Editorial

Distinct benefit from crizotinib in lung cancer patients carrying distinct ALK translocations: is fluorescent hybridization *in situ* testing still sufficient to guide clinical decisions?

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ALK rearrangements in lung cancer (LC) were discovered in the year 2007 upon the systematic search for novel LC-associated oncogenes (1,2). Fortunately, an experimental MET inhibitor, PF-2341066 (crizotinib), was by then known to have a concurrent ALK-inhibiting activity and its clinical profile was already under phase I evaluation (3-6). It was quickly revealed that the status of ALK, but not MET, is a primary determinant of tumor sensitivity to crizotinib (5), and a number of subsequent studies heralded a real breakthrough in the treatment of ALK-rearranged cancers (6-9).

Almost all pivotal trials involving ALK inhibitors relied on a companion fluorescent hybridization *in situ* (FISH) break-apart assay for the detection of ALK rearrangements. FISH is perfectly compatible with the routine of histopathological diagnosis of LC and is capable to detect all variants of ALK translocations. However, FISH is cumbersome and prohibitively expensive, therefore many laboratories now utilize immunohistochemical (IHC) prescreening for ALK-overexpressing LC in order to reduce the number of tumors forwarded to FISH-testing. For the time being, the majority of clinical decisions regarding the administration of ALK inhibitors is based on FISH or IHC/FISH testing, with thousands of patients receiving ALK-specific treatment worldwide. It is important to bear in mind that IHC/FISH, being proficient in establishing the mere fact of the presence of ALK translocation in the tumor, are unable to inform on the exact molecular structure of the detected ALK rearrangements (10-14).

There are a few dozen of distinct variants of ALK fusions and the novel types of chimeras continue to be identified (6,15-17). All ALK rearrangements preserve tyrosine kinase domain, with the breakpoint usually occurring before the exon 20. However, the gene partners and the composition of 5'-terminal part of the chimeric protein vary substantially, and at least some translocation variants demonstrate significant differences in sensitivity to crizotinib in laboratory experiments (18). The potential clinical significance of these differences remains largely uncertain, owing to the fact that ALK-specific inhibitors are usually prescribed solely on the basis of FISH-test result, and the ALK variant subtyping is not required for the drug administration (10-14).

Recently published study of Yoshida *et al*. (19) demonstrates that the diagnostic attitude towards ALK translocations has to change, at least on the level of clinical investigations. Yoshida *et al*. (19) analyzed crizotinib treatment outcomes in 35 patients with distinct EML4-ALK translocations. The median progression-free survival (PFS) in 19 patients with the variant 1 fusion (E13;A20) approached to 11.0 months, while PFS in 16 patients carrying other EML4-ALK rearrangements was only 4.2 months. Statistical analysis confirmed the significance of this difference. These data have potential practical importance, as they may impact the sequence of targeted and cytotoxic therapies. For example, there are two major types of *EGFR* mutations in LC, ex19del and L858R, with the former rendering more pronounced tumor response to EGFR inhibitors than the latter. Accordingly, patients with EGFR ex19del survive significantly longer when afatinib is administered in the first line,

whereas a chemotherapy may be considered as an upfront treatment option for the patients carrying the L858R (20). It remains to be addressed whether similar trend is applicable to the patients with distinct ALK translocations.

The study of Yoshida *et al.* (19) considered only known EML4-ALK fusions, while some other gene partners may be involved in ALK rearrangements as well (6,15-17). The mechanistic basis for the distinct duration of clinical response to crizotinib for LC carrying distinct ALK translocations is unknown. One hypothesis relies on the role of 5'-terminal portion of ALK chimeras in the protein oligomerization. It is also possible that the genetic variants of ALK translocations may have distinct propensity to acquire secondary mutations or provoke the bypass signaling pathways associated with the drug resistance. In addition, there is a question whether the correlations described by Yoshida *et al.* (19) are applicable to the novel ALK inhibitors, such as alectinib, ceritinib, brigatinib, lorlatinib, etc. (17).

The study of Yoshida *et al.* (19) illustrates an important gap in current diagnostic practices towards ALK translocations. Although polymerase chain reaction (PCR)-driven detection of ALK fusions is appreciated by many investigators due to its high sensitivity and ability to identify the translocation variant, its use in clinical routine is somehow discouraged (10-14). To our knowledge, Japan is the only country where the use of PCR for ALK detection is considered non-inferior to other testing methods (21); therefore it is not surprising that the first study emphasizing the significance of ALK genotyping came from this country (19). It is fair to acknowledge that commercial PCR kits usually target only the most common variants of ALK rearrangements, therefore, in contrast to FISH, rare ALK translocations are likely to be missed [for example, see descriptions for the Entrogene EML4-ALK Fusion Gene Detection Kit (http://entrogen.com/web3/eml4-alk-fusion-gene-detection-kit/), AmoyDx® EML4-ALK Fusion Gene Detection Kit (http://www.mobitec.com/cms/products/bio/09_ivd/Real-Time_PCR_Cancer_Diagnostic_Kits.html?pdf=ADx-AE02.pdf), QuanDx EML4-ALK Fusion Gene Detection Kit (http://www.quandx.com/sites/quandx.com/files/images/EML4-ALK%20flyer%20v3.0.pdf), Diacarta QFusion™ EML4-ALK and KIF5B-ALK Fusion Gene Detection Kit (http://www.diacarta.com/products/fusion-gene-tests/alk-fusion-gene-detection-kit/), etc.]. This limitation, however, can be overcome by PCR test for unbalanced ALK 5'/3'-end expression, which detects all types of rearrangements (15). Opponents of PCR-based ALK testing also frequently state that this methodology is less standardized as compared to the FISH analysis. Furthermore, FISH, but not PCR, was used as a companion test in the registration trials of ALK inhibitors, therefore some commercial agreements between diagnostic companies and drug manufacturers are also likely to play a role.

As a result, there is a drastic difference in the knowledge on clinical use of EGFR and ALK inhibitors. Ample experience has been accumulated for LC carrying distinct *EGFR* mutations and their response to distinct EGFR inhibitors (22,23). In contrast, despite the fact that ALK variant typing is no more complicated than *EGFR* mutation analysis, the data on genotype-response correlations for ALK-specific drugs remain very scarce. Similar limitations apply to the newly approved indication for crizotinib, i.e., ROS1-rearranged LC (24,25). We call to reconsider current approaches to the diagnostic translocation testing in human tumors and to encourage the identification of the involved gene fusion variants.

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