Therapeutic management of ALK+ nonsmall cell lung cancer patients

Boris Duchemann1,2, Luc Friboulet3 and Benjamin Besse4,5

Affiliations:
1Dept of Medical Oncology, Hopital Avicenne, Bobigny, France.
2Paris 13 University, Paris, France.
3Dept of Medicine, Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA, USA.
4Dept of Cancer Medicine, Gustave Roussy, Villejuif, France.
5Paris-Sud University, Paris, France.

Correspondence: Benjamin Besse, Dept of Cancer Medicine, Gustave Roussy, 114 rue Edouard Vaillant, Villejuif 94805, France. E-mail: benjamin.besse@gustaveroussy.fr

ABSTRACT With therapeutic approaches based on oncogene addiction offering significant anticancer benefit, the identification of anaplastic lymphoma kinase (ALK) rearrangements is a key aspect of the management of lung cancers. The EML4-ALK gene fusion is detected in 4–8% of all lung cancers, predominantly in light smokers or nonsmokers. Crizotinib, the first agent to be approved in this indication, is associated with a median progression-free survival of 10.9 months when given as first-line treatment and 7.7 months when administered after chemotherapy. Median overall survival with crizotinib in the second-line setting is 20.3 months. Second-generation ALK inhibitors are currently being evaluated, with early studies giving impressive results, notably in patients resistant to crizotinib or with brain metastases. Among available chemotherapies, pemetrexed appears to be particularly active in this population. Despite this progress, several questions remain unanswered. What detection strategies should be favoured? What underlies the mechanisms of resistance and what options are available to overcome them? What are the best approaches for progressing patients? This review provides an overview of current data in the literature and addresses these questions.

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ALK defines patients who benefit from ALK inhibitors so should be systematically tested in advanced nonsquamous NSCLC http://ow.ly/KQ2oS

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Introduction
Lung cancer is a major cause of death worldwide, with 1.6 million new cases and 1.4 million deaths recorded annually [1]. Patients with metastatic disease are rarely cured, and while chemotherapy has led to significant advances over the last few years, this has unfortunately not translated into an improved prognosis for these patients.

The identification of oncogenic drivers is one of the most significant discoveries in cancer research. In nonsmall cell lung cancer (NSCLC), rearrangements of the anaplastic lymphoma kinase (ALK) represent the second major oncogenic driver after epidermal growth factor receptor (EGFR) mutations. Like EGFR, therapeutic approaches targeting ALK genetic abnormalities have given impressive outcomes in terms of response rates and response duration.

Individually, ALK alterations are only present in a small portion of the overall population; however, specifically targeting this population is essential, along with an efficient strategy for screening and confirming diagnosis. Therapeutic management for patients harbouring this oncogene addiction should be personalised, taking into account the biological specificities of the patient and the tumour.

ALK functions and genetic abnormalities
ALK is a tyrosine kinase receptor belonging to the insulin receptor superfamily. It is composed of an extracellular transmembrane domain and an intracellular tyrosine kinase domain. Principally expressed in the central nervous system [2], the exact physiological role of ALK in humans and mammals remains unclear. Studies in zebrafish suggest it plays a role in neural crest development [3]. Defining features of the extracellular domain include a glycine-rich portion, along with an LDLa (low-density lipoprotein class A) domain and MAM (meprin, A5 protein, and receptor protein tyrosine phosphatase μ) segments [4]. Ligands include pleiotrophin [5] and midkine [6], both of which are known to play a role in neural development, as well as cell survival and tumorigenesis [7, 8].

The first evidence of the involvement of ALK abnormalities in cancer dates from over 20 years ago, when Morris et al. [9] reported a rearrangement involving the nucleophosmin gene (NPM1) (5q35) and ALK (2p23) in non-Hodgkin lymphoma. Activation of ALK can be due to a translocation (aberrant association of two genes resulting in a fusion protein), amplification (increased expression of ALK transcripts) or mutation. The ALK locus is a translocation hotspot, with over 22 fusions identified to date. Mutations are most common in the kinase domain. Abnormalities implicating ALK in tumorigenesis have been reported in different tissues, with a variety of activating mechanisms (supplementary fig. S1).

