ALK inhibitors and advanced non-small cell lung cancer (Review)

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Abstract. Treatment of unselected patients with advanced non-small cell lung cancer (NSCLC) receiving third-generation platinum-based chemotherapy has reached a plateau of effectiveness. Histology and molecular analyses are the cornerstone in the initial diagnosis of NSCLC and are key determinants to address the appropriate strategy of treatment. In non-squamous histology the combination of cisplatin plus pemetrexed or carboplatin plus paclitaxel plus bevacizumab are considered today the best regimens yielding better activity and efficacy. Epidermal growth factor receptor (*EGFR*) tyrosine kinase inhibitors (TKIs), such as gefitinib, erlotinib or afatinib are the standard-of-care for patients with advanced NSCLC harbouring activating *EGFR* mutations. The identification of anaplastic lymphoma kinase (*ALK*) rearrangements in 2-5% of NSCLC patients led to the rapid clinical development of its oral TKI, crizotinib, also targeting the proto-oncogene *MET* and *ROS1*. The results reported from the first phase III trial showed superiority of crizotinib compared with standard chemotherapy in second-line treatment of *ALK*-positive NSCLC, which was recently approved in several countries in this setting. Unfortunately, after initial activity of crizotinib, patients will ultimately develop acquired resistances within 1 or 2 years of therapy. A second generation of ALK inhibitors, such as LDK378, alectinib and AP26113 may represent a promising treatment approach: they are under investigation with very promising early results. This review discusses *ALK* rearrangements, the clinical development and use of crizotinib, and other ALK-TKIs in advanced NSCLC.

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1. Introduction

The outcome for unselected patients with advanced non-small cell lung cancer (NSCLC) remains dismal with third-generation platinum-based chemotherapy as the cornerstone of treatment. However, the more recent successful development of therapies targeting NSCLC histotype or oncogenic drivers led to survival improvements in selected populations (1).

In first-line treatment of advanced non-squamous NSCLC, cisplatin plus pemetrexed is considered the best chemotherapeutic regimen (2). Bevacizumab, a pure humanized anti-vascular endothelial growth factor (VEGF) monoclonal antibody (mAb), when combined with carboplatin plus paclitaxel, improved the survival compared with chemotherapy alone, as first-line therapy for non-squamous NSCLC patients (3). The identification of somatic mutations in the epidermal growth factor receptor (*EGFR*) gene in a subset of patients with NSCLC led to the treatment of these patients with EGFR tyrosine kinase inhibitors (TKIs), such as gefitinib, erlotinib or afatinib. Several randomized phase III trials, addressed to this subset of patients, showed a superior response rate, prolonged progression-free survival (PFS), and improved quality of life (QoL) of EGFR-TKIs when compared with platinum-based chemotherapy. Although no overall survival (OS) advantage was demonstrated, because all the trials suffered of a high post-progression treatment cross-over which inevitably undermined the results, global OS was not observed in NSCLC before the introduction of EGFR-TKIs in the treatment of *EGFR* mutant patients. Unfortunately, both *de novo* and acquired resistance to targeted therapies limit the duration of their clinical benefit (4).

Recently, the anaplastic lymphoma kinase (*ALK*) gene fusion emerged as an important biomarker for selecting a further subgroup of NSCLC patients to be treated with corresponding inhibitors. The state-of-the-art of this approach and its future developments are discussed here.

2. ALK gene alterations in NSCLC

ALK is a transmembrane receptor tyrosine kinase member of the insulin receptor superfamily. Chromosomal rearrangement of the *ALK* gene and its activation, generally takes place through the position of one of several different 5' fusion partners and their associated promoter region upstream of the kinase domain of *ALK*, inducing its transcription and protein expression. Approximately 2-5% of patients with NSCLC have tumours with an inversion in the short arm of chromosome 2 [inv (2)(p21p23)] which results in the fusion of exons 1-13 of the echinoderm microtubule-associated protein-like 4 (EML4) gene with exons 20-29 of the *ALK* gene, leading to the production of an EML4-ALK fusion tyrosine kinase which is involved in cell proliferation, differentiation and anti-apoptosis (5). Thus, the EML4-ALK protein contains the intracellular catalytic domain of ALK and the amino-terminal half of EML4 including the hydrophobic echinoderm microtubule-associated protein-like protein (HELP), which is critical for dimerization of EML4-ALK, resulting in an aberrant constitutive activity of downstream signalling such as Akt, STAT3, and extracellular signal regulated kinase 1 and 2 (ERK1/2) (6-8). Other less-frequent fusion partners of *ALK*, such as TFG and KIF5B, have since been reported (9,10). A higher probability to detect *ALK* translocations seems to be associated with specific clinical NSCLC patients characteristics including never or light smoking history, young age and adenocarcinoma histology with signet rings (11-13).

