Anaplastic lymphoma kinase (ALK) inhibitors for second-line therapy of non-small cell lung cancer

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Abstract: Targeted therapies are nowadays a treatment option in metastatic non-small cell lung cancer, for which oncogenic drivers have been identified. The epidermal growth factor-receptor tyrosine kinase inhibitors gefitinib and erlotinib, are the standard of care for patients in whom tumors are presenting with an activating epidermal growth factor-receptor mutation, with new active agents like afatinib reaching clinics in the near future. Other genetic abnormalities have been documented in squamous and non-squamous lung cancer. The EML4–ALK gene fusion is a rare event, occurring in around 5% of lung cancer, quite exclusively in adenocarcinoma with a predominance of young non/light smokers. Detection of ALK-positive tumors is challenging, as there is no gold-standard technique. Fluorescence in situ hybridization is the method used in prospective trials assessing the activity of crizotinib and is recommended by the American FDA. Crizotinib is the first orally active inhibitor of receptor tyrosine kinases, including ALK and ROS1, in clinical practice. Impressive results came from a phase I study and are now confirmed in a large phase II study with response rate of 60%, whatever the number of previous lines of chemotherapy. Other ALK inhibitors are currently in the preclinical phase, and some are showing promising results in early phase I/II studies. This review aims to present the current knowledge on the EML4–ALK gene fusion, the pitfalls for the pathologist and the clinician in searching this abnormality, and to review the existing literature on ALK inhibitors under development, focusing their role compared to chemotherapy in non-small cell lung cancer patients.

Keywords: non-small cell lung cancer, EML4–ALK, crizotinib

Introduction
Lung cancer, the leading cause of cancer death worldwide, has long been considered for therapeutic purposes as composed of two distinct entities, small and non-small cell lung carcinoma (NSCLC), the latter accounting for 85% of the cases. At diagnosis, the majority of NSCLC present with locally advanced or metastatic disease,1 for which only palliative chemotherapy can be proposed, while survival advantage has systematically been observed in comparison with supportive care.2 During the last few decades, improvements in chemotherapy administration and development of new cytotoxic agents have led to increased survival rates despite considerable acute and chronic toxicity from first-line platinum-based regimens. With time, more patients became eligible for salvage chemotherapy. The most common drugs given in second line for relapsed or progressing NSCLC are currently docetaxel and pemetrexed, while other drugs have shown some efficacy in a few studies.3

Over the last decade, better biology knowledge has led to differentiating NSCLC in subgroups with variable susceptibility to anticancer drugs. Histology was a first step,
showing, for example, with pemetrexed a differential sensitivity to the drug in favor of nonsquamous NSCLC. Further studies demonstrated that genomic alterations are different when looking at squamous and nonsquamous histology. Currently, at least 50% of adenocarcinomas present with a known genomic abnormality or an oncogenic driver, the most frequent being KRAS and EGFR mutations. The latter now has clinical implications with the development of the highly active EGFR tyrosine kinase inhibitors gefitinib, erlotinib, and afatinib.

A few years ago, a new gene alteration was discovered from a surgically resected lung adenocarcinoma, the EML4–ALK fusion gene, leading rapidly to the fruitful search for a selective inhibitor that is now reaching the clinic. The aims of this article are to present the current knowledge on this fusion gene, the pitfalls for the pathologist and the clinician in searching for this abnormality, and to review the existing literature on ALK inhibitors, focusing on their role in the therapeutic algorithm of NSCLC patients.

The EML4–ALK fusion gene

The anaplastic lymphoma kinase gene (ALK) is located on chromosome 2p23. Its normal expression is restricted to the brain, testes, and small intestine, having similarity to the insulin-receptor subfamily of kinases. This gene was originally established for its implication in the pathogenesis of rare diseases, including inflammatory myofibroblastic tumors and ALK-positive anaplastic large-cell lymphomas. In 2007, the fusion of ALK with the echinoderm microtubule-associated protein-like 4 gene (EML4) was first identified in Japanese NSCLC. This gene fusion is due to small inversions on chromosome arm 2p. Further publications underlined the importance of ALK gene fusion in other cancers (breast, colon, esophagus), while point mutation or amplifications have been reported in neuroblastoma. Translocations in NSCLC are created between ALK and a gene partner. EML4 is the main partner, but does not appear to be the exclusive one. Two other fusions have been described in NSCLC tumor samples, including TRK-fused gene (TFG, located at 3q12.2) and the kinesin family member 5 gene (KIF5B, located at 10p11.22).