Somatic ALK rearrangements associated with bronchopulmonary cancers were first reported in 2007 [10]. Several genes may be fused with the ALK gene, generating different ALK variants. In the case of NSCLC, partner genes include echinoderm microtubule-associated protein-like 4 (EML4), kinesin family member (KIF5B) [11], TRK-fused gene (TFG) [12], kinesin light chain 1 (KLC1) [13], protein tyrosine phosphatase non-receptor type 3 (PTPN3) and striatin calmodulin-binding protein (STRN) [4]. Interestingly, the ALK fusion point is the same in all cases [14]. In lung cancer, rearrangements are the primary source of ALK activation. In addition, amplifications and mutations have been reported, generally in cases of resistance to ALK tyrosine kinase inhibitors (TKIs). ALK rearrangements are referred as ALK+ throughout this review.

Signalling pathways downstream of ALK are complex and differ according to the mechanism of activation. Pathways implicated include RAS/mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/mammalian target of rapamycin (mTOR), phospholipase C gamma, RAS-related protein (RAP)1, Janus kinase (JAK), JUN and signal transducer and activator of transcription (STAT). ALK regulates several genes at the transcriptional level, including MYCN, JUNB, B-cell lymphoma (BCL)2A, BIM and BCL2 [4]. Activation of ALK results in cell growth and differentiation, while limiting apoptosis.

The role of the EML4-ALK fusion protein in tumour development has been widely reported in vitro as well as in vivo in murine models [15]. Pre-clinical studies demonstrated that ALK-driven tumours were highly sensitive to ALK TKIs in vitro in a panel of human tumour cell lines including NSCLC, and that these cells were dependent on ALK for their growth and survival [16].

Predictive and prognostic impact of ALK activation
The predictive value of ALK translocations in response to the ALK inhibitor (ALKi) crizotinib has been clearly established. ALK also appears to play an independent prognostic role. In a recent European study including 1281 patients with resected stage I–III lung adenocarcinoma, patients with ALK+ tumours (according to fluorescence in situ hybridisation (FISH) and/or immunohistochemistry (IHC)) were paired with ALK− patients [17]. Among the 80 IHC+ patients (6.2% of the population), progression-free survival (PFS) and overall survival were longer compared with IHC− patients, with a hazard ratio (HR) of 0.61 (95% CI 0.41–0.90; p=0.012) for overall survival and 0.65 (95% CI 0.46–0.93; p=0.018) for PFS. When
only FISH was taken into account (2.2% of the population), a significant difference was only seen with overall survival.

Data in advanced stage patients are less conclusive and only retrospective studies are available. In a study by SHAW et al. [18] in crizotinib-naïve patients, no significant difference in survival was observed in the 36 ALK+ (FISH) patients treated with crizotinib compared with the 253 wild-type control patients who did not receive crizotinib. However, contradictory data have been published in Asian populations, in which ALK rearrangements were associated with an unfavourable prognosis in terms of overall survival [19, 20].

Characteristics of ALK+ populations

EML4-ALK translocations represent 4–8% of non-epidermal lung cancers, equivalent to approximately 50 000 patients worldwide [21, 22], representing a major epidemiological concern. Histologically, ALK+ tumours are most typically acinous adenocarcinomas, frequently expressing thyroid transcription factor-1 (TTF-1) [23]. They may also present specific morphological characteristics such as a signet ring cell (SRC) component. In tumours harbouring SRC, some genetic abnormalities are present at a high frequency, with up to 26% for ALK and 6% for ROS1 [24].

A number of clinical characteristics have been systematically found in several studies worldwide, including American [25], European [26] and Chinese [27] populations. The majority of patients are never/light smokers (≤10 pack-years), with ALK translocations present in up to 15% of nonsmoking patients versus only 2% of smokers [25]. Patients also tend to be younger and express wild-type tumoral EGFR. Reports of higher frequency in males are inconsistent. In contrast to EGFR mutations, ethnic origins do not appear to have any influence. The main clinical particularity associated with ALK+ is a higher frequency of brain metastases at diagnosis (40%) [26].

The American College of Pathology recommends testing all patients with NSCLC tumours presenting an adenocarcinoma component or when this histology cannot be completely ruled out due to a limited tissue sample. Clinical characteristics should not be a limiting factor for exploratory analyses, and testing should be particularly encouraged in the case of never/light smokers [28]. ALK abnormalities in epidermoid lung cancers are extremely rare (one (0.2%) out of 523) [28], and the few studies reporting higher incidences should not, at this stage, change the above recommendations about screening [29].

