evaluating entrectinib in ROS1 positive NSCLC and in NTRK positive solid tumors have been recently published in Lancet Oncology [62, 63] (**Table 1**).

Overall, 53 patients with *ROS1*-fusion positive NSCLC have been treated with entrectinib as the first line TKI. As per blinded independent central review (BICR), the ORR was 77% (95% CI 64–88) with 6% of complete responses (CR). The mDOR was 24.6 months (95% CI 11.4–34.8). The intracranial (IC) ORR was 55.0% (95% CI 32–77) and median IC DOR was 12.9 mo (95% CI 5.6–not estimable) [62].

Ten patients with *NTRK*-fusion positive NSCLC have been treated with entrectinib. In this population, BICR ORR was 70.0% (95% CI 34.75–93.33) with 10.0% of CR. Four patients out of six with CNS secondary lesions achieved an intracranial response (2 complete, 2 partial) [63–65].

5. **Safety and tolerability**

Entrectinib toxicity profile is favourable. The most common treatment-emergent adverse events (AEs) are summarized in **Table 2**, compared with other Trk and ROS1 inhibitors available (**Figure 1**). Among 355 patients, treated with entrectinib regardless of molecular profile, age or cancer type, the most of AEs were grade 1-2, with dysgeusia occurring in 41% of patients, followed by fatigue, dizziness, nausea and diarrhea occurred (20-30% of cases). Only one patient experienced a G4 AE (AST increase), while no grade 5 AEs were reported. Dose reductions and treatment discontinuation were necessary in 27% and 4% of the patients, respectively [63].

The broad spectrum of nervous system adverse reactions, including cognitive impairment, increased appetite, dizziness, or sleep disturbances, reflect the capability of entrectinib to cross the BBB and the role of Trk receptors in normal function of the nervous system. In particular, loss-of-function mutations in *NTRK2* have been associated to loss of appetite control and obesity [13], therefore weight gain is a peculiar AE with Trk-inhibitors. With entrectinib, it is reported as G1-2 in 14%, and G3 in 5% of patients. Elevation of creatinine levels is a side-effect unrelated to Trk inhibition which has been observed with entrectinib. Notably, entrectinib is thought to inhibit MATE1-mediated transport of creatinine [66], a mechanism already known with others TKIs [67], hence the rise of creatinine serum levels may not actually reflect a renal failure.

6. **Regulatory affairs**

Entrectinib received its first regulatory approval in Japan, by the Japanese Ministry of Health, Labour and Welfare (MHLW) in June 2019, for the treatment of adult and paediatric patients with NTRK-positive solid tumours, and it is under regulatory review for ROS1-positive NSCLC [68]. Entrectinib has been approved by FDA for treatment of ROS1 gene fusion-positive metastatic NSCLC and NTRK gene fusion-positive solid tumours in August 2019 [69]. Moreover, entrectinib is under regulatory review in European Union (Priority Medicines designation by EMA) for NTRK-positive solid tumours and ROS1-positive NSCLC.

7. Conclusion

The landscape of druggable molecular alterations of NSCLC has been relentlessly reshaping in recent years and includes now the low prevalence gene fusions of *ROS1* or *NTRK1/2/3.* Entrectinib is an orally bioavailable tyrosine kinase inhibitor of TrkA, TrkB, TrkC, and ROS1, with the ability to cross the blood-brain barrier. Results of two phase 1 trials (ALKA-372-001 and STARTRK-1) and of the phase II STARTRK-2 basket trial showed dramatic and durable responses, including high intracranial activity in patients with TKI-*naïve* tumours harbouring *ROS1* or *NTRK1/2/3* gene fusions. Entrectinib was overall well-tolerated and based on these studies received regulatory approval in Japan and USA and it is under regulatory review in European Union.