ALK translocations typically occur independently of *EGFR* or *KRAS* mutations, although they are not mutually exclusive, and predict for a poor response to EGFR-TKIs, less responsiveness to platinum-based chemotherapy, and a lower OS in patients with advanced NSCLC (13-16). Recently, thousands of NSCLC patients were analyzed for biomarkers characteristics by two platforms: the French and the Lung Cancer Mutation Consortium. In both series, some *ALK*-positive specimens were found positive also for either an *EGFR* or *KRAS* mutation (17,18). In a phase III randomized trial comparing erlotinib versus platinum-based chemotherapy as first-line treatment of patients with advanced NSCLC harbouring activating *EGFR* mutations, 15.8% of patients were found to have also the *EML4-ALK* translocation. Despite this, no negative impact on outcome in terms of PFS, primary objective of the trial, was found, with erlotinib scoring statistically better than chemotherapy (19,20). Overall, patients with activating *EGFR* mutations and previous objective response to EGFR-TKIs, should not be excluded from *ALK* screening even though a difference to EGFR-TKI susceptibility was not observed in the EURTAC trial (20).

ALK translocation diagnostic tools. The major recommendations, by the International Association for the Study of Lung Cancer (IASLC), are to use testing for *ALK* fusions to guide patient selection for therapy with an ALK inhibitor in all patients with advanced-stage adenocarcinoma, regardless of sex, race, smoking history or other clinical risk factors (21). Of importance is the method which should be used for *ALK* testing. To date, the fluorescent *in situ* hybridization (FISH) assay using dual-labelled break-apart probes for selecting patients for ALK-TKI therapy is the diagnostic gold standard approved also by FDA (21). This methodology was used in the initial studies that demonstrated improved clinical response of patients with *ALK*-rearranged tumours to treatment with crizotinib, an ALK-TKI (12,13,22). As reported by this test, the indicative cut-off of an ALK rearrangement is the observation of >15% split nuclei (22). *ALK* immunohistochemistry (IHC) being an easier and cheaper diagnostic tool is currently under investigation to be considered as a pre-screening methodology to select specimens for ALK FISH testing (21). Until now, many reports demonstrated a strong correlation between ALK IHC expression and ALK FISH positivity or negativity (23-27); however, further confirmation is needed to definitely include the ALK IHC detection as the initial step in the algorithm for clinical ALK testing in NSCLC. Reverse transcriptase polymerase chain reaction (RT-PCR) is not currently recommended as a first-line diagnostic method for determining *ALK* fusion status. A higher failure rate of a ribonucleic acid (RNA)-based assay in routine formalin fixed paraffin-embedded (FFPE) pathology material might affect sensitivity, with the risk of false-negatives, due to variability in the *EML4-ALK* fusion structure and to the existence of other *ALK* fusion partners (21).

3. Crizotinib clinical development

Crizotinib (PF-02341066) is a first-in-class oral adenosine triphosphate (ATP)-competitive selective inhibitor of the ALK and MET tyrosine kinases with additional ROS1 and RON kinase inhibitory activity (28,29). In 2006 began the first-in-man phase I crizotinib study with a dose escalation undertaken in 37 patients with advanced solid tumours. The escalating doses of crizotinib were from 50 mg once daily to 300 mg twice daily, using a standard dose-escalation design. Dose-limiting fatigue in the cohort receiving 300 mg twice daily led to the establishment of a regimen of 250 mg twice daily in a 28-day cycle as the recommended phase II study dose. This part of the trial was followed by protocol-defined patient prescreening for evidence of *ALK* or *MET* activation in specific tumour types. The discovery of *ALK* gene rearrangements in NSCLC and the promising results reported in two patients with *ALK*-positive NSCLC led, in 2008, to expand the cohort of *ALK*-positive NSCLC to enrol (30,31). The objective response rate from the first 19 evaluable patients with *ALK*-positive NSCLC and who had been heavily pretreated was 53% (30). These interesting data were subsequently confirmed in the first 82 patients in which an objective response rate of 57% was reached with a 33% of stable disease. Treatment was well tolerated with grade 1 or 2 gastrointestinal side effects the most frequent adverse events, mild visual disturbances were reported in 41% of cases, and grade 3-4 transaminases elevation in 6% of patients. All toxicities reversed on cessation of crizotinib (31). An expanded cohort of patients with *ALK* translocated NSCLC was enrolled within the PROFILE 1001 trial. In a total of 149 patients, prevalently never smokers

