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Targeting *ALK*-positive non-small-cell lung cancer–novel inhibitors beyond crizotinib

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Abstract

ALK-positive Non-Small-Cell Lung Cancer (NSCLC) is a defined subgroup of lung cancer. Crizotinib was the first ALK-inhibitor introduced in clinical practice after two phase III trials demonstrated its superiority over chemotherapy both in second and in first line of treatment. Approximately within ten months patients developed acquired resistance to crizotinib and relapse. Second and third generation ALK-inhibitors are more potent molecules designed to overcome crizotinib resistance. Ceritinib, alectinib and brigatinib are approved by FDA as subsequent therapy in patients who have progressed after crizotinib. Lorlatinib and entrectinib are in different phases of clinical development. Moreover some of these agents are compared to crizotinib in first line setting to evaluate if an upfront more potent inhibitor could control disease longer than a sequential strategy. Despite the efficacy of second-generation ALK inhibitors, patients relapse. Each ALK-inhibitor is characterized by a distinct resistence profile with important clinical consequences in the choice of subsequent therapy.

Introduction

Unselected patients with metastatic Non-Small-Cell Lung Cancer (NSCLC) treated with conventional chemotherapy present a median survival of 10-12 months. According to the oncogene-addiction paradigm, the inhibition of molecular drivers by target agents could reduce tumor burden and improve patient survival [1,2]. Oncogenic *ALK* gene rearrangements are one of the several molecular alterations described in NSCLC especially in adenocarcinoma. Other molecular drivers are sensitizing *EGFR* gene mutations, *ROS1* gene rearrangements, *BRAF V600E* target mutations. Emerging biomarkers, for which targeted agents are under investgation, include *HER-2* mutations, *RET* gene rearrangements, high-level *MET* amplification or *MET* exon 14 skipping mutations (*MET*ex14) [3]. ALK is an insulin receptor tyrosine kinase with unclear physiologic functions. In humans, ALK expression is limited to the adult brain, no expression has been evidenced in normal lung tissue [4]. *ALK* gene rearrangements, occurring approximately in 4% of lung adenocarcinoma, define a distinct molecular subtype of NSCLC. The diagnosis of this gene alteration, although rare, offers patients the opportunity to receive highly effective target therapy [5]. Chromosomal rearrangements involving *ALK* gene were described in Non-small cell lung cancer (NSCLC), anaplastic large cell lymphoma (ALCL), and inflammatory myofibroblastic tumor (IMT). These rearrangements lead to the expression of *ALK* fusion genes in which the fusion partner mediates ligand-dependent oligomerization of *ALK*, resulting in constitutive ALK kinase activation [6-18]. In NSCLC the major fusion partner is echinoderm-microtubule-associated protein-like 4 (*EML4*), with the formation of the EML-ALK fusion protein. More than other 20 *ALK* fusion partners have been identified in lung cancer but the clinical significance of these fusion protein requires further investigations [7]. *ALK* rearrangements are mostly found in non-squamous lung histology, never- or light smokers and in younger patients. These clinical-pathologic features should not be utilized in selecting patients for testing [3]. Therefore *ALK* testing is recommended for patients with adenocarcinoma, for lung cancer of mix histology with an adenocarcinoma component, for limited specimens such as biopsy and cytology specimens where adenocarcinoma component cannot be completely excluded and for never-smokers who are younger than 50 years and have tumor of squamous histology [3,5-8]. *ALK* IHC assays are validated, standardized and cost-effective screening method to detect *ALK* rearrangement to select *ALK*-positive NSCLC . FISH can be use to confirm ALK positivity detected by an IHC assay. A practical cutoff value of 15% has been established to discriminate *ALK*-rearranged and *ALK* wild type NSCLCs [3,9-11].

Crizotinib

Crizotinib, a selected, first generation tyrosine kinase inhibitor (TKI) of *ALK*, *ROS1* and *MET*, was the first *ALK* inhibitor introduced into clinical practice. The first phase I clinical trial of crizotinib (PROFILE 1001) was initially designed to test the activity of crizotinib in patients with *MET* deregulation (*MET* amplification or *MET* mutation). A significant tumor shrinkage was observed in patients with *MET*-amplification and in patients with NSCLC harboring *ALK* rearrangement. So the protocol was amended to screen simultaneously patients for both *ALK* translocation and *MET* amplification. In *ALK*-positive NSCLC updated results showed a response rate (RR) of more than 60%, a median progression free survival (PFS) of 9 months [9,12]. Similar findings have been observed in the phase II study PROFILE 1005 [13]. Two phase III studies highlighted the advantage of crizotinib over standard chemotherapy [14,15]. In the PROFILE 1007 study, second-line crizotinib was compared to standard chemotherapy (pemetrexed or docetaxel) in patients with advanced *ALK*-positive NSCLC

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progressing after one prior platinum-based chemotherapy. Crizotinib was associated with a RR of 65% and a median PFS of 7.7 months while chemotherapy showed a RR of 20% and a median PFS of 3.0 months [14]. The PROFILE 1014 trial demonstrated improvement in PFS of crizotinib over standard platinum-based chemotherapy in first-line advanced non-squamous *ALK*-positive NSCLC. In the crizotinib group median PFS was 10.9 months, RR was 74%, in chemotherapy arm PFS was 7.0 and RR was 45% [15]. In both studies the improvement in PFS did not translated into an advantage in overall survival (OS) because of confounding effect of cross-over. Based on these data crizotinib was approved both as first-line and as subsequent therapy in patients with *ALK*-positive NSCLC [3].

