Letters to the Editor

Comment on 'A diagnostic algorithm to distinguish desmoplastic from spindle cell melanoma'

To the Editor: I read with interest the article by Weissinger *et al*[1] 'A diagnostic algorithm to distinguish desmoplastic from spindle cell melanoma' published in the September 2013 issue. They propose MelanA as a first and, if positive, exclusionary step in their algorithm. This appears to be based on their cluster analysis of 38 cases (16 spindle cell, 18 desmoplastic, 4 mixed). All 4 of their mixed and all 18 of their desmoplastic melanomas were negative for MelanA. Their meta-review (Supplementary Table 9) is a summary of published immunoprofiles comparing spindle cell to desmoplastic melanoma. It is based on much larger number of cases and suggests that MelanA is not quite that specific and that HMB-45 may actually be of superior discriminatory value than MelanA.

	Spindle cell			Desmoplastic		
	+	tested	%	+	tested	%
HMB-45	54	118	45.8	49	559	8.8
MelanA/MART-1	29	66	43.9	44	234	18.8

A comment on the difference between their cluster analysis and meta-review of published literature in this regard would be appreciated.

Disclosure/conflict of interest

The author declares no conflict of interest.

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Comparison of MelanA/MART-1 and HMB45 labeling in desmoplastic melanoma

To the Editor: We thank Dr Parsons[1] for his comment and would like to clarify our decision to include and prefer MelanA/MART-1 in our diagnostic algorithm to distinguish spindle cell from desmoplastic melanoma.[2]

The summary of our literature meta-review that Dr Parsons refers to tabulates the MelanA/MART-1 and HMB45 labeling fraction in spindle cell and desmoplastic melanoma derived from 67 different studies. The apparent difference of marker fractions in desmoplastic melanoma (8.8 *vs* 18.8%; $P < 0.0001$; $n = 54$ studies) derives in large parts from studies ($n = 37$) where only one of the two biomarkers was assessed. The key limitation of this approach to summarize data for the assessment of discriminatory value is, that the percentage of positive cases is based on a theoretical summary of separate studies on different cases rather than a direct comparison of two markers in the same samples. In other words, we do not know the MelanA/MART-1 status in the ~300 more desmoplastic melanomas that have only been stained for HMB45 (but not for MelanA/MART-1; ie, discrepant analysis). To assess and compare the discriminatory value of these two biomarkers, it is crucial to restrict the comparison to studies where samples have been assessed with both markers (Figure 1a). When restricting the tabulation to those nine studies that allow analysis of both markers on a case-by-case basis (Figure 1b),[2–11] there is no significant difference in the labeling fraction of HMB45 and MelanA/MART-1 in desmoplastic melanoma ($P = 0.79$).
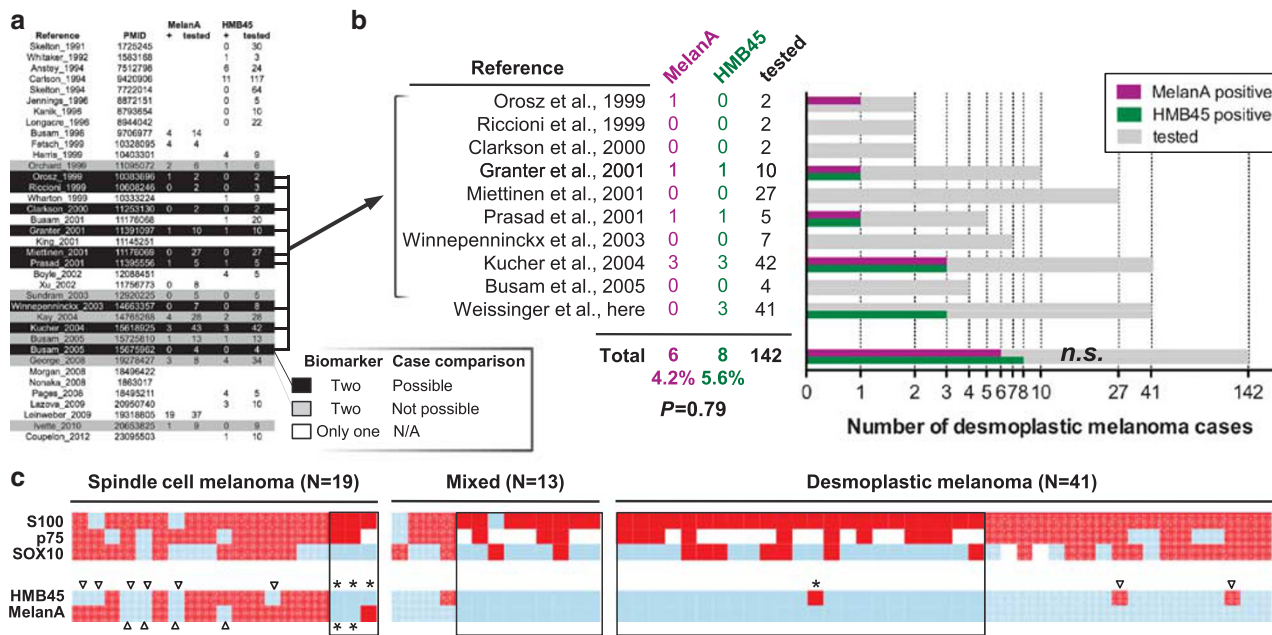
Figure 1 Assessment of discriminatory value of MelanA/MART-1 and HMB45 in desmoplastic melanoma. (**a**) Screenshot of the 37 desmoplastic melanoma studies used to compose the overall labeling fraction of MelanA and HMB45. Shading (gray and black) is visualizing those 15 studies that assessed both markers and black indicates that only 9 studies allow comparison of staining pattern of both markers on a case-by-case basis (see **b**). For a legible and more detailed tabulation see Supplementary Table 7 in our original report.[2] (**b**) Summary of studies and staining results on a case-by-case basis demonstrate no significant difference in MelanA- and HMB45-labeling fractions (P-value from Fisher's exact test). (**c**) Staining results by marker and histotype in our original study cohort (pale)[2] and 35 new cases (black squares). Top three rows demonstrate staining pattern to reach or confirm the diagnosis of melanoma, whereas bottom two rows demonstrate staining results of HMB45 and MelanA. When specifically comparing the discriminatory value of HMB45 and MelanA in spindle cell vs desmoplastic melanoma, there are twice as many misclassifications with HMB45 when compared to MelanA: in our initial cohort (triangles) as well as in the additionally examined cases of spindle cell and desmoplastic melanoma (asterisks).

