ROS-1 Rearrangements in Circulating Tumor Cells

To the Editor:

ROS1 is a receptor tyrosine kinase of the insulin receptor family, and *ROS1* gene fusions are uncommon oncogenic drivers of NSCLC. Liquid biopsy represents a valuable alternative for molecular analyses when a traditional biopsy of the primary tumor yields insufficient tissue.[1] Moreover, liquid biopsies can detect aberrations missed in tissue testing of heterogeneous tumors. Here, we report the pioneering detection of *ROS1* rearrangements in circulating tumor cells (CTCs) in cases in which next-generation sequencing (NGS) of plasma failed to identify a genetic alteration. Lung adenocarcinoma in a right pleural effusion was diagnosed a 44-year-old male Hispanic nonsmoker. Molecular testing of collected fluid failed to reveal a genetic aberration. Palliative chemotherapy was initiated; it consisted of carboplatin/pemetrexed/bevacizumab for six cycles, followed by maintenance chemotherapy with pemetrexed/bevacizumab for 23 cycles. At the time of disease progression, the patient's tumor was insufficient for further molecular tests. Blood analysis was performed using the VeriStrat test (Biodesix, Boulder, CO). The patient began second-line erlotinib therapy, which was continued for 22 months until disease progression with peritoneal carcinomatosis. NGS done on plasma failed to reveal actionable gene aberrations; a biopsy was done, and a *ROS1* gene translocation was identified in tissue and concordant with the results of subsequent fluorescence in situ hybridization analysis of blood CTCs (Fig. 1). The patient began crizotinib therapy with disease stabilization. Brain metastases were detected 21 and 34 months later, and both were treated with stereotactic brain radiation. Because of the emergence of resistance, the patient was switched to ceritinib and has been stable for 6 months.

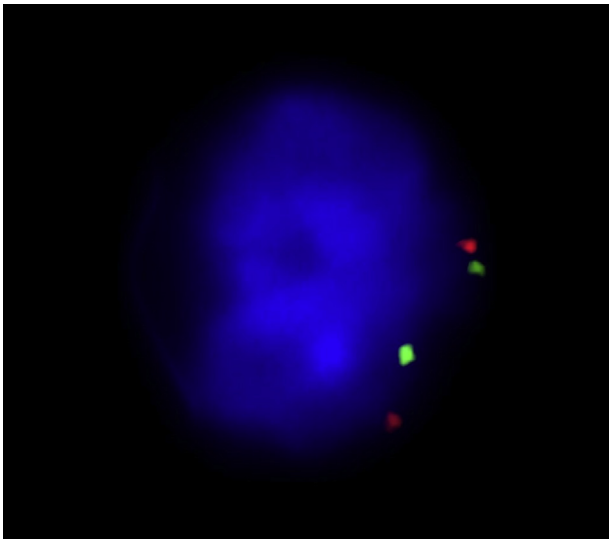

Figure 1. ROS 1 fluorescence in situ hybridization (FISH) break -apart.

ROS1 rearrangements have been detected in CTCs from four patients known to harbor *ROS1* translocations in tumor tissue.[2] However, our case is the first in which *ROS1* rearrangements were detected in CTC analysis of a peripheral blood sample when NGS evaluation of plasma failed to reveal genetic alterations. Thanks to confirmation of the *ROS1* rearrangement, the patient has been alive for 40 months while being treated with anaplastic lymphoma kinase inhibitors (criztonib first and cetinib later). Moreover, detection of a *ROS1* rearrangement in CTCs but not by NGS analysis of plasma suggests that CTC analysis may improve detection of this alteration, as we have seen with other genetic aberrations. We therefore encourage future comparisons of *ROS1* detection techniques. Whether tumor tissue is truly the criterion standard for molecular analysis is currently disputed, as blood tests can reveal alterations not discovered in tumor tissue.[3] Our study underscores the need to define an analytical criterion standard for identifying the largest possible amount of druggable alterations. In conclusion, we report that CTC analysis can identify *ROS1* rearrangements. This and other liquid biopsies can improve patient clinical outcomes (i.e., expand therapeutic options) compared with tissue testing alone.

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VIT-ALK, a Novel Alectinib-Sensitive Fusion Gene in Lung Adenocarcinoma

To the Editor:

In March 2017, a 64-year-old Chinese women was admitted to the hospital with a cough, sputum, and hemorrhage lasting 3 months. The changes on her chest computed tomography (CT) scans included a multiple, bilateral lung nodule; a narrowing of left superior lobar bronchi, and slight bilateral pleural effusion (Fig. 1*A*). We then performed a biopsy through fiberoptic bronchoscopy, and pathological analysis showed an invasive adenocarcinoma of the solid predominant type with mucin production (Fig. 2*A*). Immunohistochemistry and fluorescent in situ hybridization analyses revealed positive ALK receptor tyrosine kinase gene (*ALK*) expression (Fig. 2*B* and *C*).

Echinoderm microtubule associated protein like 4 gene (*EML4*) is the most common fusion partner for *ALK*, so we searched for it with reverse-transcriptase polymerase chain reaction, but the result verified that the patient had no *EML4-ALK* fusion. Next-generation sequencing (NGS) of the tumor DNA (Geneseeq Technology Inc., Nanjing, People's Republic of China) was further conducted to detect the potential mutation. We found a novel *ALK* partner gene, vitrin gene (*VIT*). NGS revealed that *VIT* intron 7 was fused to *ALK* intron 19, leading to a novel *VIT-ALK* fusion gene on chromosome 2. The fusion product was composed of *VIT* exons 1 to 7 and *ALK* exons 20 to 29 (Fig. 3).

We treated the patient with alectinib at a dose of 300 mg twice a day. After 2 months of follow-up, CT scans revealed that the sizes of both the primary and metastasis tumors were significantly reduced (Fig. 1*B*), and the patient's clinical symptoms were improved. About 5 months later, chest CT scans showed that the primary tumor had almost disappeared (Fig. 1*C*). The patient is still being treated with alectinib without disease progression and development of obvious toxicity.

It is well established that *ALK* rearrangement and clinical application of anaplastic lymphoma kinase (ALK) inhibitor play a landmark role in targeted NSCLC therapy. To date, more than 10 different *ALK* fusion partners have been discovered.[1] *VIT*, a gene located in 2p22.2, encodes an extracellular matrix protein named vitrin. Therefore, it is possible that *VIT*, as a constitutively expressed housekeeping gene, may drive the expression of *ALK* as indicated in other *ALK* fusion events. In addition, prior studies have suggested that *VIT* gene plays a role in neural development.[2] The protein contains a single LCCL domain and two von Willebrand factor A domains. Whittaker and Hynes reported that most Willebrand factor A–containing proteins participated in cell adhesion and migration,[3] which are crucial for tumor metastasis. It is thus interesting that additional mechanisms may be involved and remain to be understood. This patient has benefitted from alectinib treatment, which indicates that the novel fusion gene

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