Resistance to crizotinib

After a median time of 10 months, patients become refractory to crizotinib and relapse on therapy [2,3,5-17]. A deeper understanding of molecular processes of acquired crizotinib resistance results from analysis of post-progression biopsy specimens. Central nervous system (CNS) is the first site of progression in approximately 50% of patients, suggesting inadequate penetration into the CNS by crizotinib [18-20]. Mechanisms responsible of acquired resistance are target-dependent (50%), non target-dependent (30%) and unknown (20%) (Figure 1). The main mechanisms of acquired resistance are: genetic alteration of the drug-target (point mutation and/or gene amplification) and activation of bypass signaling with the activation of a parallel pathway obviating the need for the drug [17]. Target-dependent mechanisms preserve the domain of ALK signaling and can occur through mutations in the kinase domain. The most common *ALK* resistance mutations were the gatekeeper *L1196M* substitution (which is analogous to *T790M* in epidermal growth factor receptor) [17] and *G1269A*. These alterations were present in only 7% and 4% of all of the crizotinib-resistant specimens, respectively. The remaining *ALK* resistance mutations included: *C1156Y* (2%), *G1202R* (2%), *I1171T* (2%), *S1206Y* (2%), and *E1210K* (2%) [2,16] (Figure 2). Another mechanism of acquired resistance to crizotinib is an increase or amplification of the number of rearranged echinoderm microtubule-associated protein-like 4 *(EML)-ALK* genes. Therefore not all EML-ALK fusion proteins are inhibited by clinically achievable doses of crizotinib [2,17]. Non target dependent acquired resistance involved the activation of a parallel or bypass signaling pathways obviating the need for the original drug. Epidermal growth factor receptor (EGFR) activation, *KIT* amplification, *KRAS* mutations may mediate acquired crizotinib resistance [17] although their role is unclear [17]. *ALK*-rearranged-NSCLC treated with crizotinib might develop *KRAS* and *EGFR* mutated *ALK*-negative tumors [21]. Heat shock protein 90 (Hsp90) is a chaperone protein assisting other proteins in proper folding, stability and function

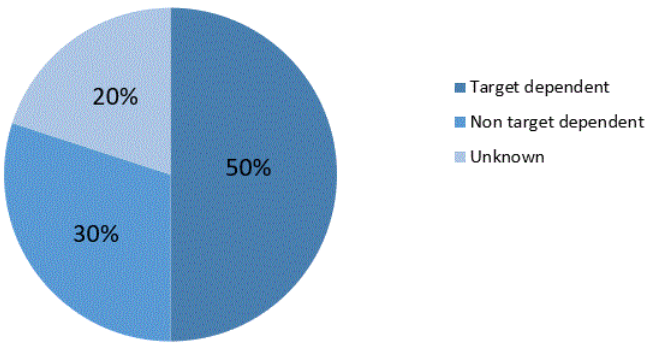

Figure 1. Mechanisms underlying acquired resistance to crizotinib [2]

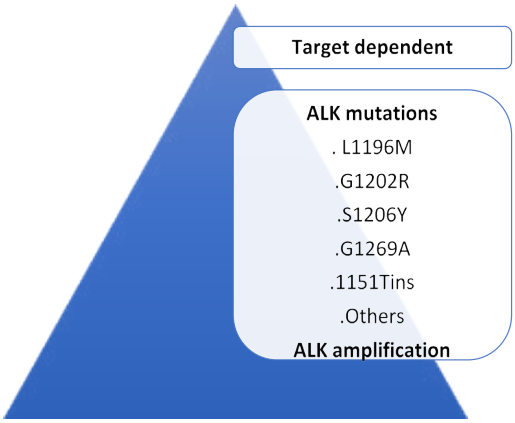

Figure 2. Target-dependent acquired resistance to crizotinib [2]