8. Expert opinion

Entrectinib enriches the therapeutic armamentarium for metastatic NSCLC by widening treatment options against ROS1-positive tumours and encompassing *NTRK* fusions into the spectrum of druggable alterations for this and other solid malignancies. Currently, crizotinib is the only ROS1 inhibitor approved by both FDA and EMA as a first-line treatment choice, but entrectinib is already approved in the US and Japan, and the EMA granted entrectinib Priority Medicine designation. Treatment with crizotinib is supported by a large amount of data, including long follow-up results and it is approved in 70 countries worldwide. Entrectinib can be an alternative first-line treatment for patients with *ROS1*-rearranged NSCLC based on its higher intracranial activity, similar duration of response and manageable toxicity profile, although side-effects mediated by Trk inhibition should be monitored. It must be considered that entrectinib has not the potential to overcome the most common resistance mutations acquired under crizotinib treatment, thus TKI-*naïve* patients could be the most appropriate population for therapy with entrectinib. In this setting, entrectinib should be the preferable choice for patients with brain metastasis, considering the lower capability of crizotinib to cross the BBB. In this way, the necessity of brain radiation therapy could be deferred or avoided, even more in the case of disseminated brain metastasis for which the toxicity on cognitive function of whole-brain radiation is more prominent, especially considering the extended survival of these patients [8]. In some

circumstances, entrectinib could also be considered as a sequential TKI after crizotinib failure. The central nervous system is indeed a common first site of progression in patients who are taking crizotinib [31] and, in this situation when cerebral oligoprogression is diagnosed, a therapeutic switch to entrectinib could be considered based on the better capability of entrectinib to cross the BBB.

In contrast, in case of systemic progression after crizotinib with evidence of secondary mutations in the ROS1 kinase domain, a sequential therapy with entrectinib should not be pursued. Other next-generation TKIs could be considered, taking into account that lorlatinib does not appear to overcome the most frequent mutation G2032R, while preliminary data with repotrectinib or DS-6051b against this resistance mechanism are promising (**Figure 2)**.

Interestingly, no secondary mutations in the ROS1 kinase domain have been reported in cell models exposed to entrectinib, but acquisition of KRAS G12C mutation or amplification of KRAS and FGF3 [70], while clinical data are still lacking. Acquisition of KRAS G12C is an already reported mechanism of resistance to crizotinib [71], and it could be a potential therapeutic target of a new class of G12C inhibitors [72]. A combination of targeted therapy against ROS1 and KRAS G12C may be a potential strategy for overcoming crizotinib or entrectinib resistance in *ROS1*-rearranged NSCLC.

Entrectinib has also been approved in Japan and in the US by the FDA based on an agnostic indication, i.e. for solid tumours harbouring a chromosomal rearrangement involving *NTRK* genes, regardless of the histologic type of tumour. The EMA also granted entrectinib Priority Medicine designation for the treatment of *NTRK* fusion-positive tumours. *NTRK* fusions are very rare in NSCLC, but the increasing use of NGS panels with the support of rational diagnostic/prioritizing algorithms can facilitate their identification and clinical targeting. NCCN guidelines recommend entrectinib, as well as larotrectinib, as a first-line treatment choice for patients with any performance status affected by NTRK-positive NSCLC.

Considering the available data based on short follow-up of a small number of patients with NSCLC treated with the two TKIs, and in absence of head-to-head comparison, no definitive conclusions can be taken about the best Trk inhibitor. Both of them demonstrated a high response rate with intracranial

activity and a favourable safety profile. Similar mechanisms of resistance to entrectinib and larotrectinib have also been described within different tumor types, as well.

Comparably to ROS-1 driven tumors, next-generation Trk inhibitors with the ability to overcome acquired mutations are already under development. The subsequent important step will be to assess the efficacy of next-generation molecules, both ROS-1 and Trk inhibitors, in first-line setting. As already happened with osimertinib in the setting of *EGFR*-mutated NSCLC [73], the availability of more potent inhibitors will likely impact on the best treatment strategy (use in first-line vs sequencing) for these patients.

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Declaration of interest

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FIGURE LEGEND

Figure 1. Synopsis of main adverse events with relative frequencies of different NTRK- or ROS1-targeted tyrosine kinase inhibitors.

Ref: [12, 35, 38, 45, 47, 62, 73].

* = not reported.

Figure 2. Activity of different tyrosine kinase inhibitors in second-line setting after crizotinib/entrectinib (ROS-1+ NSCLC) or entrectinib/larotrectinib (NTRK+ NSCLC) failure according to the molecular mechanisms of acquired resistance (secondary mutations) or the clinical evolution (CNS oligoprogression). *Single check= positive/limited preclinical data; Double check= validation in clinical setting; ?= conflicting preclinical data; X= negative data; nd=no data available. Homologous acquired alterations in ROS1 and Trk proteins are displayed in the same column. CNS oligoPD = central nervous system oligo-progressive disease.*

Ref: [40, 44, 51–53, 56, 58, 75].

