Cancer Chemotherapy and Pharmacology (2019) 84:679–688
https://doi.org/10.1007/s00280-019-03902-6

REVIEW ARTICLE

Resistance mechanisms and potent-targeted therapies of ROS1-positive lung cancer

Annie Roys[1] · Xing Chang[1] · Yang Liu[1] · Xiaobo Xu[1] · Yingliang Wu[1] · Daiying Zuo[1]

Received: 21 May 2019 / Accepted: 18 June 2019 / Published online: 29 June 2019

Abstract

The discovery of targetable mutations, which cause gene rearrangement, led to a major advancement in the treatment of patients with non-small cell lung cancer (NSCLC), and cancers with such mutations can be paired with drugs which specifically target them. c-ros oncogene (ROS1) positive NSCLC is one molecular subtype of NSCLC with a therapeutic target. Currently, different targeted therapies and ROS1 inhibitors have been discovered, but all are in different investigational phases, with only one (crizotinib) which is FDA approved. Crizotinib is a small-molecule tyrosine kinase inhibitor (TKI) which was discovered to actively inhibit ALK, MET, and ROS1. Crizotinib has shown to be remarkably efficacious against ROS1 lung cancer, prompting ROS1 detection in lung cancer to be quite significant. Sadly, crizotinib resistance in ROS1 is a frequent occurrence which poses a major clinical challenge in the successful treatment of ROS1 lung cancer; hence, the discovery of the second and third generation ROS1 inhibitors is of utmost importance. In this review, we discuss the underlying mechanisms through which ROS1 tumor cells acquire resistance to crizotinib—the first-line drug for ROS1-positive NSCLC, and summarize various new potent drugs which can overcome this resistance and serve as viable alternatives.

Keywords ROS1 · TKI (tyrosine kinase inhibitor) · NSCLC (non-small cell lung cancer) · Resistance mechanisms · Targeted therapies

Introduction

Lung cancer is the major cause of cancer deaths in both men and women worldwide [1]. Cigarette smoking is the principal risk factor for the development of lung cancer [1]. It can be treated or controlled using a combination of surgery, chemotherapy, targeted therapy, immunotherapy, and radiation therapy as well as newer experimental methods [2]. There are three types of lung cancer: non-small cell lung cancer (NSCLC), small cell lung cancer, and lung carcinoid tumors [1, 2]. NSCLC is the most common histological lung cancer subgroup, which accounts for 80–85% of lung cancers [1]. Squamous cell carcinoma, adenocarcinoma, and large cell carcinoma are all subtypes of NSCLC. Currently, there are several molecular subtypes of NSCLC that have targeted therapies approved for their treatment: mutations in the epidermal growth factor receptor (EGFR), KRAS mutations, ROS1 rearrangements, and gene rearrangements in the anaplastic lymphoma kinase (ALK) gene; tumors harboring these genetic alterations respond well to specific tyrosine kinase inhibitors (TKIs) [3–6].

ROS1 gene rearrangement

ROS1 is a gene that encodes for receptor tyrosine kinase, and it is located at chromosome 6q22 and has a structural similarity to the ALK [7]. ROS1-positive lung cancer is a type of NSCLC which tests positive for a ROS1 gene rearrangement which is one of the known "driver mutations" found in lung cancer [8–10]. In 2007, gene rearrangements involving the ROS1 gene were first detected in glioblastoma tumors (a type of brain cancer) and cell lines, and have also been found in some other cancers, including ovarian cancer, colorectal carcinoma, gastric adenocarcinoma, cholangiocarcinoma, gastric ovarian serous carcinoma, colonic adenocarcinoma, inflammatory myofibroblastic tumor, angiosarcoma, and epithelioid hemangioendothelioma [7, 11–15]. ROS1 rearrangements

✉ Daiying Zuo
zuodaiying@163.com

[1] Department of Pharmacology, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenhe District, Shenyang 110016, China

account for 1–2% of lung adenocarcinomas and approximately 1.4% of all NSCLC [9, 16]. ROS1 NSCLC was found to be prevalent in middle-aged women who are never to light smokers [17]. It is important to emphasize that the ROS1 gene rearrangement is not an acquired genetic change and cannot be inherited. Studies have shown that the inhibition of tumor cell-bearing ROS1 gene fusions by crizotinib or other ROS1 TKIs was effective in vitro [18–20].

The ROS1 gene is involved in chromosomal translocation in lung cancer, it codes for a protein that acts as a driver, and it can run the show when it comes to cell growth and division. When the gene is rearranged, the abnormal protein may thus drive abnormal growth and division of the cell [17]. These proteins are called tyrosine kinases [21]. ROS1 and ALK are receptor tyrosine kinases (RTK), and both belong to the insulin receptor superfamily. Tyrosine kinases are involved in many cell functions, including cell signaling, growth, and division. These enzymes may be too active or found at high levels in some types of cancer cells, and blocking them helps stop the cancer cells from growing. Thus, drugs have been developed to target the extracellular domain or the catalytic domain, therefore, inhibiting ligand binding and receptor oligomerization [9]. These drugs are called TKIs. TKIs are types of targeted therapy substances that block the action of tyrosine kinases. Some TKIs are used to treat cancers [9, 19, 22].

ROS1 detection

Anyone with NSCLC, especially lung adenocarcinoma, should have genetic testing (molecular profiling) done on their lung tumors. Testing is especially important for young adults with lung cancer, who have a high incidence of treatable mutations and may respond well to one of the medications in the category of TKIs. ROS1 rearrangement is detected using two approaches, in situ or non-in situ/extractive [23]. Fluorescent in situ hybridization (FISH) using a dual colour 'break-apart' probe approach is considered the "gold standard" in detecting ROS1 rearrangement [24]. Immunohistochemistry (IHC) is a cost-effective method that can be used to efficiently test patients with lung cancer for ROS1 rearrangement [23]. In addition to FISH and IHC, a number of extractive or non-in situ approaches based on real-time polymerase chain reaction (RT-PCR) or next-generation sequencing (NGS) have been developed for the detection of ROS1 gene rearrangements [23].

Effective regimens against ROS1-positive lung cancer

ROS1, like every other form of cancer, can be treated or controlled using a combination of surgery, chemotherapy, targeted therapy, immunotherapy, and radiation therapy as well as newer experimental methods.