of several molecules some of which involved in tumor growth. ALK fusion proteins are Hsp 90 clients and Hsp90 inhibitors have shown activity against EML4-ALK in clinical and preclinical studies suggesting their use in crizotinib-resistance *ALK*-positive NSCLC [17,22-24]. Preclinical studies highlight that Epithelial-Mesenchymal Transition (EMT), which enhances cell motility and invasiveness [25], is associated with crizotinib resistance [26-28]. Transformation to Small-Cell Lung Cancer (SCLC) is a mechanism of target-independent acquired resistance in *EGFR*-mutation-positive NSCLC which is also rarely described in *ALK*-rearranged lung cancer [29-31]. Presumably in patients experiencing slow progression, in limited pre-existing sites, or in a single new site without worsening of clinical status, the main mechanism of resistance is ALK-dominant. In this condition the discontinuation of ALK inhibition is associated with the risk of disease flare [32,33]. Continuation of crizotinib beyond progression in association with local therapies (radiotherapy, local ablation or surgery against only the sites of disease progression) may extend disease control by more than 6 months [34]. In 120 patients enrolled in PROFILE 1001 and 1005 who continued crizotinib for >3 weeks post-RECIST progression, the median duration of crizotinib treatment beyond progression was 19.4 weeks and median OS from the time of first progression was significantly longer for patients continuing crizotinib compared with patients who stopped [35]. Patients selection is essential because subjects who benefited from continuing crizotinib have a good performance status, had achieved an objective response to crizotinib, and had a site of progressive disease that was manageable with local therapy [36]. Patients undergoing rapid radiological progression and impairing in clinical conditions have become completely refractory to crizotinib or addicted to another driver. Crizotinib should therefore be replaced by conventional chemotherapy or a next generation ALK inhibitor [35]. Primary resistance is the lack of response to ALK-inhibitors. Mechanisms underling primary resistance are not clearly defined [2].

Second generation ALK inhibitors

Second- and third-generation ALK-inhibitors, developed to overcome acquired crizotinib resistance, have been investigated in clinical trials both in crizotinib-refractory and in crizotinib-naïve settings.

Ceritinib

Ceritinib (LDK378) is a second generation *ALK* and *ROS1* inhibitor [37]. ASCEND-1 is a phase I study aimed to assess activity of ceritinib in both ALK inhibitor-pretreated and ALK inhibitor-

naive patients with ALK-rearranged NSCLC. An overall response was reported in 72% of 83 ALK inhibitor-naive patients and in 56% of 163 ALK inhibitor-pretreated patients. Median duration of response was 17.0 months in ALK inhibitor-naive patients and 8.3 months in ALK inhibitor-pretreated patients. Median progression-free survival was 18.4 months in ALK inhibitor-naive patients and 6.9 months in ALK inhibitor-pretreated patients [38]. Based on this study, ceritinib was approved by FDA in *ALK*-positive NSCLC who have progressed on crizotinib [3,38]. ASCEND-2 is a phase II study evaluated efficacy and safety of ceritinib in *ALK*-positive NSCLC previously treated with chemotherapy and crizotinib. Patients should have received cytotoxic therapy (1–3 lines, including 1 platinum doublet) and progressed on crizotinib as the last treatment prior to study entry. The overall response rate (ORR) by investigators was 38.6% (35.7% by blinded independent review committee), with a PFS of 5.7 months [39]. ASCEND-3 is a single arm phase II study of ceritinib in ALK-inhibitor naive patients in ALK-positive NSCLC. 98.4% of patients had received at least 1 line of prior chemotherapy and 25% of patients had received ≥3 prior antineoplastic regimens. Whole-body ORR of 63.7% (by blinded independent review committee of 58.9%), median PFS was 11.1 months with a median follow-up of 8.3 months [40].ASCEND-4 is phase 3 study in untreated patients with stage IIIB/IV *ALK*-rearranged non-squamous NSCLC randomized to receive ceritinib or platinum-based chemotherapy. Median progression-free survival (assessed by blinded independent review committee) was 16.6 months in the ceritinib group and 8.1 months in the chemotherapy group [41]. Based on this phase III trial FDA approved ceritinib as first-line therapy for patient with *ALK*-positive metastatic NSCLC [3]. ASCEND-5 is a phase III trial aimed to assess efficacy and safety of ceritinib versus single-agent chemotherapy (pemetrexed or docetaxel) in patients with advanced *ALK*-rearranged NSCLC who had previously progressed following crizotinib and platinum-based doublet chemotherapy. Patients treated with ceritinib showed a significant improvement in median progression-free survival compared with chemotherapy (5.4 months for ceritinib vs 1.6 months for chemotherapy [42].

Alectinib

Alectinib (CH5424802/RO5424802) is an oral TKI inhibitor of *ALK* and *RET* rearrangements which is now recommended as the preferred first-line therapy in patients with previously untreated advanced *ALK*-positive NSCLC based on results of ALEX and J-ALEX trial [3]. ALEX trial is a randomized, open-label, phase 3 trial comparing alectinib with crizotinib in patients with previously untreated, advanced *ALK*-positive NSCLC, including those with asymptomatic brain or leptomeningeal metastases. Investigator assessed progression-free survival was 68.4% with alectinib and 48.7% with crizotinib. The median progression-free survival with alectinib was not reached as compared with 11.1 months with crizotinib. Independent review committee–assessed progression-free survival was also significantly longer with alectinib than with crizotinib (median progression free survival was 25.7 months and 10.4 months respectively). The time to CNS progression was significantly longer with alectinib than with crizotinib in the intention-to-treat population: 12% in the alectinib group had an event of CNS progression, as compared with 45% in the crizotinib group. Median duration of response was not estimable with alectinib and 11.1 months with crizotinb. Among patients with measurable CNS lesions CNS response rate was 81% in alectinib group and 50% in crizotinib group. The median duration of intracranial response was 17.3 months for alectinib and 5.5 months for crizotinib. The 12-month survival rate was 84.3% with alectinib and 82.5% with crizotinib. Overall survival data are currently immature. Alectinib demonstrated an intersting safety profile: despite the longer duration of treatment with alectinib (median 17.9 months vs 10.7 months with crizotinib), grade 3 to 5 adverse events occurred in 41% of the patients treated with alectinib and 50% of the patients treated with crizotinib [43]. The results of this trial are supported by those of the J-ALEX trial involving Japanese patients with *ALK*-positive previously untreated advanced NSCLC. Median PFS had not yet been reached with alectinib versus 10.2 months with crizotinib. Grade 3 or 4 adverse events were less frequent with alectinib when compared with crizotinib [44]. Alectinib is also approved by FDA for *ALK*-positive NSCLC who have progressed on are intolerant to crizotinib [45]. Shaw et al. evaluated alectinib in 87 patients with *ALK*-positive crizotinib-resistant NSCLC. ORR was 48%, median Duration Of Response (DOR) was 13.5 months [46]. In 138 patients who have progressed on crizotinib Ou et al. demonstrated a response rate of 50% and a median duration of response of 11.2 months [47]. In both trials alectinib showed an impressive activity in control of central nervous system (CNS) disease and a good safety profile with most adverse events of grade 1 or 2 [46,47].

