Current state of the art on the diagnosis and the role of target therapy for treatment of \textit{ROS1}-rearranged non-small cell lung cancer: a narrative review

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\textbf{Background and Objective:} Strategies for diagnosis and treatment of oncogene-addicted non-small cell lung cancer (NSCLC) are constantly evolving. In particular, the development of novel techniques for the molecular classification of NSCLC lead to detect many molecular aberrations of therapeutic interest, even in peripheral blood, including ROS proto-oncogene 1, receptor tyrosine kinase, encoded by \textit{ROS1} gene. Currently there are few drugs targeting \textit{ROS1} and most of available data on their activity comes from non-randomized studies, considering the low incidence of \textit{ROS1} alterations. Only three drugs are registered for FDA (crizotinib, entrectinib and ceritinib), with similar safety profile; no study comparing these two drugs is available yet.

\textbf{Methods:} This narrative review was conducted by gathering all the relevant literature in PubMed from 2007 to 2021 on evolving techniques for the molecular detection of \textit{ROS1} rearrangements and also on main mechanisms of resistance with consequent developments of more selective drugs. Research was carried out at both preclinical and clinical levels. For the preclinical part, we selected more than 50 publications on the implications of in situ laboratory and molecular biology techniques comparing these approaches; for the clinical part we collected the main publications on \textit{ROS1} rearranged oncogene-addicted NSCLC and reported international guidelines. We included data from ten phase I/II trials testing efficacy and safety of TKIs targeting \textit{ROS1} rearrangement.

\textbf{Key Content and Findings:} This narrative review analysed literature data on the current standard in detection of \textit{ROS1} rearrangements in NSCLC, focusing on the value and benefits of next generation sequencing (NGS) that are not universally applicable as standards in clinical practice. The key points addressed are the available therapeutic options and the benefit of new agents for treatment of \textit{ROS1}-positive lung advanced disease, which are not yet used in clinical practice.
Introduction

Background

Non-small cell lung cancer (NSCLC) is currently classified according to histology and to molecular features. Approximately two-thirds of advanced NSCLCs are identified as oncogene-addicted disease, due to the occurrence of driver genetic alterations at the time of diagnosis, including mutations (e.g., EGFR, KRAS, BRAF, MET, ERBB2) or genomic rearrangements (e.g., ALK, ROS1, RET, NTRK) (1).

Many of these driver genetic alterations are currently targetable with specific therapy such as tyrosine kinase inhibitors (TKIs), that have significantly improved the prognosis of NSCLC.

Oncogene-addicted disease have distinctive clinical features; in some cases, different genetic aberrations are overlapping although some of these alterations are usually mutually exclusive.

Significant prognostic changes in recent years occurred for NSCLC oncogenes addicted, even for uncommon oncogenic driver including ROS1 rearrangements. Next generation sequencing (NGS) has important role in ROS1-rearrangements detection and also for identification of resistance mutation to specific target agents, however this approach is not yet routinely available in clinical practice. The goal for the near future is detection of novel selective drugs for uncommon oncogene drivers and their optimal sequencing by evolving methods of molecular diagnostics.

This narrative review aims to outline the methods of ROS1 rearrangements detection and their potential evolution for the future. We also analysed the main therapeutic implications for NSCLC with ROS1 rearrangements. We present the following article in accordance with the Narrative Review reporting checklist.

Conclusions: The diagnosis and treatment of ROS1-positive NSCLC, despite the rarity of this molecular alteration, have reached important developments. The contribution of this review would be to explore the main developments on the diagnosis of ROS1 rearrangements and to identify therapeutic opportunities both those currently available in clinical practice and those not currently available but promising for the near future.

Keywords: Non-small cell lung cancer (NSCLC); ROS proto-oncogene 1 receptor tyrosine kinase rearrangements (ROS1 rearrangements); next generation sequencing (NGS); targeted therapy

Methods

In this narrative review we focused on detection pattern of ROS1 rearrangements and the consequent therapeutic implication in lung cancer. To this aim, we have analysed all the relevant literature in PubMed from 2007 to 2021 on evolving techniques for the molecular detection of ROS1 rearrangements and on the main mechanisms of resistance with consequent developments of more selective drugs (Table 1). The PubMed database was searched principally using the keywords “Non-small cell lung cancer”, “ROS1 rearrangements”. We excluded articles not published in English.

