Medical Oncology (2018) 35:72
https://doi.org/10.1007/s12032-018-1133-4

REVIEW ARTICLE

Precision medicine against *ALK*-positive non-small cell lung cancer: beyond crizotinib

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Received: 2 April 2018 / Accepted: 11 April 2018 / Published online: 17 April 2018

Abstract
Anaplastic lymphoma kinase (*ALK*) rearrangements represent the molecular driver of a subset of non-small cell lung cancers (NSCLCs). Despite the initial response, virtually all *ALK*-positive patients develop an acquired resistance to the ALK inhibitor crizotinib, usually within 12 months. Several next-generation ALK inhibitors have been developed in order to overcome crizotinib limitation, providing an unprecedented survival for this subset of patients. The aim of this review to summarize the current knowledge on ALK tyrosine kinase inhibitors (TKIs) in the treatment of advanced *ALK*-positive NSCLC, focusing on the role of novel ALK inhibitors in this setting. In addition, we will discuss their role in the pharmacological management of *ALK*-positive brain metastasis. Next-generation ALK inhibitors showed an impressive clinical activity in *ALK*-positive NSCLC, also against the sanctuary site of CNS. Sequential therapy with ALK TKIs appears to be effective in patients who fail a first ALK TKI and translates in clinically meaningful benefit. However, these agents display different activity profiles against crizotinib resistance mutation; therefore re-genotyping the disease at progression in order to administer the right TKI to the right patient is going to be necessary to correctly tailor the treatment. To avoid repeated invasive procedure, noninvasive methods to detect and monitor *ALK* rearrangement are under clinical investigation.

Keywords NSCLC · *ALK* rearrangement · Tyrosine kinase inhibitors · Brain metastasis

Introduction

In 2017, lung cancer remained the leading cause of cancer-related death worldwide, breaking up more lives than all other main cancers coupled [1]. Approximately 80% of lung cancers fall into non-small cell lung cancer (NSCLC) subgroup, with small cell lung cancer (SCLC) accounting for remaining 20%. Unfortunately, the majority of these patients are diagnosed with an advanced stage of disease (IIIB/IV), which makes their disease incurable [1]. Despite the advances in diagnostic procedures and therapeutic approaches, the prognosis of these patients has not greatly improved, with an overall 5-year survival slightly increasing over the past decade from 15.7 to 17.4%, but falling to 4% for advanced stages [2]. On the other hand, the impressive advancements over the understanding the molecular processes driving the development and progression of NSCLC yielded a dramatic impact on the way we treat patients with advanced NSCLC. In fact, the discovery of targetable genetic alterations (e.g., *EGFR, MET, ROS1, HER2, BRAF*) that promote cancer growth and survival has paved the way to personalized therapy for different molecularly defined subsets of patients harboring such genetic alterations.

In this scenario, *ALK* rearrangement is just one of the latest identified driver mutations in NSCLC, but it has already had a striking impact in the treatment of patients with advanced NSCLC carrying this specific mutation. The

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aim of this review is to summarize the current knowledge of treatment of *ALK*-positive NSCLC, focusing on the novel ALK inhibitors under clinical and preclinical developments, but also touching upon the efficacy of ALK inhibitors in the management of central nervous system (CNS) metastasis, which represent one of the most common sites of progression or relapse of *ALK*-rearranged NSCLC patients.

ALK rearrangements in NSCLC

Located at 2p23.2 and with a length of 729 kb, *ALK* gene encodes for a highly conserved type-I transmembrane tyrosine kinase protein belonging to the insulin receptor superfamily, which, similarly to other tyrosine kinase receptors, holds an extracellular domain, a transmembrane portion and a cytoplasmatic transducer segment [3]. Based on its expression pattern, it is believed that in physiological condition ALK is involved in brain development, maturation and maintenance of neuron homeostasis, becoming epigenetically silenced after early phases of embryogenesis [4].

Nonetheless, genetic alterations affecting the *ALK* gene confer high oncogenic properties. *ALK* mutations usually consist in translocation with partner genes, leading to the formation of fusion oncogenes which are overexpressed in cancer cells, in turn allowing the constitutive activation of ALK downstream signaling pathways, including RAS/MEK/ERK, which is involved in cell growth and proliferation, PI3 K/AKT/mTOR and JAK3/STAT3, which are responsible for cell survival and apoptosis escape [4–6].

ALK mutation as driver of human malignancies was first reported in 1994 by Morris and colleagues in a subgroup of patients with anaplastic large cell lymphomas [7] who harbored *NPM–ALK* rearrangement resulting from a reciprocal translocation, t(2;5)(p23; q21). Since then, an increasingly number of *ALK* partner genes have been discovered, such as *TMP3*, *CLTCL1*, *ATIC* and *TFG* [8]. When it comes to NSCLC, the first evidence of *ALK* gene mutation dates back to 2007, when Soda and coworkers unveiled the presence of echinoderm microtubule-associated protein-like 4 *(EML4)–ALK* rearrangement in a subset of Japanese patients [5]. Among the eleven *ALK* fusion variants reported in the literature so far, *EML4–ALK* is the most frequently documented rearrangement in *ALK*-positive NSCLC. According to the breakpoint on *EML4* gene, a number of *EML4–ALK* fusion variants have been further characterized, each with a different prevalence (V1 54.5%, V3a/V3b 34%, V2 10%, V5 1.5%) [5, 9]. *EML4–ALK* fusion oncogene results from an inversion rearrangement on chromosome 2, inv(2) (p21; p23) [5]. As a consequence, the resulting chimeric protein holds the N-terminal derived from EML4 and the C-terminal encompassing the whole intracellular tyrosine kinase domain of ALK. The increased tyrosine kinase activity resulting from this translocation brings about to cell proliferation and migration, and sustains pro-survival pathways.

Similarly to what has been reported with epidermal growth factor receptor (*EGFR*)-mutant NSCLCs, patients harboring *ALK* rearrangement exhibit distinct clinical and pathological features, including never or light smoking history, younger age and adenocarcinoma with either signet ring or acinar histology. Of note, *ALK* gene rearrangements are generally mutually exclusive with other driver mutation, such as *EGFR* or *KRAS*, except for very rare cases of coexistence of both mutations [9, 10].

ALK detection methods

The eligibility for treatment with ALK TKIs in advanced NSCLC requires tumor genotyping for *ALK* mutation. Currently, several molecular methods are commonly used for this purpose, including immunohistochemistry (IHC), fluorescent in situ hybridization (FISH) and polymerase chain reaction-based techniques (PCR).

FISH analysis is considered the gold standard for the detection of *AKL* mutation in NSCLC, as it is able to detect virtually all *ALK* rearrangements regardless of the fusion partner and is an accurate and reliable technique. The Vysis LSI ALK Dual Color, Break-Apart Rearrangement Probe (Abbott Molecular, USA) received FDA approval following the results of clinical trials that used FISH as companion test for the detection of *ALK*-positive cases. In spite of the advantages offered by this method for the molecular diagnosis of *ALK* rearrangements, FISH remains a challenging technique because it has high cost and requires expertise and experience for an accurate interpretation, and it does not identify a specific translocation [11].

IHC has many advantages over FISH, including the low cost, easy implementation and interpretation. Moreover, IHC allows to the identification of abnormally expressed ALK protein which represents the real target of ALK inhibitors, thus avoiding the false negative of FISH. Consistently, some authors have reported a clinical benefit by using crizotinib in IHC+/FISH− patients [12, 13]. On the other hand, ALK protein levels in *ALK*-rearranged NSCLCs are low, and to date there is not a standard protocol to detect ALK in NSCLC through IHC analysis. At the present time, two ALK IHC clones (D5F3 by Roche-Ventana, USA/Cell Signaling Technology, USA; 5A4 by Novocastra, UK) have demonstrated a sensitivity and specificity of 90–100% for the detection of ALK expression. Hopefully, in the near future the standardization of tissue preparation, the choice of antibody and signal enhancement system as well as the definition of the optimal scoring system will improve the performance of IHC [14].

ALK expression in NSCLC can also be detected by PCR-based methods, including reverse-transcriptase

PCR (RT-PCR) and 5′-rapid amplification of cDNA ends (RACE) which are specific and sensitive techniques, able to detect *EML4–ALK* fusion transcripts even if diluted in 90% of wild-type RNA. PCR-based methods require a specific technical expertise to derive and interpret results. Besides, a visual morphological control of the sample is mandatory to verify the presence and percentage of cancer cells to avoid a false negative. On the other hand, RT-PCR is free of subjectivity in assessment of the analysis as compared to IHC and FISH. Although these methods are less expensive compared to other techniques, they can fail to detect rare or novel translocations and RNA degradation which implies that RT-PCR has to be multiplexed in order to detect common and rare *ALK* variants. Moreover, the poor sample quality might prevent the detection of target sequences [15, 16].

