

Recent studies have demonstrated the benefit of EGFR tyrosine kinase inhibitors in the treatment of advanced non-small-cell lung cancer (NSCLC). The role of activation of the anaplastic lymphoma kinase (ALK) pathway and the presence of the fusion gene *EML4-ALK* are new molecular targets in studies into the pathogenesis and treatment of NSCLC. *ALK* gene rearrangement is observed in 3–5% of NSCLC patients. Crizotinib is an oral inhibitor of ALK kinase activity, approved for the treatment of NSCLC patients with *ALK* gene rearrangement. Crizotinib treatment has resulted in a progression-free survival of 7–10 months with 50–60% objective response rate. The present paper gives an overview of literature reports on the role of crizotinib in the treatment of NSCLC patients harbouring a molecular defect in the *ALK* gene. Molecular diagnosis of *ALK*-associated aberrations, results of clinical trials of different phases assessing the efficacy and safety profile of crizotinib are also discussed. Attention is given to the likely causes of drug resistance and management strategies in patients with treatment failure.

**Key words:** crizotinib, ALK, fusion gene, non-small-cell lung cancer, tyrosine kinase inhibitor.

# Crizotinib in the treatment of non-small-cell lung carcinoma

Adam Płużański, Aleksandra Piórek, Maciej Krzakowski

Lung Cancer Department, Maria Skłodowska-Curie Memorial Cancer Centre and Institute of Oncology, Warsaw, Poland

## Introduction

With ca. 1.6 million of new cases identified worldwide every year, lung cancer is the most commonly diagnosed malignancy. At the same time, it is the leading cause of cancer mortality among both men and women (ca. 1.4 million deaths annually) [1]. Ca. 70–80% of patients are diagnosed with non-small-cell lung cancer (NSCLC). Over 40% of NSCLC patients present with metastatic disease at first diagnosis. Some of the patients undergo systemic therapy with a median survival time of 6–10 months [1].

The choice of chemotherapy type was not previously determined by histological type or other pathomorphological factors. In recent years, however, certain molecular aberrations have been shown to play a role in the pathogenesis of NSCLC, and the importance of EGFR (*epidermal growth factor receptor*) tyrosine kinase inhibitors in therapy has been demonstrated, thus highlighting the benefit of a personalized approach to ensure optimum patient management. The first registration of EGFR tyrosine kinase inhibitors (TKI) authorized the use of TKI in all patients regardless of molecular characteristics. However, when the predictive value of the presence of *EGFR*-activating mutations in NSCLC cells was discovered, the indications were revised and a group of patients deriving a major clinical benefit from therapy was identified. Retrospective reviews and a number of prospective studies have shown that treatment with EGFR tyrosine kinase inhibitors in patients with advanced NSCLC and *EGFR*-activating mutation provides an objective response rate (ORR) of over 60% and extends progression-free survival to 12 months, compared to standard chemotherapy [2–5]. Observed outcomes of molecularly targeted therapy demonstrate the importance of searching for other potential molecular targets.

## *ALK-EML4* fusion gene

A novel molecular target is the anaplastic lymphoma kinase (ALK) pathway. Since the 1980s attention has been given to the role of changes in the *ALK* gene in the pathogenesis of anaplastic large-cell lymphoma (ALCL), B-cell lymphoma and neuroblastoma [6]. The relationship between *ALK* pathway aberrations and the pathogenesis of solid tumours, however, was poorly understood.

In 2007, two independent research teams reported rearrangement in the *ALK* gene in a small proportion of NSCLC patients [7, 8]. Major rearrangements associated with NSCLC result from the process of gene fusion between *ALK* and *EML-4* (*echinoderm microtubule-associated protein-like 4*) occurring as a consequence of intrachromosomal inversion of the short arm of chromosome 2 [Inv(2) (p21p23)] and leading to the fusion of exons 1–13 of the *EML-4* gene with exons 20–29 of the *ALK* gene. The resulting fusion protein *EML4-ALK* contains the N-terminal portion of *EML4* and the C-terminal fragment of the intracellular domain of *ALK* tyrosine kinase [8]. Multiple variants of the *EML4-ALK* fusion gene have now been described. All of them encode a fusion between the same cytoplasmic portions of the *ALK* but contain different truncations of *EML4* [9, 10]. Other *ALK* fusion variants (e.g. with *TGF*, *KIF5B*) are

less common than *EML4-ALK* and their clinical significance has not yet been determined [7, 11]. The *EML4-ALK* fusion mediates ligand-independent dimerization of the ALK kinase domain, resulting in cross-phosphorylation of ALK molecules and self-activation. The processes discussed above produce a constant activation of the intracellular signalling pathway, induce proliferation and inhibit apoptosis [8].