Besides targeted therapy, some chemotherapy agents are also effective in ROS1 positive tumors. ROS1-positive lung cancer appears to be particularly sensitive to the chemotherapy drug Alimta (pemetrexed) with over 58% of ROS1-positive patients responding to the drug in one study [22, 25]. Patients with ROS1 fusion had a better overall response rate (57.9%), disease control rate (89.5%), and longer progression-free survival (7.5 months) compared with patients harboring other driver mutations making ROS1 fusion positivity a favorable factor for patients with lung adenocarcinoma on pemetrexed-based therapy [25]. As discussed earlier, RTKs are active targets for drug therapy, hence the use of TKIs such as crizotinib as targeted therapies [19]. Because TKIs only inhibit cells with certain genomic alterations (i.e., they do not act on all fast-growing cells as seen in chemotherapy), this leads to the existence of fewer side effects compared to chemotherapy. However, they do not cure the cancer, but only inhibit the cancer cells. Almost all patients treated with TKIs experience tumor progression, because the cancer cells develop new acquired mutations [24].

Brain metastases in ROS1-positive lung cancer

Central nervous system (CNS) metastasis is a common cause of death in lung cancer, and the same case is seen in ROS1-positive lung cancers. Brain metastasis was found to be quite common in stage IV ROS1-positive lung cancer. In a study conducted by Patil et al., 36% of stage IV ROS1 positive patients tested positive for brain metastasis [26]. Due to crizotinib's inability to penetrate the blood–brain barrier (BBB), it is not ideal for ROS1 cases with brain metastasis [27]. However, some new TKIs with better CNS penetrating ability have been developed, drugs such as lorlatinib, ceritinib, and ropotrectinib are smaller in size and able to penetrate the BBB, but they are all yet to be FDA approved. In addition, chemotherapy drugs are unable to penetrate the BBB; hence, radiation therapy is the treatment path usually followed for cancer cases with brain metastases. Luckily, it was observed that ROS1 rearrangement tumors were particularly sensitive to these treatments [28]. There are a couple of ways through which radiation

can be given, depending on the degree of metastases or the number of 'spots' (area of metastasis). When multiple metastases are noticed, whole brain radiotherapy is the technique used, and it involves the treatment of the entire brain with radiation. However, when there are a few spots, i.e., three or four metastases present, stereotactic radiation is often performed, this approach (also called gamma knife), involves the treatment of localized spots on the brain with radiation. Both approaches have their pros and cons, whole brain radiotherapy may reduce the possibility of the reoccurrence of metastasis, while stereotactic radiotherapy presents fewer side effects, since only a localized portion of the brain is treated with radiation [17, 28, 29].

Targeting the ROS1 oncogene

Crizotinib (Xalkori) is an orally available TKI that was originally developed as an anti-MET molecule [30]. It demonstrated significant activity in ALK-driven diseases and was approved for ALK-positive lung cancer, and it was also shown to express relevant activity in ROS1-rearranged NSCLC [6, 18]. Crizotinib was the first TKI approved by the US FDA for the treatment of ROS1-positive advanced (metastatic) NSCLC [31, 32]. It has become the standard of care for metastatic ROS1-positive NSCLC in the USA [29]. The mechanism of action of crizotinib is by blocking cell signaling of ROS1/ALK tyrosine kinases [24]. Tumor growth appears addicted to stimulation by the fusion gene, and blocking it stops cell signaling. Being strictly dependent on ROS1 signaling for survival, growth, and progression, the inhibition of this signaling brings about apoptosis of tumor cells thus generating positive clinical effect [24].

Mechanisms of resistance to crizotinib

The average progression-free survival of ROS1-positive NSCLC patients using crizotinib has been 19.2 months [19]. However, cancer cells eventually develop a resistance to the effects of the drug and the patients invariably undergo disease progression. Most mechanisms explaining the resistance of cancer cells to kinase inhibitors reside within one of the two categories: genetic alteration of the drug target (that is, point mutations and/or gene amplification) or activation of bypass signaling [33–35]. The mechanisms of acquired resistance of crizotinib, the ALK/MET/ROS1 TKI, in ROS1 fusions are not completely understood. However, further studies and preclinical evidence support the idea of the generation of point mutations (missense mutations) within the ROS1 kinase domain (Fig. 1), and this effectuates the genetic alteration of the drug target causing an acquired resistance to crizotinib [33, 36]. These mutations notably decrease the potency of the inhibitor against the kinase and it can also lead to different extents of sensitivity (an increase or decrease) or resistance to other ROS1 TKIs [37, 38]. One method of overcoming this type of resistance is to identify more potent inhibitors capable of inhibiting the mutated target.

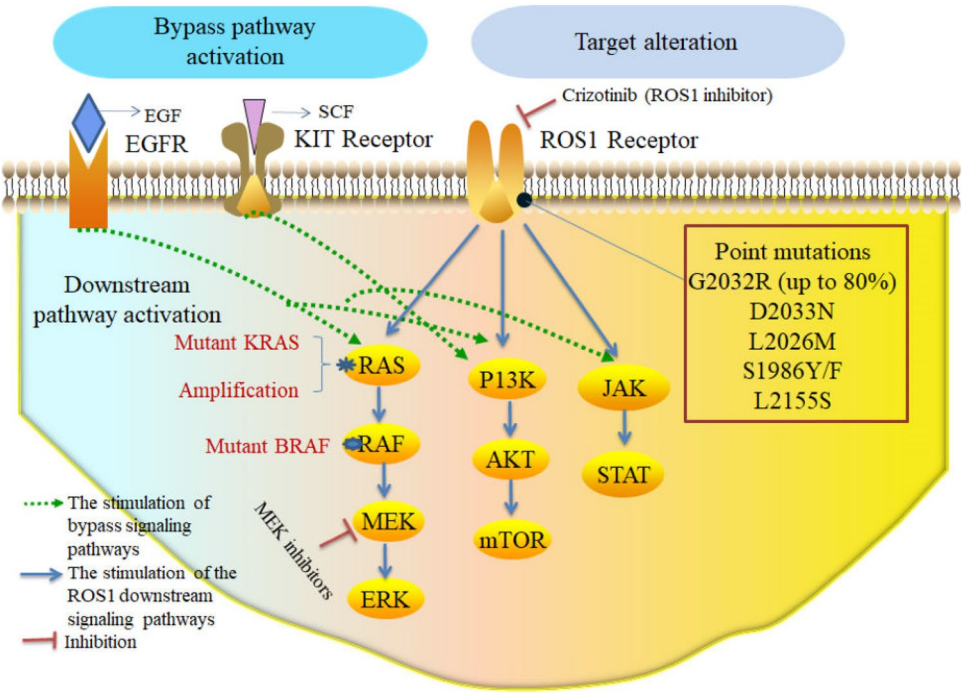

Fig. 1 Molecular mechanisms of crizotinib resistance in ROS1, depicting the two main patterns