with adenocarcinoma histology and a median age of 52 years, crizotinib confirmed a noticeable efficacy, with an objective response rate of 60.8%, including three complete and 84 partial responses. The median time to response was 7.9 weeks with a median duration of response of 49.1 weeks independent of age, sex, performance status (PS) or line of treatment. The stable disease was achieved in 21.7% of patients for a disease control rate of 82.5%. Twenty-four patients (16%) were any-therapy-naïve and reported a better median PFS which was 18.3 months versus 9.2 months achieved by the patients who received crizotinib as second-line or later treatment. Median PFS of the entire group was 10 months with an estimated 1-year OS of 75%. Also in this larger number of patients treated with crizotinib, side effects were mainly grade 1 and 2 and recovered after stopping crizotinib treatment (32). Median OS was not reached; thus, a retrospective analysis was performed in order to estimate the possible median OS. Patients with *ALK*-positive NSCLC and treated with crizotinib (crizotinib group) were compared with both *ALK*-positive, crizotinib-naïve patients (*ALK*-positive controls) and those without *ALK* rearrangement (*ALK*-negative controls, screened also for *EGFR* mutational status and included only if EGFR was wild-type). The results were very interesting, as in fact, among the 82 patients of the crizotinib group, median OS was not reached; 1- and 2-year OS were 74 and 54%, respectively, independent of age, sex, smoking history or ethnic origin. Thirty *ALK*-positive patients received crizotinib in the second- or third-line setting and reported a median OS significantly longer than that showed by the 23 *ALK*-positive controls receiving any second-line therapy (median OS not reached versus 6 months), with 1-year OS of 70% versus 44%, and 2-year OS of 55 versus 12% [hazard ratio (HR) 0.36, 95% confidence interval (CI) 0.17-0.75; p=0.004], respectively. A further comparison was performed between 36 crizotinib-naive, *ALK*-positive controls versus 253 wild-type controls, lacking *ALK* or *EGFR* alterations, with a median OS of 20 versus 15 months (p=0.244), respectively. Moreover, 56 *ALK*-positive patients treated with crizotinib had similar OS to 63 *EGFR*-positive patients treated with EGFR-TKIs with a median OS not reached versus 24 months, 1-year OS 71 versus 74%, and 2-year OS 57 versus 52%, respectively (33). Overall, these retrospective data are very interesting but should be interpreted with caution because deriving from indirect comparisons with potential biases.

PROFILE 1005 is a phase II trial of *ALK*-rearranged NSCLC designed as an open-label, single-arm study to evaluate the efficacy and safety of crizotinib in patients who had failed more than two lines of chemotherapy (34). PROFILE 1005 was a compendium trial for patients who were randomized to and progressed on the chemotherapy arm of the second-line randomized trial PROFILE 1007 which did not allow patients on the chemotherapy arm to crossover to crizotinib after disease progression (35). Instead, these patients could be enrolled into PROFILE 1005. A total of 901 patients received crizotinib and the first 261 patients were considered to be the mature population. Among all 901 patients, 15% discontinued treatment due to adverse events and 10% had a dose reduction due to toxicity. The most frequent side effects, mostly grade 1-2, were vision disorder (54%), nausea (51%), diarrhoea (44%), vomiting (44%) and constipation (37%). In the mature population, the objective response rate was 60% with a disease control rate at 6 weeks of 86% and at 12 weeks of 75% with a median PFS of 8.1 months (34).

PROFILE 1007 compared crizotinib at the dose of 250 mg, orally, twice daily to chemotherapy, docetaxel or pemetrexed, in 347 advanced *ALK*-positive NSCLC previously treated with platinum-based chemotherapy. The primary endpoint was median PFS which was statistically better for the crizotinib arm with 7.7 months compared with the chemotherapy arm with 3.0 months (HR 0.49, 95% CI 0.37-0.64; p<0.001). An impressive objective response rate in the second-line setting was observed with crizotinib (65 versus 20% in the chemotherapy arm; p<0.0001). As expected, at the first interim analysis, no OS difference was reported between the two arms, with a median OS of 20.3 months for the crizotinib group versus 22.8 months for the chemotherapy group, respectively (HR 1.02, 95% CI 0.68-1.54; p=0.54). The significant crossover of the study influenced inevitably these results however, the data are still immature. Crizotinib common adverse events, mostly grade 1-2, were visual disorder, gastrointestinal side effects, and elevated liver aminotransferase levels, which were grade 3-4 in 16% of cases. Common adverse events with chemotherapy were fatigue, alopecia and dyspnea. Patients on crizotinib reported improved QoL, with time to deterioration in lung cancer symptoms significantly longer with crizotinib than with chemotherapy, with medians of 5.6 and 1.4 months (HR 0.54, 95% CI 0.40-0.71; p<0.0001), respectively (35) (Table I).

Recently, crizotinib was shown to decrease testosterone in male patients (36). This issue was confirmed also in a larger sample of patients. In fact, the mean total testosterone levels, compared between 32 crizotinib-treated patients and 19 non-crizotinib-treated patients, was statistically lower in those receiving crizotinib (p=0.0012). Most of patients with low total levels had symptoms of androgen deficiency, such as fatigue, depression, sexual dysfunction, which improved with testosterone supplementation. The mechanism is not known, but it is interesting to note that *MET* and *ALK* are both expressed in testes (37). When treating patients with new biologic agents, oncologists must face with the possibility of new toxicities, thus it is important to have a degree of adverse event monitoring above the 'standard'.