Brigatinib

Brigatinib (AP26113) is a potent and selective second generation *ALK* and *ROS1* inhibitor designed to overcome first generation crizotinib resistance. In preclinical models it showed activity against mutant form of *EGFR* [35]. Brigatinib displays superior in vitro and in vivo potency in NSCLC models compared with crizotinib: in *ALK*-positive cell lines and in xenograft mouse models brigatinib inhibited native *ALK* with 12-fold greater potency than crizotinib. Several secondary mutations at 11 different amino acid residues in *ALK*-sequence (*G1123*, *T1151*, *L1152*, *C1156*, *I1171*, *F1174*, *L1196*, *G1202*, *D1203*, *S1206*, and *G1269*), have been associated with clinical resistance to crizotinib and/or the second-generation ALK inhibitors ceritinib and alectinib. Brigatinib is a more potent inhibitor of native *EML4-ALK* than crizotinib, ceritinib, and alectinib and exhibits substantial activity against all secondary *ALK* mutations at clinically achievable concentrations. In vivo Brigatinib demonstrates antitumor activity against *L1196M*, the most common mutation associated with resistance to crizotinib, and *G1202R* which is associated with clinical resistance to crizotinib, ceritinib, and alectinib [48]. In April 2017, based on the results from ALTA trial, brigatinib received accelerated approval for the treatment of patients with *ALK*-positive metastatic NSCLC who have progressed on or are intolerant to crizotinib [3]. ALTA is a prospective, open-label, randomized, phase II trial assessing brigatinib efficacy and safety at 90 mg once daily (arm A) and 180 mg once daily with a 7-day lead-in at 90 mg (arm B) in 222 patients with crizotinib-refractory advanced ALK-positive NSCLC. Objective response rate (ORR) was 45% in arm A and 54% in arm B. In patients with measurable brain metastases the intracranical overall response rate was 42% and 67% respectively. Median progression-free survival was 9.2 months and 12.9 months in arm A and B respectively [49]. ALTA-1L study (ALK in lung cancer trial of brigatinib in first-line), is an ongoing phase III, randomized, open-label trial evaluating efficacy and safety of brigatinib versus crizotinib in *ALK*-positive locally advanced or metastatic NSCLC who have not previously been treated with an ALK inhibitor [50]. At first interim analysis progression-free survival was significantly longer in brigatinib than in crizotinib arm. At a median follow up of 11 months the median progression-free survival was not reached in brigatinib arm and 9.8 months in crizotinib arm. The one-year PFS was 67% in patients receiving brigatinib and 43% in patients receiving crizotinib. The objective response rate was 71% with brigatinib treatment compared to 60% with crizotinib. There was a clear

benefit for brigatinib across all prespecified patient subgroups including patients with brain metastases. In patients with measurable brain metastases at baseline the confirmed ORR was 78% with brigatinib and 29% with crizotinib [74].