EML4–ALK translocation can also be detected by other emerging methods including next-generation sequencing (NGS) and exon-array profiling. NGS analysis has revolutionized the diagnostic approach to advanced NSCLC, as with a single analysis we can determine the mutational status of multiple genes, including other actionable fusion genes such as *RET, ROS1*, *NTRK1* and other, which avoid sequential time- and tissue-consuming analysis. An additional advantage is that NGS-based techniques can unveil *ALK* point mutations which are often the putative mechanism of resistance to ALK TKIs and that may drive the subsequent therapies [17]. In this regard, Peled and colleagues reported a case of a patient with lung adenocarcinoma who initially tested negative for *ALK* with FISH analysis, while NGS showed the canonical *EML4–ALK* breakpoint, thus allowing the authors to hypothesize that *EML4* and *ALK* genes were separated by small rearrangements that prevented detection with FISH and suggesting that NGS may be useful to identify cancer driver mutations that cannot be detected with other methods [12]. On the other hand, virtually all lung cancer biopsies are stored in formalin-fixed paraffin-embedded (FFPE) tissue. As a consequence, the quality of the extracted RNA should be considered as a low quality and excessive degradation can lead to false negative. Exon-array profiling (Affymetrix Human Exon 1.0 Arrays) is a novel method to identify *ALK* rearrangement in solid tumors. In a large study which screened a broad collection of patient tumor samples, the presence of *EML4–ALK* fusion was observed in 2.4% of breast (5 of 209), in 2.4% of colorectal (2 of 83) and in 11.3% of NSCLC (12 of 106), and an additional novel variant (E21;A20) was found in colorectal carcinoma, confirming that, though expensive and technically challenging, exon-array profiling can be used to detect common and uncommon *EML4–ALK* variants [18].

ALK tyrosine kinase inhibitors in NSCLC

Efficacy of crizotinib in NSCLC: a brief overview

Crizotinib is a small oral multitargeted TKI that causes a dose-dependent inhibition of ALK and ROS1 fusion proteins, c-MET, HGFR and MST1R. In the pivotal single-arm phase I trial PROFILE 1001, crizotinib demonstrated an impressive objective response rate (ORR) of 61% with a median progression-free survival (PFS) of 9.7 months in *ALK*-positive NSCLCs across multiple lines of therapy. Of note, among 24 patients (16%) who received crizotinib as first-line therapy, the authors reported a median PFS of 18.3 months. Although median overall survival (OS) was not reached, the authors estimated an OS rate at 6 and 12 months of 87.9 and 74.8%, respectively, and the majority of patients had radiological evidence of tumor shrinkage [19]. The most common adverse events (AEs) were of grades 1 and 2 and included visual disorders, nausea, diarrhea, constipation, vomiting and peripheral edema [19]. Further corroboration of crizotinib's activity in *ALK*-positive NSCLC derived from a multicenter, open-label, single-arm phase II trial (PROFILE 1005) in which crizotinib confirmed an astounding activity in patients with advanced *ALK*-positive NSCLC who progressed after ≥ 1 chemotherapy. Recently, the final results of this study have been presented and confirmed an impressive investigator-assessed ORR of 54 and 41% in the central-testing and local-testing subgroups, respectively. The most common treatment-related any grade AEs were vision disorder (58%), nausea (51%), diarrhea (47%) and vomiting (47%) [20, 21]. In a subsequent phase III clinical trial (PROFILE 1007), 347 *ALK*-positive NSCLC patients who had progressed on or following platinum-based chemotherapy were randomized to receive crizotinib or licensed second-line cytotoxic agents. Crizotinib arm was associated with an ORR of 65% in contrast to 20% of the chemotherapy arm ($P < 0.001$). The median PFS assessed by independent radiological review was 7.7 months in patients receiving crizotinib compared to 3 months of chemotherapy arm ($P < 0.001$). Although there was a numerical, but not statistically significant, improvement in the secondary endpoint of OS for crizotinib versus chemotherapy (HR 0.85; 95% CI 0.66–1.10; $P = 0.11$; median 21.7 and 21.9 months), the investigators reported an improvement in symptoms control and quality of life in crizotinib arm. Importantly, patients who received pemetrexed in the chemotherapy arm experienced a higher ORR (30 vs. 9%) and longer PFS (4.2 vs. 2.6 months) compared to docetaxel [22, 23]. Grade 3–4 AEs occurred in 16% of cases and included visual disorder, gastrointestinal side effects, and elevated liver, aminotransferase levels [22].

PROFILE 1014 is a phase III open-label clinical trial that randomized 343 chemotherapy-naïve patients with advanced *ALK*-positive NSCLC to receive crizotinib or pemetrexed plus platinum chemotherapy. Consistently with data from previous trials, PFS was significantly longer in crizotinib arm, compared to chemotherapy (10.9 vs. 7.0 months, HR 0.45, $P < 0.001$). ORRs were, respectively, 74 and 45% in crizotinib and chemotherapy arm ($P < 0.001$), but no difference in OS was observed [24]. The safety profile of crizotinib was in line with the previous studies.

Noteworthy, PROFILE 1007 and PROFILE 1014 showed no survival benefit in patients receiving crizotinib. However, these apparently unsatisfactory results can be attributed to the confounding effect of crossover to crizotinib, which was permitted upon progression to chemotherapy in both studies.

Following these results, crizotinib granted worldwide approval for the treatment of advanced *ALK*+ NSCLC (Table 1).

Mechanism of resistance to crizotinib

Similarly to what has been observed in patients with *EGFR*-mutant NSCLC treated with EGFR TKIs, despite initial response to crizotinib the majority of patients develop resistance to treatment. On the other hand, a variable portion of patients do not respond to initial therapy with crizotinib which indicates primary resistance.

With regard to primary resistance, preclinical data indicate that specific protein-folding properties in *ALK* fusion gene products may, at least in part, be responsible for heterogeneous response to crizotinib or eventually false-positive results at FISH assay used to detect *ALK* rearrangements [25]. Besides, not all *ALK* translocations generate functional rearrangements [25]. Additionally, although considered generally mutually exclusive, alterations in *KRAS* gene have increasingly been associated with primary resistance to crizotinib in a subset of patients harboring concomitant *ALK* rearrangement and *KRAS* mutation [26].

Though most of patients with *ALK*-positive NSCLC initially respond to crizotinib, the routinary clinical practice has showed us that resistance to treatment invariably occurs, usually within 12–14 months [27]. Currently, acquired resistance includes either biological or pharmacological mechanisms. Biological mechanisms cover three categories: *ALK* secondary mutations, *ALK* amplification and compensatory activation of bypass signaling pathways, the latter also referred as *ALK* non-dominant resistance. Among *ALK*-dominant mechanisms, the occurrence of secondary mutations is responsible for approximately 45% of cases of acquired resistance to crizotinib and consists into the development of novel point mutations in the *ALK* gene. Among them, the gatekeeper L1196M mutation is the most prevalent and well characterized. Similarly to T790M *EGFR*-positive NSCLC and T315I in CML, this mutation results in the impairment of crizotinib bound to ATP pocket of the target *EML4–ALK* fusion protein. The second most frequent secondary mutation is a glycine-to-alanine substitution at codon 1269 (G1269A) at the ATP-binding site, which interferes with crizotinib ability to block the tyrosine kinase domain of the target chimeric protein. Up to now, many other point mutations have been described in the literature including L1171T, L1152R, C1156Y, G1202R and S1206Y, with most of them affecting the P-loop, the beta sheet or the alpha-helix domain surrounding the gatekeeper area of ALK-rearranged protein, which explains the steric hindrance with crizotinib bound [28]. Noteworthy, different clones harboring different *ALK* resistance mutations can coexist in the same patient, which translates in heterogeneous response to crizotinib observed in *ALK*-positive NSCLCs. Importantly, acquired secondary mutations confer different degrees of resistance to structurally different ALK TKIs, which underscores the crucial role of the identification of the secondary mutation at the time of progression to crizotinib, in order to allow the oncologist to choose the most appropriate ALK TKI for each patient.