At least eleven variants of the EML4–ALK fusion gene (Figure 1) have been reported to date, all of which contain the same breakpoint in the ALK gene but a different breakpoint in the EML4 gene. Most of them are oncogenic, as assayed in NIH-3T3 cells or in Ba/F3 cells. The most common variants are E13, A20, and E6a/b; A20 is also referred to as variants 1 and 3a/b, which have been detected in 33% and 29% of NSCLC patients, respectively. The clinical significance, if any, of the different variants is currently not defined. Whatever considering a translocation or an inversion, the EML4–ALK gene fusion results in overexpression of the ALK protein, and subsequently overactivity of the ALK tyrosine kinase. This ALK protein tyrosine kinase is the target of inhibitors, of which crizotinib is the most advanced in clinical evaluation.

At the difference of EGFR mutations, which are found in around 10% of adenocarcinoma in Caucasian patients, up to 30%–40% in East Asian ones, and virtually never in squamous histology, the EML4–ALK fusion gene is generally observed in less than 5% of all NSCLC histological types, while some heterogeneity appears across the studies (Table 1). Histology is a main predictor of EML4–ALK positivity as it is essentially detected in adenocarcinoma (and adenosquamous carcinoma) and rarely squamous cell carcinoma or in other histological subtypes (Table 1). Among adenocarcinomas, ALK-positive tumors have been detected in all histological subtypes, but especially in solid signet-ring cell and mucinous cribriform patterns.

Figure 1 Graphic representation of the main variants of the ALK fusion genes. Adapted from Atlas Genet Cytogenet Oncol Haematol. Perner S, Wilberz T, Stiedl AC, Rubin MA. EML4 (echinoderm microtubule associated protein like 4). Webpage on the internet available at: http://atlasgeneticsoncology.org/Genes/EML4ID44353ch2p21.html. The clinical significance, if any, of the different variants is currently not defined. Whatever considering a translocation or an inversion, the EML4–ALK gene fusion results in overexpression of the ALK protein, and subsequently overactivity of the ALK tyrosine kinase. This ALK protein tyrosine kinase is the target of inhibitors, of which crizotinib is the most advanced in clinical evaluation.

| Variant | EML4 | ALK |
|---------|------|-----|
| Variant 1 | ![image] | ![image] |
| Variant 2 | ![image] | ![image] |
| Variant 3a | ![image] | ![image] |
| Variant 3b | ![image] | ![image] |
| Variant 4a | ![image] | ![image] |
| Variant 4b | ![image] | ![image] |
| Variant 5a | ![image] | ![image] |
| Variant 5b | ![image] | ![image] |
| TFG | ![image] | ![image] |
| KIF5B | ![image] | ![image] |
### The pitfalls in detecting the ALK mutations

As new therapeutics is currently available, it is critically important to select those patients who are more likely to have the ALK gene abnormality. There is currently no gold-standard technique for detecting EML4–ALK in NSCLC. Several methods are available: FISH, reverse-transcriptase (RT)-PCR and immunohistochemistry (IHC).

**ALK** positivity by FISH is used as an eligibility criterion in clinical trials with crizotinib. The presence of more than 15% tumor cells with an ALK FISH-positive pattern using break-apart probes was the reference to prove the presence of an ALK rearrangement in the patients enrolled in those studies. It is the first FDA-approved method for detecting the ALK fusion gene, and in the US, prescription of crizotinib is dependent upon the use of the Vysis ALK Break Apart FISH Probe Kit (Abbott Molecular, Des Plaines, IL, USA). Nevertheless, this technique has limitations for use in routine practice. The break-apart signal pattern resulting from the intrachromosomal deletion and inversion event in the setting of polysomy, typical of lung cancer, is subtle and easily missed. The morphologic features are lost with FISH analysis, which requires specialized equipment and highly trained readers for interpretation and remains time-consuming and costly.