PROFILE 1014 is an ongoing phase III randomized trial comparing, as first-line therapy, crizotinib to platinum/pemetrexed in *ALK*-positive non-squamous NSCLC patients. Crossover is allowed at progression and PFS is the primary endpoint (38).

After a rapid clinical development period and based on these results, crizotinib was approved almost worldwide for the use in previously treated *ALK*-rearranged advanced or metastatic NSCLC.

4. Crizotinib resistance

Inevitably, after an initial dramatic response to crizotinib, *ALK*-positive patients developed an acquired resistance to the treatment as with other TKIs. The onset of the resistance is on average within 1 or 2 years of TKI therapy. Mechanisms of acquired drug resistance might be classified into 2 main categories: the appearance of either a mutation or amplification

Table I. Results from PROFILE studies in previously pretreated *ALK*-positive NSCLC patients.

Study	Phase of study	Treatment	No. pts	ORR (%)	PFS (months)	Toxicity
PROFILE 1001 (32)	I	Crizotinib	149[a]	60.8	10	Grade 3-4 neutropenia (n=9), raised alanine aminotransferase (n=6), hypophosphataemia (n=6), and lymphopenia (n=6)
PROFILE 1005 (34)	II	Crizotinib	261[b]	60	8.1	15% discontinued treatment due to adverse events and 10% had a dose reduction due to toxicity[c]
PROFILE 1007 (35)	III	Crizotinib vs docetaxel or pemetrexed	173 174	65 20	7.7 3.0	Grade 3-4 treatment related toxicities were 33% versus 32%, serious adverse events were 12% versus 14%, adverse events leading to permanent discontinuation of the study drug were 6% versus 10%, respectively

[a]16% were therapy-naive; [b]mature population; [c]among 901 enrolled patients. NSCLC, non-small cell lung cancer; no. pts, number of patients; ORR, objective response rate; PFS, progression-free survival.

which alter the target gene; activation of alternative signalling pathways with tumour cells losing their dependency on the *ALK*-signalling pathway (Table II).

ALK mutation or amplification. The first evidence of mechanisms of resistance to crizotinib were described for a 28-year-old man, never smoker with advanced pretreated *EGFR* wild-type and *ALK*-positive lung adenocarcinoma who relapsed after crizotinib therapy and was biopsied at progression. In this patient, two secondary point mutations (L1196M and C1156Y) within the kinase domain of the *EML4-ALK* were described, each mutation developing independently in subclones of the tumour and conferring marked resistance to two different ALK inhibitors (39). Furthermore, several reports showed that in approximately one-third of relapsing patients, the crizotinib resistance is mediated by the appearance of mutations located in the ALK TK domain. The most commonly identified resistance mutation is the 'gatekeeper mutation' L1196M which hinders crizotinib binding at its active site on ALK kinase (40-45). Some series reported that the amplification of the *ALK* fusion gene with or without concurrent *ALK* mutation leads to drug resistance (43,44).

New pathway activation. One of the potential new pathway activation, which might mediate crizotinib resistance is that of *EGFR* signalling. In this setting, a double inhibition, of *ALK* and *EGFR*, was effective (40,43). Activation of *KRAS* might represent another resistance mechanism in *ALK*-positive tumours due to the detection of new *KRAS* mutations in *ALK*-positive NSCLC resistant to crizotinib (43).

An analysis performed on 16 *ALK*-positive NSCLC patients treated with crizotinib and re-biopsied at progression, suggested that crizotinib-resistant patients might be subdivided into two main groups. The *ALK* dominant group, which represents approximately 50% of cases and includes *ALK* kinase mutation (31%) and *ALK* fusion gene copy number gain (13%), and the *ALK* non-dominant group, which represents the other half, including emergence of alternate *EGFR* or *KRAS* mutations (31%) and in 19% of cases in which the oncogene remained unknown and could rely on KIT, EGFR or HER-2 variants (46).

Table II. Main mechanisms of acquired crizotinib resistance (46).

NSCLC still addicted to *ALK* signalling (50%)	
	Secondary *ALK* mutations (main L1196M and C1156Y) (31%)
	ALK copy number gain (13%)
	Both (6%)
Activation of alternative signalling pathways (50%)	
	EGFR mutations (19%)
	KRAS mutations (12%)
	Unknown (including possible KIT, EGFR or HER-2 variants (19%)

5. Therapeutic strategies to overcome crizotinib resistance

The knowledge of some mechanisms of resistance to crizotinib led to the development of new treatment strategies which are currently in clinical phase of investigation. Retrospective case series reported that in the presence of an oligo-progression crizotinib can be continued or resumed after local ablative therapy and still result in prolonged PFS (47). On the other hand, patients with significant and symptomatic progression during crizotinib treatment need an immediate change of therapy.