Lorlatinib

Lorlatinib (PF-06463922) is a third generation, reversible, ATP-competitive inhibitor of *ALK* and *ROS1*. In the ALK tyrosine kinase domain lorlatinib selectively inhibits leucine at position 1195 (*L1195*). Lorlatinib has shown superior potency compared with clinically available ALK inhibitors and it also addresses the two major mechanisms of clinical relapse: *ALK* resistance mutations and brain metastasis [51]. In biochemical studies lorlatinib has proven to be more potent than crizotinib, ceritinib and alectinib against wild type *ALK*. It is also the most potent inhibitor against all clinically relevant crizotinib-, certinib- and alectinib-resistant *ALK* mutations [51]. It showed activity against *L1196M* and *G1269A*, two of the most frequently detected crizotinib-resistant mutations observed in clinical practice [51-53]. Moreover PF-06463922 inhibits *1151Tins* and *G1202R ALK* mutants that confer a high-level of resistance to all second generation ALK inhibitors [51,54,55]. Lorlatinib is the most potent and brain penetrable ALK-Inhibitor designed to efficiently penetrate the blood barrier brain. Crizotinib is active against brain metastases but CNS is a common site of relapse [18-20]. Probably resistance is related to the inability of the drug to achieve therapeutic concentration in CNS compartment because of its high efflux by P glycoprotein (PGP) [56]. Second generation ALK inhibitors have shown moderate CNS activity [57]. Ceritinb is a PGP substrate and has limited brain penetration [58], alectinib is not a PGP substrate [57]. Patients treated with either ceritinib and alectinb relapse with brain metastases. Therefore it was necessary to design a more potent inhibitor which is not a substrate of PGP, which efficently penetrates the blood brain barrier with an increased CNS availability [51]. Patient with metastatic *ALK*-rearranged lung cancer received multiple ALK inhibitors during treatment course, including first-, second-, and third-generation inhibitors. Shaw et al. described resensitization to crizotinib after acquired resistance to lorlatinib. A patient with *ALK*-rearranged lung cancer, developed acquired resistance to crizotinib because of the substitution of cysteine by tyrosine at amino acid residue 1156 (*C1156Y*) in the ALK kinase domain. The disease did not respond to second generation ALK-inhibitors, but it responds to lorlatinib. When the tumor relapsed, sequencing of the resistant tumor revealed an ALK *L1198F* mutation in addition to the *C1156Y* mutation. The *L1198F* substitution confers resistance to lorlatinib but enhances binding to crizotinib resensitizing resistant cancers to crizotinib, a less potent and less selective first-generation inhibitor [59]. In a first-in man, dose-escalation phase I study, lorlatinib showed both systemic and intracranial activity in patients with advanced *ALK*-positive or *ROS1*-positive NSCLC, most of whom had CNS metastases and had previously been treated with two or more TKIs [60]. Lorlatinib might be an effective therapeutic strategy for patients with *ALK*-positive NSCLC who have become resistant to currently available TKIs, including second-generation ALK TKIs. A phase 3 trial is ongoing to to demonstrate whether lorlatinib is superior to crizotinib in advanced, treatment-naive, *ALK*-positive NSCLC [61].

Entrectinib

Entrectinib (RXDX-101) is a small molecule which inhibits tropomyosin-related kinase (TRK) TRKA, TRKB, TRKC, *ROS1* and *ALK* rearrangements. Clinical activity of entrectinib has been assessed in 4 clinical trials [62-64]. Combined results derive from two phase 1 basket trials (ALKA-372-001 and STARTRK-1) conducted in 119 patients with advanced solid tumor harbouring a recurrent gene fusion involving *NTRK1/2/3*, *ROS1*, or *ALK*. Entrectinib demonstrated interesting antitumor activity in TKI-naïve patients harboring gene rearrangements involving *NTRK*, *ROS1*, or *ALK* genes. Clinical benefits were observed abroad a range of solid tumors regardless of histology, particularly in patients with *NTRK*-rearranged tumors. No responses were observed in patients with recurrent gene rearrangements previously treated with ROS1 or ALK inhibitors. Further investigation will be required to determine the activity of this drug in TKI pre-treated patients considering that RXDX-101 has shown preclinical activity against potential resistance mutations such as the *ALK L1196M* mutation that can emerge after crizotinib therapy in ALK-rearranged lung cancers. Entrectinib proved interesting intracranial activity against both metastatic disease and primary brain tumors [62]. STARTRK-2 is an ongoing phase 2 basket trial of entrectinib for the treatment of patients with solid tumors that harbor an NTRK1/2/3, *ROS1*, or *ALK* gene fusion designed to confirm the results of STARTRK-1 and ALKA [62,63]. The STARTRK-NG trial is a Phase 1/1b study of entrectinib in pediatric patients with cancer, including primary brain tumors, neuroblastoma, and other non-neuroblastoma, extracranial solid tumors harboring *NTRK*, *ROS1*, or *ALK* gene fusions [64].

Resistance to second generation ALK inhibitors

Despite the efficacy of second-generation ALK inhibitors, patients invariably relapse. While only 20% of ALK-positive patients developed *ALK* resistance mutations on crizotinib, almost 56% of patients progressing on second-generation ALK inhibitor developed *ALK* resistance mutations (ceritinib 54%, alectinib 53%, and brigatinib 71%) reflecting the greater potency and selectivity of these agents compared with crizotinib (Table 1) [65]. *ALK G1202R* is the most common *ALK* resistance mutation after treatment with second-generation ALK inhibitor. The spectrum of other *ALK* resistance mutations differs across agents. Each ALK inhibitor is associated with a distinct range of *ALK* resistance mutations which provides differential sensitivities to second-generation ALK inhibitors with important clinical implications. Resistance profiles may evolve over time and in response to sequential ALK inhibitors, particular *ALK* resistance mutations inform the choice of subsequent ALK targeted therapies, especially after failure of two ALK inhibitors. Lorlatinib has shown to be active against all single *ALK* resistance mutations and was the only ALK inhibitor with significant activity against *ALK G1202R*. Compound resistance mutations are principally described in patients who had received multiple ALK