Methods of detection

FISH is currently the reference detection method for ALK fusions [25]. This technique uses two specific DNA probes, each coupled to a fluorescent marker, one green and one red, which cover the 2p23 ALK region. In the wild-type scenario, the red signal (3′ ALK) and the green signal (5′ ALK) are adjacent. When the distance between these two signals is more than twice the signal diameter, they are considered separated, reflecting a physical separation of the two DNA regions and hence a translocation.

FISH is considered to be positive if >15% of the tumour cells counted in four fields show either a separation between the green and red signals or a single red signal with loss of the associated green signal. This 15% threshold allows for errors due to background noise, reading or aberrant hybridisation [30]. At least 50 cells must be counted, with a second count of another 50 cells by a second reader if there are between 10% and 50% positive cells.

The strengths of FISH lie in its ability to detect ALK rearrangements irrespective of the variant or the fusion protein, as well as its correlation with clinical efficacy. It has been approved by the US Food and Drug Administration (FDA) for crizotinib (Vysis ALK Break-Apart FISH Probe kit; Abbott Molecular, Inc., Des Plaines, IL, USA).

IHC is another method for detecting ALK rearrangements in lung cancer. Initially, it was associated with sensitivity issues [31–34] and occasional false-positive results; however, newer ultrasensitive IHC techniques appear to offer a more reliable and sensitive screening method [35]. The positivity threshold is typically visual, requiring moderate to intense staining in 5–10% of cells [36]. Advantages of IHC are mainly its low cost in terms of both time and manpower, but standardisation of the test is difficult. A summary of IHC and FISH performed in several studies in lung cancer patients can be found in supplementary table S1.

While IHC is a reliable screening tool, FISH confirmation is required in the event of positive IHC and even in some cases for negative IHC in patients presenting predictive rearrangement markers, including younger age, light smokers (≤10 pack-years) and testing negative for other mutations, notably EGFR and KRAS [34, 37].

Finally, reverse transcriptase PCR may become a routine rapid diagnostic technique for ALK translocations. This highly specific technique offers the additional advantage of identifying the fusion gene
associated with ALK. Its limited use to date is due to the requirement of a quality DNA sample from fresh or frozen tumour tissue. However, new platforms allowing use of routine tissue samples, including paraffin-embedded samples, have been reported [38, 39].

Current therapeutic agents
Targeted ALK inhibitors
Knowledge gained from studies with EGFR TKIs accelerated the development of ALK TKIs. The principal studies published to date in lung cancer patients are summarised in table 1 and ongoing studies are presented in supplementary table S2. In lung cancer, ALKi are currently only used to manage patients with metastatic cancers, and no data are available in the adjuvant setting or combined with radiotherapy. Results from studies of the main ALKi currently under development are presented below.

Crizotinib
Crizotinib, a multiple TKI, was the first available treatment for ALK+ patients. Initially developed to block c-MET, it was subsequently found to also target ALK, ROS1 and the "récepteur d’origine Nantais" (RON) [44]. In the first phase I study using crizotinib (PF-02341066), 37 patients with advanced cancers were treated, including ten NSCLC patients with ALK+ tumours. Among them, one patient had a complete response, two had partial response and four patients reported stable disease [45]. This led to an extension of the study with a dedicated expansion cohort of 149 patients with advanced ALK+ lung cancers, 143 of whom were evaluable [46]. The recommended dose of 250 mg twice daily, established in the dose escalation part of the phase I study, was used. Objective responses were reported in 87 patients in this extension cohort (60.8%, 95% CI 52.3%–68.9%), including three with complete response and 84 with partial response, a spectacular improvement over the expected rate of <10% with classic cytotoxic agents. PFS was 9.7 months, with estimated 6- and 12-month overall survival of 87.9% and 74.8%, respectively [47]. These results led to fast-track approval by the FDA in August 2011 for ALK+ patients, irrespective of the number of prior treatment lines. Side-effects were mainly grade 1–2 (72%) and included nausea/vomiting, digestive toxicity (notably diarrhoea or constipation) and peripheral oedema. Neutropenia and transaminase elevations were also reported and were the most common grade 3–4 toxicity (24%). This hepatic toxicity has also been reported with other MET inhibitors and thus may be related to the MET inhibitor activity of crizotinib. The safety profile of crizotinib was confirmed in subsequent phase III studies (table 2) [40, 48].