The second *ALK*-dominant mechanism of resistance to crizotinib is represented by a gain in *ALK* fusion gene copy number and has been reported as a mechanism of acquired resistance in barely 8% of cases. It is thought that the enhanced ALK downstream signaling, which results from

Table 1 Efficacy of crizotinib in patients with advanced *ALK*-positive NSCLC

Study [R]	N	ORR (%)	PFS (months)	OS (months)
PROFILE 1001 [19]	82	57%	9.7	1 year 76% 2 year 54%
PROFILE 1005 [20, 21]	1066	54%	8.4	21.8
PROFILE 1007 [22, 23]	Crizotinib: 173 Chemo: 174	65% 29%	7.7 3 $P < 0.001$	21.7 21.9 $P = 0.11$
PROFILE 1014 [24]	Crizotinib: 171 Chemo: 172	74% 45%	10.9 8.3 $P < 0.001$	NR NR

NR not reached

ALK gene amplification, is not sufficiently inhibited by crizotinib, regardless of adequate drug exposure and concomitant *ALK* rearrangement susceptibility.

Conversely, *ALK* non-dominant mechanisms involve the activation of different "bypass tracks." Within this class, hyper-activation of EGFR pathway that is based on either the increased phosphorylation of the intracellular TK domain or upregulation of its ligands has been observed in preclinical models and confirmed in clinical setting [29]. Infrequently, *EGFR*, *MET* and *KRAS* mutations have been observed in *ALK*-positive NSCLCs who progressed to crizotinib, likely as a result the emergence of preexisting clones following prolonged exposure to crizotinib [30, 31]. Of note, preclinical data from crizotinib-resistant cell lines showed that the combined administration of crizotinib and gefitinib results in suppression of cell growth and proliferation, providing a solid basis for future clinical application [32].

In addition to EGFR axis, the activation of PI3 K–AKT–mTOR pathway has been proven to favor the development of acquired resistance to crizotinib, probably through the induction of autophagy of ALK receptor [33]. Consistently, a synergistic effect of co-administration of crizotinib and mTOR inhibitors was observed in term reduction of cell viability in crizotinib-resistant cell lines [33]. Along with the aforementioned mechanisms, it is worth remarking the possible role of transition to small cell lung cancer, which has already been reported as mechanism of acquired resistance to treatment in two patients who progressed on crizotinib and second-generation ALK TKI alectinib [34].

Pharmacological resistance refers to the insufficient drug exposure which in turn allows tumor progression. In *ALK*-positive NSCLCs, this pharmacokinetic failure reflects the poor penetration rates of crizotinib within central nervous system (CNS) which represent a typical site of relapse in patients who progress to crizotinib. In fact, although it is still not extensively evaluated, the estimated penetration rate of crizotinib in CNS is disappointing, ranging from 0.06 to 0.26% [35, 36]. A combined retrospective analysis of PROFILE 1005 and PROFILE 1007 showed that among patients without baseline brain metastases (BMs) who developed disease progression ($n = 253$) after initiation of crizotinib, 20% were diagnosed with BMs. Of note, patients with BMs prior to crizotinib experienced a 71.1% rate of CNS progression [35]. Importantly, a study previously conducted by Weickhardt and colleagues showed that CNS was the first site of progression in 46% of cases in a cohort of patients with *ALK*-positive NSCLC treated with crizotinib, and 85% of them lacked coincident systemic progression [37].

Mechanism of acquired resistance to crizotinib is heterogeneous, and in approximately 20% of patients who develop such resistance a clear mechanism of resistance cannot be identified. Up to now, lots of effort with the aim to overcome resistance to crizotinib have been made, leading to the development and consequent approval of two second-generation ALK TKIs (ceritinib and alectinib), with many other are under clinical investigation with encouraging results (Table 2).

Next-generation ALK inhibitors

Ceritinib (LDK378)

Ceritinib is an orally available ALK inhibitor, 20 times more potent than crizotinib in xenograft models of *ALK*-rearranged NSCLC, which has shown marked antitumor activity against both crizotinib-sensitive and crizotinib-resistant tumor [38, 39].

In *ALK*-positive cell-line models, ceritinib was able to efficiently inhibit ALK harboring the crizotinib-resistant mutations L1196M, G1269A, I1171T and S1206Y, but it was ineffective against the G1202R and F1174C [40]. Ceritinib has also been reported to inhibit the insulin grow factor 1 (IGF-1) receptor and ROS1 although it has no activity against MET. In cell-based assays, ceritinib had an IC50 of 0.2 nM against the EML4–ALK and NPM–ALK fusion kinases, while the IC50s for IGF-1R and ROS1 are approximately fivefold–11-fold higher [41]. The efficacy of ceritinib has been demonstrated in a robust developmental program, as outlined below.

ASCEND-1 is an open-label, phase I study that recruited 255 patients (of whom 246 *ALK*-positive) with locally advanced or metastatic NSCLC that had progressed despite standard therapy [42]. In this study, ceritinib was shown to be highly effective against *ALK*-positive NSCLC, both in the crizotinib-naïve and in crizotinib-pretreated settings. An overall response was reported in 60 (72%) out of 83 ALK inhibitor-naive patients and 92 (56%) of 163 ALK inhibitor-pretreated patients. Median PFS was 18.4 months in ALK inhibitor-naive patients and 6.9 months in ALK inhibitor-pretreated patients, while median duration of response reached 17 months in ALK TKI-naive patients and 8.3 months in ALK inhibitor-pretreated patients. Regarding safety, serious AEs were reported in 117 (48%) of 246 patients. The most common grade 3–4 laboratory abnormalities were increased alanine aminotransferase (73 [30%] patients) and increased aspartate aminotransferase (25 [10%]). The most common grade 3–4 AEs were diarrhea and nausea, both of which occurred in 15 patients (6%). According to the activity profile showed in ASCEND I, two phase II trials (ASCEND II and ASCEND III) confirmed that ceritinib treatment provides clinically meaningful and durable responses with manageable tolerability in chemotherapy- and crizotinib-pretreated patients, including those with brain metastases.

ASCEND-2 is a phase II trial in which ceritinib's efficacy and safety were evaluated in 140 patients with NSCLC

Table 2 Efficacy and safety of next-generation ALK inhibitors

Drug name	Trial.Gov ID	Ph	Comparator	ORR (%)	PFS (months)	OS (months)	Most common AEs
Ceritinib	ASCEND-1 NCT01283516	I	No	72%	Crizotinib-pre-treated: 6.9 Crizotinib-naïve: 18.4	Crizotinib-pre-treated: 16.7 Crizotinib-naïve: NR	Nausea [82%)] Diarrhea [61%] Vomiting [65%] Fatigue [47%] Increased ALT [35%]
	ASCEND-2 NCT01685060	II	No	38.6%	5.7	NA	Nausea [81.4%] Diarrhea [80%] Vomiting [62.9%]
	ASCEND-3 NCT01685138	II	No	63.7%	11.1	NA	Diarrhea [82.3%] Nausea [74.2%] Vomiting [66.9%]
	ASCEND-4 NCT01828099	III	Platinum + Pemetrexed	Ceritinib: 72.5% Chemo: 26.7%	Ceritinib: 16.6 Chemo: 8.1 $P < 0.00001$	Immature	Diarrhea [85%] Nausea [69%] Vomiting [66%]
	ASCEND-5 NCT01828112	III	Pemetrexed or docetaxel	42.6%	Ceritinib: 5.4 Chemo: 1.6 $P < 0.0001$	18.1	Diarrhea [72%] Nausea [66%] Vomiting [52%]
	ASCEND-7 NCT002336451	II	No	–	–	–	–
Alectinib	AF-001 JP JapicCTI-101264	I/II	No	93.5%	2-year PFS: 76%	2-year OS: 79%	Dysgeusia [30%] Increased AST [28%] Increased blood bilirubin [28%] Increased blood creatinine [26%] Rash [26%] Constipation [24%]
	NCT01588028	I/II	No	55%	NA	NA	Fatigue [30%] Myalgia [17%] Peripheral edema [15%]
	NCT01801111	II	No	50%	8.9	NR	Myalgia [17%] Constipation [15%] Fatigue [14%] Asthenia [11%] Increased AST [10%]
	NCT01871805	II	No	50.8%	8.9	1-year OS: 71%	Constipation (38%) Fatigue (31%) Peripheral edema [30%] Myalgia [22%] Increased AST [21%]
	ALEX NCT02075840	III	Crizotinib	82.9%	Alectinib: NR Crizotinib: 11.1 $P < 0.001$	Immature	Anemia [20%] Peripheral edema [17%] Myalgia [16%] Blood bilirubin increase [5%] ALT/ALT increase [15%]
	J-ALEX JapicCTI-132316	III	Crizotinib	85%	Alectinib: NR Crizotinib: 10.2 $P < 0.0001$	Immature	Constipation [35%] Nausea [11%] Diarrhea [9%] Vomiting [6%]

