Rimkunas and colleagues report on the development and validation of an immunohistochemical assay evaluating non–small cell lung cancers (NSCLC) for the presence of ROS1 fusions. The diagnostic was validated in a screen of 556 NSCLCs, identifying 9 (1.6%) tumors with oncogenic ROS1 rearrangements. These patients are candidates for ROS1-targeted therapies. Clin Cancer Res; 18(16); 4222–4. ©2012 AACR.

In this issue of Clinical Cancer Research, Rimkunas and colleagues report on a novel immunohistochemical assay designed to identify the expression of ROS1. Using this assay, the authors identified a small but therapeutically significant percentage of non–small cell lung cancers (NSCLC) harboring genomic ROS1 rearrangements (1).

ROS1, a gene coding for a receptor tyrosine kinase of the insulin receptor family, is involved in chromosomal translocations in a number of cancers (2–4) and has recently been shown to undergo a rearrangement leading to the formation of a fusion oncogene in NSCLC (refs. 5, 6; Fig. 1). ROS1 chromosomal rearrangements were first described in glioblastomas, where ROS1 is fused with FIG as a result of an interstitial chromosomal deletion (3), and the chimeric protein was confirmed to be oncogenic in a transgenic mouse model (7). Very recently, a number of novel ROS1 fusion and translocation products have been identified as potential oncogenic drivers in primary NSCLC tumors (Fig. 1) and in the HCC78 cell line harboring the SLC34A2–ROS1 fusion (1, 5, 6). Within these chimeric proteins, ROS1 is fused with its partners at exons 32, 34, 35, or 36 of ROS1. These breakpoints allow for the retention of the ROS1 kinase domain, constitutive kinase activity, and inferred transforming potential. Activation of the ROS1 kinase is brought about by the dimerization of the ROS1 fusion mediated by the N-terminal fusion partner. Subsequently, the activated ROS1 kinase stimulates downstream signaling, resulting in enhanced cell growth, proliferation, and decreased apoptosis.

To date, ROS1 rearrangements are not found to overlap with other oncogenic mutations commonly detected in NSCLC, including EGF receptor (EGFR) mutations, KRAS mutations, or ALK rearrangements (5, 8). Preclinical studies indicate that ROS1 fusion harboring tumors and cell lines are sensitive to the dual ALK/MET inhibitor crizotinib, an observation also confirmed by a partial response of a patient with treated CD74-ROS1–positive NSCLC to crizotinib therapy and more recently in a larger cohort of patients with NSCLC harboring ROS1 rearrangements (8, 9). Furthermore, preclinical studies show that cells harboring ROS1 fusions do not respond to treatment with EGF-targeted kinase inhibitors. These preliminary results define ROS1-rearranged NSCLC as a discrete druggable entity and underscore the importance of conclusive identification of ROS1-rearranged patients with lung cancer who may benefit from ROS1-targeted therapy.

In this issue, Rimkunas and colleagues describe a highly sensitive and specific ROS1 antibody developed to analyze ROS1 fusion expression in NSCLC, with the intent to rapidly screen and identify patients for ROS1-targeted therapy. Described in this study, the team validated the use of ROS1 (D4D6) rabbit monoclonal antibody for immunohistochemistry (IHC) in a total of 556 NSCLC samples. Four hundred and nine of these were screened by IHC as tumor microarrays, with any positive sample further validated using a whole section of each respective tumor, with break-apart FISH and formalin-fixed, paraffin-embedded (FFPE) reverse transcription PCR (RT-PCR) for FIG–ROS1, CD74–ROS1, and SLC34A2–ROS1. In their study, Rimkunas and colleagues observed CD74-ROS1 as the most common ROS1 fusion, accounting for 1.6% of NSCLC tumors and 3.3% of all screened adenocarcinomas, the most represented histologic subtype among ROS1-positive tumors. Furthermore, they identified 1 case of FIG–ROS1(S)–expressing tumor, the first time this specific ROS1 fusion has been reported in NSCLCs (Fig. 1). As expected, the team’s concurrent analysis of the samples for other oncogenic drivers revealed no concomitant expression of ROS1 fusion with ALK rearrangements. Intriguingly, of the 9 ROS1-expressing tumors, 2 samples were double positive for ROS1 and the EGFR<sup>L858R</sup> or EGFR<sup>E746-A750del</sup> mutant as detected by EGFR mutant–specific IHC. The authors show that the expression of ROS1 and mutant EGFR was in the
same region of the tumor. This finding has a potential implication for patients with NSCLC with concurrent ROS1 fusion and EGFR mutation. If these indeed occur in the same cells of the tumor, for such patients, an EGFR inhibitor in combination with crizotinib may be a more appropriate clinical therapy than crizotinib alone.