RT-PCR is a rapid diagnostic method and highly sensitive, as a very low RNA copy number is needed. The chromosomal inversion that characterizes the ALK fusion makes the sequence unique, and the RT-PCR primer only hybridizes with the fusion chimeric transcript. This unique primer is responsible for the high sensitivity of this method. In order to target all the potential fusion variants that have been reported, RT-PCR has to be multiplexed, ie, several forward primers on EML4 (±on other fusion partners) are required. Further, the diagnostic relevance of PCR directly relies on the quality of the extracted RNA, requiring a proper cryopreservation of tumor samples, and in this way is difficult to apply to archival tissues. These disadvantages make it unlikely that PCR will become the standard test for detecting the EML4–ALK fusion.

IHC has the advantage of being a cheap, easy-to-use, and routine pathological technique. Theoretically, it has the ability to identify all EML4–ALK variants, allowing a comparison with morphology, and is applicable on archival tissues. IHC positivity is dependent on the degree of expression of the EML4–ALK protein and commercially available ALK antibodies have been reported to lack the sensitivity to detect the fusion protein. Agreement between the three detection methods is relatively poor and variable according to the EML4–ALK variant. A recent effort from the European Thoracic Oncology Platform allowed a better understanding of the role of IHC for ALK translocation detection. From this consortium, 1099 stage I–IIIB

| Reference          | n   | EML4–ALK n (%) | ADC (n/+) | SCC (n/+) | Test | Clinical characteristics associated with EML4–ALK |
|--------------------|-----|----------------|-----------|-----------|------|--------------------------------------------------|
| Soda et al[^9]     | 65  | 5 (7.7%)       | 4         | 1         | PCR  | –                                                |
| Koivunen et al[^17]| 305 | 8 (2.6%)       | 8/208 (3.8%) | 0/88       | FISH | Nonlight smoking (P = 0.049)                      |
| Inamura et al[^13] | 221 | 5 (2.3%)       | 5/149 (3.4%) | 0/72       | PCR  | –                                                |
| Perner et al[^17]  | 603 | 16 (2.7%)      | –         | –         | FISH | –                                                |
| Wong et al[^21]    | 266 | 13 (4.9%)      | 11/209 (5.3%) | 0/34       | PCR  | Nonsmoking (P = 0.009), age (P = 0.018)          |
| Shaw et al[^18]    | 141 | 19 (13.5%)     | 18/130 (13.8%) | 0/2       | FISH | Age (P = 0.005), male (P = 0.036), nonlight smoking (P < 0.001) |
| Inamura et al[^14] | 363 | 11 (3%)       | 11/253 (4.3%) | 0/72       | PCR  | Age (P = 0.006), light smoking (P = 0.04), T status (P = 0.039), histological differentiation (P = 0.008) |
| Takahashi et al[^20]| 313 | 5 (1.6%)       | 5/211 (2.4%) | 0/75       | PCR  | Light smoking (P < 0.001), histological differentiation (P < 0.001) |
| Sun et al[^19]     | 52  | 3 (5.8%)       | 3/52 (5.8%) | –         | PCR  | –                                                |
| Martelli et al[^29]| 120 | 9 (7.5%)       | 3/60 (5%) | 4/48 (8.3%) | PCR  | –                                                |
| Zhang et al[^22]   | 103 | 12 (11.7%)     | 10/62 (16.1%) | 2/29 (6.9%) | PCR  | Nonsmoking (P = 0.03)                            |
| Kwak et al[^26]    | 1500| 82 (5.5%)      | 79        | 1         | FISH | –                                                |

**Abbreviations:** ADC, adenocarcinoma; FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction; SCC, squamous cell carcinoma; n/+n, number of patients with positive ALK testing between all tested patients.
NSCLCs were tested by IHC. Sixty-nine samples turned positive and were matched with 138 IHC-negative testing. From 60 IHC positive tumors (nine could not be tested by FISH), 22 were confirmed FISH-positive, while among 138 IHC-negative, only one was FISH-positive. The sensitivity and specificity of IHC were 37% and 90%, respectively, but increased to 90.5% and 97.7% if only IHC 3+ was considered. IHC could be viewed as a screening test with a high negative predictive value. If IHC is negative, the tissue is considered negative, but if it turns positive confirmation by another method is mandatory. This algorithm remains to be validated.

