

Diagnostic and therapeutic issues for patients with advanced non-small cell lung cancer harboring anaplastic lymphoma kinase rearrangement: European vs. US perspective (Review)

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Abstract. The recent availability of crizotinib in clinical practice, for the treatment of patients with advanced non-small cell lung cancer (NSCLC) selected by the presence of anaplastic lymphoma kinase (ALK) rearrangement, has relevant implications for both the diagnostic phase and the treatment choices. In the United States, crizotinib was approved by the Food and Drug Administration (FDA) in 2011 for patients with ALK positivity detected by FDA-approved companion diagnostic test. As of January, 2014, the only FDA-approved diagnostic test is Vysis ALK Break-Apart FISH Probe Kit. In Europe, European Medicines Agency (EMA) approved crizotinib for ALK-positive patients in 2012, without specifying the type of test used for determining the positivity. FISH remains the reference technique for ALK determination, but, if fully validated, immunohistochemistry could challenge the current ALK screening practice. Given the robust evidence of activity of crizotinib in ALK-positive patients both pretreated and chemotherapy-naïve, and the favourable tolerability profile of the drug, many oncologists would prefer to administer the drug as early as possible. This is technically feasible in the United States, where crizotinib was approved well before the availability of the results of the randomized phase III trial comparing the drug with standard second-line chemotherapy, and the use of crizotinib in ALK-positive patients is not restricted to a specific line of treatment. On the contrary, in Europe, differently from the FDA decision, crizotinib cannot be used in chemotherapy-naïve patients. In both realities, a deeper knowledge of mechanisms of resistance, the role of repeated biopsies, the treatment strategy for patients experiencing disease progression with crizotinib, the choice of the

best chemotherapy regimen are challenging topics for the management of ALK-positive patients in clinical practice.

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1. Introduction

In 2007, a small inversion within chromosome 2p, resulting in a fusion gene comprising portions of the echinoderm microtubule-associated protein-like 4 (*EML4*) gene and the anaplastic lymphoma kinase (*ALK*), was described in a subgroup of patients with advanced NSCLC and was immediately considered a promising candidate for a therapeutic target as well as for a diagnostic molecular marker (1). *EML4-ALK* translocation can be detected in a limited percentage of advanced NSCLC, representing about 5-6% of adenocarcinomas (2). Although ALK-positive cases are a small proportion of patients with advanced NSCLC, they represent a non-negligible number in absolute terms. In the United States, considering that about 228,190 new cases of lung cancer (both small cell and non-small cell) were expected for 2013 (3), it can be estimated that between 2,700 and 8,100 cases of ALK⁺ advanced NSCLC (representing 2-6% of all cases of advanced NSCLC) are diagnosed every year. Similarly, in Europe, where about 410,000 new lung cancer cases are diagnosed every year (4), between 5,000 and 14,500 cases of ALK⁺ advanced NSCLC are expected every year.

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Table I. Main clinical trials with crizotinib in patients with advanced NSCLC harboring ALK rearrangement.

Trial (Refs.)	Phase	No. of patients treated with crizotinib	Previous treatments	ORR	PFS
PROFILE 1001 - A8081001 (26)	Early phase (phase I followed by expanded cohort study)	143	Most patients were pre-treated with one or more lines of chemotherapy	60.8%	Median 9.7 months
PROFILE 1005 - A8081005 (6)	Phase II	259	Most patients were pre-treated with ≥ 2 prior chemotherapy regimens	53%	Median 8.1 months
PROFILE 1007 - A8081007 (7)	Phase III (vs. pemetrexed/docetaxel)	173	Progressive disease after one prior platinum-based chemotherapy regimen	65%	Median 7.7 months (Hazard ratio vs. chemotherapy 0.49; 95% CI 0.37-0.64, $p < 0.001$)
PROFILE 1014 - A8081014 (ClinicalTrials.gov Identifier NCT01154140)	Phase III	334 (estimated)	Treatment-naïve	Not yet available	Not yet available

ORR, objective response rate; PFS, progression-free survival.