The *EML4-ALK* fusion gene is identified in 3–5% of NSCLC patients [12, 13]. NSCLC patients with ALK rearrangement are younger than patients without such ALK aberrations (the median age being 52 years). In a group of 141 patients selected for the study on the basis of two (or more) demographic/clinical characteristics (female sex, Asian ethnicity, never/light smoking history or adenocarcinoma diagnosis), the probability of ALK rearrangement was 13% [14]. The presence of signet-ring cells also probably has predictive significance. ALK rearrangement is mutually exclusive with mutations affecting the *KRAS* and *EGFR* genes [15].

Recent reports, however, have documented that ca. 8% of patients with ALK gene rearrangement also have *EGFR* or *KRAS* mutations [16]. Following patients with *EGFR*-activating mutations, patients with the *EML4-ALK* fusion gene are the second group for which a molecularly targeted drug was approved.

In a panel of over 120 kinases assessed for the activity of the selective oral ALK and c-MET (c-mesenchymal-epithelial transition) inhibitor – crizotinib – a 20-fold greater selective affinity for the inhibition of ALK and MET kinases was demonstrated, as compared with other kinases [17]. Both *in vitro* and *in vivo* studies have shown crizotinib to inhibit ALK phosphorylation and signal transduction, which results in the arrest of the cell cycle in the G1-S phase and in the induction of apoptosis [18].

### Diagnosis of ALK rearrangement

FISH (fluorescence in situ hybridization) analysis is a laboratory technique which utilizes DNA probes stained with fluorescent dye for the detection of ALK gene rearrangements on chromosome 2 in NSCLC tissue samples. The method involves an assessment of integrity of the ALK gene. FISH is performed in formalin-fixed, paraffin-embedded (FFPE) fragments of cancer tissue. The green probe hybridizes to the region located in the immediate vicinity of 5'ALK, and the red probe to the 3'ALK area. Separation of the red and green signals or an isolated red signal are indicative of the presence of rearrangements. Close proximity of the red and green signals, on the other hand, indicates the presence of a correct copy of the ALK gene [19].

The FISH test for ALK gene rearrangement is considered positive if at least 15% of cells out of a minimum of 50 cells counted in the test material show an intense signal [20].

Camidge *et al.* [21] have shown that an average of 54% of cells investigated in the cancer tissue of ALK-positive patients demonstrate a positive signal pattern in the FISH analysis, compared to only 5–7% of signal-emitting cells in adjacent normal tissue and tumour areas in ALK-negative patients. In addition, 100% sensitivity and 100% specificity occurred when at least 60 cells were counted.

*Break-Apart* FISH is a diagnostic test approved by the FDA for the detection of ALK rearrangement in NSCLC in connection with crizotinib approval in the USA.

There is ongoing research to detect ALK rearrangements by immunohistochemistry (IHC) with antibodies specific for the human ALK protein. Since no ALK expression is detected in normal lung tissue, ALK detection by IHC theoretically provides evidence for ALK gene rearrangement [19]. Contrary to anaplastic large-cell lymphoma (ALCL) cells, ALK expression levels in ALK-rearranged NSCLC patients are nearly five times lower, which – despite good specificity (97%) – significantly reduces the sensitivity of assay (67%) [22]. This is likely to be caused by lower transcriptional activity of the *EML4* promoter compared to NPM (nucleophosmin) implicated in the NPM-ALK fusion in ALCL [20].

