


Comprehensive histopathological comparison of epidermotropic/dermal metastatic melanoma and primary nodular melanoma

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Aims: Metastatic melanoma involving the epidermis and/or upper dermis may show significant histological overlap with primary cutaneous melanoma, especially the nodular subtype. Proper histopathological classification is crucial to appropriate staging and management, but is often challenging. The aim of this study was to identify helpful histopathological features for differentiating epidermotropic/dermal metastatic melanoma (EDMM) and primary nodular melanoma (PNM).

Methods and results: A cohort of EDMMs ($n = 74$) and PNMs ($n = 75$) was retrospectively reviewed for various histopathological features, and the data were compared between groups by the use of univariate analysis. Features significantly associated with EDMM included a tumour size of <2 mm, an absence of tumour-infiltrating lymphocytes and plasma cells, monomorphism, and involvement of adnexal epithelium. Features associated with PNM included a polypoid (exophytic) configuration, prominent tumour-infiltrating plasma cells (TIPs), a tumour size of >10 mm, ulceration, epidermal collarettes, a higher mitotic rate,

necrosis, multiple phenotypes, significant pleomorphism, and lichenoid inflammation. In multivariate analysis, a logistic regression model including large tumour size, ulceration, prominent TIPs, lichenoid inflammation and epidermal collarettes was highly predictive of PNM. Six (8%) EDMMs from three patients showed an 'epidermal-only' or 'epidermal-predominant' pattern closely simulating *in-situ* or microinvasive melanoma. Two of these cases were tested by fluorescence *in-situ* hybridisation, which confirmed clonal relationships with their corresponding primary melanomas.

Conclusions: This is the first comprehensive histopathological comparison of EDMM and PNM. Recognition of the above histopathological associations should aid in the correct classification and staging of cutaneous melanoma. Epidermotropic metastatic melanomas may occasionally show an epidermal-only/epidermal-predominant pattern; accurate diagnosis requires prudent clinical correlation and, when necessary, ancillary molecular tests.

Keywords: epidermotropic, metastatic melanoma, nodular melanoma, staging

Introduction

Distinguishing between metastatic and primary melanomas is crucial, as the therapy and prognosis differ widely. Whereas primary melanoma is treated with wide excision, metastatic melanoma is often treated with systemic therapy.¹ Also, a patient with multiple primary melanomas is staged on the basis of the lesion with the worst prognostic parameters, while patients with satellite and in-transit metastases are considered to have stage IIIB disease, and those with distant cutaneous metastases are considered to have stage IV disease with a 10% 5-year survival rate.²

Because the skin is a common site for melanoma metastasis, histological differentiation between primary and metastatic melanomas presents a rather common diagnostic dilemma. Primary nodular melanoma (PNM) typically only minimally involves the epidermis, frequently raising consideration for metastatic melanoma even in patients without a prior history of melanoma, as metastatic melanoma may develop following complete regression of the primary tumour.^{3–5} Conversely, an epidermotropic/dermal metastatic melanoma (EDMM) may closely mimic a primary melanoma and be misdiagnosed as such, particularly when a prior history of melanoma is not known. Clinical correlation is often imperative in rendering the correct diagnosis. EDMM usually occurs near the site of a primary melanoma, sometimes presenting in crops.⁶ In contrast, it is rare for a patient to present with multiple synchronous primary melanomas.^{7,8}

Historically, epidermal involvement was considered to be pathognomonic for primary melanoma,⁹ but this belief was subsequently disputed.¹⁰ Microscopic features frequently reported in cutaneous metastatic melanoma included thinning of the epidermis, widening of the papillary dermis by aggregates of atypical melanocytes, flanking epidermal collarettes, a lack of inflammation, monomorphism, and fibrotic stroma.^{10,11} Lymphovascular invasion was also thought to favour EDMM; however, more recent studies have demonstrated this feature in up to 30% of primary melanomas.¹² Similarly, extension of intraepidermal melanocytes beyond dermal melanocytes (architectural 'shoulder') was once considered to be a unique feature of primary melanoma, but was later also reported in EDMM.^{8,13,14} The absence of an architectural shoulder in PNM further blurs their distinction.