Our reason to tabulate all available studies (listed separately in Supplementary Tables 6–8)[2] into one combined table (Supplementary Table 9)[2] was to assess individual labeling fractions of markers that allow reaching or confirming the diagnosis of melanoma in this specific setting. Briefly, we found that markers otherwise reliable in the setting of conventional melanoma (among these HMB45 and MelanA/MART-1) are with 8.8 and 18.8%, respectively, not as useful for this task in desmoplastic melanoma. In contrast, our meta-review revealed S100, SOX10 and p75 as reliable markers for the positive diagnostic identification of melanoma in the setting of spindle cell as well as desmoplastic melanoma. Once the diagnosis 'melanoma' is reached or confirmed, our algorithm can help in subclassification of spindle cell vs desmoplastic melanoma, when appropriate. Rarely, if ever, studies report staining results on a case-by-case basis and it is difficult to assess technical and diagnostic variability between reports. Therefore we based our algorithm strictly on our own analysis of test performance on cases derived from two different institutions.[2] When specifically assessing the discriminatory value of the two biomarkers in spindle cell and desmoplastic melanoma, we found eight misclassifications using HMB45, whereas MelanA showed only four misclassifications in our original series (Figure 1c, triangles). In the meantime, we had the opportunity to expand

our study cohort (Figure 1c, black squares). Again, with respect to discriminatory value, we found four additional misclassifications using HMB45, whereas MelanA showed two additional misclassifications (Figure 1c, asterisks). These findings in new independent samples validate our prior analysis. Despite these test-performance considerations and the 'exclusionary' appearance of our diagnostic algorithm, a biomarker status should not replace careful histomorphological assessment and clinicopathological correlation.

In sum, when assessing the discriminatory value of two biomarkers, it is—in our opinion—not sufficient to summarize literature data of marker fractions. It is rather necessary to compare test performance in a combined assessment of both markers in the same samples.

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Disclosure/conflict of interest

The author declares no conflict of interest.

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Comment on 'Testing for ALK rearrangement in lung adenocarcinoma: a multicenter comparison of immunohistochemistry and fluorescent *in situ* hybridization'

To the Editor: I read with great interest the recent study performed by Selinger *et al.*[1] exploring the potential utility of immunohistochemical screening for ALK rearrangements. Although the results of the study are convincingly portrayed, I did note some potential omissions worth pointing out, especially given the major impact that the study might have on the logistical approach that many laboratories might choose to take as part of the very important work-up of non-small-cell lung cancers (NSCLCs).

Although the methods outline the origin of the sample, the authors do not elaborate upon any form of *a priori* sample size calculations. These calculations can be easily performed using web-based tools; alternatively, a number of medically oriented reviews are available explaining the calculations involved. Using the worked examples of Jones *et al.*,[2] given the assumption of ALK rearrangement in 3–4% of cases of NSCLC,[1] and presuming test sensitivity of at least 95% (which is often assumed in sample size calculations for studies of screening tests[2]), I calculated a minimum sample size of 2086. This suggested that sample size is much larger than that used by Selinger *et al.*[1] and relates to the very low prevalence of ALK rearrangements in NSCLC.

Another vitally important omission is the complete lack of any statements relating to the statistical quantification of uncertainty (eg, 95% confidence intervals); this violates one of the major tenets of diagnostic testing research, as set out by the STARD group.[3] Admittedly, the lack of any false-negative immunohistochemical results in this study makes accurate calculation of confidence intervals difficult. Nevertheless, estimates are possible: if one uses the online tool provided by the KT-clearinghouse group[4] (with the caveat that a 0 false-negative value be estimated by a small non-zero value, eg, 0.01), the sensitivity in the current study can be estimated at 100% with 95% confidence interval ranging from as low as 64 to 100%. This wide confidence interval also likely relates to undersampling.

A final comment highlighting the importance of follow-up FISH studies pursuant to a positive ALK immunohistochemical result is warranted. Such a statement is an important one in order to ensure that laboratories hoping to optimize their efficiencies not