G2032R

An example of a mutation in the ROS1 kinase domain is the G2032R mutation [39], which was observed in a patient with a crizotinib-sensitive NSCLC harboring a CD74-ROS1 fusion, and the G2032R mutation was not detected in the sample of malignant cells assessed before treatment with crizotinib [39]. The G2032R mutation leads to a glycine-to-arginine substitution at codon 2032 in the ROS1 kinase domain. It causes resistance to ROS1 kinase inhibition through steric interference with drug binding (Table 1) [39]. G2032R is the most commonly observed mutation conferring crizotinib resistance in ROS1 positive NSCLC patients, and it occurs in four out of ten cases and is present in four out of five biopsies harboring ROS1 acquired mutations [24]. It also presents from a pharmacological point of view as the most difficult mutation (leading to crizotinib resistance) to treat [33, 40]. Studies conducted by our group show that G2032R increases TWIST1 expression and induces epithelial–mesenchymal transition (EMT) leading to crizotinib resistance and a combination of TWIST1 siRNA and ROS1 inhibitor may help in cases of crizotinib resistance due to G2032R [41].

D2033N

In another patient harboring crizotinib-sensitive NSCLC CD74-ROS1 fusion, an ROS1 D2033N mutation which was not present pre-crizotinib treatment was subsequently detected at the time of progression [42]. This mutation is due to an aspartic acid-to-asparagine substitution occurring at ROS1 codon 2033 [24], and it occurs at the solvent-front region of the ATP-binding site (kinase hinge) of ROS1, just like in the G2032R mutation and induces a high-level resistance to crizotinib in vitro, as a result of a series of change in the electrostatic potential and reorientation of surrounding residues in front of the ATP-binding pocket [24, 42].

L2155S

Acquired crizotinib resistance was noticed in two HCC78 cell lines harboring the SLC34A2-ROS1 fusion, and the cells were found to have the novel L2155S mutation which was not present pre-crizotinib treatment in the original cell line. A structural model for ROS1 L2155S mutation suggests that its mechanism for resistance to crizotinib is through protein malfunction [43].

S1986Y/F

In an EZR-ROS1 NSCLC patient with evident crizotinib resistance, an S1986F/Y substitution in the ROS1 kinase domain was noticed. In this case, the serine at 1986 ROS1 position can be substituted by either tyrosine (S1986Y) or phenylalanine (S1986F) amino acid residues. The mechanism of crizotinib resistance by S1986Y/F substitutions appears to be by obstructing its access to the enzyme active site and by increasing kinase activity [24, 33].

L2026M

In addition, the gatekeeper mutation L2026M has been found to confer crizotinib resistance. This mutation involves leucine and methionine and is located at the "gatekeeper" position of the inhibitor-binding pocket [36, 44].

Table 1 ROS1 mutations conferring crizotinib resistance and ROS1 inhibitors capable of combating the mutations

Mutation	ROS1 fusion	Location	Mechanism	Potent TKIs
G2032R	CD74-ROS1	Solvent front of the kinase hinge	Glycine-to-arginine substitution at codon 2032 in the ROS1 kinase domain causes resistance to ROS1 kinase inhibition through steric interference with drug binding	Cabozantinib Ropotrectinib
D2033N	CD74-ROS1	Solvent front of the kinase hinge	Aspartic acid-to-asparagine substitution at codon 2033 leads to a modification of electrostatic forces at the exterior surface of the ATP-binding site and reorientation of surrounding residues in front of the ATP-binding pocket	Lorlatinib Cabozantinib Ropotrectinib
L2155S	SLC34A2-ROS1	Not reported yet	Protein malfunction	Lorlatinib Cabozantinib Ropotrectinib
S1986F/Y	EZR-ROS1	Not reported yet	Serine to tyrosine (S1986Y)/serine to phenylalanine (S1986F) substitution leads to an obstruction in the path to the enzyme active site and an increase in kinase activity	Lorlatinib Cabozantinib Ropotrectinib
L2026M	CD74-ROS1	Gatekeeper position of inhibitor-binding pocket	Leucine-to-methionine substitution at codon 2026	Lorlatinib Ceritinib Cabozantinib Ropotrectinib

Activation of bypass-signaling pathways

Activation of bypass-signaling pathways is another occurrence that accounts for crizotinib resistance in ROS1 cancer cells, the activation of a different signaling pathway thwarts the need for the original drug target [36, 38]. The expression of the mutant KITD816G receptor in ROS1-positive NSCLC cell lines (Fig. 1) led to a KIT activating mutation. This expression engenders the resistance to crizotinib by the HCC78 and CUTO2 cell lines [35]. The KITD816G mutation is in itself oncogenic by and can promote auto-phosphorylation and proliferation in cells. An activation of KIT mutation can induce resistance to crizotinib and other TKIs in vitro [38]. Dziadziuszko and colleagues demonstrated that this cellular resistance can be overcome by the addition of a KIT inhibitor (e.g., ponatinib) to crizotinib [35].

EGFR pathway activation in ROS1-positive HCC78-TR cells was reported by Davies and colleagues [34], and they accounted a decrease in ROS1 fusion protein levels and that the growth and survival of signaling pathways switched from being mainly dependent on ROS1 activity to being mainly dependent on EGFR activity. This signaling switch led to the acceleration of the HCC78-TR cells becoming partly sensitive to only EGFR inhibition, leading us to believe that EGFR pathway activation may underlie crizotinib resistance and resistance to ROS1 inhibition as a whole [34, 43, 45, 46].

Combination therapies

Resistance as a result of a new mutation can be treated with another inhibitor capable of combatting said mutation; therefore, the discovery and development of other ROS1 inhibitors besides crizotinib are vital [39]. However, in resistance driven by bypass mechanisms, such as EGFR and KIT activation, targeting both ROS1 and the bypass pathway (e.g., EGFR/KIT) inhibition helps overcome resistance, and this can be done by combining crizotinib with another KIT/EGFR inhibitor [35]. In addition, the combination of targeted therapies with immunotherapy or chemotherapy has been thought to result in more improved responses [25]. In addition, up-front combination therapy is thought to be effective in delaying resistance [47]. Combinatorial approaches are thought to have superior efficacy compared to monotherapy, but caution has to be taken in such cases due to toxicity. Toxicity usually increases when two or more drugs are combined, and even unanticipated toxicities may be seen [48].