Research was performed at both preclinical and clinical levels. For the preclinical part, we selected more than 50 publications on the implications of in situ laboratory and molecular biology techniques comparing these approaches; for the clinical part we collected the main publications on ROS1 rearranged oncogene-addicted NSCLCs and reported international guidelines. We included data from ten phase I/II trials testing efficacy and safety of TKIs targeting ROS1 rearrangement.

Biology, clinical and pathological features of ROS1 rearrangement

ROS1 gene is located on the short arm of chromosome 6 (6q22) and included in the subfamily of insulin tyrosine kinase receptors. Physiological role of ROS1 is still unclear, it is known to have homology with anaplastic lymphoma kinase protein (ALK) greater than 80% in the ATP binding

Keywords: Non-small cell lung cancer (NSCLC); ROS proto-oncogene 1 receptor tyrosine kinase rearrangements (ROS1 rearrangements); next generation sequencing (NGS); targeted therapy

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site and kinase domains, but function and expression in adult tissue are uncertain (2). Genomic alterations of \( \text{ROS1} \) lead to fusion with different gene partners (e.g., \( \text{CD74}, \text{CCDC6}, \text{EZR}, \text{FIG}, \text{KDEL2}, \text{LRIG3}, \text{MSN}, \text{SDC4}, \text{SLC34A2}, \text{TMP3}, \text{TPD52L1} \)) encoding for oncogenic driver proteins; the fusion usually results in constitutive activation of \( \text{ROS1} \) receptor tyrosine kinase and consequently upregulation of downstream signaling pathways (JAK/STAT, PI3K/AKT and MAPK/ERK) involved in the control of cell proliferation (1-6).

\( \text{ROS1} \) fusions are implicated \textit{in vivo} and \textit{in vitro} in the pathogenesis of several tumors; notably, such fusions were described for the first time in glioblastoma and subsequently observed in other tumors including NSCLC, angiosarcoma, colon and gastric carcinoma and ovarian cancers (3,7-12).

In NSCLCs, \( \text{ROS1} \) rearrangements occur in 1–2% of cases, and are generally related to some clinical-histological features as adenocarcinoma histology with psammomatous calcification and solid growth with mucinous/cribriform patterns. Patients with \( \text{ROS1} \) positive NSCLC, are often young and non-smokers or low smokers; brain metastases are present in approximately 40% of cases at diagnosis. With regards to molecular setting, \( \text{ROS1} \)-positive NSCLCs are often wild-type for \( \text{EGFR} \) mutations, \( \text{ALK} \) rearrangements and other driver mutations, the rare cases of overlap between \( \text{ROS1} \) rearrangements and other driver molecular alterations should be assessed by further analysis with molecular testing, and usually are not confirmed (13-18). Moreover, in recent years more rigorous genetic analyses of complete molecular profile identified the coexistence of \( \text{ROS1} \) and other driver alterations in NSCLC, such as \( \text{RET} \) rearrangements and \( \text{MET} \) amplification, despite their low frequency, with important clinical and therapeutic implications (19-22).