**ALK inhibitors for the treatment of NSCLC**

The first ALK inhibitor, PF-02341066 or crizotinib, was developed by Pfizer (Table 2). It is an orally active inhibitor of receptor tyrosine kinases, including ALK and mesenchymal epithelial growth factor (c-MET)/hepatocyte growth factor receptor (HGFR). It blocks ALK and c-MET/HGFR phosphorylation showing in vitro antiangiogenic and anti-metastatic effects, inhibiting ALK-positive cell growth and inducing apoptosis of those cells. In vivo models in athymic mice showed reduction of ALK-positive or activated c-MET/HGFR tumor xenografts. Crizotinib is metabolized by the cytochrome P450 3A4/5 (CYP3A) so that inducers or inhibitors of this enzymatic system can impact on drug exposure. A different pharmacologic profile has been reported between Asian and non-Asian patients.

### Table 2 Response rates reported in clinical trials testing ALK or Hsp90 inhibitors in ALK-positive NSCLC

| Reference   | Drug     | Previous therapy                  | n     | Phase | Response rate |
|-------------|----------|-----------------------------------|-------|-------|---------------|
| Camidge et al** | Crizotinib | First-line and salvage therapy     | 143 crizotinib-naive | I     | 60.8%         |
|             |          |                                   | First-line therapy    |       | 63.6%         |
|             |          |                                   | Salvage therapy (2–3 lines) | II    | 58.7%–64.5%  |
|             |          |                                   | 803 crizotinib-naive |       | 46%           |
|             |          |                                   | 261 “mature population”* |       | 60%           |
|             |          |                                   | 173 crizotinib-naive | III   | 65.7%         |
|             |          |                                   | 59     | I     | 41%           |
|             |          |                                   | Crizotinib-naive (n = 14) |     | 21%           |
|             |          |                                   | Crizotinib-pretreated (n = 45) |    | 47%           |
| Kim et al** | Crizotinib | Salvage therapy                  | 20 crizotinib-naive | I     | 85%           |
|             |          |                                   | 45 crizotinib-naive | II    | 85%           |
|             |          |                                   | 11     | I/II  | 73%           |
|             |          |                                   | Crizotinib-naive (n = 2) |     | 100%          |
|             |          |                                   | Crizotinib-pretreated (n = 9) |     | 67%           |
|             |          |                                   | 22     | II    | 32%           |
|             |          |                                   | Crizotinib-naive (n = 8) |     | 50%           |
|             |          |                                   | Crizotinib-pretreated (n = 14) |   | 21%           |
| Shaw et al**| Crizotinib | Second-line therapy               | 59     | I     | 41%           |
|             |          |                                   | Crizotinib-naive (n = 14) |     | 21%           |
|             |          |                                   | Crizotinib-pretreated (n = 45) |    | 47%           |
| Nishio et al** | CH5424802 | Salvage therapy                  | 20 crizotinib-naive | I     | 85%           |
|             |          |                                   | 45 crizotinib-naive | II    | 85%           |
|             |          |                                   | 11     | I/II  | 73%           |
|             |          |                                   | Crizotinib-naive (n = 2) |     | 100%          |
|             |          |                                   | Crizotinib-pretreated (n = 9) |     | 67%           |
|             |          |                                   | 22     | II    | 32%           |
|             |          |                                   | Crizotinib-naive (n = 8) |     | 50%           |
|             |          |                                   | Crizotinib-pretreated (n = 14) |   | 21%           |

**Notes:** *The mature population concerned the first 261 patients included and treated in the study as of February 1, 2011; salvage therapy refers to patients treated with at least one previous therapy.