Table 2 (continued)

Drug name	Trial.Gov ID	Ph	Comparator	ORR (%)	PFS (months)	OS (months)	Most common AEs
Lorlatinib	NCT01970865	I	No	46% (ALK-positive)	NA	NA	Hypercholesterolemia [72%] Hypertriglyceridemia [39%] Peripheral neuropathy [39%]
Brigatinib	NCT01449461	I/II	No	100% in crizotinib-naïve 72% in crizotinib-pretreated	Crizotinib-pretreated: 13.4 Crizotinib-naïve: NR	NA	Nausea [53%] Fatigue [43%] Diarrhea [41%] Headache [33%] Cough [31%]
	NCT02094573	II	No	Arm A: 46% Arm B: 54%	Arm A: 8.8 Arm B: 11.1	NA	Arm A/B increased CPK [3/8%], hypertension [4/5%], pneumonia [3/5%], rash [1/4%], increased lipase [3/2%], and pneumonitis [2/3%]
Entrectinib	NCT02097810	I/II	No	NA	NA	NA	Paresthesia [42%] Nausea [37%] Myalgia [34%] Asthenia [27%] Dysgeusia [27%] Vomiting [21%] Arthralgia [19%] Diarrhea [19%]
	NCT02568267	II	No	NA	NA	NA	NA

NR not reached, *NA* not yet assessed

harboring *ALK* translocation, previously treated with at least one platinum-based chemotherapy and who had experienced disease progression during crizotinib treatment as their last prior therapy [43]. With the primary endpoint to demonstrate antitumor activity, this study showed an ORR of 38.6%. Secondary endpoints included disease control rate (DCR) (77.1%) time to response (median 1.8 months), duration of response (median 9.7 months) and PFS (median 5.7 months). Treatment with ceritinib was well tolerated, and the majority of AEs (mainly grade 1 or 2) were gastrointestinal disorders (nausea 81.4%, diarrhea 80.0% and vomiting 62.9%). Grade 3–4 AEs occurred in 71.4% of patients, with 45.7% of them experienced grade 3–4 AEs suspected to be drug-related. Patient-reported outcomes showed a trend toward improvement in cancer-related symptoms and quality of life with ceritinib [43].

ASCEND-3 evaluated efficacy and safety of ceritinib in 124 ALK inhibitor-naïve *ALK*-rearranged NSCLC patients [44]. Patients were divided into two cohorts depending on the presence of BMs. Preliminary results showed an ORR of 63.7% (58% among patients with BMs and 67.6% in the cohort of patients without BMs), a whole-body DCR of 89.5% (86 and 91.9% in the two groups), a median DOR of 9.3 months (9.1 and 10.8 months, respectively, in the two cohorts) and a median PFS 11.1 months (10.8 vs. 11.1 months). The treatment was well tolerated with common AEs of gastrointestinal type and not severe (diarrhea G1–G2 [82.3%], nausea G1–G2 [74.2%]), vomiting G1–G2 [66.9%]). Only 7.3% of patients discontinued the treatment because of development of AEs [44].

ASCEND 4 is an open-label, phase III, multicenter study in which 376 untreated patients with advanced *ALK*-rearranged non-squamous NSCLC were randomized to ceritinib or standard chemotherapy. The primary outcome was PFS which was 16.6 months in the ALK inhibitor arm versus 8.1 months in the chemotherapy arm (HR 0.55, 95% CI 0.42–0.7, $P < 0.00001$). At the time of publication, the median OS was not reached in the ceritinib group against 26.2 months (27.8 to not estimable) in the chemotherapy group (HR 0.73, 95% CI 0.50–1.08]. The benefit of ceritinib was maintained in patients with or without baseline brain metastases; in fact, median PFS in the subset of patients with brain metastases was 10.•7 months in the ceritinib group versus 6.7 months in the chemotherapy group (HR 0.70, 95%

CI 0.44–1.12). The safety profile of ceritinib was consistent with that of ASCEND 1 and 2 studies, with principally G1–G2 gastrointestinal AEs [45].

The randomized, open-label, phase III trial ASCEND 5 compared the activity of ceritinib to standard chemotherapy, in patients with advanced *ALK*-rearranged patients who had received crizotinib and almost one line of previous chemotherapy and had successive disease progression. In this study, ceritinib was superior in terms of median PFS (5.4 vs. 1.6, $P < 0.0001$) and ORR (42.6 vs. 6%). No differences were found in median OS (18.1 vs. 20.1 months), presumably as a consequence of the high rate of crossover. Although virtually all the patients in the ceritinib arm experienced a GI side effect, they were predominantly of grade 1 or 2 [46]. To address the issue of the GI toxicity, which is commonly experienced by patients taking ceritinib, a phase I trial has been specifically designed. ASCEND 8 is a phase I study aimed to assess the systemic exposure, efficacy and safety of 450 mg ceritinib taken with a low-fat meal and 600 mg ceritinib taken with a low-fat meal as compared with that of 750 mg ceritinib taken in the fasted state in adult patients with *ALK*-positive metastatic NSCLC. The primary endpoint was to evaluate the plasma concentration of ceritinib and the pharmacokinetics parameters, including AUC0-24 h and Cmax, which were ultimately similar between the 450 mg fed and the 750 mg fasted arm. The median Tmax after multiple dose administration was comparable in all three arms. With regard to the safety profile, the 450 mg fed arm had a lower incidence of GI adverse events, mostly of grade 1–2, with no grade 3–4 events or drug discontinuation registered [47].

Ongoing clinical trials ASCEND 7 is a phase II, multicenter, open-label, five-arm study in which the efficacy and safety of oral ceritinib treatment will be assessed in patients with NSCLC metastatic to the brain and/or to leptomeninges harboring a confirmed *ALK* rearrangement by FDA-approved Vysis ALK Break Apart FISH Probe Kit (Abbott Molecular Inc.). The primary endpoint is ORR, whereas secondary endpoints include DCR, OIRR, IDCR, OS PSF and DOR. This study is currently recruiting patients, and the estimated primary completion date is April 2018 [NCT02336451].

Alectinib (CH5424802)

Alectinib is a benzo[b]carbazole derivative, orally available, potent (IC50 1.9 nM), and highly selective, ATP-competitive second-generation ALK TKI. Differently from crizotinib, alectinib does not inhibit MET and ROS1. However, it inhibits RET with a similar potency to ALK, which is five times higher than crizotinib [48–51]. Furthermore, alectinib's activity against LTK and GAK has also been reported [48].

Preclinical studies have revealed that alectinib is active against the gatekeeper mutation L1196M, along with other clinically relevant crizotinib-resistant mutations, including C1156Y, F1174L and G1269A [50, 52].

In clinical setting, alectinib's activity was first investigated in a multicenter, single-arm, open-label, phase I/II trial conducted in a crizotinib-naive Japanese population. In the phase 1 portion, 24 patients received alectinib at doses of 200 or 300 mg twice daily. Because no DLTs and grade 4 adverse events (AEs) were recorded to the highest dose, 300 mg twice daily was subsequently the phase 2 dose advised. In the phase 2 portion, 43 (93.5%) out of 46 patients treated with the recommended dose of 30 mg twice daily achieved an objective response, with 2 complete response (4.3%) and 41 partial responses (89.1%). Again, no grade 4 AEs not even treatment-related deaths were observed. However, grade 3 AEs occurred in 12 (26%) patients, with 5 experiencing serious AEs [51].

A second phase I/II study conducted in the USA evaluated 47 *ALK*-positive NSCLC patients who progressed on or following crizotinib. In the dose-escalation portion of this trial, DLTs were recorded in two patients receiving the highest dose of 900 mg twice daily, who developed grade 3 neutropenia and headache, respectively. Thus, the recommended phase II dose of alectinib was 600 mg twice daily, which showed good clinical activity with an ORR of 55%, including one CR (2%) and a DCR of 91%. Importantly, in this study alectinib showed impressive activity against BMs. In detail, 45% of patients enrolled had asymptomatic brain metastases or brain metastases with no need of intervening therapy and 17 of them had already received previous brain radiotherapy. As stated by independent radiological review, the intracranial overall response rate was reported in 52% with 6 patients (29%) experiencing complete responses, 5 (24%) partial responses, and 8 (38%) achieving tumor stabilization [52]. Both phase I studies showed that alectinib was well tolerated and no disease-limiting toxicities were reported.