### ROS1 detection methods

Currently, a number of methods are used to detect \( \text{ROS1} \) rearrangements, which vary depending on the molecule being studied such as protein (IHC), DNA (fluorescence in situ hybridization (FISH); next-generation sequencing (NGS)), and RNA (reverse transcription polymerase chain reaction (RT-PCR); NGS) (23,24). Generally, \textit{in situ} analysis methods such as FISH and IHC, are routinely used in clinical practice. FISH is traditionally considered the gold standard for \( \text{ROS1} \) and \( \text{ALK} \) rearrangement identification in lung cancer, using the same criteria; this technique is moderately expensive, laborious and inclined to false negative results. IHC is an efficient tool for the selection of \( \text{ROS1} \)-positive lung cancer, with higher sensitivity (over 90%) as compared to FISH, which is instead characterized by high specificity (more than 90%), but low specificity (less than 60%). Furthermore, IHC needs low operator requirements and has a shorter turnaround time for results, but it reveals to be less specific than FISH. Therefore, detection of \( \text{ROS1} \)-negative NSCLC (whether non-smokers or smokers with non-squamous NSCLC) by IHC, may not require a validation by FISH, thus reducing testing costs (21,22,24-38). On the other hand, FISH is traditionally considered the gold standard for the validation of \( \text{ROS1} \) rearrangements in lung cancer. IHC is generally preferred to FISH on formalin-fixed and paraffin-embedded (FFPE) tissue biopsy, as well as on few tumor cells obtained from pleural or pericardial effusion (39,40). Conversely, FISH can be performed on both tissue and cytological samples and it is superior compared to IHC on non-bloody cytological swipes. Although FISH is characterized by higher specificity (more than 90%) compared to IHC, this method has the limit to be somewhat expensive, laborious and inclined
to false negative results and it is for these reasons that molecular analyses by RT-PCR and NGS are additionally executed (39,40).

**ROS1 rearrangements diagnosis in FISH**

Two main patterns of ROS1 rearrangements can be detected by FISH: “break-apart” pattern and “atypical pattern” using one isolated fusion signal. The “break-apart” FISH pattern, that uses dual color probes, is the conventional approach for ROS1 identification. In presence of a rearrangements in tumor cells, two ends of ROS1 gene are spliced and the part containing the tyrosine kinase domain is fused with another partner to create a ROS1 fusion gene. This technique involves two probes that lap with a green fluorochrome the 3’ (centromeric) part of the fusion breakpoint and with an orange fluorochrome the 5’ (telomeric) part; the green fluorochrome results by the kinase-fusion domain of ROS1 gene (typical rearranged pattern). In the atypical pattern the single green signal (5’ end) does not show a corresponding orange signal (3’ end) in conjunction to a fused and/or split signals (isolated 3’ ROS1 signals). To detect ROS1 fusion gene positivity the signal of rearrangements recorded in FISH has to reach a cut-off of 15% or more among 50 neoplastic cells (24,38,40,41). Some “break-apart” FISH assay for ROS1 fusion have the limit to fail in detecting intrachromosomal deletions which is emerging as an acquired resistance mechanism to osimertinib (EGFR inhibitor) in lung adenocarcinoma (42,43).

**ROS1 rearrangements diagnosis in IHC**

A consensus of experts reports that ROS1 IHC can be used as a screening test in advanced stage lung adenocarcinoma patients but that positive ROS1 IHC results should be confirmed by FISH or other molecular methods (44).

IHC analysis for ROS1 rearrangements can be performed by different amplification kits and detection systems and the monoclonal antibody D4D6 (cell signalling technology) is generally used. A relatively recent multicenter study provided real-world data on ROS1 rearrangements in patients with NSCLC, demonstrating that the new SP384 ROS1 IHC clone (Ventana medical systems) showed excellent sensitivity. Although to date, no FDA-approved IHC assay is advised for clinical routine, the two available antibodies (i.e., D4D6 and SP384) have demonstrated high performance in most studies (45).

Two different score systems have been developed to define tumor ROS1-positivity. The first one is based on IHC staining intensity according to a semi-quantitative scoring system as follows: (I) negative (score 0), (II) weak signal (1+), (III) moderate signal (2+), (IV) strong positive signal (3+). Tumor cells with ROS1-rearrangements have intense immunohistochemical staining, resulting 2+/3+ score. Another employed score system is the H-score, ranging from 0 to 300. This score is obtained by multiplying percentage of positive tumor cells and IHC staining intensity (from 0 to 3+), with positivity threshold for ROS1 rearrangements greater than 100 (24,26). At IHC, neoplastic cells with ROS1 rearrangements present finely granular cytoplasmic staining, that changes according to different fusions. An important feature in the ROS1 detection by IHC is the possibility to insert an external positive control; indeed, although ROS1 protein is absent in normal adult tissues, it can be detected in reactive alveolar type II pneumocytes and macrophages (24,41). Despite higher sensitivity but lower specificity of IHC compared to FISH, the two techniques correlate well when D4D6 clone is combined with high sensitivity amplification kits in IHC. For immunohistochemistry diagnosis of ROS1 rearrangement there are other promising but less investigated monoclonal and polyclonal anti-ROS1 antibodies (24).