**Phase I study with crizotinib**

Crizotinib demonstrated effectiveness during a dose-escalation phase I study in two patients with NSCLC presenting with ALK rearrangement. In an expanded cohort study, impressive response and 8-week disease-control rates were observed of 57% (95% confidence interval [CI] 46%–68%) and 87%, respectively. The results of this phase I study were recently updated. One hundred forty-three evaluable patients were included. The response rate (RR) was confirmed at 60.8% (95% CI 52.3%–68.9%), with a median duration of 49.1 weeks (95% CI 39.3–75.4 weeks). Most of the patients received crizotinib for salvage treatment after one (32%), two (21%), three (13%), or more than three (19%) previous chemotherapy lines. Nevertheless, results were similar whatever the number of previous treatments, with an RR of 63.6% in untreated patients and 59.1%, 64.5%, and 58.7% for those having received one, two, or more than two previous chemotherapy treatments. Sensitivity (RR) to crizotinib appears independent of age (<65 years 60.2% versus >65 years 65%), sex (male 64.8% versus female 56.9%), and performance status (Eastern Cooperative Oncology Group [ECOG] score 0, 54.7% versus ECOG 1, 63.9% versus ECOG 2, 66.7%), while Asian patients seem more sensitive to the drug (RR 76.9%, 95% CI 60.7%–88.9%) than non-Asian ones (RR 54.8%, 95% CI 44.7%–64.6%). The estimated median progression-free survival was 9.7 months (95% CI 7.7–12.8 months). At the time of analysis, median survival was not yet reached, and estimated 6- and 12-month survival rates were 87% and 74.8%, respectively.
Phase II study with crizotinib

A phase II study (Profile 1005) in advanced ALK-positive NSCLC is ongoing. First results were presented at the 2012 American Society of Clinical Oncology (ASCO) and European Society for Medical Oncology (ESMO) meetings.\(^4\) Crizotinib 250 mg twice daily was orally administered to patients progressing after at least one previous chemotherapy regimen. Patients with brain metastases were not excluded. As of January 2012, 901 patients had been registered, of whom 803 were assessable for response. A subgroup including a so-called mature population of 261 patients (those enrolled and treated before February 2011) was evaluated. Demographics were similar to previous epidemiological studies (Table 1) with a predominance of nonsmokers (66%), adenocarcinoma (92%), and good ECOG performance status of 0/1 (82%). The median duration of treatment was 20 weeks in the whole group, but 48 weeks (range 1–94) in the mature population. Results of the previous study were confirmed either in the whole group or in the mature population, with respective RRs of 46% (95% CI 42%–50%) and 60% (95% CI 54%–66%), disease-control rates at 6/12 weeks of 80% (95% CI 77%–83%)/61% (95% CI 58%–64%) and 86% (95% CI 82%–90%)/75% (95% CI 70%–80%), and median progression-free survival of 8.1 months (95% CI 6.9–9.5) and 8.1 months (95% CI 6.8–9.7). While 15% of the patients discontinued treatment due to adverse events, these were generally of low grade, confirming the phase I data.\(^5\) They mainly consisted of visual disturbances, gastrointestinal disorders (nausea, vomiting, constipation, or diarrhea) and transaminase abnormalities.

Visual disorders are an uncommon side effect observed with other targeted therapies, such as mitogen-protein activated kinase (MEK) inhibitors, and occurred in up to 60% of the patients treated with crizotinib.\(^3,13\) They are generally mild and can reverse while on treatment. Most commonly, they present as flashing lights, streamers, or overlapping images without impact on daily living. The impact of crizotinib on quality of life (QOL) was assessed as a secondary end point in the Profile 1005 study.\(^10\) It was confirmed that a clinically significant deterioration was due to constipation and diarrhea. Despite these adverse events, a significant improvement in the QOL European Organisation for Research and Treatment of Cancer QLQ-C30 questionnaire was found for global QOL, physical, role and, social functioning, and for some patients, self-reported symptoms included cough, pain, dyspnea, and fatigue.