Crizotinib, an anticancer drug that acts as a protein kinase inhibitor by competitive binding within the ATP-binding pocket of target kinases, initially developed as a c-MET inhibitor, has demonstrated a relevant activity in patients with ALK-positive advanced NSCLC, even if heavily pretreated (Table I) (5,6). Within the first-in-man phase I study, the activity of crizotinib was tested in a large cohort of 149 patients with advanced NSCLC selected for the presence of ALK rearrangement (5). In these patients, although many of them had already received more than one line of chemotherapy, the administration of crizotinib was associated with an impressively high rate of rapid and durable objective responses (60.8%). Subsequently, a randomized phase III trial compared crizotinib vs. standard second-line chemotherapy (pemetrexed or docetaxel) in ALK-positive patients after failure of a first-line platinum-based chemotherapy (7). Crizotinib was associated with a significant prolongation of progression-free survival (PFS), that was the primary endpoint of the trial (median PFS was 7.7 with crizotinib vs. 3.0 months with chemotherapy, hazard ratio 0.49; 95% CI, 0.37-0.64, $p < 0.001$). A similar benefit was observed also in response rate, confirming the brilliant activity shown in the phase I trial: the objective response rate was 65 vs. 20%, with crizotinib and chemotherapy respectively ($p < 0.001$). Furthermore, patients treated with crizotinib reported greater reductions in symptoms of lung cancer and greater improvement in global quality of life, compared to those assigned to chemotherapy. The recent availability of crizotinib in clinical practice, for the therapy of patients with advanced NSCLC selected by the presence of ALK rearrangement, has

relevant implications for both the diagnostic phase and the treatment choices.

2. Approval of crizotinib by regulatory agencies in United States and Europe

In the United States, crizotinib was approved by the regulatory agency in 2011, well before the availability of the results of the randomized phase III trial comparing its efficacy vs. chemotherapy in the second-line setting. In detail, based on the early demonstration of activity, the US Food and Drug Administration (FDA) granted accelerated approval to the drug on August 26, 2011, for patients with locally advanced or metastatic NSCLC that is ALK-positive as detected by an FDA-approved test. According to this approval, the use of crizotinib is not restricted to a specific line of treatment, and both patients chemotherapy-naïve and those pretreated with chemotherapy are eligible for crizotinib, if ALK-positivity is demonstrated.

In Europe, on 19 July, 2012, the Committee for Medicinal Products for Human Use (CHMP) of European Medicines Agency (EMA) adopted a positive opinion, recommending the granting of a conditional marketing authorisation for crizotinib. This approval followed the demonstration of clinical benefit in the phase III trial that compared crizotinib to chemotherapy as second-line treatment, after failure of platinum-based first-line chemotherapy. Consequently, the EMA-approved indication is for 'the treatment of adults with previously treated ALK-positive advanced NSCLC'. According to this marketing authorization, differently from the FDA decision, crizotinib cannot be used in chemotherapy-naïve patients.

3. Which patients should be tested for ALK-positivity, and which test should be used?

In the United States, as described above, crizotinib was approved by FDA for the treatment of patients with ALK-positive advanced NSCLC together with the approval of a companion diagnostic test. This procedure was similar to the decision made by the FDA for other drugs, requiring FDA-approved companion diagnostics. In the case of crizotinib approval, FDA did not explicitly specify what is the acceptable test to identify patients eligible for the drug. However, as of January 2014, the only FDA-approved diagnostic test is Vysis ALK Break-Apart FISH Probe Kit (Abbott Molecular, Inc.), that is a fluorescence *in situ* hybridization (FISH) test designed to detect ALK gene rearrangements (8). If an immunohistochemistry (IHC) kit will be approved in the US by FDA as companion diagnostic, the eligibility for treatment with crizotinib could be based on techniques other than FISH. It should be kept in mind that all patients enrolled in the first pivotal trial leading to FDA approval were all selected by FISH positivity. In Europe, European Medicines Agency (EMA) approved crizotinib for ALK-positive patients, without specifying the type of test used for determining the positivity.