**Molecular technologies: RT-PCR and NGS**

An alternative method to validate IHC-based ROS1 positivity, is the RT-PCR, although this technique is at time less reliable when used alone, unless RNA-based anchored multiplex polymerase chain reaction library preparation followed by NGS is performed.

RT-PCR, starting from RNA, first converts RNA to complementary DNA (cDNA); then the cDNA is amplified to allow the detection of gene fusions. Despite high sensitivity, specificity and rapidity, the availability of good quality RNA samples may limit the application of RT-PCR for the detection of ROS1 rearrangements and prevent the identification of atypical ROS1 fusion patterns in routine practice. For these reasons, a combined assay involving IHC followed by multiplex RNA-based PCR and NGS was recently evaluated (24,46-49).

NGS is a technology that enables nucleic acid sequencing of multiple genes simultaneously and can be performed with both DNA and RNA. Although it is affected by the need for appropriate bioinformatic equipment and staff skilled in data testing and analysis (50-56), this technology has
proved to be an essential tool in all areas of genomic and epigenomic basic and clinical research.

Indeed, NGS has high sensitivity and specificity, and can allow the identification of gene alterations with low sample amount from both FFPE tissue and circulating tumor DNA (ctDNA) in plasma. Notably, NGS can simultaneously detect known or novel ROS1 fusions, as well as rearrangements by analyzing short intron region of DNA (57-64).

However, RNA-based NGS is preferable to DNA-based NGS in that sequencing can be focused on coding sequences instead of introns (63). To note that, although high quality RNA is needed, particularly if FFPE samples are used, the RNA based NGS can also allow the detection of any fused genes or novel ones such as ROS1-GOPC, overcoming the limits of DNA-based NGS not covering intronic breakpoints (43).

Therefore, NGS provides many advantages and is a noteworthy tool in molecular diagnosis of lung cancer, even in analysis of small sample amounts that cannot be analyzed by traditional methods. Indeed, Interestingly, Lim et al. reported that 58% of 51 patients with wild-type lung cancer, diagnosed by standard molecular testing, had gene alterations as ROS1 and others (e.g., EGFR, MET, RET, ALK, BRAF, NTRK) found by using an NGS approach (57,62).

Another emerging technology for ROS1 rearrangements detection is represented by NanoString nCounter platform. Specifically, nCounter platform is able to detect multiple gene fusions simultaneously including ALK, RET and ROS1, using limited amounts of RNA. However, similarly to the previous RNA-based tools, very short or highly fragmented RNA samples (35).

In several studies, ROS1 positivity rates in the same cohort of NSCLC patients were different when evaluated by multiple approaches other than IHC; ROS1 positivity in IHC has also been reported in non-neoplastic conditions, such as bronchial metaplasia, in reactive type II pneumocytes or macrophages, confirming that the sensitivity of this methodologies is higher than its specificity.

Therefore, the detection of ROS1 rearrangements in IHC should be confirmed by further analyses with different techniques such as FISH or NGS, although some disadvantages limit its routine application in the clinical practice. The main advantages and disadvantages of NGS and other technologies for ROS1 detection are summarized in Table 2 (22,24,33,35,65).

**Comparison of IHC, FISH, RT-PCR and NGS in detection of ROS1-rearrangements**

Despite, to date a number of approaches are used for the detection of ROS1 rearrangements, some drawbacks need to be considered.

In a retrospective study, a total of 107 patients with NSCLC, including 92 adenocarcinomas, 12 squamous-cell carcinomas and 2 adeno-squamous carcinomas, 11 samples resulted positive for ROS1 by IHC, only two of which were confirmed with NGS (36,65). In the same study, there was evidence of a high concordance in terms of sensitivity between RT-PCR and NGS in 12 samples resulted positive for ROS1 and ALK rearrangements (65). Similarly, Reguart et al. demonstrated a concordance of 87% and 86% in the evaluation of ROS1 between nCounter platform vs. IHC and FISH, respectively (35).