Drugs targeting ROS1

Acquired resistance to crizotinib is a recurring challenge when dealing with patients with ROS1-positive lung cancer, and the existence and discovery of the next-generation ROS1 inhibitors are eminent in finding lasting treatment and solutions for patients with ROS1. So far, crizotinib is the only FDA-approved targeted therapy against ROS1, but several other molecules are currently undergoing research and clinical trials to evaluate their therapeutic effects and efficacy against ROS1.

Lorlatinib

Lorlatinib is an investigational ALK/ROS1 TKI for the treatment of ALK/ROS1-positive NSCLC patients previously treated with an ALK/ROS1 TKI (Fig. 2) [49]. In 2017, it was granted breakthrough therapy designation for ALK-positive metastatic NSCLC from the FDA [50]. Lorlatinib was particularly designed to inhibit tumor mutations that confer resistance to other ALK inhibitors and to penetrate the BBB. It was granted priority review by the FDA in ALK-positive NSCLC in February 2018 [50]. Lorlatinib has shown impressive potency against ROS1 L2026M, D2033N, and S1986F/Y (Table 1) substitutions which confers crizotinib and ceritinib resistance [42, 44]. It was reported that it is able to sustain strong growth inhibition of ROS1 S1986F/Y cells. Facchinetti and colleagues performed structural studies that revealed a conformational shift in the alpha-C helix and the glycine-rich region close to the active site of the enzyme which influences crizotinib binding, but permits lorlatinib inhibitory activity into the kinase domain [33]. In addition, lorlatinib has also shown to be efficacious towards overcoming crizotinib resistance due to bypass-signaling activation [33].

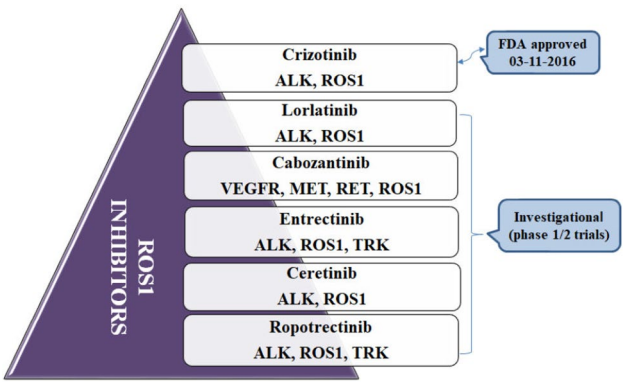

Fig. 2 Summary of all the current ROS1 inhibitors, their major targets, and investigational status

Cabozantinib

Cabozantinib is a small-molecule TKI that inhibits the activity of multiple tyrosine kinases, including the RET proto-oncogene (RET), MET proto-oncogene (MET), and vascular endothelial growth factor receptor 2 (VEGFR2) (Fig. 2). Cabozantinib is currently an FDA-approved drug for the treatment of medullary thyroid carcinoma and more recently metastatic renal cancer carcinoma [51, 52]. Cabozantinib inhibits wild-type ROS1 and has shown potency against crizotinib resistant G2032R and D2033N ROS1 mutations (Table 1) [42]. In addition, its ability to sustain a majority of the interactions at the enzyme active site accounts for its potent inhibition of wild-type and crizotinib-resistant ROS1 [53]. Cabozantinib is required in dosages inferior to crizotinib this makes administration of the drug difficult due to an increase in side effects. It has been reported to be able to overcome all resistance conferred by newly identified secondary mutations [54].

Ceritinib

Ceritinib is a second-generation ALK inhibitor approved for crizotinib-resistant ALK-positive NSCLC in preclinical studies [55, 56]. It is a selective oral ALK TKI which is supposedly more potent than crizotinib due to a better BBB penetrating ability [57, 58]. In ALK-positive NSCLC, ceritinib shows potency in both the crizotinib-naïve and the crizotinib-resistant settings, but ceritinib's activity in ROS1-positive NSCLC is restricted just to crizotinib-naïve patients [57]. In addition, ceritinib shows no potency towards most ROS1 resistant mutations making it ineffective towards crizotinib-resistant tumors, since most of the mutations account for acquired crizotinib resistance in tumors. Another disadvantage of ceritinib is that it causes significantly more side effects (nausea, vomiting, and diarrhea) than seen with crizotinib [57], making crizotinib a more preferable choice despite its increased potency.

Entrectinib

Entrectinib is an investigational, orally available small-molecule TKI [59]. It is clinically active in ROS1 inhibitor-naïve ROS1-rearranged lung cancers and demonstrates CNS activity which makes it advantageous compared to crizotinib in that aspect. Given the propensity of brain metastases in ROS1 NSCLC, entrectinib was designed to penetrate the blood–brain barrier, permitting it to both address preexisting CNS lesions and giving it the ability to prevent or delay the onset of metastases to the brain [60]. In ROS1-rearranged lung cancers, entrectinib displayed significant disease control and prolonged progression-free survival, but it is not effective against crizotinib-resistant ROS1 tumors. It demonstrated a lack of activity in tumor cells harboring the gatekeeper mutation, L2026M, and the solvent-front mutations, D2033N and the G2032R mutation which is the primary mutation conferring crizotinib resistance [53, 61].

Ropotrectinib

Ropotrectinib (TPX-0005) is a brain-tumor penetrable ROS1/TRK/ALK kinase inhibitor which was designed not only to target abnormal activity of ROS1, TRK, and ALK, but also to overcome drug resistance caused by the previous ROS1, neurotrophic tyrosine kinase (NTRK), and ALK inhibitors, it received FDA orphan drug designation in June 2017 for treatment of NSCLC harboring ALK, ROS1, or NTRK oncogenic rearrangements (Fig. 2) [62]. It is distinctly smaller in size than the prevalent ROS1/ALK inhibitors. It can target the ATP-binding site center and is able to successfully bypass steric interference caused by mutations such as G2032R which engenders clinical resistance [63]. As a result, ropotrectinib is an active inhibitor of both wild-type and mutant ALK/ROS1 fusions. It displayed the potential to fight against multiple resistance mechanisms, including mutations, bypass mechanisms, and even metastasis [64]. It is currently in clinical trial and the results from the phase 1 trial would be released later this year.

Others

There are a few other drugs currently under clinical trials that are also thought to be potential ROS1 inhibitors, drugs such as ensartinib which is an orally available small molecule currently in clinical trial for pediatric ROS1 positive patients [29, 65], and DS-6051b which is also an orally available ROS1 TKI [29].

Although the development of foretinib has been discontinued since 2015, Davare and colleagues in a study conducted in 2013 found foretinib to be a highly effective inhibitor of ROS1 in humans and that it demonstrated greater potency both in vitro and in vivo compared to crizotinib [37]. Foretinib showed efficacy towards inhibiting the G2032R ROS1 mutation which confers crizotinib resistance. Hence, it was found to be effective against both wild-type ROS1 and the crizotinib-resistant ROS1 mutations [37].