The screening strategy for ALK positivity might have relevant implications in terms of economic, logistic and technical aspects. Given that thousands of patients with advanced NSCLC need to be tested for the presence of ALK translocation, the costs of the diagnostic phase can be very high, because FISH technique (not only the instrument itself, but also probes) is quite expensive. Furthermore, FISH requires highly skilled expertise, it is not feasible in all laboratories and the results are not always easy to interpret (9). On the other hand, immunohistochemistry is easier from a technical point of view, less expensive and seems to be more reproducible than FISH (9,10). IHC is today a routine diagnostic procedure in almost every clinical-pathology laboratory in most of the (western) world.

If IHC becomes fully validated in the diagnostic phase of ALK rearrangement, a first scenario could imply the use of both immunohistochemistry and FISH, within an operational algorithm similar to what is currently established for the screening of HER2 positivity in breast cancer patients. Immunohistochemistry could be used for a screening of all cases: negative cases could be classified as ALK-negative, while the positive cases (1+, 2+ or 3+) could be confirmed with FISH. However, more clinical data are needed before applying the 'HER2 breast cancer model' of diagnostic screening to ALK test in advanced NSCLC. In a second scenario, immunohistochemistry could completely replace FISH in the diagnostic phase, representing a reliable screening tool in routine pathology laboratories for identification of patients with ALK rearrangement (11). When 196 cases of lung adenocarcinoma were tested for the presence of ALK rearrangement by different diagnostic techniques (two immunohistochemistry assays, FISH and real-time reverse transcription-PCR), the Ventana ALK-IHC kit (antibody D5F3) showed excellent sensitivity (100%) and specificity (98%) compared to FISH. Interestingly, in that series, two cases that were ALK-positive at immunohistochemistry but ALK-negative by FISH, were confirmed to be positive by RT-PCR and direct sequencing. Some of these patients might obtain clinical benefit with crizo-

tinib, but could be classified as false negative cases by FISH. The FDA approved FISH assay (Vysis ALK Break-Apart FISH Probe Kit) has been approved based on certain defined criteria; at least 15% of the tumor cells should have the characteristic 'split-apart' phenomena between the probes. However, in some cases an 'atypical' pattern occurs (i.e., single red signal), and in some cases a 'borderline' pattern is seen (12). These cases will be classified as 'ALK-negative' according to the FDA approved test, but some of these tumors have been reported with 'dramatic' effect to crizotinib therapy (13,14). Thus, the defined FDA criteria might be a limitation for the use of crizotinib in the broader community practices.

4. Which line of treatment for crizotinib?

The PROFILE 1014 trial (ClinicalTrials.gov Identifier NCT01154140) was designed to compare crizotinib to platinum-based chemotherapy as first-line treatment. Results of this trial are still unpublished, although in March 2014 a press release announced that the study met its primary endpoint, with a significant prolongation of PFS in favor of crizotinib. However, well before the completion of this study, the drug was approved in the US for any line of treatment. On the contrary, its use in Europe is limited to pretreated patients, coherently with the results of the randomized trial demonstrating its better efficacy compared to second-line chemotherapy. Given the robust evidence of activity of crizotinib in ALK-positive patients, both pretreated and chemotherapy-naïve, and the favourable tolerability profile of the drug, many oncologists would prefer to administer the drug as early as possible. This is technically feasible in the US, while in Europe clinical practice should be conducted within the restrictions imposed by regulatory agencies.