Further complicating this issue are rare reports of purely intraepidermal epidermotropic metastatic melanoma.^{11,14,15} Despite their striking resemblance to

melanoma *in situ*, the clinical presentation of numerous lesions and their small size, circumscription and symmetry supported the interpretation of epidermotropic metastatic melanomas with an 'epidermal-only' pattern.

In this study, we performed a comprehensive histopathological analysis of EDMM and PNM in order to identify the most helpful features for differentiating the two.

Materials and methods

This study has been approved by the Institutional Review Board at the University of Michigan. Our pathology database was searched for EDMM and PNM by the use of word searches for 'nodular' + 'melanoma', 'epidermotropic' + 'melanoma', and 'metastatic melanoma' from 2000 to 2016. Final classification as EDMM or PNM was based on careful clinicopathological correlation. Patients with prior history of melanoma were excluded from the PNM group, whereas patients without a previously diagnosed primary melanoma were excluded from the EDMM group. Metastases distant from the previous primary melanoma site were also excluded from the EDMM group; only locoregional metastases (e.g. from the same extremity) were included. To qualify as EDMM, a metastatic melanoma should involve the epidermis/adnexal epithelium and/or the upper dermis (papillary dermis or superficial reticular dermis). Haematoxylin and eosin (H&E)-stained slides of the final cohort were reviewed by two dermatopathologists (D.P.A. and M.P.C.) and a pathology resident (S.L.S.) for various histopathological features, as listed in Tables 1 and 2. Selected features are defined below.

A tumour was polypoid (exophytic) if its epicentre was above the skin surface. An architectural shoulder was defined as an intraepidermal component extending at least three rete ridges beyond the dermal component. Pleomorphism was scored as follows: 0, mostly monomorphic cells; 1, pleomorphism appreciated at medium to high magnification; and 2, pleomorphism readily appreciated at low magnification, often with bizarre-looking cells. Tumours consisting of two or more morphologically distinct cell populations were considered to have multiple phenotypes. Mitotic rate was the number of dermal mitotic figures in a 1-mm² 'hot spot' (i.e. most mitotically active area). As per the American Joint Committee on Cancer guidelines for melanoma,² tumour-infiltrating lymphocytes (TILs) were either absent (0), non-brisk

Table 1. Histopathological features showing statistically significant differences between epidermotropic/dermal metastatic melanoma (EDMM) and primary nodular melanoma (PNM) by univariate analysis

Histopathological features	EDMM (<i>n</i> = 74)	PNM (<i>n</i> = 75)	<i>P</i> -value	Specificity (%)
Greatest diameter <2 mm, no. (%)	19 (26)	0 (0)	<0.0001	100
Absence of tumour-infiltrating plasma cells, no. (%)	67 (91)	38 (51)	0.0036	49
Monomorphism, no. (%)	41 (55)	24 (32)	0.0309	68
Absence of tumour-infiltrating lymphocytes, no. (%)	9 (12)	2 (3)	0.0348	97
Involvement of adnexal epithelium, no. (%)	32 (43)	18 (24)	0.0417	76
Polypoid (exophytic) configuration, no. (%)	0 (0)	16 (21)	<0.0001	100
Prominent tumour-infiltrating plasma cells, no. (%)	1 (1)	22 (29)	<0.0001	99
Greatest diameter >10 mm, no. (%)	2 (3)	31 (41)	<0.0001	97
Ulceration, no. (%)	4 (5)	36 (48)	<0.0001	95
Epidermal collarettes, no. (%)	18 (24)	53 (71)	<0.0001	76
Mean mitotic rate (median, range)	4 (2, 0–23)	13 (11, 1–53)	<0.0001	–
Tumour necrosis, no. (%)	2 (3)	19 (25)	0.0002	97
Multiple phenotypes, no. (%)	6 (8)	24 (32)	0.0012	92
Significant pleomorphism, no. (%)	13 (18)	31 (41)	0.0073	82
Lichenoid inflammation, no. (%)	3 (4)	14 (19)	0.0088	96