Several monoclonal antibodies are currently in the development stage. The novel antibody D5F3, which has a higher specificity to ALK, has demonstrated 100% sensitivity and 99% specificity, a positive predictive value of 96% and a negative predictive value of 100% [22]. A comparison of new antibody (IHC) assays and *Break-Apart* FISH has demonstrated that positive IHC scores (IHC 3+) or an absence of expression (IHC 0) are consistent with corresponding FISH outcomes. However, in cases with equivocal IHC results (IHC 2+ or IHC 1+) FISH has sometimes indicated the presence of the fusion gene [23, 24]. The usefulness of IHC assays and the algorithm for handling equivocal staining cases require verification and validation in further studies.

The detection of ALK rearrangements using another proposed method, Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), requires larger quantities of the tissue material than FISH, and the sequence of the molecular aberration in the ALK gene must be known [20].

A comparison of RT-PCR with FISH and IHC in anaplastic large-cell lymphoma has revealed the greatest consistency of results between IHC and FISH, with RT-PCR demonstrating lower sensitivity than other assays [20, 25].

### Crizotinib – phase I trials

The first multicentre trial designed to investigate the pharmacokinetics, maximum tolerated dose (MTD) and adverse event characteristics of crizotinib was launched in 2006 [17]. The trial involved patients with advanced-stage colorectal and pancreatic cancer, sarcoma, anaplastic large-cell lymphoma and NSCLC. The MTD for further study was set at 250 mg BID. A steady-state concentration of crizotinib was reached after 15 days of treatment, while the half-life was 43–51 hours. No effects of food on the pharmacokinetics of crizotinib was observed [26]. Considerable response to crizotinib therapy was observed in two patients with advanced NSCLC. Molecular tests revealed that both patients had ALK-rearranged tumours. The trial was expanded to include a cohort of patients with advanced ALK-rearranged NSCLC (verified by FISH). Other eligibility criteria included ECOG performance status 0–2, absence of previously untreated brain metastases and normal organ function. The number of previous lines of treatment was not important for determining patient eligibility for enrolment. Between August 2008 and May 2011, a total of 149 patients with a ver-

ified rearrangement in the *ALK* gene were enrolled for the trial [12]. Among the trial patients with molecular aberrations, 97% were adenocarcinoma patients, 71% were never smokers and 28% former smokers, divided between female and male. Patients aged below 65 years constituted 83% of the study population, the median age being 52 years. Among 143 subjects evaluated during the follow-up (median period of 16.3 months), objective response to treatment was observed in 61% of the patients (partial response in 84 and complete response in 3 patients). The median period until the achievement of an objective response was 8 weeks, i.e. a response was confirmed during the first assessment by imaging techniques, as required by the trial protocol. In some patients, radiological response was noted in a previously performed additional imaging study as early as after several days of crizotinib therapy [27]. An analysis of additional predictive factors revealed no correlations between the ORR and demographic/clinical parameters (sex, age, performance status and number of previous treatment lines). The median duration of response was 49 weeks. The trial demonstrated *ALK* gene rearrangements to be the most significant predictive factor of response to crizotinib therapy irrespective of other clinical features. The median progression-free survival in crizotinib-treated patients was 9.7 months. According to Camidge *et al.* [12], the duration of progression-free survival was significantly longer in the group of 24 patients receiving crizotinib as the first-line treatment option compared to patients treated with the drug in subsequent lines of therapy. The duration of progression-free survival was 18.3 months and 9.2 months, respectively. The median overall survival was not reached at the time of publication. The estimated 6-month and 12-month survival rates were 88% and 75%, respectively.

Because of the lack of comprehensive data on overall survival, Shaw *et al.* [28] conducted a retrospective analysis of overall survival rates in a cohort of 82 crizotinib-treated patients with NSCLC harbouring *ALK* gene rearrangement, 36 *ALK*-positive patients who were not given crizotinib, 67 patients without *ALK* rearrangement but positive for the *EGFR* activating mutation, and 253 patients lacking any molecular modifications in the *ALK* and *EGFR* genes, who enrolled in the phase I clinical trial of crizotinib. A significantly higher rate of 2-year survival was observed among patients with *ALK* rearrangement receiving crizotinib compared to subjects who were not treated with the drug (57% and 36%, respectively). Differences in 2-year survival among *ALK*-positive patients in the second and subsequent lines of treatment were significantly greater (55% and 12%, respectively), demonstrating the superiority of crizotinib. However, there was no difference in survival in groups of patients with *ALK* rearrangement who were given crizotinib and patients with *EGFR*-activating mutation and without *ALK* rearrangement receiving gefitinib or erlotinib. Objections can be raised with regard to the retrospective nature of the analysis and differences in the number of patients included in the compared groups.