Table 1. Frequence of *ALK*-resistance mutations after first and second generation ALK-inhibitors [66]

***ALK* Resistance Mutations**	**Crizotinib (N=55)**	**Ceritinib (N=24)**	**Alectinib (N=17)**	**Brigatinib (N=7)**
1151Tins	*2%*	0%	0%	0%
C1156Y	*2%*	8%	0%	0%
I1171T/N/S	*2%*	4%	12%	0%
F1174L/C	*0%*	17%	0%	0%
V1180L	*0%*	4%	6%	0%
L1196M	*7%*	8%	6%	0%
G1202R	*2%*	21%	29%	43%
G1202del	*0%*	8%	0%	0%
D1203N	*0%*	4%	0%	14%
S1206Y/C	*2%*	0%	0%	14%
E1210K	*2%*	0%	0%	29%
G1269A	*4%*	0%	0%	0%
ALK Mutations	**20%**	**54%**	**53%**	**71%**

inhibitors, suggesting that their development may be facilitate by sequential use of ALK inhibitors. Compound resistance mutations in ALK-positive NSCLC are analogous to drug resistant *T790M/C797S* described following sequential treatment with first- and third-generation EGFR inhibitors in *EGFR*-mutant NSCLC.

Clinical strategies

Based on the results of clinical trials and FDA approval, alectinib is the preferred therapy for untreated patients with *ALK*-positive metastatic NSCLC. Crizotinib and ceritinib are also recommended in first-line setting [3]. In the phase III trial comparing crizotinib with chemotherapy (pemetrexed or docetaxel) as second line treatment, patients who continued crizotinb beyond disease progression showed a median duration of further treatment of 16 weeks (range 3-73 weeks) [5]. In a retrospective analysis of two single-arm trials, patients with disease progression who still obtain clinical benefits from crizotinib and who were allowed to continue treatment, showed a significantly longer overall survival than those who stopped the drug (16.4 vs. 3.9 months) [36]. Based on this results patients with asymptomatic progression can continue crizotinb. Patients experiencing "oligo-progressive disease" (progression in a single site or in up to five sites) can continue crizotinib in association with local therapy (surgery and/or ablative treatments) [3]. The availability of second- and third-generation ALK-inhibitors has extended the therapeutic options both for patients experiencing disease progression on crizotinib and for crizotinib-naive patients. Subsequent treatments for patients who progress on first-line crizotinib include alectinib or ceritinib or brigatinib. Similarly to crizotinib, patients with asymptomatic progression or oligoprogression on alectinib or ceritinib can continue the drug in association with local therapy. Cytotoxic therapy can be proposed to patients with systemic progression in multiple sites on crizotinib, alectinib or ceritinib [3] (Figures 3-5).

Toxicity profile of ALK inhibitors

ALK-inhibitors demonstrated an overall interesting toxicity and safety profile. Regarding alectinib most adverse events were grade 1–2, the most frequent were constipation (36%), fatigue (33%), peripheral edema (25%) and myalgia (21%). Grade 3–4 events were mainly asymptomatic laboratory abnormalities: elevated gamma-GT, neutropenia, and hypophosphataemia [43,47]. The most common all-grade toxicity of ceritinib was diarrhea and nausea, reported in approximately 80% of patients. The most common grade 3–4 events were laboratory abnormalities: increased alanine and aspartate aminotransferase [38,40]. With regards to brigatinib in ALTA trial the most common any-grade adverse events included nausea (arm A/B, 33%/40%), diarrhea (arm A/B, 19%/38%), headache (arm A/B, 28%/27%). A subset of pulmonary adverse events (AE) caracterized by early onset (median time to onset 2 days, range 1 to 9 days), dyspnea, hypoxia, cough, pneumonia or pneumonitis occurred in 6% of patients. These AEs, occurring at 90 mg in boths arms without further events after escalation to 180 mg, requires early diagnoses and treatment [49]. As reguard to lorlatinib, in phase I clinical trial the most common treatment-related adverse events among the 54 enrolled patients were hypercholesterolaemia (72%), hypertriglyceridaemia (39%), peripheral neuropathy (39%), and peripheral oedema (39%). The most common

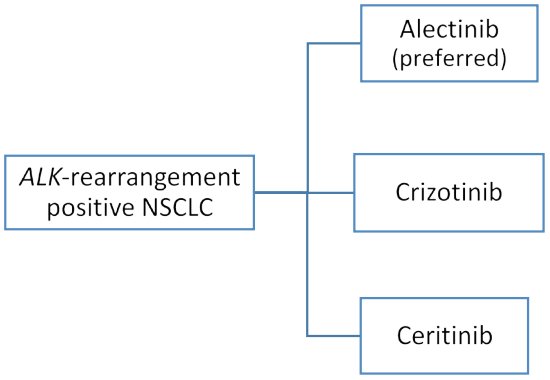

Figure 3. *ALK*-rearrangement positive NSCLC: first-line therapy [3]