(1), or brisk (2). Tumour-infiltrating plasma cells (TIPs) were scored as follows: 0, no intratumoral plasma cells; 1, rare aggregates of intratumoral plasma cells only appreciable upon close inspection; and 2, prominent, readily appreciable aggregates of intratumoral plasma cells. Regression was defined as a discrete fibrotic area with melanophages, lymphocytes, and increased vascularity. Tumoral melanosis refers to large aggregates of melanophages replacing a portion of the melanoma. Unlike lymphovascular invasion, in which melanoma cells are present within a vascular lumen, angiotropism refers to extravascular melanoma cells bulging into the vascular lumen but covered by endothelial cells.

In univariate analysis (performed by M.P.C.), comparison of each feature between EDMM and PNM was performed with chi-square tests (categorical) or two-sample *t*-tests (continuous). Multivariate analysis was conducted by L.Z. with SAS (version 9.4; SAS Institute, Cary, NC, USA). A logistic regression model was

obtained by a stepwise variable selection procedure based on a significance level of 0.15. The parameter estimates, *P*-values from Wald chi-square tests and the area under the receiver operating characteristic (ROC) curve were reported. A *P*-value of <0.05 was considered to be significant.

Fluorescence *in-situ* hybridisation (FISH) was performed by A.A.A., P.W.H. and M.W. on selected EDMM cases with an unusual 'epidermal-only' or 'epidermal-predominant' pattern and their corresponding primary tumours to confirm clonal relationships. Four-micrometre sections were prepared from each selected formalin-fixed paraffin-embedded tissue block; one was stained with H&E, and two were hybridised by use of the Vysis Melanoma FISH probe kit, including probes 6p25 (*RREB1*), 6q23 (*MYB*), CEP6, and 11q13 (*CCND1*), and Vysis LSI probes 8q24 (*MYC*) and 9p21 (*CDKN2A*)/CEP9, according to the manufacturer's instructions. Thirty cells were evaluated, and the percentage of nuclei with copy

Table 2. Histopathological features without statistically significant differences between primary nodular melanoma (PNM) and epidermotropic/dermal metastatic melanoma (EDMM) by univariate analysis

Histopathological features	PNM (<i>n</i> = 75), no. (%)	EDMM (<i>n</i> = 74), no. (%)	<i>P</i> -value
Associated naevus	8 (11)	2 (3)	0.0578
Perineural invasion	12 (16)	5 (7)	0.0991
Symmetry*	12/74 (16)	20/69 (29)	0.1036
Infiltrative borders	38 (51)	52 (70)	0.1238
Lymphovascular invasion	18 (24)	10 (14)	0.1405
Regression	13 (17)	7 (9)	0.1946
Expansion of papillary dermis by melanoma	69 (92)	54 (73)	0.2004
Pseudomaturation	15 (20)	9 (12)	0.2364
Brisk tumour-infiltrating lymphocytes	8 (11)	5 (7)	0.4054
Tumoral melanosis	14 (19)	10 (14)	0.4379
Pseudoepitheliomatous hyperplasia	10 (13)	7 (9)	0.4970
Architectural 'shoulder'	18 (24)	14 (19)	0.5017
Lentiginous growth	57 (76)	62 (84)	0.5949
Pagetoid spread	48 (64)	52 (70)	0.6455
Junctional nests	55 (73)	51 (69)	0.7559
Sheet-like growth	31 (41)	33 (45)	0.7642
Angiotropism	11 (15)	10 (14)	0.8614
Involvement of preserved rete ridges	48 (64)	49 (66)	0.8709

*Some cases were partial biopsies in which symmetry could not be determined.