Rimkunas and colleagues used 2 ROS1 fusion gene–expressing cell lines as positive controls to develop their IHC staining protocol. Whereas the SLC34A2–ROS1 harboring HCC78 produced a strong cytoplasmic signal using the ROS1 antibody, the U-118 MG cell line expressing the Golgi apparatus–localized FIG–ROS1(L) stained very weakly, requiring the application of a special signal boosting reagent. This raises some concerns about the sensitivity of the antibody for detecting the expression of different ROS1 fusion isoforms and/or its ability to reliably detect ROS1 fusion proteins with distinct subcellular localizations. In light of a recent report by Takeuchi and colleagues describing several novel ROS1 rearrangement products in NSCLC using the split probe FISH and 5' RACE assays, these concerns could be addressed by validating the ROS1 antibody for IHC staining for the novel ROS1 fusions at the genetic level (6). Even though the full potential of the ROS1 antibody as a reliable diagnostic tool for all rearranged ROS1 products in NSCLC is yet to be determined, the authors present a strong case for the ROS1 antibody as being highly sensitive in identifying even low-expressing ROS1 tumors for at least 3 of the known ROS1 fusion proteins.

There are several advantages to a standardized IHC screening method to identify patients with ROS1 rearrangements in NSCLC, glioblastoma, or cholangiocarcinoma.
Compared with many other diagnostic techniques, IHC testing is simple, inexpensive, and available in every pathology laboratory worldwide. It is a fast screening test, ideal for a low-incidence genetic alteration such as ROS1. FISH, on the other hand, requires specialized equipment, expensive reagents, long processing times, and a high level of expertise and cannot be conducted in all clinical laboratories. The advantage of FISH compared with IHC lies in its more objective nature in terms of scoring. For IHC, testing criteria can vary significantly between different testing sites. Another drawback to conducting IHC lies in its inability to differentiate between the wild-type (WT) and rearranged ROS1, as well as between various rearranged species of ROS1. Even though there is currently no study evaluating the prognostic or predictive value of knowing the type of ROS1 rearrangement in cancer, it is conceivable that different ROS1 fusion partners and/or the subcellular localization may impart differential oncogenic and/or therapeutic potentials. Similarly, even though WT ROS1 is not widely expressed in normal tissue, Rimkunas and colleagues pointed out that in some cases, non-neoplastic cells, such as macrophages and bronchial epithelial cells, may stain positive with the ROS1 antibody. This nonselectivity between oncogenic and WT ROS1 may pose difficulties especially when analyzing tumors with a substantial immune infiltrate. For tumors that are difficult to evaluate, FISH may prove to be a better diagnostic than IHC and remains an important method to provide a genetic validation of all ROS1-positive IHC tests.

In summary, with the identification of novel oncogenic drivers in NSCLC, it has become imperative to identify patients who may benefit from new therapies designed to specifically target these oncogenes. ROS1 fusions have emerged as a new molecular subtype in NSCLC, and preclinical and clinical data indicate that ROS1-positive tumors are sensitive to crizotinib. In the work presented by Rimkunas and colleagues, a ROS1 IHC assay was developed to detect NSCLC tumors harboring ROS1 fusions. This assay provides a fast, broadly applicable, and inexpensive way to screen for patients with cancer who may benefit from therapy with crizotinib.

Disclosure of Potential Conflicts of Interest
P.A. Janne serves as a consultant and member of the advisory board for Pfizer. No potential conflicts of interest were disclosed by the other author.

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