Additionally, a second phase II study was conducted in the USA and Canada. In this trial, 87 patients with ALK-positive NSCLC who had progressed on or after crizotinib received alectinib 600 mg twice daily until disease progression or drug withdrawal. Consistently with previous data, updated analysis from this study showed an ORR of 52% with a median duration of response of 13.5 months in 67 out of 87 patients who had baseline measurable disease. The median PFS reported was 8.1 months, whereas estimated 1-year OS was 71%. Within this trial, 16 patients had measurable CNS lesions at baseline, and 11 had undergone brain radiotherapy. According to independent review committee, the overall intracranial response rate was 75% with an astounding CNS DCR of 100% and median duration of CNS response of 11.1 months. Among 52 patients with

measurable or non-measurable CNS disease, 40% experienced an objective response, which raised to 67% in the subgroup of radiotherapy-naive patients. In the overall population with CNS disease, the median DOR was 11.1 months and the DCR 89%. With regard to safety profile, alectinib was shown to be well tolerated with predominantly grade 1 or 2 toxicities, which consisted in constipation (36%), fatigue (33%), myalgia (24%) and peripheral edema (23%) [53].

In order to further assess the safety and efficacy of alectinib in advanced *ALK*-positive NSCLC, a global phase II study has been conducted more recently by Ou and colleagues. One hundred and thirty-eight patients were enrolled in this trial and received alectinib 600 mg twice daily. Among patients evaluable for response, the authors reported an ORR of 50%, and a median duration of response (DOR) of 11.2 months, which were in line with data from previous phase I/II studies. The median PFS was 8.9 months. Of note, in chemotherapy-naive patients, the median PFS reached 13.0 months [54]. Among 84 patients with brain metastases at baseline, the CNS DCR was 83%, with a median duration of response reaching 10.3 months. In the cohort of patients with baseline measurable CNS lesions, the intracranial response rate was 57%. Intriguingly, 10 (43%) patients out of 21 with baseline measurable or non-measurable CNS metastases and radiotherapy-naive achieved a complete CNS response. Beyond the astounding clinical activity, alectinib also showed a favorable safety profile, with most AEs of grade 1–2. The most common AEs reported were myalgia (17%), constipation (15%), fatigue (14%) and asthenia (11%). Only 8% of patients enrolled in the study permanently discontinued alectinib due to an AE [54].

On the heels of these promising results, two phase III studies have been designed with the aim to directly compare alectinib and crizotinib for advanced ALK-positive NSCLC.

The ALEX trial randomized 303 advanced *ALK*-positive NSCLC treatment-naïve patients to alectinib (600 mg twice daily) or crizotinib (250 mg twice daily). The primary endpoint was met with an investigator-assessed median PFS not reached in the alectinib arm at the data cutoff (95% CI 17.7 months to not estimable), compared to 11.1 months (95% CI 9.1–13.1) with crizotinib, with an HR for disease progression or death of 0.47 (95% CI 0.34–0.65). Of note, alectinib showed an impressive intracranial activity which was superior to crizotinib. The comparison between the safety profiles has shown a less gastrointestinal toxicity, referring to any grade adverse events, for the alectinib arm (nausea 14 vs. 48%, diarrhea 12 vs. 45%, vomiting 7 vs. 38%). Laboratory alterations of any grade as anemia (20 vs. 5%) or bilirubin elevation (15 vs. 1%) were more frequent with alectinib, like myalgia (16 vs. 2%), increased weight (10 vs. 0%) and photosensitivity (5 vs. 0%). Forty-one percentage of the patients experienced a grade 3–5 adverse events related to alectinib (versus 50% with crizotinib), more frequently a blood-test abnormality, and 16% needed to discontinue the cure (versus 25% with crizotinib) while 3% had a drug-related death (vs. 5% with crizotinib) [55]. J-ALEX is the second phase III study that compared alectinib and crizotinib in ALK TKI-naïve Japanese patients with advanced NSCLCs harboring *ALK* rearrangements. Again, this study met its primary endpoint with a significantly longer median PFS (not reached) for alectinib (95% CI 20.3 months-not estimated) compared with 10.2 months reported in crizotinib arm (95% CI 8.2–12.0) and a 66% reduction in the probability of progression or death (HR 0.34, 99% CI 0.17–0.70; $P < 0.0001$). Consistently with the ALEX trial, alectinib exhibited better safety profile than crizotinib with grade 3–4 AEs occurring in, respectively, 27 and 51% of patients. Certainly, this study presents some limitations, including an overestimation the HR due to the still immature data, the addition of an interim analysis after the 33% of required progression-free survival events, and lastly the utilization of a 300-mg twice daily dose for the Japanese population based on the safety and pharmacokinetics data from the phase 1 Japanese AF001JP trial [56]. Updated results are eagerly awaited and will provide new information of alectinib efficacy in this subset of patients.

Lorlatinib (PF-06463922)

Lorlatinib is a highly selective ALK and ROS1 inhibitor which has shown dose-dependent activity against all known crizotinib-resistant *ALK* mutations in preclinical studies [57]. In biochemical assays, lorlatinib inhibited the catalytic activity of recombinant human wild-type *ALK* with a mean Ki of < 0.07 nM. In addition, lorlatinib showed a range of mean Ki values of < 0.1 to 0.9 nM against the following crizotinib-resistant *ALK* mutants: L1196M, G1269A, 1151Tins, F1174L, C1156Y, L1152R and S1206Y. Besides, lorlatinib is tenfold more potent against wild-type *EML4–ALK* and 40-fold more potent against *EML4–ALK* L1196M compared with crizotinib in vitro [58]. Furthermore, in biochemical studies, lorlatinib also resulted more potent than ceritinib and alectinib against wild-type *ALK*. In addition to its high potency against *ALK*, lorlatinib has previously demonstrated sub-nanomolar cell potency against *ROS1* and demonstrated > 100-fold selectivity against nontarget kinases, relative to the ALKL1196M gatekeeper mutant, for > 95% of the 206 kinases tested [59]. In order to directly compare the potencies of lorlatinib, crizotinib, ceritinib and alectinib in cell assays, Zou and colleagues engineered NIH3T3 and Ba/F3 cells to express either wild-type or the crizotinib-resistant mutants 1151Tins, L1152R, C1156Y, L1196M, G1269A, G1202R, F1174L or S1206Y [60]. The authors showed that lorlatinib was the most potent inhibitor against all clinically relevant crizotinib-, ceritinib- and alectinib-resistant

ALK mutants. In the same study, lorlatinib showed strong ALK phosphorylation potency against the L1196M (IC50 = 15–43 nM) and G1269A (IC50 = 14–80 nM) *ALK* mutants, which are two of the most frequently detected crizotinib-resistant mutations observed in clinical practice [60, 61]. Moreover, lorlatinib demonstrated potent ALK phosphorylation activity against G1202R (IC50 = 77–113 nM) and the 1151Tins (IC50 = 38–50 nM) *ALK* mutants, that confer a high level of resistance to all second-generation ALK inhibitors [60]. Of note, the physicochemical properties of lorlatinib were specifically optimized to increase its CNS availability. Lorlatinib demonstrated 21–31% free brain drug exposure relative to free plasma concentration in non-tumor-bearing rats and dogs and is predicted to penetrate the intact blood–brain barrier (BBB) in humans [58]. Recently, Shaw and colleagues have reported the results of a multicenter, single-arm, first in human phase I trial that evaluated the safety, efficacy and pharmacokinetic of lorlatinib in patients with *ROS1-/ALK*-rearranged NSCLC. For *ALK*-positive patients, the proportion of patients who experienced an objective response was 19 (46%) of 41 patients, while for those who had received two or more TKIs, the proportion of patients with an objective response was 11 (42%) of 26 patients. Of the 41 *ALK*-positive patients, the estimated median PFS was 9.6 months. Noteworthy, median PFS was 13.5 months in the subset of patients who had received one previous ALK TKI, and 9.2 months for the 26 patients who had received two or more ALK TKIs. The most common treatment-related AEs among the 54 patients were hypercholesterolemia (39 [72%] of 54 patients), hypertriglyceridemia (21 [39%] of 54 patients), peripheral neuropathy (21 [39%] of 54 patients) and peripheral edema (21 [39%] of 54 patients). Patients were treated with a dose ranging from 10 to 200 mg, while the established phase II dose was 100 mg once daily, based on the safety profile seen across all doses and the expected plasma concentration predicted to inhibit the *ALK* Gly1202Arg mutation [62]. More recently, Solomon et al. presented the preliminary results from the phase II part of the trial in which the ORR was 69% in patients previously treated with crizotinib with or without chemotherapy. Importantly, the ORR was 33 and 39% in patients previously treated with a non-crizotinib ALK inhibitor with or without chemotherapy and in those previously treated with 2 or 3 ALK inhibitors with or without chemotherapy, respectively. No treatment-related deaths and a low (3%) rate of discontinuation due to AEs were reported in this study. The tolerability was consistent with that of the phase I trial and no novel safety concerns emerged [63].