Second-generation ALK inhibitors. If the crizotinib resistance is mediated by *ALK* mutation or amplification it means that the NSCLC is still addicted to *ALK* signalling: this is the case

Table III. Clinical results of main second-generation ALK inhibitors in advanced NSCLC.

Characteristics	LDK378 (48)	Alectinib (50)	Alectinib (51)
Phase of study	I	I/II	I
Escalating oral dose	≤750 mg/day	≤300 mg BID	≤900 mg BID
No. of patients	114	46	47
Crizotinib-naive	35	46	0
Crizotinib-resistant	79	0	47
ORR (%)	58	93.5	54.5
PFS (months)	8.6	Not yet reached	Not yet reached
Grade 3-4 toxicity (%)	Diarrhoea (8) Transaminase increase (10-19)	Serious (11)	GGT, neutrophil, hypophosphatemia in 2 pts each
Recommended oral dose	750 mg/day	300 mg BID	600 mg BID

NSCLC, non-small cell lung cancer; ORR, objective response rate; PFS, progression-free survival; BID, bis in die; GGT; γ-glutamyl transferase.

in which a second generation ALK inhibitors might represent a promising treatment approach. These new *ALK* inhibitors are structurally distinct from crizotinib and with the capability to inhibit also secondary acquired mutations (40,43). Among the most promising next generation *ALK* inhibitors, LDK378 is active in conventional *ALK*-positive tumours and in those expressing the mutation C1156Y. In a dose-escalating phase I single-arm study, the maximum tolerated dose, safety, pharmacokinetics and preliminary antitumour activity of LDK378 were investigated in *ALK*-positive solid tumours of any type. A total of 114 patients had *ALK*-positive NSCLC, 78 patients taking LDK378 at 750 mg/day and 36 additional patients taking 400-750 mg/day. All 114 patients were evaluable for response, with 79 patients progressing during or following treatment with crizotinib, and 35 patients were crizotinib-naïve. The objective response rate was 60% in the 78 patients who received LDK378 at 750 mg/day. In the 114 patients treated with LDK378 at doses ≥400 mg/day, the objective response rate was 58%, with a median duration of response of 8.2 months and a median PFS of 8.6 months. The most frequent adverse events were nausea (73%), diarrhoea (72%), vomiting (58%) and fatigue (41%). The most frequent grade 3-4 adverse events were increased alanine aminotransferase (19%), increased aspartate aminotransferase (10%) and diarrhoea (8%) (48). These very impressive results led to the design of further trials which are actively recruiting worldwide. Among these a phase II multicenter, open label, single-arm study is evaluating the efficacy and safety of oral LDK378 750 mg once-daily in patients with ALK-positive advanced NSCLC who received 1-3 lines of therapy (including 1 platinum doublet) and progressed on crizotinib as the last therapy prior to study entry. The study was designed to enrol 137 patients (49).

Alectinib (CH5424802) was a potent, selective, ALK inhibitor with 10-fold more potency than crizotinib and effectives against most of the mutations of the ALK domain. A multicentre, single-arm, open-label, phase I/II study, *ALK*-rearranged inhibitor-naïve advanced NSCLC patients received alectinib orally twice daily by dose escalation. In the phase I study, 24 patients were treated with 20-300 mg twice daily. No dose limiting toxicities or grade 4 adverse events were reported, even with the highest dose. Thus, 300 mg twice daily was the recommended dose for the phase II study. A total of 46 patients were treated with the recommended dose, with an objective response rate of 93.5% including two complete responses (4.3%). Grade 3 treatment-related adverse events were reported in 12 (26%) patients, serious side effects occurred in 5 patients (11%). Median PFS has not been reached yet, since 40 of the 46 patients in the phase II portion remain on treatment (50). Another phase I study enrolled 47 *ALK*-positive NSCLC patients who failed crizotinib and chemotherapy. No dose limiting toxicities were observed up to the highest dose tested (900 mg twice daily). Grade 3-4 adverse events included γ-glutamyl transferase increase (n=2), neutrophil decrease (n=2), hypophosphatemia (n=2), hyperglycemia, syncope, renal failure and pericardial effusion (n=1 each), but no grade 3 nausea, vomit, diarrhoea, oedema were reported. The objective response rate was 54.5% across all dose cohorts for all patients, all partial responses, with a median duration on treatment >4 months. Alectinib at the oral dose of 600 mg twice daily was chosen as recommended phase II dose based on pharmacokinetics, efficacy and tolerability (51). In both trials, the promising antitumour activity of alectinib was observed in *ALK*-positive patients including also those who failed crizotinib. Therefore, a global single-arm phase II study of alectinib in crizotinib-resistant *ALK*-positive NSCLC patients is ongoing (52) (Table III).