Phase III trials with crizotinib

Two phase III trials comparing crizotinib to chemotherapy have been designed. Profile 1007\(^37\) is comparing crizotinib to docetaxel or pemetrexed in advanced ALK-positive NSCLC in a second-line setting. Patients presenting with ALK-positive NSCLC by central FISH testing progressing after one previous platinum-based chemotherapy, ECOG performance status 0–2, and a measurable disease were eligible. Brain metastases were allowed, provided that they have been previously treated. Patients were randomized between oral crizotinib at 250 mg twice daily or monotherapy with pemetrexed (500 mg/m\(^2\) with folate and vitamin B\(_12\) supplementation) or docetaxel (75 mg/m\(^2\)) every 3 weeks. In case of progression on chemotherapy, patients were allowed to cross over on crizotinib. The primary end point was progression-free survival, assessed by an independent radiological board. One hundred seventy-three patients were randomized to crizotinib and 174 to chemotherapy, of whom 99 and 72 received pemetrexed and docetaxel, respectively. As for previous populations, there was a high proportion of nonsmokers and a large predominance of adenocarcinoma (95%). Median progression-free survival was substantially improved from 3.0 months for the chemotherapy arm to 7.7 months in the crizotinib one (hazard ratio [HR] 0.49, 95% CI 0.37–0.64; \(P < 0.0001\)). The comparison of crizotinib with each chemotherapeutic drug also showed a significant improvement with respective median progression-free survival of 4.2 (\(P = 0.0004\)) and 2.6 months (\(P < 0.0001\)) for pemetrexed and docetaxel, respectively. An impressive RR in the second-line setting was observed with crizotinib (65.7%), while it was higher, as expected, for pemetrexed (29.3%) and in the classical range for docetaxel (6.9%). This effectiveness did not result in prolonged survival at the first interim analysis with medians of 20.3 and 22.8 months (HR 1.02, 95% CI 0.68–1.54; \(P = 0.54\)) for the crizotinib and chemotherapy arms. However, 111 patients crossed over to crizotinib after chemotherapy failure. When performing survival analysis adjusting for crossover, the HR was 0.83 (95% CI 0.36–1.35). As expected from previous publications, the most frequent adverse event related to crizotinib administration was visual disorders of grade 1–2. Nevertheless, some important signals came from this study concerning nonhematological adverse events. These included grade 3–4 transaminase elevation in up to 16% of the cases, but also pulmonary embolism, pneumonia, hypokalemia and QTc prolongation in 4% of the patients treated with crizotinib. It must be stressed that one patient died from arrhythmia in the crizotinib group. Despite these adverse events, a statistically significant improvement in global QOL, physical, emotional, and social functioning, as well as for cough, dyspnea, fatigue, alopecia, insomnia, and pain (all \(P < 0.0001\)) were noted with crizotinib, confirming the first results reported in Profile 1005. Importantly, time to deterioration in lung cancer
symptoms was 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.

A phase III randomized trial is ongoing, comparing crizotinib to a combination of cisplatin and pemetrexed in chemotherapy-naive ALK-positive NSCLC patients, with crossover allowed at progression. The primary end point is progression-free survival.