Brigatinib (AP26113)

Brigatinib is a potent, orally available ALK inhibitor with an IC50 of 0.62 nM in cell-free assay. The drug exerts activity against crizotinib resistance mutations, including G1202R, and also against ROS1 (IC50 of 16–41 nM) [64, 65].

Moreover, this compound inhibits mutant EGFR, including T790M, making it a suitable therapeutic option for patients progressing to crizotinib because of the activation of EGFR pathway as mechanism of resistance [66].

Brigatinib is currently being evaluated in a phase I/II trial for advanced malignancies (n: 137), including *ALK*-mutated NSCLC (n: 79) [NCT01449461]. Among patients with advanced *ALK*-positive NSCLC with prior exposure to crizotinib, the ORR was 72% (51/71, including 44 confirmed responses), while for crizotinib-pretreated patients who received dosing regimens explored in phase II, 90 mg q.d., 90 mg q.d. for 7 days followed by 180 mg q.d. (90 → 180 mg q.d.), and 180 mg total daily, the ORR reached 77% (10/13, including 7 confirmed responses), 80% (20/25, including 19 confirmed responses) and 65% (15/23, including 14 confirmed responses), respectively. Median duration of response and median PFS were 11.2 months (95% CI 7.8 to not reached [NR]) and 13.2 months (95% CI 9.2–NR). All crizotinib-naive (n: 8) patients had confirmed objective responses, including three complete response, while median PFS was not reached. Importantly, in a post hoc analysis of patients with brain metastases at baseline, 53% had an intracranial objective response. The most common treatment-emergent adverse events (TEAEs) in ≥ 30% of patients were generally grade 1–2 and included nausea (53%), fatigue (43%), diarrhea (41%), headache (33%) and cough 31%. Serious TEAEs in ≥ 2% of patients were dyspnea (7%); pneumonia (7%); hypoxia (5%); pulmonary embolism (3%); pyrexia (2%); and 9% of all 137 patients discontinued due to an AE [67].

A phase II trial (ALTA) has terminated the enrollment of *ALK*-positive NSCLC patients pretreated with crizotinib, and preliminary outcome evaluation has been presented. Two hundred and twenty-two patients were randomized 1:1 to receive brigatinib at 90 mg q.d. (arm A) or 90 mg q.d. for 7 days followed by 180 mg q.d. (arm B). Investigator-assessed ORR in arm A was 46%, while ORR in arm B was 54%. Median PFS was, respectively, 8.8 and 11.1 months in arms A and B. The drug produced a clinical meaningful benefit in terms of responses and PFS with an acceptable safety profile. Most common grade ≥ 3 treatment AEs observed according to the dose schedule (A/B) were: increased CPK (3/8%), hypertension (4/5%), pneumonia (3/5%), rash (1/4%), increased lipase (3/2%) and pneumonitis (2/3%) [68].

Of note, a head-to-head comparison of crizotinib versus brigatinib is currently ongoing (NCT02737501, ALTA-1L trial), and brigatinib will be soon evaluated in sequential strategy after ceritinib or alectinib in a phase II study [NCT02706626].

Entrectinib (RXDX-101, NMS-E628)

Entrectinib is an orally available tyrosine kinase inhibitor that currently represents the best antagonist of NTRK1-3 fusion proteins, an emerging molecular target in NSCLC which is detectable in approximately 3% of patients [69, 70].

Entrectinib has been initially developed as an ALK inhibitor showing remarkable in vitro and in vivo activity in preclinical models also against L1196M and C1156Y crizotinib-resistant mutations [71]. Entrectinib also demonstrated antitumor activity against *TRK*-, *ROS1*- and *ALK*-driven xenograft models of different human cancers (*NPM–ALK*-driven lymphoma and *EML4–ALK*-driven NSCLC). Moreover, it has been reported to efficiently cross the blood–brain barrier in mice with intracranially injected NCIH2228 *EML4–ALK*-rearranged cells [71, 72]. A combined analysis of two phase I studies evaluating the safety and activity of entrectinib in patients with advanced solid tumors and harboring *NTRK1/2/3*, *ROS1* or *ALK* gene fusions has been recently published [73]. In this study, entrectinib was well tolerated, with predominantly grade 1 or 2 AEs that were reversible with dose modification. Of note, responses were observed in patients with TKI-naive NSCLC, colorectal cancer, mammary analog secretory carcinoma, melanoma and renal cell carcinoma. On this basis has been designed a phase II basket trial (STARTRK-2), with the aim of evaluating the efficacy of entrectinib in patients screened for *NTRK*, *ROS1* and *ALK* mutations by NGS [NCT02568267].

Mechanisms of resistance to second-generation ALK TKIs

Although second-generation ALK TKIs have been proven to be more potent and highly selective inhibitors, virtually all patients develop resistance to them.

G1202R and F1174C/V secondary mutations have increasingly been reported to emerge under selective pressure of ceritinib. Mechanistically, the G1202R substitution results in a steric hindrance that prevents the proper binding of ceritinib to the affected protein [41]. In addition, many other secondary mutations including C1156Y, 1152Tins, and L1152R, G1123S have also been demonstrated to induce resistance to ceritinib [74, 75]. Of note, although several studies have demonstrated that alectinib can overcome resistance to crizotinib and ceritinib in clinical setting, two resistant mutations, namely I1171T and V1180L, have been reported to emerge under the selective pressure of alectinib in vitro and in vivo [76]. Similarly to what has been observed with crizotinib and ceritinib, the emergence of the G1202R secondary mutation leads to resistance to alectinib as well. On the other hand, several point mutations including L1122 V, F1174V+ L1198F, S1206C and L1198F have been shown to confer resistance against brigatinib in ALCL cell lines [77] and in patients with advanced *ALK*-positive NSCLC [78]. It should be noticed that patients with acquired resistance to second-generation ALK inhibitors can be reversed by switching back to crizotinib or other ALK TKIs according to the mechanism of resistance. Of note, the G1202R confers resistance to all clinically available ALK TKIs, except for the third-generation ALK TKI lorlatinib.

Together, these data further underscore the primary importance of testing each patient at disease progression in order to provide the most effective treatment option on the basis of the molecular determinants of resistance.

Novel ALK inhibitors in clinical development

ASP3026 is selective, ATP-competitive, potent inhibitor of ALK and ROS1. In NSCLC xenograft models, ASP3026 oral administration resulted in complete responses and reached tumor drug concentration levels 100-fold higher than in plasma. Notably, ASP3026 also led to tumor regression in xenograft models harboring the crizotinib-resistant gatekeeper L1196M mutation, even though a threefold weaker inhibitory activity was reported compared to models without the L1196 mutation [79]. In the phase I study, the maximum-tolerated dose (MTD) was 525 mg/day, with the most frequent AEs reported as fatigue (44%), vomiting (39%), nausea (37%) and constipation (24%). Among 15 ALK+ NSCLC patients enrolled in the dose-escalation portion of the study, the ORR was 44%, with 50% achieving a SD. The median PFS was 5.9 months (95% CI 3.8–9.4 months), and eight patients were still on treatment at the time of the analysis [80]. However, in February 2015, Astellas Pharma stopped the developmental program of ASP3026 due to strategic reasons.

Belizatinib (TSR-011) is a novel inhibitor of ALK and TRK A/B/C and is currently being evaluated in a phase I/II trial [NCT02048488]. Preliminary results presented at ASCO 2015 showed the occurrence of dysesthesia and QTc prolongation as DLTs at 120 mg once daily schedule. However, fractionated dosing at 40 mg q8h was recommended to minimize peak exposure associated with QTc prolongation. At a total daily dose of 120 mg or more, responses occurred in 60% of ALK inhibitor-naive patients (3/5) and in 50% (3/6) who had progressed following crizotinib as the only ALK inhibitor previously received. Three patients who progressed after ceritinib/alectinib achieved SD as the best response. The most common grade 1–2 AEs included fatigue (17.4%), constipation (15.9%), QTc prolongation (15.9%), diarrhea (14.5%) and headache (13%). On the other hand, grade 3–4 treatment-related AEs were fatigue (5.8%), anemia (5.8%) and QTc prolongation (4.3%) [81].