AP26113 is a novel dual ALK-/EGFR-TKI that potently inhibits mutated forms including the ALK L1196M and EGFR T790M mutations. A phase I/II open-label, multicenter study enrolled 44 patients with advanced solid tumours, including 37 patients with NSCLC, refractory to available therapies or for whom no standard treatment exists. AP26113 was administered with escalating once-daily oral doses. Most common 3-4 treatment-related adverse event was diarrhoea, reported in 5% of cases. Two dose limiting toxicities were observed at 240 mg (grade 3 alanine aminotransferase increase) and at 300 mg (grade 4 dyspnea). Thus, doses <300 mg are

being explored further in the ongoing phase II expansion, which includes 4 cohorts: *ALK*-positive NSCLC naïve or resistant to prior ALK-targeted therapy, *EGFR* mutated NSCLC resistant to EGFR-targeted therapy, other cancers with abnormalities in *ALK* or other AP26113 targets (53).

Heat-shock protein 90 inhibitors. ALK fusion proteins, including those with resistance mutations, are known heat shock protein 90 (HSP90) clients. HSP90 inhibitors bind in the ATP-binding pocket of the enzyme, and prevent it from regulating the activation and stability of its client proteins, including *ALK*. Thus, inhibition of HSP90 resulted in reduction of the expression of *EML4-ALK* through proteasome-mediated degradation (41,54). Unfortunately, to date no clinical trial has been conducted to investigate HSP90 inhibitors specifically addressed to patients with *ALK*-positive NSCLC, but retrospective analysis showed promising results of HSP90 inhibitors in this setting (55-57). Ganetespib (STA-9090) an HSP90 inhibitor, was administered as monotherapy at the dose of 200 mg/m^2 weekly, for 3 weeks with a one-week rest, to 99 patients heavily pretreated for advanced NSCLC. Patients were subdivided in 3 cohorts: *EGFR*-mutated, *KRAS*-mutated, no *EGFR*- or *KRAS*-mutated. In this last cohort, 4 partial responses were reported, and all in *ALK*-positive crizotinib-naïve patients. However, *ALK* gene rearrangements were retrospectively detected by FISH in 1 case or PCR-based assays in the other 3 cases. The most common adverse events reported in all patients were diarrhoea, fatigue, nausea and anorexia (55). Based on these earlier results, to evaluate whether or not this approach will be effective the CHIARA trial (CHaperone Inhibition in ALK Rearranged lung cAncer) is ongoing. In this phase II trial, ganetespib monotherapy is administered to previously treated patients with stage IIIB/IV NSCLC harbouring an *ALK* gene rearrangement and who have not been previously treated with a direct ALK inhibitor. Primary endpoint is objective response rate with approximately 100 patients planned to be enrolled (58). To mitigate the development of acquired resistance, a dual inhibition of critical signalling pathways is being investigated. A phase I/II study is evaluating the combination of crizotinib and ganetespib in previously treated patients with NSCLC *ALK*-positive not pretreated with any specific inhibitor, with the primary endpoint to define the maximum tolerated dose to be investigated in the subsequent phase II trial (59). This trial should give more information on whether this approach is more effective than either therapy alone, and whether dual treatment prevents development of acquired resistance.

Another HSP90 inhibitor is retaspimycin hydrochloride (IPI-504), which was investigated in a phase II trial at the starting dose of 400 mg/m^2 on days 1, 4, 8 and 11 of a 21-day cycle and then at the dose of 225 mg/m^2 due to hepatotoxicities observed at the highest dose in another trial. A total of 76 patients with advanced NSCLC and heavily pretreated including a line with EGFR-TKI were enrolled. Among these patients, 3 were *ALK*-positive, two had partial responses and the third had prolonged stable disease (7.2 months, 24% reduction in tumour size). The most common grades 1 and 2 adverse events included fatigue, nausea and diarrhoea. Grade ≥3 hepatotoxicities were observed in nine patients (11.8%) (56).

AUY922, is a highly potent, non-geldanamycin, HSP90 inhibitor which was tested in a phase II study including also *ALK*-positive NSCLC patients. AUY922 was administered at the weekly dose of 70 mg/m^2 to 121 previously treated NSCLC patients. In the total of 22 *ALK*-positive patients, 7 objective responses (32%) were reported, 3 of which among the 14 crizotinib-resistant NSCLC patients; the disease control rate was 59% (100% in the crizotinib-naive group and 36% in the crizotinib-resistant group). The most frequent adverse events, mainly grade 1-2, were eye disorders (77%), diarrhoea (74%) and nausea (46%) (57). AUY922 is currently being investigated in *ALK*-positive patients in two trials. A phase II study is evaluating AUY922 in *ALK*-positive patients resistant to an ALK-TKI therapy with the objective response rate as primary endpoint and the estimated enrolment of 20 patients (60). LDK378 and AUY922 were administered within a phase Ib study to patients with *ALK*-positive NSCLC already pretreated with crizotinib. The primary endpoint is the incidence rate of dose limiting toxicities with the estimated enrolment of 142 patients (61).

6. Special topics

Further interesting considerations arose during crizotinib biological and clinical investigations.