A phase III PROFILE 1007 study was then performed, comparing crizotinib with pemetrexed or docetaxel combined with platinum as second-line therapy in ALK+ patients [40]. A total of 347 patients were included and the study was positive for the primary objective, with PFS of 7.7 months in the experimental arm compared with 3.0 months in the control arm (HR 0.49; p<0.001). The response rate was 65% versus 20% (p<0.001). An interim survival analysis (96 of the 241 events required) did not demonstrate a survival advantage (20.3 versus 22.8 months, respectively); however, this outcome is difficult to interpret given the likely exposure of patients in the control arm to crizotinib during follow-up. This study formed the basis for the marketing approval of crizotinib in the second-line setting in Europe.

<table>
<thead>
<tr>
<th>Study name [ref.]</th>
<th>Phase</th>
<th>Patients n</th>
<th>Comparison drug</th>
<th>Experimental drug</th>
<th>Treatment setting</th>
<th>Efficacy outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROFILE 1007 [40]</td>
<td>III</td>
<td>347</td>
<td>Pemetrexed or docetaxel</td>
<td>Crizotinib</td>
<td>After chemotherapy</td>
<td>Positive PFS 7.7 versus 3 months; response rate 65% versus 20%</td>
</tr>
<tr>
<td>PROFILE 1014 [41]</td>
<td>III</td>
<td>343</td>
<td>Cisplatin/carboplatin +pemetrexed</td>
<td>Crizotinib</td>
<td>First-line</td>
<td>Positive PFS 10.9 versus 7 months; response rate 74% versus 45%</td>
</tr>
<tr>
<td>NCT01283516 [42]</td>
<td>I</td>
<td>246</td>
<td>Ceritinib</td>
<td>Ceritinib</td>
<td>After chemotherapy or ALKi</td>
<td>Post-crizotinib PFS 6.9 months, crizotinib-naive PFS ≥18.4 months*, all patients PFS 8.2 months; response rates 55%, 66% and 59%, respectively</td>
</tr>
<tr>
<td>JapicCTI-101264 [43]</td>
<td>I/II</td>
<td>46</td>
<td>Alectinib</td>
<td>Alectinib</td>
<td>After chemotherapy or ALKi</td>
<td>Response rate 87%</td>
</tr>
</tbody>
</table>

PFS: progression-free survival. *: median PFS not reached at the time of analysis.
The impact of crizotinib in ALK+ patients in the first-line setting was evaluated in the phase III PROFILE 1014 study [41]. A total of 343 untreated patients received either crizotinib (n=172) or pemetrexed with a platinum (cisplatin 75 mg·m$^{-2}$ or carboplatin, target area under the curve of 5–6 mg·mL$^{-1}$·min every 3 weeks, for up to six cycles; n=171). The primary objective was reached, with PFS of 10.9 months in the experimental arm versus 7.0 months in the control chemotherapy arm (HR 0.45, 95% CI 0.35–0.60; p<0.0001). The response rate was higher with crizotinib (74% versus 45%; p<0.0001). Again, no significant difference was seen in overall survival in the interim analysis (HR 0.82, 95% CI 0.54–1.26; p=0.18), probably due to crossover to crizotinib as mentioned for the PROFILE 1007 study. The results of this recent PROFILE 1014 study are expected to lead to a rapid extension of the European marketing approval to include the first-line setting.

Crizotinib has been prescribed as either first- or second-line therapy in the USA since 2011. In Europe, authorisation for second-line therapy only was obtained in 2012 [49]. Prescription is irrespective of the method of detection. Several studies with crizotinib in a range of settings are ongoing, including in the early stage adjuvant setting (NCT02201992), in combination with chemotherapy (NCT02134912), with radio-chemotherapy (NCT01822496) or with other targeted therapies (supplementary table S2).

Ceritinib

Following the early success of crizotinib, second-generation ALKi have been developed to improve outcome, one of which is ceritinib (LDK378). The first phase I clinical trial published with this agent was performed in progressing ALK+ patients [42]. Previous treatment with crizotinib was permitted but in such cases a re-biopsy was required prior to ceritinib treatment, for a study of the mechanism of resistance. A total of 59 patients were included and the recommended dose was determined to be 750 mg·day$^{-1}$. Toxicities included diarrhoea/vomiting, dehydration, hypophosphataemia and transaminase elevations, with a 57% rate of grade 3–4 toxicity (table 2).

