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Letter to the Editor

The rarity of concomitant genetic alterations in lung cancer

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To the Editor: We have been very interested by the case series reported by Tang *et al*[1] on 15 cases of lung adenocarcinomas with concomitant chromosomal alterations in *ALK*, *ROS1*, *RET* and *MET*. Indeed, lung adenocarcinomas with more than one oncogenic alteration are very rare and could cause diagnostic and therapeutic dilemmas when the two (or more) oncogenic alterations allow access to different treatments.[2] In this manner, it is worth to collect data in this field for the physicians who could have to make therapeutic choices in a patient having a cancer with concomitant oncogenic driver alterations. Nevertheless, the case series by Tang *et al*[1] also raises questions about some technical points discussed in the present letter.

First, we want to discuss about the thresholds used by Tang *et al*[1] to interpret *ALK*, *ROS1*, *RET* and *MET* fluorescent *in situ* hybridization (FISH) tests. Tang *et al*[1] used cutoffs established in their laboratory. The $\geq 15\%$ cutoff for *ALK* FISH test is consensual in comparison with other studies and guidelines as are the criteria used searching for *MET* amplification (ie, *MET*/chromosome 7 ratio ≥ 2, average *MET* copy number per nuclei ≥ 5 and/or >20 copy or clusters of *MET* signals in $>10\%$ of the tumor cells).[3,4] On the contrary, the cutoffs used for *ROS1* and *RET* FISH tests are unusually low and it would be interesting to know how Tang *et al*[1] have previously determined their cutoff values. Indeed, Tang *et al*[1] considered a tumor being positive for *ROS1* rearrangement if $>3.5\%$ of tumor nuclei showed 5′*ROS1*–3′-*ROS1* split signals or isolated 3′-*ROS1* signals and a tumor being positive for *RET* rearrangement in case of $>7.9\%$ of nuclei with 5′*RET*–3′-*RET* split signals or $>3.1\%$ of nuclei with 3′-*RET* signals.[1] The cutoffs used in the literature, clinical trials and guidelines to diagnose *ROS1* and *RET* rearrangements are $\geq 15\%$ of nuclei with split or single 3′ signals using FISH.[5,6] The reasons for using lower cutoff values is not obvious in the study by Tang *et al*,[1] and we postulate that these low thresholds could expose to overdiagnosis of some *ROS1* and *RET* rearrangements. It could be interesting to know the percentages of *ROS1*- and *RET*-rearranged nuclei in the different tumors to conclude whether they would also have been diagnosed as *ROS1*- and *RET*-positive using traditionally used cutoff values. Further analyses would be also interesting, especially in cases with low rates of FISH-positive nuclei to further confirm the rearrangements.

Immunohistochemistry (IHC) would be one of these additional analyses that could be performed to collect data about the expression of oncogenic proteins. Indeed, chromosomal rearrangements are the only mechanisms causing gene fusions and abnormal expression and oncogenic activation of the ALK, ROS1, RET and MET proteins, which are targeted by specific therapies. ALK IHC actually tends to replace *ALK* FISH as the first line diagnosis tool and, in French guidelines, for example, only cases with faint (1+) or moderate (2+) staining with ALK IHC must be further analyzed using *ALK* FISH.[7,8] In a similar manner, guidelines recommend *ROS1* FISH testing only in cases with ROS1 positive IHC (score 1+ to 3+) and ROS1 IHC is already the first-line screening tool searching for *ROS1* rearrangement in non-small cell lung cancers (NSCLC).[5] The relationship between MET overexpression and *MET* amplification remains unclear, but interestingly, in a French nationwide study using a first selection based on MET expression using IHC followed by *MET* FISH testing in IHC-positive cases, among 25 or so selected patients with advanced and *MET*-amplified NSCLC treated by crizotinib, 8 (32%) patients presented a partial response and 7 (25%) patients had a stable disease.[9] The interest of RET IHC is not established to date searching for *RET*-rearranged NSCLC. The potential discrepancies between the detection of a molecular event at the chromosome level using FISH and the lack of expression of the corresponding protein using IHC are known to be of clinical interest in the field of *ALK* testing in NSCLC. Indeed, less than half of *ALK* FISH-positive but IHC-negative tumors respond to targeted therapies in comparison with most of *ALK* FISH-positive and IHC-positive patients.[10,11] Whether these discrepancies between FISH and IHC are linked to technical artifacts or true biological events remains unanswered in most of the cases. In this manner, to further confirm the rearrangements described by Tang *et al*[1] at the protein level (ie, IHC, especially on ALK and ROS1) and/or at the RNA level (using RNA sequencing or RT-PCR, for example) could be interesting to eliminate technical artifacts in these rare cases reported with double chromosomal alterations.

Moreover, despite oncogenic alterations having traditionally been considered as mutually exclusive in NSCLC, we fully agree with Tang *et al*[1]and previous studies that in real life concomitant driver alterations are rare but real events of therapeutic interest in NSCLC.[2] In our experience, we have also encountered various combinations of molecular

co-alterations in patients including a recently case reported of double *ALK*- and *ROS1*-rearranged tumor being positive with FISH and IHC in a patient presenting a good response to crizotinib therapy.[12] In addition, exceptional cases of NSCLC with triple oncogenic alterations were also reported in the literature and are certainly even rarest than cases with double oncogenic alterations.[13] In this manner, we are very surprised by the high rate of tumors having not only double but triple oncogenic driver alterations in the series of 15 cases reported by Tang *et al.*[1] Indeed, 9/12 cases (75%, with no gene mutation result being available in 3/15 cases) had a concomitant *EGFR* or *RAS* activating mutation (ie, 5 *EGFR* mutations and 4 *RAS* mutations with 3 *KRAS* and 1 *NRAS*) in addition to the chromosomal rearrangements involving *ALK*, *ROS1*, *RET* and *MET*. Of course, the series described by Tang *et al*[1] only represents 15 very unusual cases selected among a total of 5206 cases analyzed in their institution (0.3% of cases).[1] Nevertheless, taking into account, on the one hand, the potential technical issues listed above about the FISH testing and, in the other hand, the unexpected high rate of cases with triple oncogenic alterations in this series, we greatly think that these 15 exceptional cases really merit further analyses to confirm the concomitant rearrangements and oncogenic mutations.

Because of the extreme rarity of patients with NSCLC having multiple driver molecular alterations, it is almost impossible to conduct a clinical trial to compare different therapeutic strategies in this field. Collecting data about isolated cases and series as reported by Tang *et al*[1] could consist in a valuable way to share individual experiments in the therapeutic management of these rare patients. Nevertheless, there is a need of precise and deeply validated results to take lessons for the diagnostic and therapeutic managements of these exceptional cases.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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