### Crizotinib – phase II trials

The multicentre phase II trial PROFILE 1005 was designed to assess the efficacy of crizotinib in patients who had failed two or more chemotherapy treatments. After preliminary

results of the phase I study were released, amendments were made to the trial protocol to allow *ALK*-rearranged patients to enroll regardless of the line of treatment and method employed to detect the presence of the fusion gene. In the group of 136 patients who completed preliminary evaluation in 2011 by Crino *et al.* [29], 94% had adenocarcinoma, 68% had never smoked, and 53% were female. Over 93% of patients had at least 2 prior chemotherapy regimens. Response to treatment was evaluated in 76 patients, of which 41 patients (54%) had an objective response. An important part of the trial from the viewpoint of clinical practice was the assessment of quality of life and tumour-associated symptoms. Clinically significant improvements in cough, dyspnoea, pain and fatigue were seen after two cycles of treatment.

Based on treatment outcomes achieved in 255 patients enrolled for the phase I trial A8081001 and for the PROFILE 1005 trial, FDA granted a conditional approval for crizotinib in 2011. It should be noted, though, that there are as yet no results of a direct comparison of crizotinib and first-line chemotherapy, and the effect of the drug on overall survival in patients with *ALK* gene rearrangement.

### Crizotinib – phase III trials

In addition to continued phase I and II trials, there are ongoing phase III trials evaluating the efficacy of crizotinib in patients with *ALK* gene rearrangements. PROFILE 1014 is a clinical trial conducted in previously untreated patients with advanced NSCLC, which compares crizotinib with cisplatin or carboplatin/pemetrexed chemotherapy. The trial is scheduled to be completed in December 2013 [30].

Recently, results of a randomized phase III trial assessing the efficacy of crizotinib in second-line treatment in a head-to-head comparison with pemetrexed or docetaxel [31] have been announced. The trial involved a total of 374 patients with rearrangements in the *ALK* gene. A significant extension of progression-free survival (PFS) was observed in 173 crizotinib-treated patients, which was the primary endpoint of the trial. The median PFS was 7.7 months with crizotinib vs. 3 months with standard chemotherapy. The objective response rates were 62% and 20%, respectively. A preliminary analysis showed no significant differences in overall survival between the two arms of the trial, which is probably attributable to the fact that crizotinib therapy was administered after disease progression in 62% of chemotherapy-pretreated patients. It is worthwhile to note, though, that the overall survival in both arms of the trial exceeded 20 months, which is quite uncommon in patients receiving second-line palliative chemotherapy. PROFILE 1007 is the first phase III trial providing a head-to-head comparison of crizotinib with standard chemotherapy in patients with *ALK* gene rearrangement.

### Crizotinib – toxicity

The most common adverse events (AEs) observed among patients receiving crizotinib treatment are grade 1 and 2 AEs according to the CTCAE classification [32]. The most frequent AEs are gastrointestinal disorders: nausea (53%), diarrhoea (43%), vomiting (40%), constipation (27%), fatigue (20%) and anorexia (19%). Skin rash typically associated with anti-EGFR therapy was present in ca. 10% of treated patients [12, 29].

Distinctive side effects reported in patients receiving crizotinib are grade 1 and 2 vision disorders including flashes of light, double vision, slow adaptation to light changes or blurred vision in the peripheral visual field. Such visual impairment is observed in ca. 62% of all patients, usually accompanying changes in light intensity (light/dark shifts and *vice versa*). Grade 3 and 4 AEs comprise primarily self-limiting and asymptomatic elevations in aminotransferase levels (5%) and fatigue (2%). Crizotinib has also been associated with life-threatening treatment-related pneumonitis (with a frequency of 1.6%) and QT interval prolongation, potentially predisposing to cardiac arrhythmia [33].