ROS1 targeted therapies in TKI-naïve NSCLC					
Drug	**Entrectinib**	**Crizotinib**	**Ceritinib**	**Lorlatinib**	**Repotrectinib**
Ref.	Drilon 2019 [62]	Shaw 2019 [8]	Lim 2017 [35]	Shaw 2019 [38]	Cho 2019 [45]
N	53	53	30	21	11
ORR	77%	72%	67%	62%	82%
mDOR	24.6 mo	24.7 mo	21.0 mo	25.3 mo	NE
mPFS	19.0 mo	19.3 mo	19.3 mo	21 mo	-
IC ORR	55%	-	25%	64%	100% (3/3)

NTRK targeted therapies in TKI-naïve NSCLC		
Drug	**Entrectinib**	**Larotrectinib**
Ref.	Doebele 2019 [63]	Hong 2020 [48]
N	10	12
ORR	70%	75%
mDOR	NE	NE
mPFS	14.9 mo	NE
IC ORR	67% (4/6)	-

Table 1. **Summary table of results from different tyrosine kinase inhibitors in ROS1+ and NTRK+ NSCLC.** Abbreviations: N: number of patients; NSCLC: non-small-cell lung cancer; Ref.: reference; -: data not available; ORR: overall response rate; IC: intracranic; mo: months; mDOR: median duration of response; mPFS: median progression free survival; mOS: median overall survival; NE: not estimable.

Drug	**Entrectinib**	**Larotrectinib**	**Crizotinib**
Study	ALKA, STARTRK-1, STARTRK-2, STARTRK-NG	NCT02122913, NAVIGATE	Profile 1014
Ref.	Doebele 2019 [63]	Hong 2019 [74]	Solomon 2014 [76]
N	355	125	171
Most common (≥10%) TEAEs G1-2 (%)	Dysgeusia 41 Fatigue 25 Dizziness 25 Constipation 23 Diarrhea 21 Nausea 21 Paraesthesia 19 Creatinine increase 15 Myalgia 15 Weight increase 14 Oedema 14 Vomiting 14 Arthralgia 12 AST increase 10	Fatigue 38 AST/ALT increase 35 Dizziness 30 Nausea 29 Constipation 26 Cough 24 Dyspnoea 20 Vomiting 26 Diarrhea 23 Oedema 19 Anaemia 17 Myalgia 16 Pyrexia 15 Weight increase 16 Arthralgia 14 Low appetite 14 Dysgeusia 13 Insomnia 10 Memory impairment 10 Paraesthesia 10	Vision disorder 70 Diarrhea 59 Nausea 55 Oedema 48 Vomiting 44 Constipation 41 AST/ALT increase 22 Fatigue 26 Dysgeusia 26 Dizziness 18 Neutropenia 10
Most common (≥1%) TEAEs G3 (%) ^	Weight increase 5 Anaemia 5 Fatigue 3 Diarrhea 1 AST increase 1* Arthralgia 1 Myalgia 1 Creatinine increase 1 Dizziness 1	Anaemia 13 Fatigue 5 AST/ALT increase 6 Weight increase 7 Neutropoenia 7 Low appetite 4 r Dyspnoea 2 Hypertension 3 Nausea 2 Pyrexia 2# Constipation 1 Dizziness 1 Diarrhea 1	AST/ALT increase 14 Neutropenia 11 Dyspnoea 3 Fatigue 3 QTc prolonged 2 Anaemia 2 Constipation 2 Diarrhea 2 Vomiting 2 Nausea 1 Vision disorder 1 Oedema 1
TEAEs leading to dose reduction	27%	15%	NA
TEAEs leading to permanent discontinuation	4%	0	6%

Table 2. **Main toxicities of entrectinib, larotrectinib, and crizotinib**. Abbreviations.

TEAEs: treatment-emerging adverse events; G: grade; N: number; NA: data not available.

*= 1 pt with G4 AST increase. #=2 pts with G4 pirexia. ^=G3-G4 for crizotinib.