<table>
<thead>
<tr>
<th>Adverse events#</th>
<th>All grades</th>
<th>Grade 3–4</th>
<th>Toxicity monitoring</th>
<th>Toxicity management</th>
</tr>
</thead>
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<tr>
<td></td>
<td>%</td>
<td>%</td>
<td></td>
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<tr>
<td>Crizotinib [40, 48]¶</td>
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<td></td>
</tr>
<tr>
<td>Visual effect</td>
<td>60, 64</td>
<td>0</td>
<td>AST, ALT, ALP and bilirubin every 2 weeks for 2 months then</td>
<td>Interstitial pneumonia any grade: permanent discontinuation</td>
</tr>
<tr>
<td>Nausea</td>
<td>55, 56</td>
<td>1</td>
<td>weekly</td>
<td>Hepatotoxicity: ALT/AST any grade with bilirubin &lt;G2, withhold until ≥G1 recovery; resume</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>50, 60</td>
<td>0</td>
<td>CBC monthly</td>
<td>200 mg twice daily; ALT/AST with bilirubin &gt;G1, permanent discontinuation</td>
</tr>
<tr>
<td>Vomiting</td>
<td>39, 47</td>
<td>1</td>
<td>Echocardiogram before treatment</td>
<td>QTc prolongation: G3 withhold until recovery</td>
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<td>Peripheral oedema</td>
<td>30, 31</td>
<td>2</td>
<td>Ophthalmological and respiratory examination</td>
<td>Haematological: G3–4 withhold until recovery ≥G2; resume at 200 mg twice daily if G4</td>
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<tr>
<td>Constipation</td>
<td>28, 42</td>
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<td>Dizziness</td>
<td>21, 22</td>
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<td>Hypogonadism if symptomatic</td>
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<td>22, 38</td>
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<td>Dyspnoea</td>
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<td>Rash</td>
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<td>Upper respiratory infection</td>
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<td>Interstitial pneumonia*</td>
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<td>Amylase</td>
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<td>Increased ALP</td>
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<tr>
<td>Hyperglycaemia</td>
<td>7</td>
<td>4</td>
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</tr>
</tbody>
</table>

# >5% patients; ¶: data are presented as two values from the two studies, if available; *: <5% patients. AST: aspartate transaminase; ALT: alanine transaminase; ALP: alkaline phosphatase; CBC: complete blood count; G: grade.

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TABLE 2 Toxocities associated with anaplastic lymphoma kinase inhibitors

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This study was subsequently extended to include crizotinib-naïve patients, and results were reported recently [50]. Among the 246 patients included in the study and treated with 750 mg·day$^{-1}$ ceritinib, 66 had received prior crizotinib. Median PFS was 6.9 months in patients who had received ALKi treatment, while in ALKi-naïve patients the median PFS had not been reached but was >18.4 months. PFS in the overall population was 8.2 months. Response rates were 55%, 66% and 59%, respectively. Notably, toxicity resulted in dose reductions in 39% of patients and discontinuations in 10%.

Responses to ceritinib were observed in patients with resistance mutations, including the L1196M gatekeeper mutation, as well as in patients without detectable mutations. Furthermore, responses were also seen in brain metastases in patients who received prior crizotinib. In the ASCEND-1 study, which included 124 patients with brain metastases (98 pre-treated, 26 therapy-naïve), the overall response rate was 54% and median PFS was 6.9 months [50, 51]. On the basis of these preliminary data, ceritinib received FDA approval for ALK$^+$ patients who are resistant or intolerant to crizotinib [42], and an exceptional access programme has been approved in other countries.

Several ongoing phase II and III studies are evaluating the role of ceritinib in NSCLC ALK$^+$ patients in other settings, including patients resistant to crizotinib (ASCEND-5; NCT02040870), as first-line therapy (ASCEND-4; NCT01828099) in ROS1-mutated patients (NCT02186821) and in combination with a heat shock protein (HSP)90 inhibitor (NCT01772797) (supplementary table S2).