As for the debate about an early or a delayed use of the drug, one argument favoring the delayed use of crizotinib could be the high activity even in pretreated patients (5,6), and the lack of prolongation of overall survival compared to the control arm in the randomized trial vs. second-line chemotherapy (7). In that study, an interim analysis of overall survival showed no significant improvement with crizotinib as compared with chemotherapy (hazard ratio for death in the crizotinib group, 1.02; 95% CI, 0.68-1.54; $p=0.54$). However, as a general rule, when a significant proportion of patients assigned to the control arm receives the experimental drug as crossover after disease progression, this is expected to dilute the differences in terms of overall survival between the two arms (15). This is what has been systematically observed in the trials of first-line epidermal growth factor receptor (EGFR) inhibitors vs. chemotherapy in EGFR mutated cases (16). Overall survival might not be a good end-point for trials with agents like crizotinib in advanced NSCLC. The available data demonstrate that objective response and progression-free survival are improved significantly, and dramatically compared to chemotherapy. Furthermore, there is a clear demonstration of symptomatic improvement. Even in the absence of a formal demonstration of overall survival benefit within a randomized trial, it is quite clear that the use of crizotinib produced an improvement in the prognosis of ALK-positive patients. Notably, an indirect comparison of ALK-positive patients treated with crizotinib with a cohort of ALK-positive patients who did not receive crizotinib and with a cohort of ALK-negative patients showed that prognosis of ALK-positive

Table II. Main ongoing phase II and phase III trials with second generation ALK inhibitors in patients with advanced NSCLC harboring ALK rearrangement.

Drug (company)	Trial	Phase	Primary endpoint	Inclusion criteria
LDK378 (ceritinib) (Novartis)	NCT01685138	II	Response rate	1-3 lines of previous chemotherapy, NOT pretreated with crizotinib
	NCT01685060	II	Response rate	1-3 lines of previous chemotherapy, pretreated with crizotinib
	NCT01828112	III	Progression-free survival	Pretreated with 1 platinum doublet and pretreated with crizotinib (control arm: pemetrexed or docetaxel
	NCT01828099	III	Progression-free survival	Treatment-naïve (control arm: cisplatin-pemetrexed or carboplatin-pemetrexed)
AF802 (CH5424802/ RO5424802) (Hoffmann-La Roche)	NCT01871805	II	Response rate	Pretreated with crizotinib
AP26113 (Ariad)	NCT01449461	I/II	Response rate	Expansion cohort 1: not pretreated with crizotinib Expansion cohort 2: pretreated with crizotinib Expansion cohort 5: patients with active, measurable brain metastases

Source: www.clinicaltrials.gov (accessed February 18, 2014).

patients who did not receive crizotinib was not significantly better than ALK-negative patients while, among ALK-positive cases, patients treated with crizotinib showed longer overall survival compared with crizotinib-naïve controls (17).

5. Main mechanisms of resistance to crizotinib and the role of repeated biopsy

Recently, several mechanisms of resistance to crizotinib have been identified (18,19). The mechanisms of resistance can be roughly divided between ALK-dependent and ALK-independent mechanisms. Among the ALK-independent mechanisms, occurrence of a separate oncogenic driver (EGFR mutation, KRAS mutation) without evidence of persistent ALK rearrangement, shift to small cell histology or different mechanisms have been described. Among the ALK-dependent mechanisms, increased ALK copy number or mutations in the ALK gene have been described. Mutant clones are less sensitive to crizotinib (19), and this explains the clinical finding of disease progression, although ALK remains a driver in the tumor biology. In the cases characterized by ALK-dependent mechanism of resistance, second-generation ALK inhibitors (such as AP26113, LDK378, AF802) could overcome resistance producing objective responses after progression with crizotinib (20). In a phase I trial, ceritinib (LDK378) was associated with high activity in patients with advanced, ALK-rearranged NSCLC, including a group of patients who had experienced disease progression during crizotinib treatment (21). The second-generation ALK

inhibitors that are currently tested in phase II or phase III clinical trials in patients with advanced NSCLC selected for ALK rearrangement are listed in Table II. Several other drugs are currently in phase I (20). Some of these drugs could obtain better results in the control of tumor metastases growing in the central nervous system, that are poorly controlled with crizotinib, due to the insufficient delivery past the blood-brain barrier (22).

