Malignant Pleural Mesothelioma—Does Ki67 Make the Grade?

Owen W. J. Prall, M.B.B.S., PhD*

In this issue, Belderbos et al.[1] investigate various histopathologic features of malignant pleural mesothelioma (MPM) that could help to predict the risk of recurrence and guide the appropriateness of various therapeutic strategies. They studied growth pattern (epithelioid, sarcomatoid, transitional, and mixed), degree of nuclear atypia (three-tier scale), degree of necrosis (< or >50%), mitotic count (≤4 or >4 per 10 high-power fields), proportion of cells expressing Ki67 (Ki67 labeling index, ≤10% or >10%), and the presence or absence of BAP-1 and MTAP expression. They concluded that Ki67 was the strongest prognostic factor for overall survival and progression-free survival. On the basis of their results, they suggest that patients with a Ki67 greater than 10% would be unlikely to benefit from surgery. Although this is a small retrospective study (27 patients), and all patients were somewhat unusually treated with extended pleurectomy/decortication, raising possibility of selection bias, the findings support those from multiple previous studies which also found that Ki67 had prognostic significance in MPM.[2–7]

This raises the question as whether Ki67 labeling index now be reported as part of the histopathology data set and used as the sole factor to assign a tumor grade? Ki67 has many attractions as a prognostic marker. It is an antigen that is robustly expressed in all phases of actively cycling cells, and it makes biological sense for there to be an association of a high proportion of cycling tumor cells and outcome. Dysregulated cell cycle control is a hallmark of oncogenesis,[8] and many pathogenic variants occur in genes involved either directly or indirectly in cell cycle progression, for example, receptor tyrosine kinases, MAPK pathway, p16/CDKN2A, cyclin D1, and RB1.

There are many tumors for which measures of cell proliferation have been found to be prognostic and are incorporated into grading. Nevertheless, this is not universal, as architectural growth pattern or nuclear features, or both, and not cell proliferative markers are used to grade some common tumors, including those carcinomas from lung, colorectum, kidney, uterus, ovary, and prostate. Mitotic activity and not Ki67 labeling is the most frequently used proliferative marker. Mitotic figures are typically counted manually in H&E sections, either within a given number of high-power fields, or, in recognition that this varies between microscope objectives, within a defined area. There are strong supportive data from multiple large studies for the prognostic value of mitotic counts in breast carcinoma and melanoma. Scoring criteria have been progressively refined to provide relatively clear instructions to pathologists regarding common issues that include tumor heterogeneity and sample size adequacy. Other tumors for which mitotic figures are routinely counted include sarcomas and gastrointestinal stromal tumors.

Although counting mitotic figures is reasonably reproducible and quick, it can be susceptible to significant errors. Mitotic figures can sometimes be surprisingly difficult to recognize or may be mistakenly scored in nontumor cells. Apoptotic bodies and infiltrating lymphocytes can be mistaken for mitotic figures, and variations in section thickness will modify the count. Immunohistochemistry for antigens expressed in mitosis (e.g., phosphoserine 10 on histone H3) can help with some of these issues but not with other problems including the loss of mitotic figures owing to delayed fixation, particularly for large specimens, as unfixed cells can progress through mitosis into G1 phase.

The Ki67 labeling index is less affected by delayed fixation and has more recently been incorporated into prognostic data for some tumors, including neuroendocrine

*Corresponding author.

Department of Pathology, Peter MacCallum Cancer Centre, Melbourne, Australia.

Disclosure: The author declares no conflict of interest.

Address for correspondence: Owen W. J. Prall, M.B.B.S., PhD, Department of Pathology, Peter MacCallum Cancer Centre, 305 Grattan St, Melbourne VIC 3000, Australia. E-mail: Owen.Prall@petermac.org

Cite this article as: Prall O. Malignant pleural mesothelioma—does Ki67 make the grade? *JTO Clin Res Rep* 2021;2:100170.

ISSN: 2666-3643

https://doi.org/10.1016/j.jtocrr.2021.100170

neoplasms (NENs) with some notable exceptions (e.g., those arising in lung) and gliomas. There can be significant heterogeneity in the Ki67 labeling index in NENs, and counting is directed to "hotspots" of at least 500 cells, as these are more prognostic than random or average counts.[9] Scanning at low power is very useful to identify hotspots, but eyeball estimates of Ki67 labeling are inaccurate and discouraged. Instead, detailed counts of both positive and negative tumor nuclei are necessary, either by using a calibrated objective grid, or by printing an image and manually marking off scored cells to avoid recounting positive cells and to ensure all cells are scored. This is a significant time investment for the pathologist that is unlikely to be proportionately renumerated. Accurate counting typically requires either specific training or experience. Both the mitotic count (in a 2 mm^2 area) and Ki67 index can be used to assign a grade for NENs; however, the grade attributed from Ki67 regularly "trumps" that from mitoses, suggesting theoretical advantage is borne out in daily practice.

