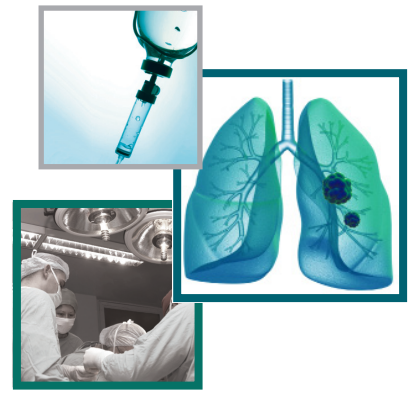
EDITORIAL



Crizotinib: hunting another piece of the lung cancer genome

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"Crizotinib appears to be a safe and manageable drug with consistent data among studies."

In the last decade a better understanding of tumor biology has led to the identification of several potential oncogenic drivers in non-small-cell lung cancer (NSCLC), including the *ALK* translocation, described in approximately 6–8% of adenocarcinomas [1]. This was recognized as a potential target early in the clinical development of crizotinib (Xalkori®, Pfizer, NY); following clinically significant objective responses seen in two *ALK*-positive patients treated in a Phase I clinical trial in solid tumors and lymphomas, in which the drug was initially investigated as a c-MET/HGF tyrosine kinase inhibitor. The study was consequently amended to include an expanded cohort of *ALK*-positive patients with lung cancer [2]. Accelerated US FDA approval of crizotinib in advanced NSCLC patients with *ALK* translocation, as identified by Vysis (Abbott Moleular) ALK break apart FISH probe kit, has been recently granted, for the first time in NSCLC, based on the results of Phase I and II trial data (PROFILE 1005, NCT00932451). The overall response rate was 61% in the Phase I portion of the study and 51% from the preliminary analysis of the Phase II trial, progression-free survival in Phase I was 10 months with an early and prolonged clinical benefit and a tolerable safety profile [3–6]. Besides this shining and very rapid ascent in the scientific scenario, there are still some noteworthy issues that should be pointed out.

To select candidate patients to receive crizotinib, the FDA has established FISH as the gold standard for detecting *ALK* rearrangements. However, other methods such as immunohistochemistry (IHC) and reverse transcriptase-PCR (RT-PCR), have been evaluated. The ALK FISH technique is cumbersome, may be associated with false negatives (splitting of red and green signals can be subtle), requires specialized technical resources and expertise, is not available or reproducible in all pathology laboratories and the cost per patient is still high [7].

IHC is widely used, rapid and the pathologist-preferred method for screening and diagnosis. The first major limitation to routine use of IHC to detect *ALK* rearrangement is the often low-level expression of ALK fusion proteins in positive NSCLC cases, which needs the development of more sensitive IHC methods and different antibodies. Several

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antibodies have been tested, some with very promising results in terms of specificity, such as D5F3, developed by Cell Signaling or the 5A4 by Ventana [8,9]. Considering that an IHC score ≥3 has demonstrated 100% concordance with FISH positivity, while a score of 0 has shown 100% concordance with FISH negativity, a two-tier screening could be a good compromise waiting for more robust data from the above-mentioned ongoing evaluations. One possible algorithm should be characterized by an initial IHC screening, followed by FISH evaluation for those IHC cases scored 1 and 2 [10].

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Minimal data comparing the results of RT-PCR screening with either ALK FISH or ALK IHC are available, and in tissues with borderline preservation RT-PCR will more likely fail compared with FISH or IHC, because RNA is typically more sensitive to degradation than DNA or protein. This method should identify the specific fusion, but requires several sets of PCR primers to cover all rearrangements: the results of a panel of RT-PCR assays for *EML4–ALK* fusion gene variants presented at the last American society of American Oncology (ASCO) meeting represent the basis for the commercial diagnostic assay for *ALK* rearrangement offered by Response Genetics Inc. [11].

Although *ALK* rearrangement is associated with certain clinical features, such as younger age and never-smoking history, these are by no means absolute associations: in the Phase II PROFILE 1005 study presented at the last ASCO meeting, the median age of the 901 patients enrolled was 53 years (ranging from 18 to 83 years old) with a non-negligible percentage of smoker or former smoker (34.3%) [12]. The amount of data currently available do not allow any specific selection to screen NSCLC patients for *ALK* rearrangement. However, because in NSCLC *EGFR* and *ALK* genomic changes are generally mutually exclusive, in newly diagnosed patients a sequential approach could be considered with *EGFR* mutation testing followed by ALK FISH, or ALK FISH could be offered to test previously treated patients who are not known to harbor mutations in *EGFR* or other oncogenic drivers such as *KRAS*. Further data coming from Phase III trials could better address this issue, establishing more precise criteria regarding the patient population to be screened.

Crizotinib appears to be a safe and manageable drug with consistent data among studies. The most recent results come from the PROFILE 1005 study, enrolling 136 patients in a Phase II study of crizotinib in recurrent, advanced or metastatic NSCLC, who progressed after previous chemotherapy. Common treatment-related adverse events were gastrointestinal (diarrhea, nausea and vomiting) and visual events (e.g., transient problems with light/dark adjustment, shimmering, streaks and/or floaters, flashing lights and/or trailing lights, in most of the cases transient and with minor impact on daily life), mainly with a grade 1 or 2 severity and often improving with continuation of therapy.