Phase I and randomized studies are not designed to answer the question of improving survival with crizotinib in comparison with conventional chemotherapy, as crossover is allowed in randomized trials. Data reporting survival improvement with crizotinib in ALK-positive NSCLC came from a retrospective case-control study. The cases consisted of the 82 ALK-positive patients included in the initial phase I study, and the controls were advanced NSCLC patients who had tested positive for EML4–ALK fusion and were not receiving crizotinib (n = 36), with EGFR mutation and negative for ALK (n = 67), or negative for both genomic abnormalities (n = 253). The median survival time for crizotinib-treated patients was not reached, with 1- and 2-year survival rates of 74% (95% CI 63%–82%) and 54% (95% CI 40%–66%). When looking at comparable groups, including non-Korean patients receiving crizotinib in second- or third-line therapy (n = 30) and crizotinib-naive patients receiving any second-line therapy (n = 23), a survival improvement was documented, with 1- and 2-year survival rates of 70% (95% CI 50%–83%) and 55% (95% CI 33%–72%) versus 44% (95% CI 23%–64%) and 12% (95% CI 2%–30%), respectively. Median survival time was not reached for the crizotinib group, while it was 6 months (95% CI 4–17 months) for the crizotinib-naive one. The HR for overall survival was 0.36 (95% CI 0.17–0.75; \(P = 0.004\)). ALK-negative patients showed similar survival to crizotinib-naive ALK-positive ones (\(P = 0.18\)), but there was reduced prognosis in comparison with those NSCLC patients receiving crizotinib for second- or third-line therapy (HR 0.49, 95% CI 0.27–0.91; \(P = 0.02\)). The incremental survival obtained with crizotinib was of the same order as the addition of an EGFR tyrosine kinase inhibitor in EGFR-mutated tumors (\(P = 0.58\)). These results suggested that EML4–ALK is not a prognostic factor for survival but is predictive of the efficacy of the ALK inhibitor crizotinib.

Based on these results, the US FDA approved in August 2011 crizotinib administration in ALK-positive (that needs to be detected by an FDA-approved test) locally advanced or metastatic NSCLC. In Europe, the European Medicines Agency approved in July 2012 crizotinib for the treatment of adults with previously treated ALK-positive advanced NSCLC.

### Resistance to crizotinib

Development of resistance is observed in virtually all patients, even with very active targeted therapies as EGFR tyrosine kinase inhibitors in EGFR-mutated NSCLC or crizotinib in ALK-positive tumors. The first case of crizotinib-resistant tumor was reported in a young NSCLC patient presenting with a 5-month response duration to the ALK inhibitor. Two mutations in the tyrosine kinase domain of EML4–ALK were documented — C1156Y and L1196M. Another case was subsequently published showing another mutation in the kinase domain (L1152R) with a coexisting activation of EGFR signaling. In vitro models suggested that different mechanisms are responsible for the resistance to crizotinib, including gene amplification and mutations in the gatekeeper kinase domain that can be overridden by other ALK inhibitors, such as TAE684, or Hsp90 inhibitors. These observations were recently confirmed in two series of NSCLC patients progressing on crizotinib. In the first one, a secondary mutation in the ALK tyrosine kinase domain or a gene amplification was found in 28% of the cases. Aberrant mutations including KIT and increased EGFR autophosphorylation were also documented, while multiple mechanisms were coexisting. In the second series, secondary mutations in the ALK tyrosine kinase domain, ALK copy number gain, and the presence of another oncogene driver (EGFR or KRAS) were responsible for the resistance to crizotinib. The same author reported during the 2012 ASCO meeting a series of 16 patients progressing on crizotinib with the same distribution of genomic alterations. He suggested a separation of resistant tumors into those having persistent ALK dominance (secondary mutations in the ALK tyrosine kinase domain with or without increased gene copy number) from those that are ALK nondominant (other oncogene driver, loss of the ALK translocation).

### New strategies for ALK inhibition

Further ALK or Hsp90 inhibitors are currently in development at a preclinical or a clinical stage, most of them having dual or multiple therapeutic targets or demonstrating activity in crizotinib-resistant ALK-positive NSCLC strains. The main compounds are developed by Novartis (NVP-TAE684, LDK378, 3-39, AUY922 [Hsp90 inhibitor]), Chugai (CH5424802), Astrella (ASP3026), Ariad (AP26113), GlaxoSmithKline (GSK 1838705A), Xcovery (X-396), Infinity (IPI504, an Hsp90 inhibitor), Nerviano Medical Science (NMS-E628), and Cephalon (CEP-14083, CEP-28122). Few clinical data are currently available.

