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Commentary

ALK Fusion Detection in Circulating Free DNA: Finding an Important Needle in the Haystack

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Disclosures of potential conflicts of interest may be found at the end of this article.

Since the initial discovery of chromosomal rearrangements involving the anaplastic lymphoma kinase (*ALK*) gene in non-small cell lung cancer (NSCLC) in 2007, *ALK* rearrangements have emerged as important therapeutic targets in oncology [1]. Indeed, within a decade of this initial description, three ALK-directed therapies have gained regulatory approval in the U.S., and multiple other ALK targeted therapies are in clinical development.

ALK rearrangements lead to expression of constitutively active fusion kinases that drive malignant growth and cellular proliferation. In NSCLC, *ALK* rearrangements are found in 3%–7% of patients and define a distinct molecular subset of the disease with characteristic clinical and pathologic features [2]. *ALK* rearrangements also confer exquisite sensitivity to treatment with ALK tyrosine kinase inhibitors (TKIs), such as crizotinib. In pivotal randomized trials, crizotinib produced significant improvements in response rates, progression-free survival, and quality of life compared to cytotoxic chemotherapy, establishing crizotinib as standard first-line therapy for ALK-positive NSCLC [3, 4]. While crizotinib has transformed the management of ALK-positive NSCLC, patients invariably develop resistance to therapy. As a result, a number of more potent and selective, next-generation ALK inhibitors (e.g., ceritinib, alectinib, brigatinib) have been developed, with each demonstrating significant antitumor activity in ALK-positive NSCLC [5–8].

Recently, the explosion of broad-based molecular testing in oncology has led to the identification of *ALK* rearrangements in malignancies beyond NSCLC and lymphoma [9]. Specifically, *ALK* fusions have now been reported in inflammatory myofibroblastic tumors (IMT) [10], colorectal cancer (CRC) [11, 12], breast cancer [11], renal cancer [13], and ovarian cancer [14]. However, ALK TKIs have yet to be approved for use in ALK-driven cancers outside of NSCLC to date.

In this issue of *The Oncologist*, Lai et al. and Wang et al. describe the presence of *ALK* rearrangements in two distinct malignancies and highlight the use of liquid biopsies in molecular diagnostics [15, 16]. Wang et al. report the case of an *ALK*-rearranged atypical neuroendocrine tumor with diffuse central nervous system metastases [15]. Of note, molecular testing could not be performed on the patient's initial diagnostic biopsy specimen due to insufficient tissue; however, genotyping of circulating free DNA (cfDNA) using a capture-based next-generation sequencing (NGS) platform revealed a novel *SMC5-ALK* fusion. Based upon this finding, the patient was treated with the next-generation ALK inhibitor alectinib, which resulted in significant systemic and intracranial responses, both of which were ongoing at the time of reporting. Likewise, Lai et al. used the same capture-based cfDNA platform to identify an *ALK* rearrangement in a patient with metastatic CRC [16]. Importantly, at the time of this patient's initial diagnosis, ALK immunohistochemistry (IHC) was negative, but parallel genomic profiling of cfDNA and available tissue using hybrid capture-based NGS identified a *STRN-ALK* fusion. These cases highlight the emerging role of liquid biopsies in molecular testing, as well as the complexities surrounding their use, particularly with respect to fusion detection.

Historically, ALK testing has been performed using fluorescence in situ hybridization (FISH) and/or IHC on formalin-fixed, paraffin-embedded tissue [2, 17, 18]. More recently, NGS, which permits the simultaneous evaluation of multiple genes, has also emerged as a promising alternative [19]. Nonetheless, tissue-based tests are not always feasible during routine clinical care due to various factors, including sites of malignant disease, insufficient tissue, or patient-specific factors, among others. As a result, liquid biopsies have gained momentum as less-invasive methods of genotyping.

The term "liquid biopsy" encompasses a range of assays aimed at evaluating circulating factors, including circulating tumor cells, cell-derived vesicles (exosomes), and cfDNA. To date, cfDNA analysis has emerged as the most common form of liquid biopsy to be used in the clinic. In general, clinically available cfDNA assays rely on either polymerase chain reaction (PCR) (e.g., allele-specific PCR, emulsion PCR) or NGS-based approaches (e.g., amplicion-based NGS, capture-based NGS) [20]. Though rapid, cost-effective, and highly sensitive, PCR-based assays evaluate only known genomic alterations and are unable to detect certain alterations, such as gene fusions. By contrast, NGS-based cfDNA assays are not as sensitive as PCR-based methods [21] and require more complex bioinformatics, but NGS has the advantage of interrogating a larger number of genomic loci. Moreover, capture-based NGS platforms are able to detect a range of genetic alterations, including gene fusions, such as *ALK*.