**Alectinib**

Alectinib (CH5424802/RO5424802) is another second-generation ALKi designed to increase selectivity and activity in patients with the L1196M, F1174L or R1275Q mutations [52]. Results from the first phase I/II study (JapicCTI-101264) were recently published [43]. The study was performed in a Japanese population of ALK$^+$ patients who had received at least one line of prior chemotherapy and were ALKi-naïve. Patients received alectinib at doses up to 300 mg twice daily. The response rate at 6 weeks was 87%, including two complete responses among 46 patients. PFS and overall survival were not reported. The main toxicities were dysgeusia (30%), elevations in aspartate transaminase (28%), bilirubin (28%) and creatinine (26%), as well as skin rash (26%). Grade 3 toxicities (37%) included liver function abnormalities in 2% of patients, neutropenia in 4% and creatine phosphokinase elevations in 4%. Although no toxic deaths or grade 4 toxicity were reported, the 48% rate of patients discontinuing treatment due to toxicity was notably high.

Results of a phase I/II study with alectinib (NCT01588028) in patients who had received prior crizotinib were recently published [53]. A 55% response rate was reported in the 44 patients evaluable for efficacy, including one complete response, 23 partial responses (14 confirmed, nine unconfirmed) and 16 patients with stable disease. Brain metastases were reported in 21 patients at study entry, including 12 with cerebral progressive disease. 11 of these 12 patients responded, including six complete responses in brain lesions; four of these patients did not require radiotherapy, two of whom had a complete response, one had a partial response and one had stable disease. Of note, among the 21 patients with brain metastases at diagnosis, none experienced central nervous system progression.

This outcome with an ALKi in the second-line setting is reminiscent of that seen with ceritinib. Studies with alectinib in patients who have received prior crizotinib (NCT01801111) or versus crizotinib in treatment-naïve patients (ALEX; NCT02075840) are currently recruiting (supplementary table S2).

**AP26113**

AP26113 was designed as a multipurpose inhibitor to block both ALK and EGFR, as well as to overcome the L1196M and T790M resistance mutations [54]. Results of a phase I/II study were recently made available (NCT01449461) [55, 56]. The recommended dose is 180 mg·day$^{-1}$. A 67% response rate among 18 ALK$^+$ patients was reported, including two complete responses, and an equivalent response rate was seen irrespective of prior crizotinib. Of note, four out of five patients with brain metastases responded. Side-effects included nausea (33%), fatigue (22%) and diarrhoea (20%), with a 7% rate of grade 3–4 events. Six out of 44 patients stopped treatment due to side-effects and two toxic deaths were reported. A specific lung toxicity, the appearance of early hypoxic dyspnoea associated with bilateral ground-glass nodules visible on scans, was observed and led to the exclusion of patients with diffuse interstitial lung disease or oxygen dependence. Despite this, respiratory toxicities persisted in four (9%) of the 44 treated patients.

**Other therapies**

Several other ALKi therapies are under development, including NMS-E628 (Nerviano Medical Sciences, Milan, Italy), X-396 (Xcovery, West Palm Beach, FL, USA), ASP-3026 (Astellas Pharma, Tokyo, Japan), CEP-37440 (Teva Pharmaceutical Industries Ltd, Petah Tikva, Israel), PF-06463922 (Pfizer, New York, NY, USA) and TSR-011 (Tesaro, Inc., Waltham, MA, USA).
Chemotherapy

While the presence of an ALK translocation is known to be a powerful predictive marker of response to an ALKi, the question has been raised as to whether it is predictive of response to other treatments. Several retrospective studies have shown evidence of potential efficacy with pemetrexed in ALK+ patients [57, 58]. With only a relatively small number of patients treated (89 (19 ALK+) patients in one study, 95 (15 ALK+) patients in another), PFS in ALK+ patients treated with pemetrexed combined with a platinum was found to be significantly higher compared with ALK− patients, with a duration of approximately 9 months.

A particularly striking result was seen in an analysis of a subgroup of patients treated in the PROFILE 1014 study (NCT00932893). The HR for progression or death in patients receiving pemetrexed or docetaxel in the second-line setting compared with patients receiving crizotinib was 0.30 for docetaxel and 0.59 for pemetrexed, with response rates of 7% (95% CI 2%–16%) and 29% (95% CI 21%–39%), respectively. This suggests that pemetrexed offers a true benefit over docetaxel.

A retrospective study by Shaw et al. [59] reported that ALK+ patients responded better than ALK− patients to a platinum/pemetrexed doublet in the first-line setting; however, this advantage was no longer apparent in never/light smokers. With platinum/pemetrexed as first-line therapy, PFS was 8.5 months for patients with mutated ALK, which was comparable to the 7-month PFS reported in the control arm (pemetrexed/platinum) of the PROFILE 1014 study [41]. In a study by Scagliotti et al. [60] of lung adenocarcinoma patients treated with pemetrexed/cisplatin, PFS was 5.3 months, and maintenance therapy with pemetrexed in the PARAMOUNT study gave a PFS of 6.9 months [61].