Some early phase I or II studies, all performed in pretreated patients, were presented at the 2012 ASCO and
ESMO meetings. In a dose-escalating phase I study including ALK-positive solid tumors of any type, LDK378 was administered to 79 NSCLC patients, of whom 56 were pretreated with crizotinib.\textsuperscript{48} LDK378 is active on conventional ALK-positive tumors but also on those expressing the gatekeeper mutation C1156Y and the insulin growth factor 1 receptor. Among 59 patients receiving an LDK378 dose between 400 and 750 mg per day, an objective response was documented in 24 cases (41%) and in 21 patients progressing after crizotinib (47%). Activity was also found on brain metastases. Phase II studies are planned either in ALK-positive NSCLC pretreated with chemotherapy and crizotinib and in those naive of ALK inhibitor. CH5424802 is presenting with different binding capacity to the adenosine triphosphate pocket of the ALK domain, explaining better inhibition of ALK-positive tumors less sensitive to crizotinib and activity in the presence of the gatekeeper mutation of the ALK domain, L1196M, and C1156Y, with tenfold more potency than crizotinib.\textsuperscript{49} This compound was tested in a phase I/II study in previously treated ALK-positive NSCLC patients naive to crizotinib. In the phase I part, an 85% response rate was observed among 20 assessable patients.\textsuperscript{50} Preliminary results of the phase II confirmed an RR of 85% (95% CI 71%–94%) among 45 evaluable patients, including regression of brain metastases.\textsuperscript{51} AP26113, which is potentially active both on ALK-positive and EGFR-mutated tumors, including the T790M mutation, showed activity in crizotinib-resistant ALK-positive NSCLC in a dose-finding phase I/II study.\textsuperscript{52} AP26113 is, in vitro, ten times more powerful for ALK and ROSI inhibition than crizotinib and is also active in the case of L1196M mutation in a model of acquired resistance to crizotinib.\textsuperscript{41} Among eleven assessable patients with ALK-positive NSCLC, eight partial responses were noted, of which six occurred in nine patients progressing on crizotinib. The drug appears to be active on brain metastases. Lastly, AUY922, an Hsp90 inhibitor, was tested in a phase II study including ALK-positive and EGFR-mutated NSCLC.\textsuperscript{53} Among 22 assessable ALK-positive tumors, seven objective responses (32%) were noted, three among 14 crizotinib-resistant NSCLC patients. The disease-control rate for the whole group was 59%, being 100% and 36% in the crizotinib-naive and -resistant groups respectively. All these preliminary data (Table 2) support the use of ALK and Hsp90 inhibitors in NSCLC patients presenting with EML4–ALK gene fusion, whether sensitive or resistant to crizotinib.

Crizotinib has other potential promise for advanced NSCLC. It has shown clinical activity in another subgroup of NSCLC patients harboring ROSI rearrangement. In an expansion cohort of the initial phase I study, 23 patients, of whom 20 were assessable for response, were included. A 50% objective response rate was noted, and recruitment is ongoing.\textsuperscript{54} This drug is also currently tested in combination as a way to overcome acquired resistance to EGFR tyrosine kinase inhibitors.\textsuperscript{55,56}

**Conclusion**

Knowledge of cancer biology and oncogenic drivers has led to a better understanding of lung cancer and the development of very active targeted therapies. Nowadays, EGFR tyrosine kinase inhibitors for tumors presenting with an activating mutation are the standard of care in NSCLC. New targets have been identified, including the EML4–ALK gene fusion, which is a relatively rare genetic abnormality predominant in adenocarcinomas. Crizotinib, a dual ALK and cMET inhibitor, showed impressive activity in this small group of patients when given as first-line or salvage therapy, whatever the number of previous chemotherapy lines. Based on available data, Figure 2 is a proposed algorithm for the therapeutic management of NSCLC. As observed with other targeted agents, resistance occurs invariably. Research has identified secondary ALK mutations, gene amplification, and other aberrant mutations (KIT, EGFR, KRAS, etc) as the main mechanisms. New ALK and Hsp90 inhibitors are under clinical investigation and have shown promising results in early phase I/II studies.

**Disclosure**

The authors have no conflicts of interest to be declared in relationship with the present manuscript.
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