**Table 2** Benefits and disadvantages of technologies for ROS1 detection

<table>
<thead>
<tr>
<th>Technologies</th>
<th>Advantages</th>
<th>Drawbacks</th>
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<tbody>
<tr>
<td>IHC</td>
<td>Low cost; high sensitivity; low number of samples needed; easy to use</td>
<td>Antibody dependence; dependence on tissue fixation; dependence on subjective error in interpretation results</td>
</tr>
<tr>
<td>FISH</td>
<td>High reliability</td>
<td>Expensive; long and difficult procedure; high risk of false negative results</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>High sensitivity; high specificity; low number of samples needed</td>
<td>Need to use FFPE samples with good quality; risk of error in the interpretation of the result</td>
</tr>
<tr>
<td>NGS</td>
<td>Several applications; available in research and in clinical practice; evolving high number of kits; limited time from sample preparation to results</td>
<td>Highly specialized equipment for data analysis; expensive in some countries; no possibility of standardization and/or application of standardized material in clinical practice</td>
</tr>
</tbody>
</table>

ROS1, ROS proto-oncogene 1 receptor tyrosine kinase; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; RT-PCR, reverse-transcriptase polymerase chain reaction; NGS, next generation sequencing; FFPE, formalin-fixed and paraffin-embedded.
Targeted agents for ROS1-positive NSCLC

Several drugs belonging to the class of TKIs are effective in NSCLC with ROS1 rearrangement (Table 3); currently crizotinib, ceritinib and entrectinib are approved by the Food and Drug Administration (FDA), while only crizotinib and entrectinib have been approved by the European Medicines Agency (EMA), and their use achieved a significant impact in terms of efficacy for these patients. The most relevant clinical studies available to date have been summarized in Table 4.

There are also emerging preclinical efficacy data, in vivo and in vitro, of new generations ROS1-inhibitors, ROS1 NUV-520 and DS-6051b, overcoming G2032R resistance mutations in kinase domain secondary to crizotinib therapy and also not accessible by others target agents as lorlatinib and entrectinib (76,77). Furthermore NUV-520 is a brain-penetrant molecule, with preliminary data on activity in the central nervous system (CNS) (77).

Crizotinib

Crizotinib is an orally available TKI that binds the tyrosine kinase domain and blocks ATP-dependent functions in tumoral cells, thus inhibiting cell proliferation activity induced by several oncogene alterations including ROS1 as well as ALK and MET (Figure 1). Crizotinib has represented for years the gold standard in first line treatment for ROS1-positive NSCLC, according to the results of several preclinical, phase I, retrospective and prospective studies (2,69,78,79). The first efficacy data derive from PROFILE 1001, a phase I trial that included 53 patients with ROS1-rearranged NSCLC treated with crizotinib; at 22.4 months of treatment the objective response rate (ORR) was 72%, of which six patients (11%) with complete response (63). In the updated trial results of 2019, 19.3 months (95% CI, 15.2–39.1 months) of median progression free survival (PFS) and 51.4 months (95% CI, 29.3 months–not reached) of median overall survival (OS) were reported; no correlation between crizotinib efficacy and ROS1 fusion partner was observed and a favorable safety profile was shown even with prolonged treatment (66). All patients had at least one adverse event (AEs), albeit mostly (n=33, 64%) of grade 1–2 according to Common Terminology Criteria for Adverse Events (CTCAE); only 19 patients (35%) had grade 3 AEs (neutropenia, hypophosphatemia, elevated transaminases) and no grade 4 events were reported. The most common side effects were vision disorder, nausea, edema, diarrhea; no AEs were related to permanent discontinuation of treatment (66).