X-376 and *X-396* are potent ALK inhibitors with less activity against MET compared with crizotinib in

biochemical- and cell-based assays. X-396 has also been documented to potently inhibit L1196M and C1156Y ALK mutations, which are commonly associated with acquired resistance to crizotinib. Moreover, preclinical data also indicate that X-396 might overcome pharmacokinetics resistance to crizotinib due to its favorable bioavailability within CNS [82]. In clinical setting, X-396 showed promising disease control in both crizotinib-naive ($n=3$) and crizotinib-resistant ($n=10$) *ALK*-rearranged NSCLCs in a phase I/II trial. Among 18 patients evaluable for response, 6 were *ALK*-positive and demonstrated an ORR of 83% and stable disease in 17% with median duration of follow-up of 20 weeks. Importantly, responses were recorded in both crizotinib-naive and in crizotinib-pretreated patients. Furthermore, 2 patients with brain metastases experienced also intracranial response, which is consistent with preclinical data indicating that X-396 can cross the BBB. Drug-related AEs in the whole population were almost exclusively of grade 1 or 2 and included rash (36%), fatigue (30%), nausea (27%), vomiting (27%), edema (20%) [83]. The expansion phase of this study in patients with *ALK*-positive NSCLC is ongoing, and results are awaited.

The phase III study of XALT3 (NCT02767804), designed to compare ensartinib and crizotinib in ALK TKI-naïve patients, is currently ongoing, and the estimated completion date is April 2020.

CEP-28122 is a novel and potent (IC50 = 1.9 nM) ALK inhibitor. Preclinical data indicate that CEP-28122 is high selectivity against *ALK* mutations among various types of tyrosine kinases, including c-MET and IGF-R1. Moreover, in NSCLC xenograft mice model oral administration of CEP-28122 resulted in sustained inhibition of tumor growth in NSCLC with complete or near-complete tumor regressions reported at a dose of 30 mg/kg [84]. *CEP-37440* is a dual ALK/focal adhesion kinase (FAK) inhibitor which is undergoing clinical development in a phase I trial in patients with advanced or metastatic solid tumors, including *ALK*-rearranged NSCLC [NCT01922752]. The study has recently been completed, but no data are available at the moment.

Tackling brain metastasis in *ALK*-positive NSCLC

Despite the impressive initial activity of crizotinib in patients with *ALK*-rearranged NSCLC, virtually all patients develop progressive disease. In addition to secondary mutation in *ALK* gene, tumor spread to the CNS represents another major determinant of acquired resistance to treatment, which in this case resides in poor penetration rates of crizotinib into the CNS [35, 36]. Nonetheless, a certain grade of crizotinib activity against brain metastasis (BMs) has been reported. A retrospective analysis of radiotherapy-naïve patients with asymptomatic brain metastases enrolled in PROFILE 1007 and 1005 showed an overall intracranial disease control rate (IDCR) at 12 weeks of 56%, whereas those with previously treated BMs achieved a IDCR of 62%. Notably, CNS appeared to be a sanctuary site, as 20% of patients with no BMs at baseline developed CNS disease, while those with pre-crizotinib BMs experienced brain progression in 71% of cases [35]. Consistently, a previous study reported that CNS was the first site of progression in 46% of patients prospectively followed on crizotinib, with 85% of them lacking concomitant extracranial progression [37].

Moving in this scenario, current research has recently focused attention on the development of next-generation ALK TKIs that can cross the BBB, in order to target BMs. As previously mentioned, ceritinib is a second-generation ALK TKIs which exerts activity against crizotinib-resistant tumors. Among 94 patients enrolled within ASCEND-1 study with retrospective confirmed brain metastases and at least one post-baseline MRI or CT tumor assessment, the intracranial disease control was reported in 79% of ALK inhibitor-naive patients and in 65% of patients pretreated with ALK TKIs. Among them, 11 had measurable brain lesions and had not received previous radiotherapy to the brain. Of them, six patients achieved a partial intracranial response [42]. In the ASCEND-2 study, intracranial responses were evaluated in 20 patients with investigator-assessed measurable brain lesions at study entry. Objective intracranial responses (OIRR) were observed in 45.0% with an IDCR of 80.0%. Of note, a pre-specified subgroup analysis of whole-body efficacy was performed in 100 patients with baseline CNS disease. Investigator-assessed ORR in patients with baseline BMs was 33.0%, the DCR was 74.0%, the median DOR was 9.2 months (95% CI 5.5–11.1 months), and the median PFS was 5.4 months [43]. Consistently, results from ASCEND-3 showed an IDCR of 80.0% (95% CI 44.4, 97.5) in 10 patients with measurable brain lesions. Notably, six patients with no prior brain radiotherapy had responses in the brain matching or exceeding the whole-body response [44]. A phase 2 study of ceritinib (ASCEND-7) for ALK-rearranged BMs and leptomeningeal disease (LM) that will collect CSF samples is currently ongoing and is expected to definitively address the intracranial penetration of this drug.

Alectinib is another second-generation ALK TKIs that demonstrated high levels of activity against brain metastases. Differently from crizotinib and ceritinib, alectinib is not a substrate of P-glycoprotein, and animal models of intracranial metastases revealed that this compound penetrates into the CNS with a brain-to-plasma concentration ratio of 0.63–0.94 at Tmax [85]. Clinically, a pooled analysis of two phase II studies evaluating the intracranial activity of alectinib in a crizotinib-pretreated population reported an OIRR of 64% in patients who had measurable

CNS disease, with a median IDOR of 11.1 months for all patients with measurable and/or non-measurable CNS disease [86]. Consistently, our group has recently presented a case series of eleven patients pretreated with ALK TKIs reporting an OIRR of 85.7% in seven patients with measurable CNS disease, with a median CNS-DOR of 8 months. Median CNS-PFS, and O-PFS were 8 months (95% CI 2–14), and 8 months (95% CI 3–13), respectively, whereas the median OS was 13 months (95% CI 7–19). Of note, two patients experiencing a brain response were assessed for alectinib's concentrations in serum and cerebrospinal fluid (CSF) and showed a low CSF to serum ratio, which ranged from 0.001 to 0.003 ng/mL, thus suggesting that measuring the concentrations of the drug in the CSF may not be a reliable surrogate of its distribution into the CNS [87]. These findings are in line with those recently reported by a large, global phase II study evaluating alectinib' activity in pretreated patients with advanced NSCLC. Among 84 patients with brain metastases at baseline, the IDCR was 83%, with a median duration of response of 10.3 months. Noteworthy, in the cohort of patients with baseline measurable CNS lesions the intracranial response rate was 57% [54]. Preliminary data regarding the intracranial efficacy of brigatinib have recently been published by Tiseo et al. in a combined analysis of data from the aforementioned phase I/II and phase II studies of brigatinib in advanced *ALK*-positive NSCLC. Sixty-three and 154% of patients of the phase I/II and II trial had brain metastasis at baseline. The confirmed OIRR in those with measurable lesions was 53% in phase I/II study and 42/67% in ALTA A/B, whereas the IDCR was 87 and 85/83%, respectively [88]. In addition to second-generation ALK TKIs, different next-generation ALK TKIs are under clinical evaluation. As detailed above, lorlatinib is a novel drug specifically designed to overcome both acquired and pharmacological resistance to either first- or second-generation ALK TKIs. Lorlatinib has the potential of crossing the BBB, and preliminary data from the phase II study have shown an impressive activity of lorlatinib against BMs [63].

The advent of next-generation ALK inhibitors is radically changing our approach to brain metastatic *ALK*-positive NSCLC as this subset of patients had traditionally been offered brain radiotherapy at first evidence of CNS involvement. To date, the use of crizotinib up-front in case of asymptomatic BMs has been proven to be efficacious and may delay brain radiotherapy. On the other hand, CNS represents a major site of disease progression in patients treated with crizotinib, often being the only site of relapse. Although radiotherapy maintains a pivotal role in this setting, sequential therapy with next-generation ALK TKIs that might overcome crizotinib limitation has already been reported to provide a re-response in the brain in crizotinib-pretreated patients, thus allowing a further delay of radiotherapy and its sequelae. Certainly, these data should be interpreted cautionary as clinical trials evaluating novel agents possess inherited discrepancy in terms of patient populations (crizotinib-naïve, crizotinib-pretreated) and study design. Besides, the temporal relationship with radiation therapy is still unclear and should be addressed in dedicated studies.