The mechanism of action for pemetrexed is not yet clearly identified, although lower expression of thymidylate synthase in ALK+ cells has been evoked [58], previously suggested to be a predictive factor for response to this agent [60]. Although the predictive role of ALK under pemetrexed is not yet clear, the characteristics of the targeted population, the low response rate with docetaxel and the trends reported all support the administration of pemetrexed in the ALK+ population.

Resistance in ALK+ NSCLC patients

While the use of ALKi is clearly fundamental to the therapeutic management of ALK+ lung cancer, issues relating to acquisition of resistance and escape have arisen [62]. The experience gained from the study of EGFR TKIs [63] is again invaluable, and resistance studies should be an integral aspect of the early development of all ALKi. Resistance mechanisms and proposed treatment options are discussed below and summarised in figure 1.

Primary resistance

Approximately 25% of patients treated with crizotinib present primary resistance [48], which may be directly related to the type of ALK abnormality. The presence or absence of resistance may be due to the fusion of ALK with proteins other than EML4 or to differences in ALK variants, as has been observed in some cell models [14]. Nonetheless, evaluation of the subgroups in the initial studies did not confirm this hypothesis, although patient numbers were small [46]. It is also important to take into consideration that, in practice, false positives may occur, linked to technical or interpretation difficulties [30]. Notably, the crizotinib response rate has never been precisely correlated with the percentage of mutated cells. The heterogeneity of response to TKI treatment may correlate with differences in the biology of apoptosis, and in particular with the pro-apoptotic BIM protein [65, 66].

Secondary resistance

Mechanisms of secondary resistance are usually classified into two distinct groups: one where the tumour remains dependent on ALK signalling, and the other ALK independent [64]. In ALK-dependent tumours, resistance is caused by a mutation or amplification of the targeted kinase itself, or alterations to drug metabolism. In ALK-independent tumours, resistance occurs via activation of alternative signalling pathways.

The first (and most common) mechanism of resistance identified involves modification of the crizotinib target [67], accounting for approximately 25% of resistance [68]. The most studied of these mutations is the substitution of a leucine by a methionine at the 1196 position (L1196M) in the ALK tyrosine kinase domain, the equivalent of the T790M mutation for EGFR. This mutation causes a change in conformation at the ATP pocket, preventing crizotinib binding. Seven other mutations have been identified to date, affecting other regions of the kinase, such as the N-terminal and the C-helix [69, 70]. ALK amplification appears to be sufficient to confer resistance to crizotinib, as shown both in vitro [71] and in the clinic [64], and may account for 15% of acquired resistance. Cases of loss of the ALK fusion gene have also been described as a source of resistance [64]. It should be noted that mutation(s) and amplification may be found simultaneously in the same patient [72]. Consistent with this, several resistance mutations can...
develop in the same ALK+ patient, such as was reported for a progressing patient in whom L1196M and C1156Y were simultaneously present [67].

Patients invariably relapse under crizotinib treatment and the second-generation ALKi have been developed to overcome this acquired resistance. Alectinib and ceritinib, the two best characterised second-generation ALKi, have been shown to overcome crizotinib resistance in preclinical models [52, 70] and have shown promising activity in patients who have progressed on crizotinib, with response rates of >50% [51].

Another mechanism of resistance relates to the emergence of clones with activation of alternative proliferation pathways such as EGFR [72–74], HSP90 [75–77], PI3K AKT mTOR [78], cKIT amplifications [72] or insulin-like growth factor 1 receptor (IGF1R) [79]. This mechanism accounts for approximately 20% of reported cases of crizotinib escape [80]. In the event of activation of another signalling pathway, combination treatment of the ALKi with another inhibitor or the development of a multi-kinase inhibitor may be effective. Several studies combining EGFR inhibitors or HSP90 inhibitors in resistant patients are currently ongoing (supplementary table S2).

As mentioned, one of the particularities of acquired resistance to ALKi is the possibility of the presence of several of these mechanisms occurring within the same tumour lesion [64, 72].