Following these results, efficacy of crizotinib in ROS1-positive, advanced NSCLC was demonstrated in other prospective and retrospective studies (67,69,79); in the EURO51 cohort retrospective study, crizotinib demonstrated disease control rate (DCR) of 87% and PFS of 44% at 12 months in 32 patients with ROS1-positive NSCLC (77). Two subsequent prospective phase II trials confirmed the efficacy of crizotinib with a DCR higher than 85%, although reporting different PFS results, respectively 5.5 months (95% CI, 4.6–9.1 months) in the French study and 15.9 months (95% CI, 12.9–24.0 months) in the Asian study (69,79). The different PFS between these trials, particularly the relatively short result in the Acsè trial, may be influenced by the small size of the study population (i.e., 37 patients in the French study vs. 127 patients in the Asian trial) (69,79). In the METROS trial, a prospective phase II study, Landi et al. analyzed the clinical role of crizotinib in 505 patients with advanced NSCLC and ROS1 rearrangements or MET amplifications (68,70); in the ROS1-positive cohort, the ORR was 65%, including 4% complete responses, with DCR of 85% and a median PFS of 22.8 months (95% CI, 15.2–30.3 months). Overall, ROS1-positive population experienced treatment-related AE mostly of grade 1–2 and only 4% of patients reported serious events (grade 3–4) as neutropenia, peripheral edema, fatigue, nausea (68,70).

In spite of these results, the efficacy of crizotinib is limited by different mechanisms of resistance, such as point mutations or activation of alternative signaling pathways, that consequently lead to loss of drug activity and disease progression. Several mutations in different domains were...
### Table 4 Efficacy of agents active on ROS1-positive NSCLC

<table>
<thead>
<tr>
<th>Agent</th>
<th>Study</th>
<th>Patients (n)</th>
<th>Outcomes</th>
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</thead>
<tbody>
<tr>
<td>Crizotinib</td>
<td>Shaw et al., 2019, PROFILE 1001 (66) (phase I)</td>
<td>53</td>
<td>ORR 72%, PFS 51.4 months (95% CI, 29.3 months–not reached)</td>
</tr>
<tr>
<td></td>
<td>Mazières et al., 2015, EUROS1 (67) (cohort retrospective trial)</td>
<td>32</td>
<td>DCR 87%, PFS 9.1 months</td>
</tr>
<tr>
<td></td>
<td>Moro-Sibillito et al., 2019, Acsè trial (68) (phase II)</td>
<td>37</td>
<td>ORR 47.2%, PFS 5.5 months (95% CI, 4.6–9.1 months)</td>
</tr>
<tr>
<td></td>
<td>Wu et al., 2018, Asian study (69) (phase II)</td>
<td>127</td>
<td>ORR 71.7%, PFS 15.8 months (95% CI, 12.9–24 months)</td>
</tr>
<tr>
<td></td>
<td>Landi et al., 2019, METROS trial (70) (phase II)</td>
<td>505</td>
<td>ORR 65%, PFS 22.8 months (95% CI, 15.2–30.3 months)</td>
</tr>
<tr>
<td>Entrectinib</td>
<td>Dziadziuszko et al., 2021 (71), ALKA-372-001, STARTRK-1, STARTRK-2 (pooled data from phase I/II studies)</td>
<td>161</td>
<td>ORR 67.1%, PFS 15.7 months (95% CI, 13.9–28.6 months); intracranial ORR 79.2%, intracranial PFS 12 months (95% CI, 6.2–19.3 months)</td>
</tr>
<tr>
<td>Cabozantinib</td>
<td>Sun et al., 2019 (72), case series</td>
<td>4</td>
<td>ORR 25%, PFS 4.9–13.8 months</td>
</tr>
<tr>
<td>Lorlatinib</td>
<td>Solomon et al., 2018 (73), phase II trial</td>
<td>47</td>
<td>ORR 26.5% (pre-treated patients)</td>
</tr>
<tr>
<td></td>
<td>Shaw et al., 2019 (74), phase I/II trial</td>
<td>69</td>
<td>ORR 62% (TKIS naïve patients), ORR 35% (pre-treated patients)</td>
</tr>
<tr>
<td>Repotrectinib</td>
<td>Cho et al., 2019, TRIDENT-1 (75) (phase I)</td>
<td>75</td>
<td>ORR 39% (pre-treated patients)</td>
</tr>
</tbody>
</table>

ROS1, ROS proto-oncogene 1 receptor tyrosine kinase; NSCLC, non-small cell lung cancer; ORR, objective response rate; DCR, disease control rate; PFS, progression free survival.