To date, clinical descriptions evaluating the use of cfDNA to identify oncogenic fusions are limited. In one recent example, Cui

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et al. performed capture-based NGS in 39 patients with stage IB-IV NSCLC, including 24 ALK-positive patients (by Ventana ALK IHC analysis and confirmed by FISH) and 15 ALK-negative patients [22]. Capture-based NGS of cfDNA identified *ALK* rearrangements in 13 patients, including two cases with rare *ALK* fusions (*FAM179A-ALK*, *COL25A1-ALK*). The overall sensitivity, specificity, and accuracy of testing for *ALK* in this study was 54.2%, 100%, and 71.8%, respectively. Of note, sensitivity was greater in cases of advanced disease versus early stage disease (64.7% vs. 28.6%). In a separate study investigating *ALK* rearrangement detection in NSCLC using capture-based NGS of cfDNA, Wang et al. reported a sensitivity and specificity of 79.2% and 100%, respectively [23]. Collectively, these studies highlight both the promise (noninvasive, high specificity, ability to detect new fusions) and shortcomings (lower sensitivity, inability to determine clinical significance of novel fusions) of the current technology.

In addition to underscoring the role of cfDNA analysis in current clinical practice, the articles by Wang et al. and Lai et al. also raise important questions about the use of targeted therapies across tumor types. First, does the same genetic alteration confer sensitivity to targeted therapy across malignancies? The success of HER2-directed therapies in both breast and gastric cancer is one notable example in which this has been observed clinically [24, 25]. However, $BRAF^{V600E}$ mutant neoplasms are important reminders that this is not always the case and that the same genetic alteration can have differential sensitivities to targeted therapy based upon the tissue of origin. Indeed, whereas BRAF +/− MEK inhibition often leads to dramatic tumor responses in both $BRAF^{V600E}$ melanoma and NSCLC [26–29], similar targeted approaches have been largely disappointing in $BRAF^{V600E}$ CRC [30, 31]—possibly due to inadequate suppression of the MAPK pathway and rapid feedback activation of epidermal growth factor receptor in CRC [31–33]. Given this experience, we are left to ask—where do *ALK* rearrangements exist on this continuum? To date, several case reports and series have demonstrated that ALK TKIs can be active in ALK-positive IMT, anaplastic large cell lymphoma, and diffuse large B-cell lymphoma [34–37]. In this issue of *The Oncologist,* Wang et al. extend this literature by demonstrating a dramatic response to alectinib in an *ALK*-rearranged atypical neuroendocrine tumor, suggesting that *ALK* rearrangements may be viable targets across malignancies [15].

More broadly, how does the oncology community at large evaluate the activity of specific targeted therapies when a given genetic alteration is rare and present in diverse malignancies? One recent approach has been the basket study. While traditional clinical trials focus on treatment of a particular tumor histopathology, basket studies evaluate therapies aimed at a specific genetic mutation regardless of where the cancer originates. This trial design enables investigators to evaluate how targeted therapies may differ across tumor types harboring similar drivers. The potential utility of basket studies is highlighted by the ongoing clinical development of larotrectinib, a potent, selective oral inhibitor of the TRK family of neurotrophin receptors. In preliminary reporting of a phase I basket study of larotrectinib, partial responses were observed in six of seven efficacy-evaluable patients with *TRK* fusion-positive tumors [38]. Based in part on these results, larotrectinib was recently granted breakthrough therapy designation by the U.S. Food and Drug Administration. In another example of a basket trial, the National Cancer Institute launched the Molecular Analysis for Therapy Choice (NCI-MATCH) in 2015 (NCT02465060). This study aims to perform DNA sequencing on tumor specimens from approximately 6,000 patients. Afterward, patients with select genetic alterations (e.g., *ALK* rearrangements, *HER2* mutations, *BRAF* mutations) will be assigned to one of 30 planned treatment arms. Of note, NCI-MATCH plans for approximately 25% of enrolled subjects to have rare cancers, thus allowing investigators to evaluate the impact of targeted therapies across a spectrum of malignancies.

In summary, advancements in precision medicine will likely continue to fuel the identification of novel genetic alterations as well as the detection of known genetic alterations in previously undescribed settings. While this may provide new therapeutic opportunities for our patients, the ever-expanding volume of genomic data encountered during routine clinical practice can also be daunting. Therefore, together with preclinical studies to functionally validate new targets, there is a growing need for innovative clinical trial designs, molecular tumor boards, and clinical reports of exceptional responders. Collectively, these efforts may help guide insights into the best use of targeted therapies across tumor types.

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Editor's Note: See the related articles, "A Case of Metastatic Atypical Neuroendocrine Tumor with *ALK* Translocaion and Diffuse Brain Metastases" by Victoria E. Wang et al. on pages 768–773 and "Detection of an *ALK* Fusion in Colorectal Carcinoma by Hybrid Capture-Based Assay of Circulating Tumor DNA" by Andrea Z. Lai et al. on pages 774–779 of this issue.