Reply to Letter "The Use of Immunohistochemistry Improves the Diagnosis of Small Cell Lung Cancer and Its Differential Diagnosis. An International Reproducibility Study in a Demanding Set of Cases."

In Response:

The use of (weighted) κ is currently the standard method of reporting, and as such there should not be any controversy.[1] *The weighted κ is preferred when the data are ordinally categorized, which is not the case in our study, allowing use of the Fleiss κ, which suitable for our data.* The limitations of κ statistics in relation to prevalence and number of categories are well known.[2] However, we take issue with his statement "They concluded that the diagnosis using hematoxylin and eosin staining alone showed moderate agreement among pathologists in tumors with neuroendocrine morphologic features, but agreement improved to good in most cases with the judicious use of [immunohistochemistry], especially in the diagnosis of SCLC. Such conclusion may be a misleading message on account of inappropriate use of a statistical test. Briefly, for reliability analysis, appropriate tests should be applied."

First of all, in our article,[3] the κ scores cited in Table 2 for the categories (combined) SCLC, large cell neuroendocrine carcinoma, atypical carcinoids, typical carcinoids, carcinoids (typical and atypical), poorly differentiated NSCLC, small round cell sarcoma, non-Hodgkin's lymphoma, and other, which ranged from 0.05 to 0.81, were calculated over two classes (specific diagnosis versus other). Thus, one of the two aforementioned limitations of the κ scores, namely, lower κ values when more categories are used, is not applicable. Also in that table, κ scores over four (nonordinal) categories are calculated, leading to the same outcome. In addition, the article states that each of the cited κ values represents the mean value of 171 comparisons—with 19 observers and (19*18)/2 combinations—for each diagnostic category, which is a high enough number to exclude large variations due to possible differences in prevalence. Therefore, the κ outcome measures in our study are based on sound application of κ statistics and thus not inappropriately used. In addition, the obtained κ values are, where available, in line with the literature.[4–6]

As for the content, the primary objective of our study was to test the hypothesis that the use of immunohistochemistry (IHC) leads to greater diagnostic reproducibility when distinguishing SCLC from its differential diagnoses. This led to moderate κ scores in SCLC, and therefore, the participating pathologists advocated the use of additional stains, resulting in increased κ values for several categories (again based on 171 comparisons). As a take-home message we stated, "In conclusion, a [hematoxylin and eosin] diagnosis of SCLC or other pulmonary neuroendocrine tumor is relatively straightforward, but IHC improves diagnostic reproducibility. IHC can aid the pathologist in cases where histologic features are considered equivocal, or in cases where the pathologist is looking for additional support." We believe that our conclusion is based on appropriate use of κ statistics that supports the use IHC, especially in the diagnosis of SCLC, leading to better management of patients in routine clinical care.

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A Rare Case of *ROS1* and *ALK* Double Rearranged Non-Small Cell Lung Cancer

To the Editor:

Lin et al. recently reported a study of a large file of 228 *ROS1*-rearranged NSCLCs and concluded that *ROS1* rearrangements rarely overlap with alterations in other oncogenes.[1] In their study, five cases and one case were also *KRAS*- and *EGFR*-mutated, respectively. To our knowledge, no case harboring *ROS1* and anaplastic lymphoma receptor tyrosine kinase gene (*ALK*) concomitant rearrangements has been reported in the literature so far. Here we are reporting the first case of double *ROS1*- and *ALK*-rearranged NSCLC.

A 77-year-old female never-smoker presented with a locally advanced tumor of the lower right lung that was causing cough, hemoptysis, and chest pain. Histopathological examination of a computed tomography–guided biopsy of the tumor resulted in a diagnosis of lung adenocarcinoma (Fig. 1*A*). The tumor expressed thyroid transcription factor 1 and was diffusely positive according to ROS1 immunohistochemistry (score 2+ with D4D6 antibody [Cell Signaling Technology, Danvers, MA, and Ozyme, Saint Quentin en Yvelines, France], dilution 1:50) (Fig. 1*B*) and anaplastic lymphoma kinase (ALK) immunohistochemistry (score 3+ with 5A4 antibody [Clinisciences, Nanterre, France], dilution 1:25) (Fig. 1*C*). Fluorescence in situ