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**Figure 1** ROS1 rearrangement-dependent mechanism of cell proliferation. Intracellular signalling mechanisms for cell proliferation activated by ROS proto-oncogene 1 protein receptor tyrosine kinase. ROS1, ROS proto-oncogene 1 receptor tyrosine kinase; SHP2, Src homology phosphatase 2; PI3K, phosphatidylinositol 3-kinase; AKT, serine/threonine protein kinase; MEK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JAK, Janus protein tyrosine kinase; STAT, signal transducer and activator of transcription.
detected (e.g., D2033N, G2032R, L2026M, L2155S) in patients with advanced NSCLC that, through various paths, lead to an increase in kinase activity and therefore in cell proliferation. The G2032R mutation, involving the kinase domain target of the drug, is apparently the most common way of resistance (80-86).

In several studies, the activation of the EGFR signaling pathway was observed in advanced ROS1-positive NSCLC treated with crizotinib, leading to loss of ROS1 pathway cell proliferation signal and consequent resistance to treatment. While in some tumoral cells KIT signaling pathway resulted activated, highlighting the possibility of an association therapy with selective alternative pathway-inhibitors, such as erlotinib or gefitinib for EGFR and ponatinib for KIT, to overcome resistance to crizotinib in selected cases. The role of human epidermal growth factor receptor 2 (HER2) and TP53 gene amplification and mitogen-activated protein kinase (MAPK) pathway upregulation in crizotinib resistance remains still unclear (86-91).

**Entrectinib**

Entrectinib is another oral TKI currently available and effective for the treatment of ROS1-rearranged NSCLC, particularly active on brain metastases due to its potential to cross the blood-brain barrier (BBB). Although there are no trials comparing entrectinib and crizotinib, a retrospective analysis showed that ROS1-positive patients treated with crizotinib, without brain metastases at diagnosis, had an encephalic progression during treatment in 42% of cases with a time to progression of 24 months (13).

Whereas, recent results from integrate analysis of phase I/II studies on entrectinib as first-line in 161 patients with advanced NSCLC, have shown high clinical benefit this TKI with a follow-up over 6 months. The objective response was obtained and confirmed in 108 patients (ORR 67.1%) in term of overall efficacy, with a median duration of response (DoR) of 15.7 months. In 24 patients with brain metastases at diagnosis, the intracranial ORR was 79.2% with median PFS of 12.9 months (95% CI, 6.2–19.3 months) and median DoR of 12.9 months. Interestingly, the time to intracranial response was short (median: 0.95 months) (71,92-94). With regard to safety profile, patients treated with entrectinib reported mostly low grade and manageable AEs, such as dysgeusia or fatigue, usually reversible (71). Therefore, entrectinib showed benefit in patients with intracranial disease, and a good safety profile, similar to crizotinib and others TKIs; nevertheless, it has limited efficacy data against ROS1 resistance mutations like G2032R, D2033N and L2026M (92).

**Other agents**

Other TKIs acting against ROS1 alterations, including brigatinib, cabozantinib, ceritinib, lorlatinib and repotrectinib shown efficacy in ROS1 positive lung cancer, but with limited activity against resistance mutations of ROS1-kinase domain following first line therapy with crizotinib; furthermore, these drugs achieved brain disease control (83,95,96). While brigatinib, lorlatinib and ceritinib do not appear to be effective in crizotinib-pretreated NSCLC when G2032R resistance mutation develops, other agents including Repotrectinib, result to have modest activity in vitro and in vivo in this setting (97).

Cabozantinib is an orally available multi-target TKI (active on AXL, KIT, MET, RET, ROS1, VEGFR2, TIE2), currently available for renal cell and medullary thyroid carcinoma. Cabozantinib showed efficacy in ROS1-positive NSCLC patients who develop resistance to crizotinib and ceritinib; more specifically, Sun et al. reported the results from a case series of four previously treated patients (three of which had received both crizotinib and ceritinib); in this series, cabozantinib achieved a 100% DCR and OR was observed in one patient, while median PFS ranged from 4.9 to 13.8 months (72). However, although cabozantinib achieved promising results in overcoming acquired resistance ROS1 mutations, this agent is characterized by a challenging toxicity profile compared to other TKIs, with the most common reported AE being neutropenia, xeroderma and pulmonary embolism (71,82).