number changes, including gain of 6p25, loss of 6q23, gain of 11q13, gain of 8q24, and homozygous loss of 9p21, was recorded and considered as positive or negative on the basis of predetermined thresholds.¹⁶

Results

The final cohort consisted of 74 EDMM specimens obtained from 44 patients, and 75 PNM specimens obtained from 75 patients. There was no overlap of the patient pools of the two groups. Epidermal

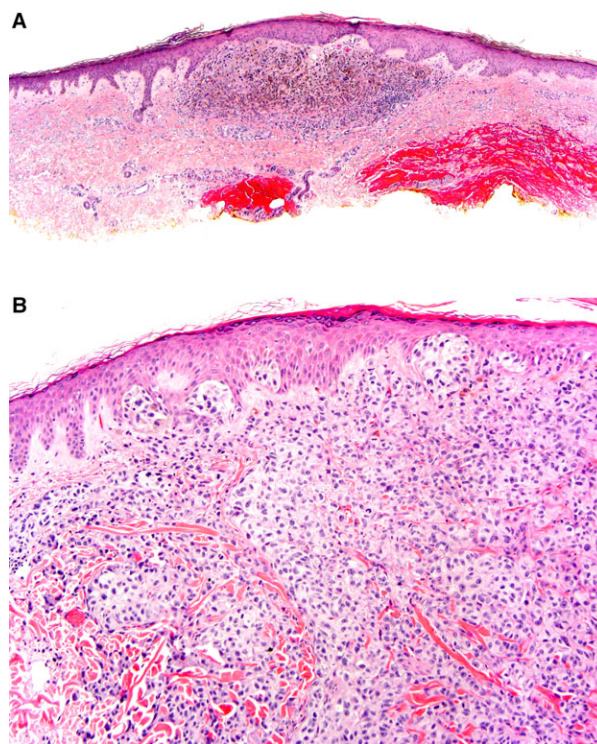


Figure 1. Representative features associated with epidermotropic/dermal metastatic melanoma. A, A small lesion in the upper dermis measuring <2 mm in greatest dimension [haematoxylin and eosin (H&E)]. B, Another lesion shows a dermal nodule with epidermotropism, cytological monomorphism, and lack of tumour-infiltrating lymphocytes and plasma cells (H&E).

involvement was observed in 62 (84%) EDMM cases and 59 (79%) PNM cases. Histopathological features showing statistically significant differences between EDMM and PNM by univariate analysis are listed in Table 1. Features associated with EDMM included a greatest dimension of <2 mm, a TIP score of 0, a pleomorphism score of 0, a TIL score of 0, and involvement of the adnexal epithelium (Figure 1). Features associated with PNM included a polypoid (exophytic) configuration, a TIP score of 2, a greatest dimension of >10 mm, ulceration, epidermal collarettes, a higher mitotic rate, tumour necrosis, multiple phenotypes, a pleomorphism score of 2, and lichenoid inflammation (Figure 2). Ulceration was seen much more frequently in lesions >10 mm (24/33; 73%) than in lesions <10 mm (16/116; 14%) ($P < 0.0001$). Other examined features did not show significant differences between groups (Table 2). Multivariate analysis resulted in a predictive model for PNM that included large tumour size, ulceration, prominent TIPs, lichenoid inflammation, and epidermal collarettes (Table 3). The area under the ROC