Figure 1

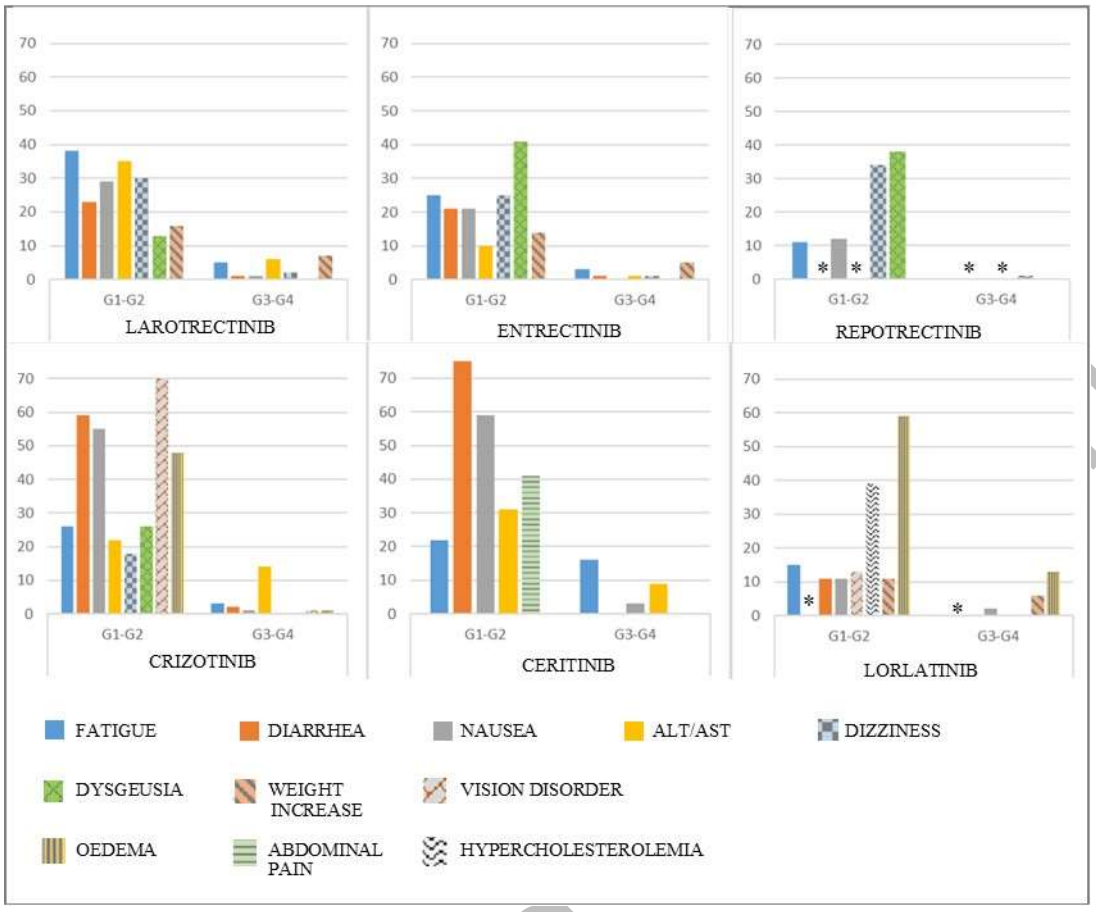

Figure 2

Second-line ROS-1 TKIs	Entrectinib	✓✓	?	nd	?	X	✓	
	Ceritinib	✓	✓	nd	X	X	X	
	Lorlatinib	✓✓	✓	✓	✓	X	✓	
	Repotrectinib	✓✓	✓✓	✓	✓	✓✓	✓	
	DS-6051b	✓	✓	nd	✓	✓	✓	
ROS-1: Reason for 1° line crizotinib or entrectinib failure		*CNS oligoPD to crizotinib*	ROS1-L2026M	ROS1-S1986Y/F	ROS1-D2033N	ROS1-G2032R	ROS1-L1951R	
NTRK: Reason for 1° line entrectinib or larotrectinib failure			TrkA-F589L TrkB-F633L TrkC-F617L			TrkA-G595R TrkB-G639R TrkC-G623R/E		TrkA-G667C TrkB-G709C TrkC-G696A
Second-line NTRK TKIs	Selitrectinib		✓✓			✓✓		✓✓
	Repotrectinib		✓✓			✓✓		✓
	DS-6051b		nd			X		X