Intratumor Distribution of Ki-67 Antigen Beyond Labeling Index for Clinical Decision-Making: A New Way of Counting

To the Editor:

Ki-67 was largely being used in the classification and clinical handling of patients with gastroenteropancreatic tract neuroendocrine neoplasms (NENs), after its pioneer introduction about 30 years ago by our research team.[1] Its assessment is being increasingly adopted by many oncologists even in the setting of lung NENs, especially metastatic carcinoids, to help with the clinical decision-making process.[2–4] The recent encasement in the new WHO classification of lung carcinoids, either primary or metastatic, as characterized by elevated proliferation rates (>10 mitoses per 2 mm^2 or Ki-67 labeling index >30 %) while retaining well-differentiated NE histology,[5] is the hallmark that an unprecedented diagnostic role[6] has been attributing to this biomarker in the refinement of new tumor entities.[7] In fact, the only, thus far, allowed application of Ki-67 to diagnostic practice was to avoid the overdiagnosis of carcinoids as small cell carcinomas on biopsy specimens,[8] in which, the defining criteria (cell morphology, mitoses, and necrosis)[7] are harder to reliably recognize.[9] The clinical relevance of Ki-67 in assessing prognosis,[10,11] grading,[12] and immunotherapy refinement[13] has recently been expanded even to malignant pleural mesothelioma (MPM), a still-hopeless disease in which new insights are clinically warranted. In general, one of the major criticisms on the use of Ki-67 in thoracic tumors has always been its purported scarce interobserver reproducibility or discrepancy between biopsy and surgical samples owing to unpredictable intratumor distribution of proliferating clones as a function of sampling randomness.[14] Although these considerations could hold true for every repeated quantitation (including mitotic counts), combining together different proliferation criteria, each reflecting different biological domains, might synergically compensate for any punctual assay error.[12] Therefore, we are wondering whether the stochastic intratumor distribution of Ki-67 owing to the existence of diversely proliferating cell clones[15] could not, instead, become a resource to unravel tumor biology and whether its claimed role as a prognosticator in patients with MPM[10–12] could be further validated by additional independent observations.

In this scenario, we were delighted by the interesting study by Belderbos et al.[16] and we congratulate the authors on the important insights of their stimulating work. Briefly, 27 patients with MPM treated with extended pleurectomy and decortication (eP/D) were assessed for several immune-histopathologic traits, including Ki-67. It was found that, not only a Ki-67 labeling index of greater than 10 % in eP/D samples was relevant to the prognosis for both progression-free (8.81 versus 25.35 mo, $p = 0.001$) and overall (19.7 versus 44.5 mo, $p = 0.001$) survival (area under the curve equal to 0.756 on receiver operating characteristic analysis, with 90 % sensitivity and 71 % specificity for a cutoff of 10 % for Ki-67), but also that there was a congruency rate of 87 % between eP/D samples and purported biopsy samples, as represented by pseudotissue microarrays constructed by 10 randomly picked-up 2-mm–sized regions for each tumor. These findings support a practical role of Ki-67 in the clinical decision-making process for selecting patients with MPM for surgery.[16] Although most of these cases remained confined to the same risk category, Ki-67 distribution, however, was somewhat heterogeneous among these pseudomicroarray tumor regions,[16] in keeping with a polyclonal derivation on the coalescence of independent cell clones.[17] This study also fits with the proposal of score-based, multiparameter grading system of patients with MPM we have recently reported in the journal on the largest series of these tumors thus far assessed for both Ki-67 and mitotic count (328 cases in the training set and 612 in the validation set), in which either marker enhanced each other independently of the type of material (small to large biopsies or eP/D) to predict the ultimate clinical outcome.[12] However, the biological implications of the intratumor distribution of Ki-67 were not addressed in the meritorious study by Belderbos et al.,[16] and it is still unassessed whether such information might be relevant to lung NENs. Recently, we have

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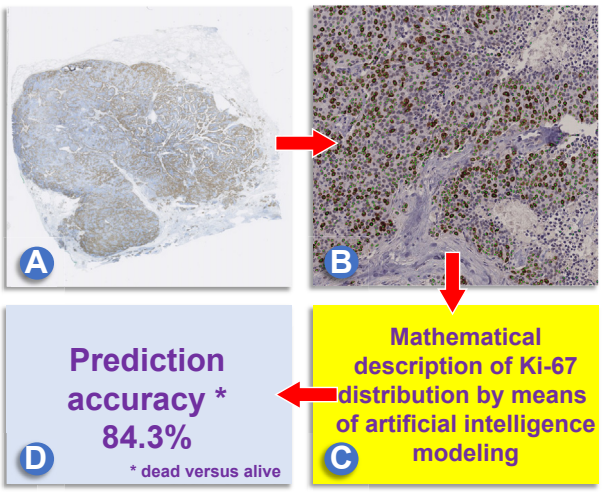

Figure 1. Artificial intelligence tools. *(A)* On whole section digitized images, *(B)* tumor cells stained for Ki-67 are identified through bioinformatics detection tools. *(C)* By means of a mathematical description of their distribution and artificial intelligence prediction algorithms, *(D)* a final result is obtained with clinical implication: 84.3 % accuracy in predicting the ultimate clinical outcome (dead versus alive patients).

bioinformatically dissected a series of 30 consecutive surgically resected lung NENs (10 typical and 14 atypical carcinoids and six large cell NE carcinomas) for Ki-67 intratumor distribution on digitized whole-section images.[18] Mathematical descriptors from multiple domains of spatial organization analysis were exploited and a prognostic model was built to account for Ki-67 intratumor distribution.[18] This model resulted in 84.3 % diagnostic accuracy in predicting the ultimate clinical outcome of patients (dead versus alive), regardless of WHO classification[18] (Fig. 1*A-D*).

Our conclusion agrees with the final remark that an old and apparently exploited biomarker such as Ki-67 could come as a surprise when its intratumor distribution is accounted for through artificial intelligence tools in the era of precision pathology and personalized medicine.

CRediT Authorship Contribution Statement

Linda Pattini: Conceptualization, Methodology, Writing–review and editing, Manuscript finalization.

Matteo Bulloni: Methodology; Writing–review and editing, Manuscript finalization.

Giuseppe Pelosi: Conceptualization, Methodology, Writing-original draft preparation; Writing–review and editing, Manuscript finalization.

Declaration of Interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Compliance With Ethical Standards

This is a comment to already existing literature data, thus no approval by Internal Review Board was required.

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