Conclusion

ALK inhibitors yielded a dramatic impact on clinical outcome of patients with *ALK*-rearranged NSCLC. Despite initial effectiveness, patients treated with crizotinib go through disease progression, usually within 1 year. As discussed above, a sequential treatment with a next-generation ALK TKIs at the time crizotinib failure seems to assure the best outcome in terms of responses, PFS and eventually OS, as novel ALK inhibitors might overcome the majority of crizotinib-resistant mutations also leading to a deeper inhibition of ALK kinase domain, with an ORR approaching 50% in crizotinib-pretreated patients [27]. Additionally, next-generation ALK TKIs have been reported to cross the BBB and produce intracranial responses in both patients with preexisting brain metastasis or those who develop CNS relapse during treatment with crizotinib [87]. Nonetheless, this approach could be questioned. In fact, crizotinib might be a precious alternative in later lines of treatment following treatment with next-generation ALK inhibitors such as alectinib or lorlatinib in case resistance to treatment depends, respectively, on the development of *ALK* L1198F secondary mutation and *MET* amplification. In order to answer the question whether crizotinib should be used in first or later lines of treatment following more potent ALK inhibitors that have the potential to induce deeper and prolonged response, different clinical trials have been designed and are currently ongoing. Among them, the ALEX trial has completed the accrual and preliminary results have been published. As discussed above, in this study alectinib was shown to prolong median PFS and the median time to CNS progression, with a better safety profile [55]. Although the OS data are still immature, based on the astounding results of the ALEX trial, alectinib can be now considered the new standard of care for patients with treatment-naïve *ALK*-positive NSCLC. In fact, the combined PFS of first-line crizotinib followed by a second-generation ALK TKI is similar if not inferior to the PFS we can achieve with up-front alectinib. Interestingly, brigatinib and lorlatinib are currently being compared with crizotinib as first-line therapy in patients with advanced ALK-positive NSCLC. These data are eagerly awaited and are expected to shine light on this corned yet unexplored.

In recent years, other approaches to overcome resistance to ALK-targeted therapy have been investigated. ALK protein is a client of heat shock protein 90 (Hsp90),

a molecular chaperone that functions to stabilize different proteins during folding in ATP-dependent way. ALK fusion variants are now established to be among the most susceptible proteins to Hsp90 inhibition, thus proving a rational for the use of Hsp90 inhibitors in *ALK*-positive NSCLC. To date, three Hsp90 inhibitors are in clinical trials for *ALK*-positive NSCLC, ganetespib, onalespib (AT13387) and NVP-AUY922. Although a full discussion of Hsp90 inhibitors in NSCLC is beyond the scope of this review, we would highlight that a phase 2 study (CHIARA) of single-agent ganetespib in *ALK*-positive NSCLC patients without prior ALK inhibitor therapy has recently completed accrual [NCT01562015], while preliminary results of a phase 1 study of ganetespib in combination with crizotinib in NSCLC have been reported at ASCO 2015 [89]. Among 12 ALK inhibitor-naive patients, no DLTs were reported, and partial response was seen in 67% of patients. Based on these encouraging data, further evaluation of this combination in patients not previously treated with crizotinib is warranted.

In the era of immunotherapy, whether checkpoint inhibitors might gain a role in the management of *ALK*-positive NSCLC is certainly a question that needs to be answered. At a preclinical level, enhanced expression of *EML4–ALK* fusion protein has been proven to increase PD-L1 expression, therefore providing an attractive rational for combination regimens of immunotherapy with ALK TKIs [90]. Consistently, treatment with alectinib has been reported to attenuate PD-L1 expression in *ALK*-rearranged NSCLC cells [90]. The combination between an immune-checkpoint inhibitor and a target therapy has been interestingly evaluated in the CheckMate 370 study [91], which was a five-cohort, phase I/II study investigating the safety and efficacy of nivolumab as maintenance after a first-line chemoregimen or as first line with other standard therapies. In particular, in the cohort E, 13 patients harboring an *ALK* translocation with an advanced or recurrent locally advanced disease were assigned to nivolumab plus crizotinib as upfront therapy. The primary endpoint of safety and tolerability was not satisfied, and the cohort was permanently closed for hepatic toxicities. In particular, five patients experienced a ≥ 3 grade liver toxicity, one death drug-related and another fatal event due a grade 4 liver failure. Similarly, 36 patients with metastatic *ALK*-positive NSCLC have been enrolled in a dose-escalation phase Ib study [NCT02393625] evaluating the combination of nivolumab plus ceritinib as first or second line. In the group 1, in which ceritinib was administered with low-fat meal at 450 mg/day, 4 patients discontinued for unacceptable toxicity, 2 for pancreatitis, 1 for lipase and transaminase increase, 1 for an autoimmune hepatitis. In the group 2, with a 300 mg/day dose of ceritinib, two patients discontinued the treatment due to ALT increase in grade 3. Overall, the most common grade ≥ 3 AEs were blood elevation of ALT (22%), GGT (17%), amylase (11%) and lipase (11%). [92]. Tough preliminary, emerging evidence indicates that a combination of immune-checkpoint inhibitors and ALK TKIs is still challenging in light of the increasingly safety concerns. However, these data are still immature, and further studies aimed to address this issue are required.

Expert commentary

Lung cancer has been considered for decades as a single disease. Nevertheless, the recent advancements in understanding the molecular mechanism underling the development of this deadly disease have led to the discovery of distinct disease genotypes, which exhibit exquisite responses to targeted therapies. Patients harboring *ALK* rearrangements have excellent sensitivity to the administration of ALK inhibitors, which translates in meaningful clinical benefit. To date, crizotinib is considered the most effective first-line option for this unique subset of patients. Unfortunately, as observed in patients with *EGFR*-mutant NSCLC, resistance to treatment occurs in virtually all patients. Available data indicate that next-generation ALK TKIs may overcome the mechanism of resistance to crizotinib, thus allowing patients to continue taking advantage from ALK inhibitors after experiencing resistance to crizotinib. However, several issues still lie ahead. In fact, not all mechanisms of resistance to crizotinib have been identified, and ALK non-dominant resistance cannot be overwhelmed with novel ALK TKIs. On the other hand, next-generation ALK TKIs have a different activity profile against *ALK* secondary mutation, making imperative to re-genotyping the disease at progression in order to administer the right TKI to the right patient. That having been said, it might appear reasonable to sequentially switch patients to next-generation ALK inhibitors upon documentation of disease progression to crizotinib. Although data are still lacking, a retrospective analysis conducted by our group on 69 patients with advanced *ALK*+ NSCLC showed that post-progression survival (PPS) significantly favored patients who were subsequently treated with a second ALK TKI (either ceritinib or alectinib) over those who transitioned to other systemic treatments but not versus those who were treated with a first ALK TKI beyond progression. This study suggests that sequential treatment with different ALK TKIs as well continuing ALK inhibitors beyond progression when clinical benefit prevails represents suitable options in treating patients with advanced *ALK*-rearranged NSCLC [93]. Similarly, Gainor and coworkers have recently shown that sequential treatment with crizotinib followed by ceritinib resulted in a median combined PFS of 17.4 months, which suggest a cumulative benefit with the addition of ceritinib. Of note, this effect was also observed among patients who directly transitioned from crizotinib to ceritinib (median combined PFS 17.0 months), indicating that these results are

unlikely due to a re-challenge effect [94]. Consistently with these findings, in a more recent multicenter retrospective analysis, Ito and colleague showed that sequential therapy with crizotinib and alectinib after crizotinib failure tended to provide a better OS benefit than therapy with alectinib alone in *ALK*-positive NSCLC patients [95]. Taken together, the aforementioned data suggest that a sequential strategy with different ALK TKIs might concretely improve the clinical outcome of patients with *ALK*-positive NSCLC. Certainly, data are limited and derive mostly from retrospectives analyses; thusly, larger prospective studies specifically aimed to address this issue are indispensable. On the other hand, the recently published ALEX trial has shown that alectinib is superior to crizotinib in terms of PFS as first-line treatment and might also potentially delay the development of CNS failure [52]. In light of these data, alectinib is gaining worldwide approval as up-front therapy advanced for *ALK*-positive NSCLC. Of note, also other next-generation ALK TKIs are being compared to crizotinib as up-front therapy in patients with newly diagnosed *ALK*-rearranged NSCLC.

However, as for crizotinib, resistance to treatment also occurs with novel ALK TKIs which further underscores the desperate need of a precise identification of the mechanisms underlying the development resistance. In this direction, several studies are evaluating the feasibility of detection of *ALK*-rearranged circulating tumor cells and circulating nucleic acids (either ctDNA or transcripts sequestered in platelets) with the first attempt to provide a noninvasive method for detecting and monitoring *ALK* rearrangements during the course of disease.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was not required for this study.

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