Thymidylate synthase and pemetrexed. In a retrospective analysis, the efficacy of pemetrexed in terms of PFS was evaluated in 89 advanced NSCLC studying the relationship with specific molecular subtype. Patients with *ALK* gene rearrangements had a longer median PFS on pemetrexed than on *KRAS* mutant, *EGFR* mutant, or triple-negative patients (9 months compared with 7, 5.5 and 4 months, respectively). The data were confirmed also by the multivariate analysis in which the only statistically significant variable associated with prolonged PFS on pemetrexed was *ALK*-positivity (HR 0.36; p=0.0051) (62). Another retrospective analysis confirmed the previous data. Fifteen *ALK*-positive patients reported a better objective response rate than 43 EGFR-mutated patients and 37 wild-type patients with 46.7, 4.7 and 16.2% (p=0.001), respectively. Time to progression (TTP) was also in favour of *ALK*-positive patients (9.2, 1.4 and 2.9 months, respectively; p=0.001) (63).

This correlation might be explained with the lower concentration of thymidylate synthase (TS), the main target of pemetrexed, in *ALK*-positive tumours (63,64).

The largest retrospective analysis compared 121 *ALK*-positive NSCLC patients with 266 patients with *ALK*-negative, *EGFR*-wild-type NSCLC, including 79 with *KRAS* mutations. Among 70 *ALK*-positive patients treated with a platinum/pemetrexed regimen, the median PFS was 7.3 months while it was 5.5 months for 51 *ALK*-positive patients treated with single-agent pemetrexed or non-platinum/pemetrexed combinations. PFS of *ALK*-negative patients, on all pemetrexed-based regimens, was similar to that of *ALK*-positive patients, except in first-line platinum/pemetrexed where the median PFS was 4.2 and 5.4 months, in patients with *KRAS*-mutation and wild-type, respectively (65).

Contrary to previous data, this large retrospective analysis did not report any statistical advantage for *ALK*-positive patients treated with pemetrexed-based therapy.

Table IV. Main clinical results of pemetrexed therapy in *ALK*-translocated NSCLC patients.

Characteristics	Camidge *et al* (62) 2011	Lee *et al* (63) 2011	Shaw *et al* (65)[a] 2013	Shaw *et al* (65)[b] 2013	Shaw *et al* (35) 2013
Type of study	Retrospective	Retrospective	Retrospective	Retrospective	Prospective
Line of pemetrexed therapy	Any line	≥2nd-line	Any line	Any line	2nd-line
No. of patients					
ALK-rearrangements	19	15	70	51	99
EGFR-mutations	12	43	-	-	-
KRAS-mutations	21	-	112	75	-
Wild-type	37	37	49	30	-
ORR (%)					
ALK-rearrangements	42	46.7	Not reported	Not reported	29
EGFR-mutations	30	4.7			-
KRAS-mutations	37	-			-
Wild-type	14	16.2			-
PFS (months)					
ALK-rearrangements	9.0	9.2	7.3[c]	5.5	4.2
EGFR-mutations	5.5	1.4	-	-	-
KRAS-mutations	7.0	-	5.9[c]	3.9	-
Wild-type	4.0	2.9	4.5[c]	7.8	-

[a]Platinum/pemetrexed regimens; [b]non-platinum/pemetrexed regimens or single-agent pemetrexed; [c]time-to-progression. NSCLC, non-small cell lung cancer; ORR, objective response rate; PFS, progression-free survival.

These contrasting results could be directly addressed by the prospective studies such as PROFILE 1007 and PROFILE 1014. To date, only the results of PROFILE 1007 are available, showing an interesting relationship between *ALK*-positivity and sensitivity to pemetrexed treatment. Patients treated with pemetrexed experienced better objective response rates as compared to those treated with docetaxel (29.3 and 6.9%, respectively) and better PFS (4.2 and 2.6 months, respectively). It is important to emphasise that the study was not designed to compare the two chemotherapeutics. Moreover, the choice between pemetrexed or docetaxel was left to the investigator and not to randomization (35) (Table IV).

The final results of the PROFILE 1014 trial are awaited also to better understand the activity of pemetrexed in *ALK*-positive NSCLC patients.

Crizotinib for ROS1-rearrangements. ROS1 is a receptor tyrosine kinase of the insulin receptor family, shown to fuse at exons 32, 34, 35 or 36 with multiple partners in NSCLC (*TPM3, SDC4, SLC34A2, CD74, EZR, LRIG3* and *FIG*). These chimeric proteins, found in approximately 1-2% of NSCLC patients, maintain constitutive ROS1 kinase activity, leading to persistent downstream signalling and transforming ability via enhanced cell growth, proliferation and decreased apoptosis (66,67). Like *ALK*-positive NSCLC patients, *ROS1*-positive NSCLC patients tend to be younger (median age, 49.5 years), never-smokers, have a histological diagnosis of adenocarcinoma, and seem to be mutually exclusive with other gene alterations (68).