Lorlatinib and repotrectinib, which are potent oral inhibitors of ALK and ROS1, among other targets, achieved promising results against several ROS1 emerging mutations (D2033N e S1986Y) resistant to crizotinib and ceritinib, but not against G2032R, common resistance mutations after previous treatment with crizotinib. In phase I/II studies, lorlatinib and repotrectinib demonstrated ORR of 26.5% and 39%, respectively, in patients pretreated with crizotinib or other TKIs; furthermore, both agents achieved important benefit for the management of intracranial disease, due to high potential to cross the BBB, with response rate with repotrectinib close to 100% in treatment-naïve patients and 75% in pretreated patients. Finally, both lorlatinib and repotrectinib are characterized by a globally manageable safety profile and high activity in ROS1-positive disease especially on CNS disease. While the use of repotrectinib.
in clinical practice is still experimental, lorlatinib currently represents the preferred second-line treatment for ROS1-positive, pretreated NSCLC, due to its ability to overcome acquired resistance (72-75,85,98-104).

Discussion

The treatment of NSCLC has undergone profound changes in recent years. The definition of “wild type” NSCLC is changing from year to year thanks to the increase of drugs active towards specific genic alterations, including known and novel oncogenic drivers; as a result, antineoplastic therapy is being increasingly tailored to each individual patient with important and prolonged benefits in terms of response and survival. In this setting, the availability of new drugs must be accompanied by a cultural change. To date, despite the consolidated availability of targeted agents, many patients are still not tested for the four most frequent molecular targets, which include EGFR, ALK, BRAF and ROS1. Quite surprisingly, only approximately 50% of patients are being tested in US for all the aforementioned targets, in addition to PD-L1 expression. ROS1 is tested in approximately 70–75% of cases (105,106). This change of perspective must be accompanied by an improvement in the knowledge of oncologists, pathologists and molecular biologists who research these alterations. As previously discussed, the appropriate identification of ROS1 rearrangement can present some diagnostic challenges depending on the method used. NGS is not widely available yet, especially in peripheric area hospitals, and other methods have non-negligible limits in terms of sensitivity and specificity.

Among the advantages of NGS approach, one specific mention should be reserved to its ability to detect acquired resistance mutations which develop during targeted treatment; while this approach is useful for virtually all cases of oncogene-addicted NSCLC, including novel emerging biomarkers (107), it appears even more appealing when ROS1 or ALK rearrangements are involved, as the identification of specific on-target resistance mutation might help select the most appropriate compound among the different available agents based on their individual ability to overcome that specific mechanism. Furthermore, NGS approaches can be employed on peripheral blood, eventually leading to decreased need of invasive tissue biopsies (108).

Notably, the increasing availability of novel effective targeted agents for ROS1-positive NSCLC may lead to two opposing strategies: one possible approach involves the sequential use of available agents based on the identified emerging mutations, while the other approach relies on the upfront use of the most active agents. While the former approach appears to be more tailored on each individual patient and provides a strong rationale for therapeutic sequences, thus offering credible therapeutic opportunities after the initial disease progression, the latter approach is based on preventing the development of resistance mechanisms in first place and implies reduced need for repeated molecular analyses throughout each patient’s history. Currently, the “best first” approach is being favored, at least partially due to its easy application compared to sequential strategies; furthermore, the activity of novel agents on preventing and treating brain metastases, compared to crizotinib, cannot be ignored, as it can translate in relevant prevention or improvement of neurologic symptoms. In contrast with other more frequently detected oncogenic drivers, such as EGFR, results from randomized controlled trials comparing different agents for ROS1-positive NSCLC are currently lacking; hence, most available evidence to date derives from single-arm phase I/II trials. While randomized trials are generally warranted, the rarity of ROS1 rearrangements implies that such trials suffer from slow enrollment, which might make their results obsolete, as additional novel agents are in the meanwhile developed. In this setting, real-life data collected with coordinated, multi-institutional efforts, might provide useful data; furthermore, in the near future, the investigation of uncommon oncogenic drivers for NSCLC, for which ROS1 represents a good model, will require novel methodic designs, in order to provide robust and useful data while at the same time keeping pace with the rapid development of novel agents and sequencing technologies.

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Footnote

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