### Crizotinib – resistance

Similarly to EGFR tyrosine kinase inhibitors, the disease progresses at some stage of crizotinib treatment despite the initially observed objective response to therapy. There is ongoing research to identify mechanisms underlying the development of resistance to crizotinib. One of the likely mechanisms is the formation of gene mutations reducing the activity of the tyrosine kinase inhibitor. The mutation can, furthermore, activate another signalling pathway, thus eliminating signal passage through the stage originally inhibited by the drug. One of the means of overcoming drug resistance in this mechanism could be to use inhibitors of subsequent stages of signal transmission. Considering the fact that in nearly all instances the presence of the *ALK* fusion gene excludes *EGFR* and *KRAS* mutation positivity, it is presumed that the development of crizotinib resistance can be a consequence of activation of the EGFR/*KRAS* signalling pathway. Some of the *ALK*-positive cells may, in fact, contain activating mutations even before the introduction of treatment because some patients exhibit primary resistance to crizotinib despite *ALK* rearrangement [20, 34]. Studies investigating second generation *ALK* inhibitors are currently under way to overcome both primary and secondary resistance [35, 36].

Another interesting proposed mechanism of eliminating the problem of crizotinib resistance is based on heat shock proteins 90 (HSP90) inhibitors which are implicated in the protection and stabilization of the fusion protein EML4-*ALK*. Heat shock proteins (HSP) are a family of “chaperone” proteins protecting other proteins from degradation and environmental stress conditions (e.g. hypoxia, free radicals, effects of ionizing radiation or chemotherapy), which in effect contributes to sustaining unstable tumour cells [37]. Theoretically speaking, the inhibition of HSP90 mediates the process of EML4-*ALK* degradation and suppresses further *ALK* signalling pathways [20, 36, 38]. There are a number of preliminary reports indicating clinical activity of HSP90 inhibitors in *ALK*-positive patients [20, 39, 40].

### Other molecular targets

Crizotinib was originally designed as an inhibitor of the MET pathway. Research is currently in progress to assess efficacy of the drug among NSCLC patients displaying amplification or mutation in the *MET* gene. Recently, there have been reports on the efficacy of crizotinib in a different molecular disorder, identified in 1% of NSCLC patients, namely rearrangement of the *ROS1* gene. A preliminary analysis of

14 patients with *ROS1* rearrangement revealed the ORR of 57%, which justified further prospective verification of the implications of that molecular aberration [41].

### Summary

Over the past dozen years or so, the standard treatment for NSCLC, irrespective of other pathomorphological and clinical features, has been multi-drug chemotherapy based on platinum derivatives. Chemotherapy is associated with moderately prolonged survival and produces often significant toxicity. Identification of molecular targets is a major step forward in the process of optimization of treatment of advanced NSCLC. The discovery of the role of the *EGFR* activating mutation as a predictive factor for the success of treatment with EGFR tyrosine kinase inhibitors was a breakthrough point for the development of personalized NSCLC treatment. Identification of *ALK* gene rearrangements and the introduction of crizotinib as another molecularly targeted drug with a proven efficacy in the treatment of the patient population with this molecular defect is another milestone in the process of individualization of therapy and may potentially improve patient outcomes, while offering an acceptable toxicity profile. Objective response rates to standard chemotherapy are 20–30%. By contrast, ORR to molecularly targeted drugs in patients with specific predictive biomarkers exceeds 50%. In clinical terms, molecularly targeted drugs can only be used in ca. 10–20% patients diagnosed with lung adenocarcinoma.

A still unresolved problem, however, remains the lack of identified molecular targets that would be useful in the therapy of squamous cell carcinoma.

Crizotinib therapy in patients with *ALK* gene rearrangement makes it possible to achieve ORR in ca. 60% of all patients and prolong progression-free survival to ca. 10 months.

An important aspect of future research should be identification of drug resistance mechanisms and development of methods of post-progression treatment.

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#### Address for correspondence

Adam Płużański

Lung Cancer Department  
Maria Skłodowska-Curie Memorial Cancer Centre  
and Institute of Oncology  
Roentgena 5  
02-781 Warszawa  
e-mail: apluzanski@coi.pl

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