Brigatinib which was approved by the FDA for the treatment of ALK-positive patients with metastatic NSCLC who have had disease progression on crizotinib or are intolerant to crizotinib [66] was also found to inhibit native ROS1 at possible concentrations in patients [53], but was ineffective against the G2032R mutation which is the predominant cause of acquired crizotinib resistance. Thus, exempting it from being a top of the line therapy in treatment of ROS1-positive NSCLC.

Crizotinib's inability to penetrate the BBB makes it unsuitable for ROS1 cases with metastases to the brain; in such cases, lorlatinib, ropotrectinib, ceritinib, and entrectinib are viable options, as they were designed with better CNS activity and hence are able to penetrate the BBB [50, 58, 60, 63]. Although ceritinib and entrectinib have better CNS activity than crizotinib, they are ineffective against crizotinib-resistant tumors and are only applicable to crizotinib-naïve patients; hence, they can be used in metastasized ROS1 patients or patients with crizotinib intolerance. So far, lorlatinib and ropotrectinib seem to be the most potent drugs in inhibiting ROS1, with good CNS activity, ability to inhibit resistance mutations, and their ability to overcome crizotinib resistance due to bypass-signaling activation. Lorlatinib is currently approved for ALK-positive NSCLC and ropotrectinib is undergoing clinical trials [50]. Hopefully, the results when released at the end of the trials will be what is needed to take it on its way to first/second line therapy for the treatment of metastatic ROS1-positive NSCLC.

Discussion

ROS1 inhibitors have shown to be highly effective against ROS1-positive NSCLC, but acquired resistance to these drugs often leads to disease progression in the patients; therefore, the design of stronger molecules is vital. Improvement of ROS1 inhibition will help in delivering better and enhanced therapy for patients with this type of lung cancer. In addition, the developments of better CNS penetrating TKIs are vital for patients, whose cancer metastasizes to the brain.

Cancer cells can acquire resistance in array of ways. The most comprehended mechanism of resistance is genetic alteration of the drug target. Gene alteration in the form of point mutations is a significant factor underlying drug resistance in ROS1, whereby the presence of one or more mutations leads to resistance of one or more inhibitors. The ability to ascertain the character of various resistance mechanisms towards ROS1-targeted therapies is imperative for enhancement of treatment against disease progression [67]. The type of targeted therapy used for treatment will depend on the existence and amount of certain mutations in the cancer patient.

Activation of bypass-signaling pathways is another leading cause of drug resistance in ROS1 lung cancer. Therefore, signal pathway investigation is important for the development of better drugs for combination therapies. In addition, a better understanding of the different signal pathways will lead to the development of drugs capable of inhibiting/blocking said pathways, consequently combatting resistance.

There is an urgent need for sensitive clinical detection methods to identify the different resistance mechanisms from blood and tissues upon disease progression. Early detection of the responsible resistance mechanism will be useful to design a treatment plan which can hinder tumor progression and thwart further drug resistance.

Conclusion

ROS1-positive NSCLC cases are not very common, but identification of such cases is eminent due to the discovery of various targeted therapies. Sadly, disease progression as a result of acquired resistance frequently occurs, this is a significant obstacle in successfully treating ROS1 lung cancer. Understanding the molecular alterations underlying the development of resistance to targeted therapies is necessary to develop therapeutic strategies against disease progression, as well as to design new and effective combination therapies. These new approaches show promise against effectively combatting ROS1 and improving treatment for patients with ROS1-positive lung cancer.

Acknowledgements This work was funded by the National Natural Science Foundation of China (81872394), young and middle age backbone personnel training program of Shenyang Pharmaceutical University (ZQN2015003) and Liaoning BaiQianWan Talents supporting Program (2016921065).

Compliance with ethical standards

Conflict of interest The authors declare that they have no financial, personal, or professional conflict of interest.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. CA Cancer J Clin 61:69–90
2. Torre LA, Siegel RL, Ward EM, Jemal A (2016) Global cancer incidence and mortality rates and trends—an update. Cancer Epidemiol Biomark Prev 25:16–27
3. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, Nishiwaki Y, Ohe Y, Yang JJ, Chewaskulyong B, Jiang H, Duffield EL, Watkins CL, Armour AA, Fukuoka M (2009) Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 361:947–957
4. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 304:1497–1500
5. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, Zhang S, Wang J, Zhou S, Ren S, Lu S, Zhang L, Hu C, Hu C, Luo Y, Chen L, Ye M, Huang J, Zhi X, Zhang Y, Xiu Q, Ma J, Zhang L, You C (2011) Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol 12:735–742

6. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, Ou SH, Dezube BJ, Janne PA, Costa DB, Varella-Garcia M, Kim WH, Lynch TJ, Fidias P, Stubbs H, Engelman JA, Sequist LV, Tan W, Gandhi L, Mino-Kenudson M, Wei GC, Shreeve SM, Ratain MJ, Settleman J, Christensen JG, Haber DA, Wilner K, Salgia R, Shapiro GI, Clark JW, Iafrate AJ (2010) Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 363:1693–1703
7. Birchmeier C, Sharma S, Wigler M (1987) Expression and rearrangement of the ROS1 gene in human glioblastoma cells. Proc Natl Acad Sci USA 84:9270–9274
8. Arai Y, Totoki Y, Takahashi H, Nakamura H, Hama N, Kohno T, Tsuta K, Yoshida A, Asamura H, Mutoh M, Hosoda F, Tsuda H, Shibata T (2013) Mouse model for ROS1-rearranged lung cancer. PLoS One 8:e56010
9. Bergethon K, Shaw AT, Ou SH, Katayama R, Lovly CM, McDonald NT, Massion PP, Siwak-Tapp C, Gonzalez A, Fang R, Mark EJ, Batten JM, Chen H, Wilner KD, Kwak EL, Clark JW, Carbone DP, Ji H, Engelman JA, Mino-Kenudson M, Pao W, Iafrate AJ (2012) ROS1 rearrangements define a unique molecular class of lung cancers. J Clin Oncol 30:863–870
10. Davies KD, Le AT, Theodoro MF, Skokan MC, Aisner DL, Berge EM, Terracciano LM, Cappuzzo F, Incarbone M, Roncalli M, Alloisio M, Santoro A, Camidge DR, Varella-Garcia M, Doebele RC (2012) Identifying and targeting ROS1 gene fusions in non-small cell lung cancer. Clin Cancer Res 18:4570–4579
11. Aisner DL, Nguyen TT, Paskulin DD, Le AT, Haney J, Schulte N, Chionh F, Hardingham J, Mariadason J, Tebbutt N, Doebele RC, Weickhardt AJ, Varella-Garcia M (2014) ROS1 and ALK fusions in colorectal cancer, with evidence of intratumoral heterogeneity for molecular drivers. Mol Cancer Res 12:111–118
12. Birch AH, Arcand SL, Oros KK, Rahimi K, Watters AK, Provencher D, Greenwood CM, Mes-Masson AM, Tonin PN (2011) Chromosome 3 anomalies investigated by genome wide SNP analysis of benign, low malignant potential and low grade ovarian serous tumours. PLoS One 6:e28250
13. Gu TL, Deng X, Huang F, Tucker M, Crosby K, Rimkunas V, Wang Y, Deng G, Zhu L, Tan Z, Hu Y, Wu C, Nardone J, MacNeill J, Ren J, Reeves C, Innocenti G, Norris B, Yuan J, Yu J, Haack H, Shen B, Peng C, Li H, Zhou X, Liu X, Rush J, Comb MJ (2011) Survey of tyrosine kinase signaling reveals ROS kinase fusions in human cholangiocarcinoma. PLoS One 6:e15640
14. Davies KD, Doebele RC (2013) Molecular pathways: ROS1 fusion proteins in cancer. Clin Cancer Res 19:4040–4045
15. Zhu VW, Upadhyay D, Schrock AB, Gowen K, Ali SM, Ou SH (2016) TPD52L1-ROS1, a new ROS1 fusion variant in lung adenosquamous cell carcinoma identified by comprehensive genomic profiling. Lung cancer 97:48–50
16. Takeuchi K, Soda M, Togashi Y, Suzuki R, Sakata S, Hatano S, Asaka R, Hamanaka W, Ninomiya H, Uehara H, Lim Choi Y, Satoh Y, Okumura S, Nakagawa K, Mano H, Ishikawa Y (2012) RET, ROS1 and ALK fusions in lung cancer. Nat Med 18:378–381
17. Eldridge L (2018) Understanding ROS1 gene rearrangement in non-small cell lung cancer. http://www.verywell.com/ros1-positive-lung-cancer-2248947. Accessed 04 Dec 2018
18. Shaw AT, Ou SH, Bang YJ, Camidge DR, Solomon BJ, Salgia R, Riely GJ, Varella-Garcia M, Shapiro GI, Costa DB, Doebele RC, Le LP, Zheng Z, Tan W, Stephenson P, Shreeve SM, Tye LM, Christensen JG, Wilner KD, Clark JW, Iafrate AJ (2014) Crizotinib in ROS1-rearranged non-small-cell lung cancer. N Engl J Med 371:1963–1971
19. Shaw AT, Solomon BJ (2015) Crizotinib in ROS1-rearranged non-small-cell lung cancer. N Engl J Med 372:683–684
20. Mazieres J, Zalcman G, Crino L, Biondani P, Barlesi F, Filleron T, Dingemans AM, Lena H, Monnet I, Rothschild SI, Cappuzzo F, Besse B, Thiberville L, Rouviere D, Dziadziuszko R, Smit EF, Wolf J, Spirig C, Pecuchet N, Leenders F, Heuckmann JM, Diebold J, Milia JD, Thomas RK, Gautschi O (2015) Crizotinib therapy for advanced lung adenocarcinoma and a ROS1 rearrangement: results from the EUROS1 cohort. J Clin Oncol 33:992–999
21. Rikova K, Guo A, Zeng Q, Possemato A, Yu J, Haack H, Nardone J, Lee K, Reeves C, Li Y, Hu Y, Tan Z, Stokes M, Sullivan L, Mitchell J, Wetzel R, Macneill J, Ren JM, Yuan J, Bakalarski CE, Villen J, Kornhauser JM, Smith B, Li D, Zhou X, Gygi SP, Gu TL, Polakiewicz RD, Rush J, Comb MJ (2007) Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell 131:1190–1203
22. Zhang L, Jiang T, Zhao C, Li W, Li X, Zhao S, Liu X, Jia Y, Yang H, Ren S, Zhou C (2016) Efficacy of crizotinib and pemetrexed-based chemotherapy in Chinese NSCLC patients with ROS1 rearrangement. Oncotarget 7:75145–75154
23. Bubendorf L, Buttner R, Al-Dayel F, Dietel M, Elmberger G, Kerr K, Lopez-Rios F, Marchetti A, Oz B, Pauwels P, Penault-Llorca F, Rossi G, Ryska A, Thunnissen E (2016) Testing for ROS1 in non-small cell lung cancer: a review with recommendations. Virchows Arch 469:489–503
24. Facchinetti F, Rossi G, Bria E, Soria JC, Besse B, Minari R, Friboulet L, Tiseo M (2017) Oncogene addiction in non-small cell lung cancer: focus on ROS1 inhibition. Cancer Treat Rev 55:83–95
25. Chen YF, Hsieh MS, Wu SG, Chang YL, Yu CJ, Yang JC, Yang PC, Shih JY (2016) Efficacy of pemetrexed-based chemotherapy in patients with ROS1 fusion-positive lung adenocarcinoma compared with in patients harboring other driver mutations in east asian populations. J Thorac Oncol 11:1140–1152
26. Patil T, Smith DE, Bunn PA, Aisner DL, Le AT, Hancock M, Purcell WT, Bowles DW, Camidge DR, Doebele RC (2018) The incidence of brain metastases in stage IV ROS1-rearranged non-small cell lung cancer and rate of central nervous system progression on crizotinib. J Thorac Oncol 13:1717–1726
27. Gainor JF, Tseng D, Yoda S, Dagogo-Jack I, Friboulet L, Lin JJ, Hubbeling HG, Dardaei L, Farago AF, Schultz KR, Ferris LA, Piotrowska Z, Hardwick J, Huang D, Mino-Kenudson M, Iafrate AJ, Hata AN, Yeap BY, Shaw AT (2017) Patterns of metastatic spread and mechanisms of resistance to crizotinib in *ROS1*-positive non-small-cell lung cancer. JCO Precis Oncol 1:1–13
28. Lukas RV, Hasan Y, Nicholas MK, Salgia R (2015) ROS1 rearranged non-small cell lung cancer brain metastases respond to low dose radiotherapy. J Clin Neurosci 22:1978–1979
29. Drugs to treat ROS1+ cancer. http://www.ros1cancer.com/treatments-and-clinical-trials/. Accessed 04 Dec 2018
30. Zou HY, Li Q, Lee JH, Arango ME, McDonnell SR, Yamazaki S, Koudriakova TB, Alton G, Cui JJ, Kung PP, Nambu MD, Los G, Bender SL, Mroczkowski B, Christensen JG (2007) An orally available small-molecule inhibitor of c-Met, PF-2341066, exhibits cytoreductive antitumor efficacy through antiproliferative and antiangiogenic mechanisms. Cancer Res 67:4408–4417
31. Roskoski R Jr (2017) ROS1 protein-tyrosine kinase inhibitors in the treatment of ROS1 fusion protein-driven non-small cell lung cancers. Pharmacol Res 121:202–212
32. U.S.F.a.D. Administration, FDA expands use of Xalkori to treat rare form of advanced non-small cell lung cancer. https://www.fda.gov/newsevents/newsroom/pressannouncements/ucm490329.htm. Accessed 04 Dec 2018
33. Facchinetti F, Loriot Y, Kuo MS, Mahjoubi L, Lacroix L, Planchard D, Besse B, Farace F, Auger N, Remon J, Scoazec JY, Andre F, Soria JC, Friboulet L (2016) Crizotinib-resistant ROS1 mutations reveal a predictive kinase inhibitor sensitivity model for ROS1- and ALK-rearranged lung cancers. Clin Cancer Res 22:5983–5991