A second biopsy at the time of disease progression could give relevant information about the occurrence of one of the above described mechanisms of resistance. However, repeated biopsy can currently be considered essential for a proper characterization of tumor biology in clinical research, but it cannot yet be considered a routine procedure in clinical practice (16). However, also in clinical practice, at least in some patients, therapeutic decision could be affected by the results of repeated biopsy: for instance, if an EGFR mutation is detected, an EGFR tyrosine kinase inhibitor could potentially be effectively used.

6. Which treatment for patients with disease progression due to resistance to crizotinib?

The majority of patients with ALK-positive advanced NSCLC treated with crizotinib obtain objective response (5,6,7), but the duration of this response over time is limited, and disease progression after the initial control is unfortunately the rule. In clinical practice, due to the absence of other standard targeted

agents, these patients who experience disease progression with crizotinib can be offered chemotherapy. Currently, there is no clear evidence to prefer one agent among the possible chemotherapy options, although pemetrexed is considered to be particularly active in ALK positive patients (23,24).

Based on current evidence, interruption of crizotinib and switch to chemotherapy is the reasonable approach for patients with frank disease progression. On the other hand, continuation of crizotinib, eventually associated with local treatment, appears to be reasonable for patients experiencing progression in a single site or in a few sites of disease (so called 'oligo-progression') (25). For instance, in a patient with progression in brain metastases but stable disease in other sites, despite the formal definition of progressive disease, crizotinib could be reasonably continued, and associated with local treatment of brain metastases. This strategy is conceptually similar to what is often made in clinical practice with EGFR tyrosine kinase inhibitors for patients with EGFR mutation positive advanced NSCLC. In the phase I trial, a relevant proportion (56.5%) of patients who experienced disease progression continued to receive crizotinib for more than 2 weeks because, they were deriving clinical benefit from the drug according to investigators' opinion (26). In some cases, administration of crizotinib beyond progression was particularly prolonged: 12 patients received crizotinib for at least further 6 months. Similarly, in the phase III trial in second-line setting (7), many patients were kept on crizotinib beyond documented disease progression, with a median duration of further treatment of 16 weeks (range, 3-73 weeks). However, these data support the tolerability and the feasibility of a prolonged administration, but they do not represent a direct evidence of the effectiveness of the strategy.

In the absence of randomized trials supporting the role of continuing crizotinib after disease progression, the efficacy of this strategy was explored in a retrospective analysis of two single-arm trials enrolling patients with ALK-positive advanced NSCLC treated with crizotinib (27). In both trials, patients who developed disease progression according to RECIST criteria were allowed to continue treatment with crizotinib if they were deriving ongoing clinical benefit. In this analysis, patients with primary resistance to crizotinib (those who did not obtain objective response or stable disease) were not included. Overall survival was significantly longer for patients who continued crizotinib beyond progression (120 patients, 62%) than for those who stopped the drug (74 patients, 38%): median overall survival from disease progression was 16.4 vs. 3.9 months, respectively (hazard ratio 0.27; 95% CI, 0.17-0.42; $p < 0.0001$). Although this finding was confirmed also at multivariate analysis adjusting for relevant prognostic factors, this result is affected by selection bias that makes difficult the comparison between the two groups of patients. The SouthWest Oncology Group is conducting a randomized phase II trial (SWOG 1300) testing the role of continuing crizotinib beyond progression in addition to chemotherapy. In that trial, patients assigned to the control arm receive pemetrexed alone, while patients assigned to the experimental arm receive pemetrexed plus crizotinib.

As for the minority of patients with ALK-positive tumors experiencing primary, intrinsic resistance to crizotinib (absence of disease control), from a biological point of view these patients are probably different from patients who obtain

an initial response followed by acquired resistance. In the absence of other drugs with proven efficacy, these patients should be treated with standard chemotherapy. Differently from patients who have initially obtained a response and present a disease progression in a few sites of disease, when continuation of crizotinib appears to be a reasonable choice, the strategy of continuing crizotinib does not appear useful in patients with primary, intrinsic resistance.

Of course, the better characterization of resistance mechanisms in both groups of patients (acquired resistance and primary resistance) could allow the availability of new drugs as an alternative to standard chemotherapy.