A direct consequence of the diversity of these resistance mechanisms is the need to enable repeated biopsies at progression. Although re-biopsying is technically feasible [81], given the tumoral heterogeneity and risks associated with repeated biopsies in patients who are often quite fragile, a noninvasive approach...
such as using DNA from circulating tumour cells would be an advantage [62, 82]. Some mutational abnormalities have been reported using this technique [83]. Discussion of re-biopsying should not affect the therapeutic strategy, however. For now, this should be reserved for exploration of resistance mechanisms in the context of a clinical trial.

Another interesting angle relates to research showing that immunomodulation and molecular signalling pathways interact. Activation of various signalling pathways increases the expression of programmed death-ligand 1 (PD-L1), which plays a key role in tumour escape from an immune response [84]. Notably, this has been shown in the case of ALK+ T-cell lymphomas, where the expression of PD-L1 is increased via STAT3 [85]. Understanding these interactions is critical for envisaging the combination of immunotherapy with targeted therapies.

**Therapeutic strategies**

Following the PROFILE 1014 study, crizotinib was considered the optimal first-line therapeutic choice in ALK+ patients in light of its good toxicity profile. However, given that progression in this treated patient population is systematic, further therapeutic options are needed. This raises the question of whether to continue crizotinib after progression [62] versus the place of second-generation TKIs, and which chemotherapies should be prioritised.

As with tumours presenting an addiction to the EGFR pathway, it may be of interest to continue treatment targeting ALK despite Response Evaluation Criteria in Solid Tumours (RECIST) progression, in the following cases. First, in the event of asymptomatic relapse and slow progression, further treatment with close surveillance may be an option in patients who tolerated the treatment well, as there is a risk of a flare-up effect, probably due to rapid growth of cell clones sensitive to ALKi, after stopping the inhibitor [86]. Secondly, in the case of oligometastatic relapse, crizotinib can be continued and may be combined with local treatment. For example, cerebral relapses affect one-third of all patients [47] and radiotherapy is often proposed [87], giving rise to prolonged responses with continued crizotinib [88]. However, increasing the dose does not appear to be clinically efficient under these circumstances [89]. A study by Costa et al. [90] may explain the importance of crizotinib in the case of cerebral relapse, with an apparently low ratio of crizotinib concentration in the cerebrospinal fluid compared with the serum suggesting poor transfer across the blood–brain barrier.

This approach of continuing crizotinib may also be useful in cases of extracerebral oligometastatic progression with the possibility of local surgery or radiotherapy, with a rationale of the emergence of ALKi-resistant clones that may be eradicated by local treatment [91]. Continued TKI administration has been successfully implemented for patients with EGFR mutations [92]. When progressive disease is not accessible to local treatment, novel ALKi are the treatments of choice, in particular alectinib [53] and ceritinib [42]. Response rates are high (58% with alectinib [93] and 59% with ceritinib [42]), and these agents have shown activity against brain metastases.

**FIGURE 2** Proposed treatment algorithm for the treatment of nonsmall cell lung cancer with rearrangements of anaplastic lymphoma kinase (ALK), taking into account local progression. C: carboplatin; P: platinum; Pem: pemetrexed; Bev: bevacizumab; CNS: central nervous system; ALKi: ALK inhibitor.

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Consequently, the role of novel ALKs as first-line therapy, rather than as a second-line post-crizotinib option, is currently being evaluated in phase III studies. In current practice, given the rate of response under these second-line therapies and the absence of clear predictive markers for response, systematic re-biopsy is not currently considered essential. In cases where these treatments are not available or when a second-line TKI fails, chemotherapy with a pemetrexed/platinum doublet is the treatment of choice, and could potentially be associated with bevacizumab. In terms of re-treatment after progression under chemotherapy, several cases have been reported of re-acquisition of sensitivity to an ALKi as third-line therapy after second-line chemotherapy [94]. Figure 2 summarises the therapeutic strategies for NSCLC patients.

Conclusion
Diagnosis and targeting of ALK-activating mutations now forms part of routine therapeutic management of lung cancer. Results obtained with the first ALKs are without precedent in terms of PFS and overall survival. An understanding of the mechanisms of resistance, as well as more specific targeting of ALK mutations, has given rise to promising results for patients progressing under ALKi. Several challenges remain in the management of these tumours, including the association of current strategies with complementary therapeutics such as anti-angiogenics and immunotherapy, in the hopes of further improving outcomes for these patients.

References


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