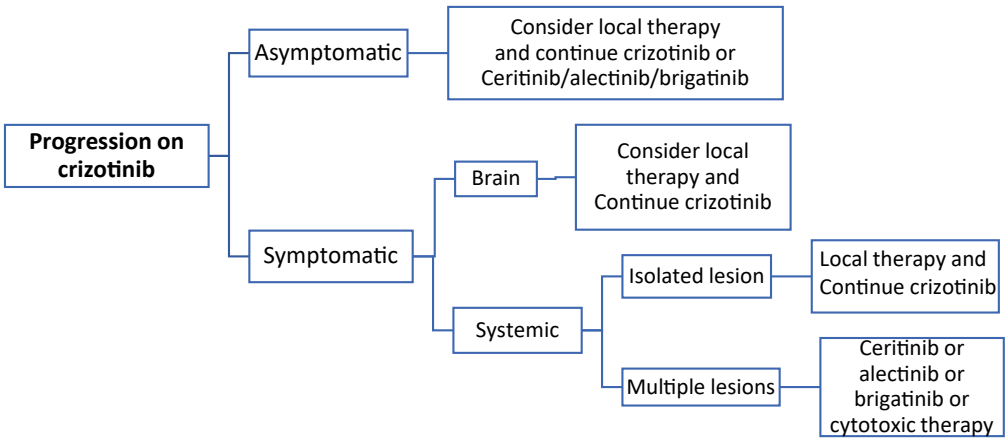

Figure 4. *ALK*-rearrangement positive NSCLC: subsequent therapy [3]

grade 3-4 adverse event was hypercholesterolaemia. Neurologic and psychiatric disorders and peripheral neuropathy, occurring in 28% to 36% of patients, represent grade 1-2 toxicity but require rapid identification and management [60]. With regard to entrectinib, in the phase I study ALKA-372-001, the most common all-grade toxicity was paresthesia (42%), while asthenia represented the dose-limiting toxicity and muscular weakness the most common grade 3-4 events [62]. In the STARTRK-1 trial fatigue (33%) was the most common all-grade toxicity, neutropenia (11%), fatigue (7%) and cognitive impairment (7%) represented the most frequent grade 3–4 events [62].

Target agents and immune check-point inhibitors

Both in oncogene-addicted and molecularly unselected advanced NSCLC, mutational load impacts on tumor immunogenicity. Tumor cell death induced by chemotherapy and target agents produces the release of neoantigen triggering the immune response [66]. Clinical trials evaluating combination strategies with target agents in association with immune check-points inhibitors, including PD-1/PD-L1 and CTLA-4 inhibitors, are ongoing despite available data from CheckMate 057 [67] and KEYNOTE 010 [68] showed statistically significant shorter PFS and borderline lower ORR in *EGFR*-mutant/*ALK*-positive patients who are generally non-smokers [69] (lower mutational load and lower immunogenecity has been observed in never-smoker population). Early results from combination trial in selected TKI-naive or TKI-pretreated *EGFR*- or *ALK*-mutated NSCLC showed increased anti tumor responses although in some cases safety questions [70]. Enrollment in a Phase 1/2 study of nivolumab plus crizotinib in previously untreated *ALK*-positive NSCLC (CheckMate 370) was closed and combination treatment was discontinued due to observed grade ≥3 hepatic toxicities [71]. In a phase 1 dose escalation study in previously treated (ALK inhibitor or chemotherapy) or untreated IIIB/IV *ALK*-positive NSCLC, the combination of nivolumab plus ceritinib showed an interesting activity but a significant toxicities [72]. JAVELIN Lung 101 is a phase 1b/2 dose-finding trial evaluating avelumab plus crizotinib or aveumab plus lorlatinib in patients with advanced *ALK*-negative/wildtype NSCLC or *ALK*-positive NSCLC, respectively. The combination of avelumab and lorlatinib showed an acceptable safety profile, distinct from avelumab and crizotinib, and promising antitumor activity in patients with *ALK*-positive NSCLC. This combination will be evaluated in treatment-naive patients in phase 2 trial [73] (Table 2).

Conclusions

Acquired resistance is the main concern of targeted-therapies in oncogene-addicted NSCLC. Even in *ALK*-rearranged NSCLC identification of mechanisms of acquired resistance, which can be