Of note, Ki67 labeling has been thoroughly investigated in breast cancer and has prognostic significance, but it is not officially recommended as there is no consensus for scoring methodology or what the cutoffs should be.[10] This experience, together with the progressively refined guidelines for scoring Ki67 in NENs, highlights the potential difficulties that will likely be faced trying to introduce it into the routine prognostic data sets for MPM. Standards for tissue processing, immunohistochemistry staining, and counting methods will need to be defined. The apparent lack of heterogeneity in Ki67 labeling that Belderbos et al.[1] described is promising, as this suggests that small biopsies of MPM will be representative and hotspots will not necessarily need to be scored. Nevertheless, this important detail will need to be confirmed in a larger series of MPM and prospectively in small biopsies and possibly cytology cell block preparation from each morphologic subtype. In small biopsy specimens, crush artifact can hinder evaluation of histological features and Ki67 labeling index, and there are greater challenges for the pathologist to distinguish malignant mesothelial cells from reactive ones and malignant sarcomatoid cells from stromal cells.

Belderbos et al.[1] scored Ki67 within a 2 mm^2 area, but as for NENs, specifying a minimum number of tumor cells to score would seem more appropriate. Low cellularity tumors may not have an adequately representative cell number in 2 mm^2. In contrast, high cellularity tumors would be very time consuming to count, as there can be up to 5000 cells in 2 mm^2. It is possible that digital pathologic findings and image analysis could help with this, and standardize counting and improve reproducibility, but this technology is not currently widely available and would need thorough independent validation.

A Ki67 cutoff value needs to be rigorously established, particularly as this may direct management. The Ki67 cutoff of 10% was found prognostic by Belderbos et al.[1] and another study,[2] although the latter study also found the combination of nuclear atypia and mitotic count to be independently prognostic. Cutoffs of 15%[3] and 20%[4] have also been proposed. A multi-institutional study of 940 patients with MPM also found a Ki67 cutoff of 30% to be prognostic in MPM, but Ki67 alone was outperformed when it was combined with histologic subtyping, necrosis, and mitotic count.[5]

Ultimately, only large well-designed prospective studies in different populations will be able to define how to grade MPM and what contribution the Ki67 labeling index will make to this. It is hoped that such studies will result in an evidence-based grading system for MPM with clear and practical guidelines for pathologists and strong prognostic significance.

References

1. Belderbos RA, Maat APMW, Baart SJ, et al. Ki67 as a prognostic marker for clinical decision making prior to extended pleurectomy decortication in malignant pleural mesothelioma. *JTO Clin Res Rep*. 2021;2:100155.
2. Kadota K, Suzuki K, Colovos C, et al. A nuclear grading system is a strong predictor of survival in epithelioid diffuse malignant pleural mesothelioma. *Mod Pathol*. 2012;25:260–271.
3. Ghanim B, Klikovits T, Hoda MA, et al. Ki67 index is an independent prognostic factor in epithelioid but not in non-epithelioid malignant pleural mesothelioma: a multicenter study. *Br J Cancer*. 2015;112:783–792.
4. Bitanihirwe BK, Meerang M, Friess M, et al. PI3K/mTOR signaling in mesothelioma patients treated with induction chemotherapy followed by extrapleural pneumonectomy. *J Thorac Oncol*. 2014;9:239–247.
5. Pelosi G, Papotti M, Righi L, et al. Pathologic grading of malignant pleural mesothelioma: an evidence-based proposal. *J Thorac Oncol*. 2018;13:1750–1761.
6. Comin CE, Anichini C, Boddi V, Novelli L, Dini S. MIB-1 proliferation index correlates with survival in pleural malignant mesothelioma. *Histopathology*. 2000;36:26–31.
7. Beer TW, Buchanan R, Matthews AW, Stradling R, Pullinger N, Pethybridge RJ. Prognosis in malignant mesothelioma related to MIB 1 proliferation index and histological subtype. *Hum Pathol*. 1998;29:246–251.
8. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144:646–674.
9. Nagtegaal ID, Odze RD, Klimstra D, et al. The 2019 WHO classification of tumors of the digestive system. *Histopathology*. 2020;76:182–188.
10. WHO Classification of Tumours Editorial Board. *Breast Tumours: WHO Classification of Tumours*. 5th ed. Vol 2. Lyon, France: International Agency for Research on Cancer; 2019.