34. Davies KD, Mahale S, Astling DP, Aisner DL, Le AT, Hinz TK, Vaishnavi A, Bunn PA Jr, Heasley LE, Tan AC, Camidge DR, Varella-Garcia M, Doebele RC (2013) Resistance to ROS1 inhibition mediated by EGFR pathway activation in non-small cell lung cancer. PLoS One 8:e82236
35. Dziadziuszko R, Le AT, Wrona A, Jassem J, Camidge DR, Varella-Garcia M, Aisner DL, Doebele RC (2016) An activating KIT mutation induces crizotinib resistance in ROS1-positive lung cancer. J Thorac Oncol 11:1273–1281
36. McCoach CE, Le AT, Gowan K, Jones K, Schubert L, Doak A, Estrada-Bernal A, Davies KD, Merrick DT, Bunn PA Jr, Purcell WT, Dziadziuszko R, Varella-Garcia M, Aisner DL, Camidge DR, Doebele RC (2018) Resistance mechanisms to targeted therapies in ROS1(+) and ALK(+) non-small cell lung cancer. Clin Cancer Res 24:3334–3347
37. Davare MA, Saborowski A, Eide CA, Tognon C, Smith RL, Elferich J, Agarwal A, Tyner JW, Shinde UP, Lowe SW, Druker BJ (2013) Foretinib is a potent inhibitor of oncogenic ROS1 fusion proteins. Proc Natl Acad Sci USA 110:19519–19524
38. Katayama R, Shaw AT, Khan TM, Mino-Kenudson M, Solomon BJ, Halmos B, Jessop NA, Wain JC, Yeo AT, Benes C, Drew L, Saeh JC, Crosby K, Sequist LV, Iafrate AJ, Engelman JA (2012) Mechanisms of acquired crizotinib resistance in ALK-rearranged lung cancers. Sci Transl Med 4:120ra117
39. Awad MM, Engelman JA, Shaw AT (2013) Acquired resistance to crizotinib from a mutation in CD74-ROS1. N Engl J Med 369:1173
40. Gainor JF, Friboulet L, Yoda S, Alghalandis LD, Farago AF, Logan J, Schultz K, Sequist LV, Engelman JA, Shaw AT (2016) Frequency and spectrum of ROS1 resistance mutations in ROS1-positive lung cancer patients progressing on crizotinib. J Clin Oncol 34:9072
41. Gou W, Zhou X, Liu Z, Wang L, Shen J, Xu X, Li Z, Zhai X, Zuo D, Wu Y (2018) CD74-ROS1 G2032R mutation transcriptionally up-regulates twist1 in non-small cell lung cancer cells leading to increased migration, invasion, and resistance to crizotinib. Cancer Lett 422:19–28
42. Drilon A, Somwar R, Wagner JP, Vellore NA, Eide CA, Zabriskie MS, Arcila ME, Hechtman JF, Wang L, Smith RS, Kris MG, Riely GJ, Druker BJ, O'Hare T, Ladanyi M, Davare MA (2016) A novel crizotinib-resistant solvent-front mutation responsive to cabozantinib therapy in a patient with ROS1-rearranged lung cancer. Clin Cancer Res 22:2351–2358
43. Song A, Kim TM, Kim DW, Kim S, Keam B, Lee SH, Heo DS (2015) Molecular changes associated with acquired resistance to crizotinib in ROS1-rearranged non-small cell lung cancer. Clin Cancer Res 21:2379–2387
44. Zou HY, Li Q, Engstrom LD, West M, Appleman V, Wong KA, McTigue M, Deng YL, Liu W, Brooun A, Timofeevski S, McDonnell SR, Jiang P, Falk MD, Lappin PB, Affolter T, Nichols T, Hu W, Lam J, Johnson TW, Smeal T, Charest A, Fantin VR (2015) PF-06463922 is a potent and selective next-generation ROS1/ALK inhibitor capable of blocking crizotinib-resistant ROS1 mutations. Proc Natl Acad Sci USA 112:3493–3498
45. Sasaki T, Koivunen J, Ogino A, Yanagita M, Nikiforow S, Zheng W, Lathan C, Marcoux JP, Du J, Okuda K, Capelletti M, Shimamura T, Ercan D, Stumpfova M, Xiao Y, Weremowicz S, Butaney M, Heon S, Wilner K, Christensen JG, Eck MJ, Wong KK, Lindeman N, Gray NS, Rodig SJ, Janne PA (2011) A novel ALK secondary mutation and EGFR signaling cause resistance to ALK kinase inhibitors. Cancer Res 71:6051–6060
46. Doebele RC, Pilling AB, Aisner DL, Kutateladze TG, Le AT, Weickhardt AJ, Kondo KL, Linderman DJ, Heasley LE, Franklin WA, Varella-Garcia M, Camidge DR (2012) Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. Clin Cancer Res 18:1472–1482
47. Rotow J, Bivona TG (2017) Understanding and targeting resistance mechanisms in NSCLC. Nat Rev Cancer 17:637–658
48. Lin JJ, Riely GJ, Shaw AT (2017) Targeting ALK: precision medicine takes on drug resistance. Cancer Discov 7:137–155
49. Pfizer (2017) Pfizer's next-generation ALK/ROS1 inhibitor, lorlatinib, granted breakthrough therapy designation from FDA for ALK-positive metastatic non-small cell lung cancer. http://press.pfizer.com/press-release/pfizers-next-generation-alkros1-inhibitor-lorlatinib-granted-breakthrough-therapy-desi. Accessed 04 Dec 2018
50. Post TA (2018) FDA grants priority review to lorlatinib in ALK-positive NSCLC. http://www.ascopost.com/News/58536. Accessed 04 Dec 2018
51. Elisei R, Schlumberger MJ, Muller SP, Schoffski P, Brose MS, Shah MH, Licitra L, Jarzab B, Medvedev V, Kreissl MC, Niederle B, Cohen EE, Wirth LJ, Ali H, Hessel C, Yaron Y, Ball D, Nelkin B, Sherman SI (2013) Cabozantinib in progressive medullary thyroid cancer. J Clin Oncol 31:3639–3646
52. Choueiri TK, Escudier B, Powles T, Mainwaring PN, Rini BI, Donskov F, Hammers H, Hutson TE, Lee JL, Peltola K, Roth BJ, Bjarnason GA, Geczi L, Keam B, Maroto P, Heng DY, Schmidinger M, Kantoff PW, Borgman-Hagey A, Hessel C, Scheffold C, Schwab GM, Tannir NM, Motzer RJ, Investigators M (2015) Cabozantinib versus everolimus in advanced renal-cell carcinoma. N Engl J Med 373:1814–1823
53. Chong CR, Bahcall M, Capelletti M, Kosaka T, Ercan D, Sim T, Sholl LM, Nishino M, Johnson BE, Gray NS, Janne PA (2017) Identification of existing drugs that effectively target NTRK1 and ROS1 rearrangements in lung cancer. Clin Cancer Res 23:204–213
54. Katayama R, Kobayashi Y, Friboulet L, Lockerman EL, Koike S, Shaw AT, Engelman JA, Fujita N (2015) Cabozantinib overcomes crizotinib resistance in ROS1 fusion-positive cancer. Clin Cancer Res 21:166–174
55. Facchinetti F, Tiseo M, Di Maio M, Graziano P, Bria E, Rossi G, Novello S (2016) Tackling ALK in non-small cell lung cancer: the role of novel inhibitors. Transl Lung Cancer Res 5:301–321
56. Muller IB, De Langen AJ, Honeywell RJ, Giovannetti E, Peters GJ (2016) Overcoming crizotinib resistance in ALK-rearranged NSCLC with the second-generation ALK-inhibitor ceritinib. Expert Rev Anticancer Ther 16:147–157
57. Dagogo-Jack I, Shaw AT (2017) Expanding the roster of ROS1 inhibitors. J Clin Oncol 35:2595–2597
58. Crino L, Ahn MJ, De Marinis F, Groen HJ, Wakelee H, Hida T, Mok T, Spigel D, Felip E, Nishio M, Scagliotti G, Branle F, Emeremni C, Quadrigli M, Zhang J, Shaw AT (2016) Multicenter phase 2 study of whole-body and intracranial activity with ceritinib in patients with ALK-rearranged non-small-cell lung cancer previously treated with chemotherapy and crizotinib: results from ASCEND-2. J Clin Oncol 34:2866–2873
59. Drilon A, Siena S, Ou SI, Patel M, Ahn MJ, Lee J, Bauer TM, Farago AF, Wheler JJ, Liu SV, Doebele R, Giannetta L, Cerea G, Marrapese G, Schirru M, Amatu A, Bencardino K, Palmeri L, Sartore-Bianchi A, Vanzulli A, Cresta S, Damian S, Duca M, Ardini E, Li G, Christiansen J, Kowalski K, Johnson AD, Patel R, Luo D, Chow-Maneval E, Hornby Z, Multani PS, Shaw AT, De Braud FG (2017) Safety and antitumor activity of the multitargeted Pan-TRK, ROS1, and ALK inhibitor entrectinib: combined results from two phase 1 trials (ALKA-372-001 and STARTRK-1). Cancer Discov 7:400–409
60. Jacob Chacko I (2017) Interim analysis of Ignyta's entrectinib suggests potential best-in-class profile as a first-line targeted therapy in patients with ROS1-positive non-small cell lung cancer. Ignyta, Inc. http://www.businesswire.com/news/home/20171017006907/en/Interim-Analysis-Ignyta%2C-Entrectinib-Suggests-Potential-Best-in-Class