Regarding gastrointestinal toxicity, the 2012 ASCO meeting results on 1054 patients treated with crizotinib have been presented showing a high percentage of AST, ALT and AP elevation (70.9% for ALT, 61.3% for AST and 64.7% AP) and also a 3.6% increase in serum bilirubine. Most of these events were reported as grade 1–2, with the first adverse event reported mainly within the first 2 months from the start of treatment; three cases met the Hy's law criteria (an index of a compromised hepatic function) and no specific risk factor was identified. Consequently, it was suggested to carefully check hepatic function and to also screen for the identification of signs and symptoms potentially indicating liver dysfunction in the first 2 months of treatment [13]. Recently, hypogonadism in 19 male patients treated with crizotinib has been reported, identifying testosterone levels significantly lower in crizotinib-treated patients compared with controls (131 vs 311 ng/dl, respectively) [14]. This adverse effect may be responsible for signs and symptoms such as fatigue (20% in Phase I and II trials, mostly grade 1 and 2), depression and sometimes insomnia, in addition to sexual dysfunction, but more data are needed before making any statements or taking into consideration therapeutic measures (for instance testosterone replacement), and some researchers are prospectively collecting further information regarding this specific finding.

The CNS is described as the most common site of relapse in crizotinib-resistant patients: in patients enrolled in PROFILE 1001 and PROFILE 1005, the brain was the most common site of progression (46%) [15,16]. Differently from standard clinical studies in NSCLC evaluating

cytotoxic drugs, clinical trials with crizotinib allow the continuation of the drug in the case of RECIST disease progression if there is a manifested clinical benefit from the treatment itself. Data regarding the benefit of continuation of crizotinib after the progression of the disease showed that 30% of patients received the drug for a period longer than 6 months and most of them were patients with cerebral metastases [15]. In crizotinib-treated patients a dramatic response to treatment is often observed, but after a variable period of time the tumor starts to slowly grow back. However, tumor size is still much smaller than at baseline. Despite the RECIST definition of progressive disease, keeping the patient on crizotinib may still continue to derive clinical benefit, and thus using progression-free survival and RECIST criteria may not truly reflect the beneficial effect of a highly effective targeted therapy, compared with classic cytotoxic drugs [17].

Data regarding drug resistance have curbed the impact of the rapid and prolonged clinical benefit of reversible EGFR tyrosine kinase inhibitors along with crizotinib in *ALK*-positive NSCLC patients. In a recent report by Doebele and colleagues, two major mechanisms of resistance to *ALK* inhibitors have been described: one so called ALK dominant (50% of the cases) and the other non-ALK dominant. In the first case, the genomic alteration is inside the *ALK* domain (for example development of mutations within the drug target that alter drug sensitivity and *ALK* fusion gene copy number gain), in the other group there is the possibility of the emergence of alternative mechanisms of resistance such as *EGFR*- or *KRAS*-activating mutations consequent to a selection of resistant clones. In approximately 20% of patients, the cause of acquired resistance to crizotinib is still unknown [18].

A paper presented at the 2012 annual ASCO meeting reported that in a cohort of 16 patients, who developed resistance to ALK inhibitors, in six cases a single mutation of the ALK kinase domain sequence was described, and none of these cases presented with the same mutation. Could we consider each of these patients as a single small population or do the presence of these mutations not affect the effectiveness of another ALK inhibitor?

A new ALK inhibitor called LDK378 showed promising results in patients with NSCLC who progressed with crizotinib (response rate of 81%) and interestingly a specific activity was described on brain metastases, representing a potential advantage compared with crizotinib even if the toxicity profile seems to not be as tolerable as it is for the FDA-approved ALK inhibitor [19].

ROS1 rearrangement in NSCLC was originally discovered in 2007; although *ROS1* shares only 49% amino acid sequence homology with *ALK* in the kinase domain, several ALK inhibitors have demonstrated *in vitro* inhibitory activity against *ROS1*. *ROS1*-rearranged and *ALK*-rearranged NSCLC patients share similar clinicopathologic characteristics such as younger median age of diagnosis and never-smoking status with a diagnosis of adenocarcinoma. Patients with advanced NSCLC harboring *ROS1* rearrangement, as determined using a break-apart FISH assay, were recruited into an expansion cohort of a Phase I study of crizotinib and 13 were available for response, reaching an overall response rate of 54% [20].

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Although further consideration regarding the optimal diagnostic test to identify this target is largely warranted, and more potent inhibitors are currently in clinical development, crizotinib and the break-apart FISH test have clearly identified a percentage of NSCLC patients with dramatically improved outcomes compared with previous experiences and other therapeutic approaches.

Continuous learning from research developments, such as for crizotinib, together with advances in cancer genome deep sequencing and tumor molecular profiling, will represent the basis for future clinical trial designs, such as small early-phase trials employing adaptive hypothesis testing conducted in molecularly defined populations enriched for the drug target.

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