assume that a positive ALK immunohistochemical test equates to a positive ALK rearrangement (especially in light of the very low positive predictive value noted in this study).

Disclosure/conflict of interest

The author declares no conflict of interest.

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Response to Mahe

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To the Editor: We thank Dr Mahe for her interest in our article and appreciate her methodological observations and thoughts on the optimal strategy for detecting ALK rearrangements in non-small-cell lung cancer (NSCLC). We acknowledge that while our study describing results of ALK IHC and FISH in a cohort of 594 NSCLC patients¹ is one of the largest studies reporting on ALK IHC, no single study on ALK IHC published to date has come close to the suggested minimum sample size of 2086 indicated by Mahe.² The single largest published study on ALK IHC examined only the 5A4 antibody in 640 patients.³ Our study is the larger of only two studies that have undertaken a direct comparison of the three most commonly used commercially available antibodies and FISH, with the only other study of this kind examining 377 cases.⁴ The findings of our study together with the results of these and other studies have recently been compiled in the 'IASLC atlas of ALK testing in lung Cancer'⁵ and are consistent in demonstrating good sensitivity and excellent specificity of IHC when used with appropriate protocols for ALK detection.

All the data required to estimate confidence intervals (CIs) are included in our paper. Using an online tool to estimate CIs⁶ based on the data in Table 3¹ if any IHC staining is considered positive (1, 2, or 3+ intensity, and using 0.001 as a substitute for 0 where required), the D5F3 antibody (and the ALK1 clone) had a sensitivity of 100% (95% CI 64.6–100%), specificity 99.0% (95% CI 97.8–99.5%), negative predictive value 100% (95% CI 99.3–100%), and positive predictive value 53.8% (95% CI 29.1–76.8%). However, as shown in Table 3, most true positive cases stained only weakly with the ALK1 clone (5 of 7 with 1+ intensity) compared to the D5F3 clone where most cases stained more strongly (only 2 of 7 with weak 1+ staining). 5A4

had a sensitivity of 100% (95% CI 64.6–100%), specificity of 98.1% (95% CI 96.7–99.0%), a negative predictive value of 100% (95% CI 99.3–100%), and positive predictive value of 38.9% (95% CI 20.3–61.4%). While this shows that the 95% CIs are relatively wide as a result of the low numbers of ALK FISH-positive cases, the very high negative predictive value of IHC (100% with 95% CI 99.3–100%) supports our conclusion that ALK IHC is a valuable screening method where false negatives are important to avoid. Screening strategies require confirmation by a more accurate testing method and the relatively low positive predictive values support confirmation by FISH testing as outlined in our paper.¹

The optimal diagnostic algorithm to detect ALK in clinical samples is still evolving and will likely be influenced not only by test sensitivity and specificity but also by local expertise and cost effectiveness. Our study and others support the inclusion of IHC within this algorithm. The recently published consensus Molecular Testing Guidelines from CAP/IASLC/AMP⁷ recommend that 'ALK immunohistochemistry, if carefully validated, may be considered as a screening methodology to select specimens for ALK FISH testing.' In addition, the IASLC Atlas of ALK Testing in Lung Cancer⁵ states that 'a high diagnostic specificity and sensitivity for ALK IHC' compared to FISH has been confirmed in many studies.' As in our paper, the authors of the ALK Atlas recommend that ALK FISH is used to verify all IHC-positive cases until further data are obtained regarding clinico-pathological associations of ALK IHC-positive cases and importantly response to ALK inhibition.⁵ Finally, we are in complete agreement that further prospective studies are required to establish the optimal diagnostic strategy for detection of ALK gene rearrangements in NSCLC.

Disclosure/conflict of interest

The author declares no conflict of interest.

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