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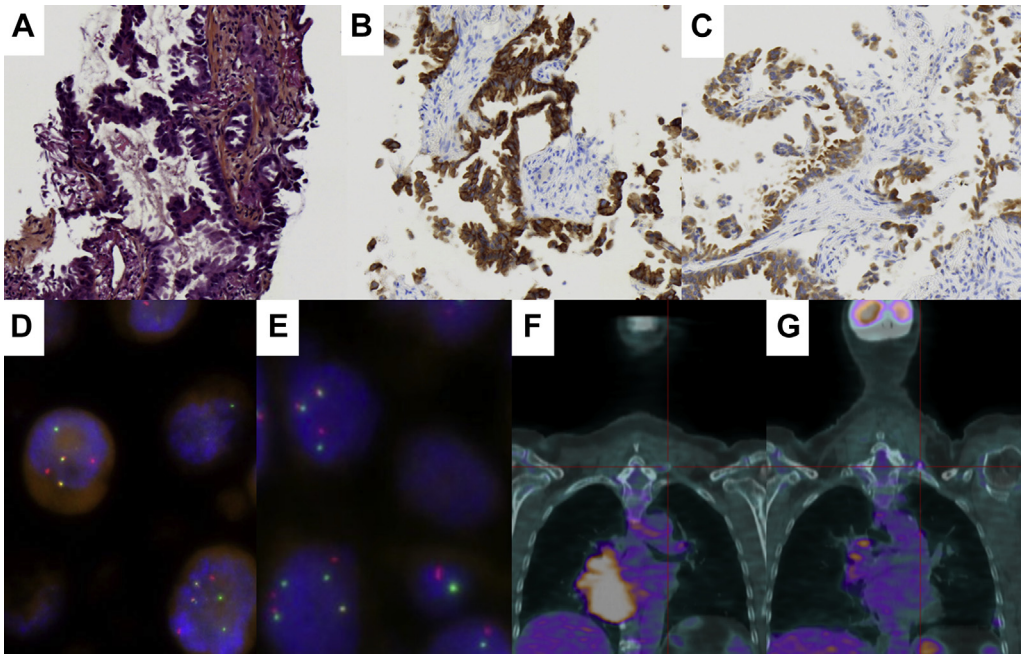

Figure 1. Illustration of the histopathological, immunohistochemical, and fluorescence in situ hybridization results and the response to crizotinib therapy. (*A*) Adenocarcinoma cells (hematoxylin-eosin-saffron; original magnification, ×25). (*B*) ROS1-positive immunohistochemistry (clone D4D6) (hematoxylin counterstaining; original magnification, ×25). (*C*) Anaplastic lymphoma kinase-positive immunohistochemistry (clone 5A4) (hematoxylin counterstaining; original magnification, ×25). (*D*) Twenty-two percent of tumor nuclei present a split positive pattern with separation between the 5′ *ROS1* red part and the 3′ *ROS1* green part of the Vysis 6q22 ROS1 Break Apart FISH probe, which is consistent with a *ROS1* rearrangement (4,6-diamino-2-phenylindole counterstaining; original magnification, ×100). (*E*) Twenty-six percent of tumor nuclei present a split positive pattern with separation between the 5′ anaplastic lymphoma receptor tyrosine kinase gene (*ALK*) green part and the 3′ *ALK* red part of the ALK Vysis Break Apart FISH probe, which is consistent with *ALK* rearrangement (4,6-diamino-2-phenylindole counterstaining; original magnification, ×100). (*F*) Coronal diagnostic computed tomography/^{18}F-fluorodeoxyglucose positron emission tomography fusion image at initial diagnosis showing the tumor in the lower right lung. (*G*) Coronal diagnostic computed tomography/^{18}F-fluorodeoxyglucose positron emission tomography image 3 months after initiation of treatment with crizotinib showing a large reduction in tumor size and metabolism.