61. Liu D, Offin M, Harnicar S, Li BT, Drilon A (2018) Entrectinib: an orally available, selective tyrosine kinase inhibitor for the treatment of NTRK, ROS1, and ALK fusion-positive solid tumors. Ther Clin Risk Manag 14:1247–1252
62. Li P (2017) TP therapeutics announces FDA orphan drug designation granted to TPX-0005 for treatment of non-small cell lung adenocarcinomas harboring ALK, ROS1, or NTRK oncogenic rearrangements. http://www.businesswire.com/news/home/20170627005421/en/TP-Therapeutics-Announces-FDA-Orphan-Drug-Designation. Accessed 04 Dec 2018
63. Drilon A, Ou SI, Cho BC, Kim DW, Lee J, Lin JJ, Zhu VW, Ahn MJ, Camidge DR, Nguyen J, Zhai D, Deng W, Huang Z, Rogers E, Liu J, Whitten J, Lim JK, Stopatschinskaja S, Hyman DM, Doebele RC, Cui JJ, Shaw AT (2018) Repotrectinib (TPX-0005) is a next-generation ROS1/TRK/ALK inhibitor that potently inhibits ROS1/TRK/ALK solvent-front mutations. Cancer Discov 10:1227–1236
64. Cui JJ, Zhai D, Deng W, Rogers E, Huang Z, Whitten J, Li Y (2016) TPX-0005, a novel ALK/ROS1/TRK inhibitor, effectively inhibited a broad spectrum of mutations including solvent front ALK G1202R, ROS1 G2032R and TRKA G595R mutants. Eur J Cancer 69:S32–S32
65. Lovly CM, Heuckmann JM, de Stanchina E, Chen H, Thomas RK, Liang C, Pao W (2011) Insights into ALK-driven cancers revealed through development of novel ALK tyrosine kinase inhibitors. Cancer Res 71:4920–4931
66. U.S.F.a.D. Administration (2017) Brigatinib. http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm555841.htm. Accessed 04 Dec 2018
67. McCoach CE, Bivona TG, Blakely CM, Doebele RC (2016) Neoadjuvant oncogene-targeted therapy in early stage non-small-cell lung cancer as a strategy to improve clinical outcome and identify early mechanisms of resistance. Clin Lung Cancer 17:466–469