Preclinical studies of cell lines harbouring *ROS1* rearrangements and clinical evidence on a single *CD74-ROS1*-positive NSCLC patient showed sensitivity to crizotinib (68,69). Thirteen *ROS1*-positive NSCLC patients, all previously treated, received crizotinib at the standard dose of 250 mg twice daily. Patients were young with a median age of 47 years, all with adenocarcinoma histology, and all but one were never-smokers. The objective response rate was 54%, with a disease control rate at 8 weeks of 85% (70). A progression of disease is reported also for *ROS1*-positive patients after a response to crizotinib treatment. A crizotinib resistance due to an acquired mutation leading to a glycine-to-arginine substitution at codon 2032 in the *ROS1* kinase domain was demonstrated in a *ROS1*-positive patient initially responding to crizotinib. This mutation confers resistance to crizotinib through steric interference with drug binding (71).

Brain metastases. The incidence of brain metastases in *ALK*-positive NSCLC patients was described to be ≤46% (72). A case report showed development of brain metastases under crizotinib therapy, despite an objective response of extracranial lesions, in an *ALK*-positive NSCLC patient. Measurement of the levels of crizotinib in the plasma and cerebrospinal fluid (CSF) indicated a poor penetration of the drug into the brain, thus potentially hindering efficacy in metastatic brain tumours

(73). Further reports underlined this aspect reporting several cases in which *ALK*-positive NSCLC patients reported a brain progression of disease despite a shrinkage of extracranial tumour sites (74-76). On the contrary, other reports showed slight reduction of brain metastases (77) or response (78) with crizotinib therapy even if with a different schedule.

Interesting results on brain metastases were gained from phase I/II trials with alectinib (50,79). Among the 46 patients enrolled in the phase II portion of the first study (50), 15 (33%) patients with known identified brain metastases, of whom 12 (26%) were previously irradiated and three (7%) were clinically stable without symptoms at baseline. No progression of brain metastases in any of the patients was reported, although previous radiotherapy might have affected the natural history of brain disease. Two of the three patients who had baseline brain metastases and had not received previous radiotherapy continued alectinib for >300 days without brain progression (50). In the second trial (79), 21 out of 47 enrolled patients had brain metastases at baseline, and 4 received no prior brain irradiation. In these 4 patients, two complete and one partial responses with one stable disease were reported. Among the 17 patients with brain metastases and previously irradiated, one patient progressed in both systemic and brain sites, three progressed only in systemic sites and the other 13 patients are still on treatment with one complete response (79).

In both trials, alectinib demonstrated consistent and rapid clinical activity in brain metastases in *ALK*-positive NSCLC patients who progressed on crizotinib. This is likely due in part to low penetration of crizotinib into the central nervous system. In fact, the blood-brain barrier (BBB) contributes to brain homeostasis by protecting the brain from potentially harmful endogenous and exogenous substances. The efflux transporter P-glycoprotein (Pgp) is a key element of the BBB that can actively transport a huge variety of lipophilic drugs out of the brain capillary endothelial cells that form the BBB (80). Crizotinib is a good Pgp substrate, but not alectinib. Moreover, preclinical studies in the central nervous system implantation models suggest a promising antitumour activity of alectinib against brain lesions (79).

Of course, further studies specifically addressing this issue must better clarify the potential role of alectinib also in this setting.

7. Conclusion

Histology and molecular analyses are the cornerstone in the initial diagnosis of NSCLC, and are key determinants to address the appropriate strategy of treatment. Among the first targeted agents approved for molecular selected NSCLC patients were EGFR-TKIs, gefitinib, erlotinib and afatinib in *EGFR*-mutated patients. *ALK* translocations were discovered as new molecular drivers for which specific inhibitors are in development. The first clinical trials provided evidence for the use of ALK-TKI crizotinib, changing the therapeutic options for this subgroup of approximately 5% of *ALK*-positive NSCLC patients. The emergence of crizotinib took place over a short period of just four years, from identification of the target to approval of the drug, leading to the availability of another potential therapeutic option and the challenge of identifying suitable patients effectively and efficiently through the integration of *ALK* identification into routine clinical practice. The dramatic clinical responses achieved in patients with *ALK*-positive NSCLC with the use of crizotinib are of limited durability, because patients will ultimately develop acquired resistance to crizotinib. Concerning this issue, in the presence of known oncogenic drivers in tumours, an important consideration is that serial biopsies should be mandatory, when possible, to detect resistance mechanisms and to define further appropriate treatment strategies for progressing patients. Therefore, additional therapeutic approaches to prevent acquired resistance to crizotinib or effectively treat crizotinib-resistant disease are greatly needed. Several drugs in early phase trials demonstrated promising results in crizotinib-resistant NSCLC patients, but further randomized trials, to date ongoing, are needed to clarify their role in this setting.

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