7. Role of chemotherapy in ALK-positive cases

In mouse models of human adenocarcinomas harboring EML4-ALK fusion, pemetrexed produced longer overall survival compared to docetaxel, suggesting that this drug could be the preferred chemotherapy in these patients (28). The level of thymidylate synthase in ALK-positive cells has been found to be significantly lower compared with control cells, and this could explain the higher sensitivity to pemetrexed of these tumors (24). Similarly, molecular analysis performed in a subset of a large cohort of North-American NSCLC patients showed that the median thymidylate synthase level in 85 ALK-positive cases was significantly lower than the level in ALK-negative lung adenocarcinomas (29).

Preliminary clinical data have suggested that pemetrexed could be particularly active in ALK-positive patients (23,24). In the randomized phase III trial PROFILE 1007, comparing crizotinib to chemotherapy as second-line treatment, patients assigned to the chemotherapy arm received pemetrexed or docetaxel, according to investigators' choice (7). Although the study design did not allow a direct comparison between the two cytotoxic drugs used in the control arm, pemetrexed produced a better outcome compared to docetaxel. In detail, median progression-free survival was 4.2 months with pemetrexed and 2.6 months with docetaxel. Similarly, objective response was obtained in 29.3 and 6.9%, with pemetrexed and docetaxel, respectively.

In a retrospective analysis of the outcome of patients with ALK positive advanced NSCLC receiving both pemetrexed and crizotinib (29 patients receiving pemetrexed before crizotinib, and 9 patients receiving pemetrexed after crizotinib), high response rate was observed with pemetrexed, both in patients receiving the drug before crizotinib (66%) and in those patients receiving the drug after crizotinib failure (75%) (30).

In order to understand if pemetrexed is particularly active in ALK-positive cases compared to ALK negative cases, an indirect, retrospective comparison between the outcome of 121 patients with advanced, ALK-positive NSCLC and 266 patients with advanced, ALK-negative, EGFR-wild-type NSCLC, both receiving pemetrexed as a single agent or pemetrexed-containing combination chemotherapy, was performed (31). The PFS obtained with pemetrexed single agent or non-platinum/pemetrexed combination was similar between ALK-positive and ALK-negative cases, while the PFS obtained with a platinum/pemetrexed regimen was longer for ALK-positive patients. However, this could be confounded by the higher proportion of never smokers in ALK-positive patients,

because when comparing PFS in never- or light-smokers, there was no difference in PFS between ALK-positive and ALK-negative patients. Furthermore, this study does not add any information about the relative efficacy of pemetrexed compared to other cytotoxic agents in ALK-positive patients.

8. Treatment of ALK⁺ cases in earlier stages of disease

Use of effective ALK inhibition in ALK-positive cases who received surgery for earlier stages of disease could be particularly relevant, because, differently from the advanced setting, use as adjuvant treatment is today a potentially curative approach. Although there are no solid data on the prevalence of ALK rearrangement in earlier stages of disease, it is relatively rare even in clinically selected patients. In a series of 162 never-smoking patients who underwent resection for stage IB to IIIA lung adenocarcinoma, ALK rearrangement was detected in 8.6% of cases (32). The low prevalence of ALK rearrangement makes the conduction of prospective clinical trials in these patients challenging and potentially costly. If the efficacy of ALK inhibition as adjuvant therapy should be proven similarly to what has been done with adjuvant chemotherapy in the last decades, the screening of many thousands of patients should be needed to obtain an adequate study sample size, even in the hypothesis of a much larger advantage compared to that obtained with chemotherapy. In the US, the National Cancer Institute launched the ALCHEMIST protocol, that plans the screening of 6,000-8,000 patients with resected NSCLC (<http://www.ascopost.com/issues/april-15,-2013/implementing-a-national-cancer-clinical-trials-system-for-the-21st-century.aspx>). Patients with ALK rearrangement will be offered the participation to the ECOG 4,517 trial, randomized to crizotinib for 2 years vs. placebo.

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