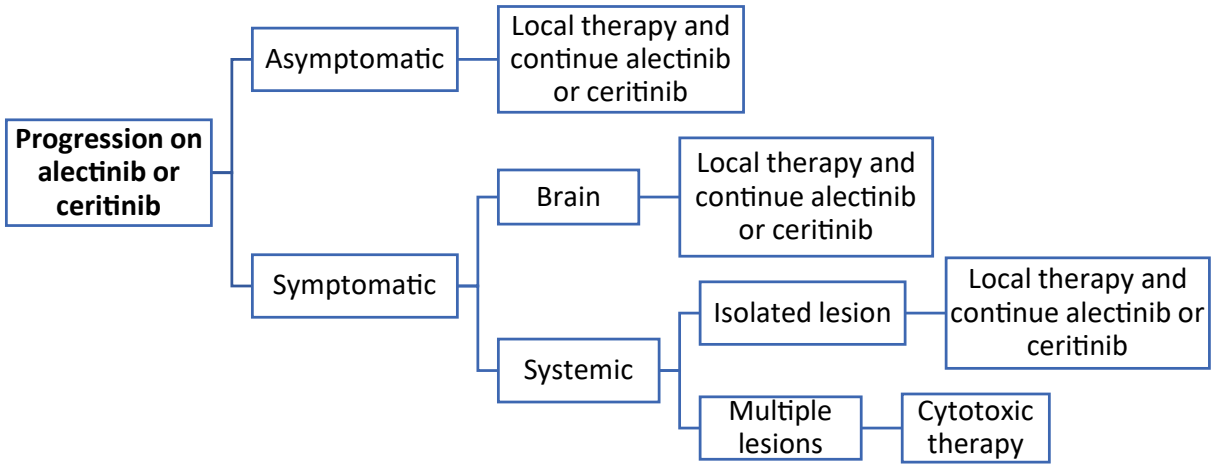

Figure 5. *ALK*-rearrangement positive NSCLC: subsequent therapy [3]

Table 2. Clinical trials of immune checkpoint inhibitors in combination with ALK TKIs in advanced NSCLC [72]

Clinical trial	Phase	Setting	Intervention	Status
NCT02574078/ CheckMate 370	I/II	Newly diagnosed/maintenance LA/ stage IV NSCLC	Nivolumab+erlotinib (group D)/crizotinib (group E)	Ongoing, not recruiting for group E
NCT01998126	I	Stages II–IV TKI-naïve or TKI-treated for less than 6 months EGFR- or ALKmutated NSCLC	Nivolumab/ipilimumab+ erlotinib/crizotinib	Ongoing, not recruiting
NCT02511184	I	Newly diagnosed LA/stage IV ALKpositive non-squamous NSCLC	Pembrolizumab +crizotinib	Recruiting
NCT02013219	I	LA/stage IV TKI-naïve EGFR-mutated and treatment-naïve ALK-positive NSCLC	Atezolizumab+erlotinib/ alectinib	Ongoing, not recruiting
NCT02584634/ Javelin Lung 101	Ib/II	LA/stage IV pretreated ALK-negative (group A) or ALK-positive (group B) NSCLC	Avelumab+crizotinib (group A)/lorlatinib (group B)	Recruiting
NCT02898116	I/II	Stage IV ALK rearranged NSCLC	Durvalumab+ensartinib	Recruiting
NCT01998126	I	Stages II–IV TKI-naïve or TKI-treated for less than 6 months EGFR- or ALKmutated NSCLC	Ipilimumab+erlotinib/crizotinib	Ongoing, not recruiting

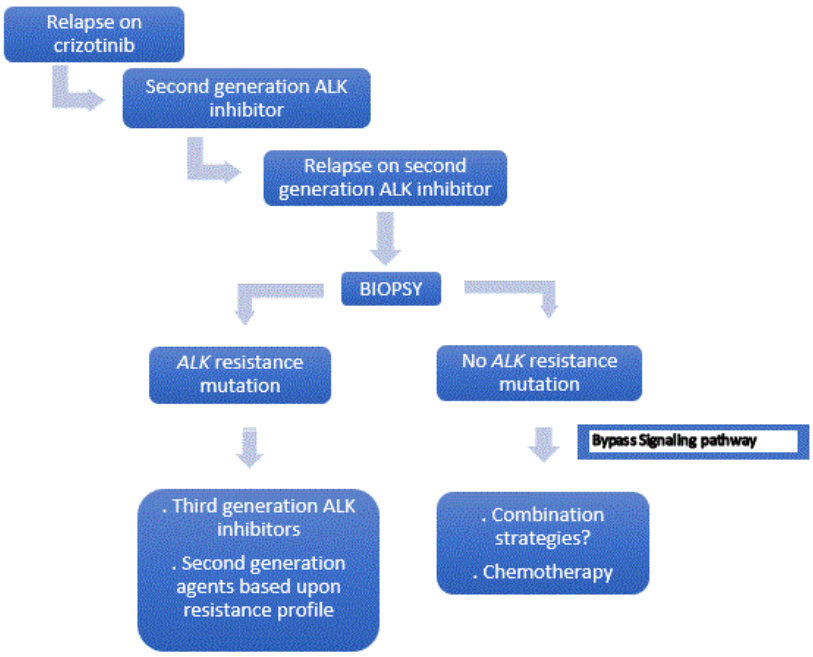

Figure 6. New paradigms and new opportunities of therapy [66]

target-dependent or target-independent, will be essential to design the best treatment strategy in each patient. Repeating biopsies after progression on TKIs will play a crucial role in treatment algorithm. Terapeutic strategies for non-target dependent acquired resistance still remain an open issue. New paradigms, including combination treatment and association with immunotherapy, are under investigation to identify new opportunities of therapy (Figure 6). The introduction of novel, more potent compound in untreated *ALK*-positive NSCLC highlights the need to develop studies evaluating sequences of targeted therapies versus upfront next-generation molecules to define which strategy could offer the longer control of disease.

Conflicts of interest

The authors have no conflicts of interest to declare.

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