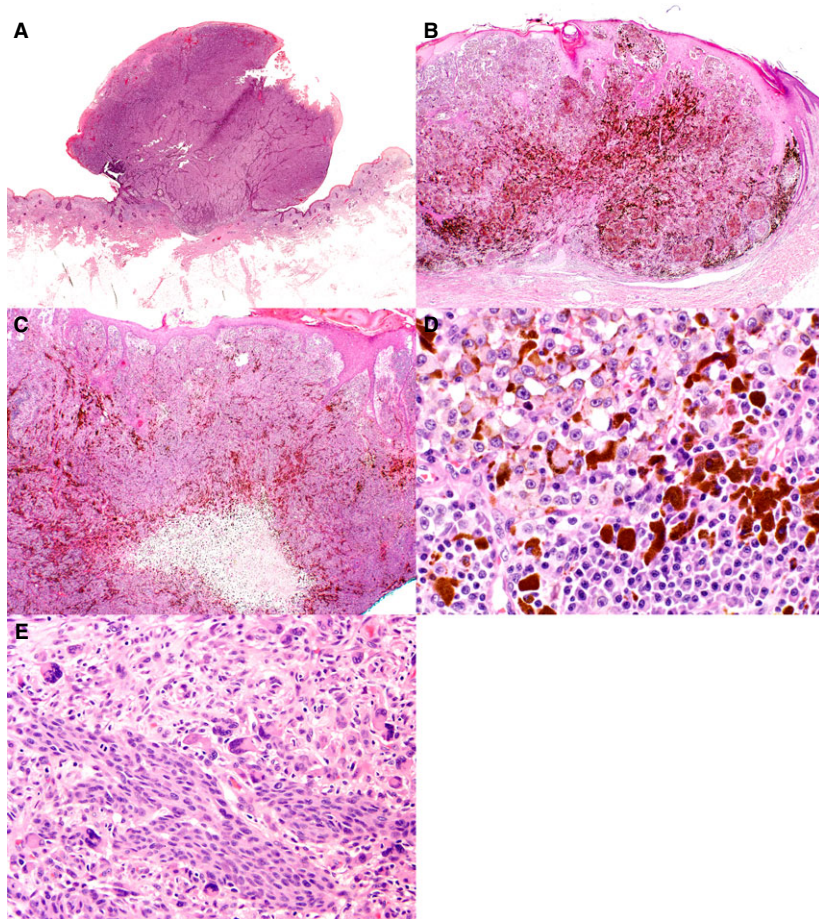


Figure 2. Representative features associated with primary nodular melanoma. A, A large, ulcerated nodular melanoma with an exophytic polypoid configuration [haematoxylin and eosin (H&E)]. B, This tumour is flanked by epidermal collarettes (H&E). C, Zonal tumour necrosis is noted in the deeper portion of this biopsy (H&E). D, Clusters of tumour-infiltrating plasma cells are present in this example (H&E). E, This nodular melanoma shows multiple distinct phenotypes and significant pleomorphism. One population of cells contains highly pleomorphic and bizarre-looking nuclei (top), whereas the other population consists of nests of relatively uniform cells (bottom) (H&E).

Table 3. Logistic regression model by multivariate analysis

Histopathological features	Estimates with PNM*	P-value
Greatest diameter of >10 mm (versus <2 mm)	2.2024	0.0023
Greatest diameter of 2–10 mm (versus <2 mm)	0.5340	0.3695
Presence of ulceration	0.8014	0.0166
Presence of prominent tumour-infiltrating plasma cells	0.9775	0.0487
Presence of lichenoid inflammation	0.7993	0.0675
Presence of epidermal collarette	0.6903	0.0028

PNM, primary nodular melanoma.

*A greater positive estimate indicates a higher probability of PNM.

curve was 0.90, indicating a high predictive accuracy of this model.

Six EDMM lesions from three patients were purely or predominantly intraepidermal, and were

microscopically indistinguishable from melanoma *in situ* and microinvasive melanoma (Figure 3). In two of these patients, multiple new pigmented lesions developed around the site of a previously resected primary scalp melanoma. Another patient presented with multiple small pigmented lesions on the right leg after excision of a primary right heel melanoma. Two epidermal-only/epidermal-predominant EDMM lesions from the two patients with scalp melanoma were studied by FISH, which showed identical copy number changes to those in their corresponding primary melanomas (Table S1), confirming a clonal relationship.

Discussion

The histological distinction between cutaneous metastatic melanoma and primary melanoma has important diagnostic, prognostic and treatment implications, but, to our knowledge, none of the previous studies have systematically compared the histopathological features of the two. We selected

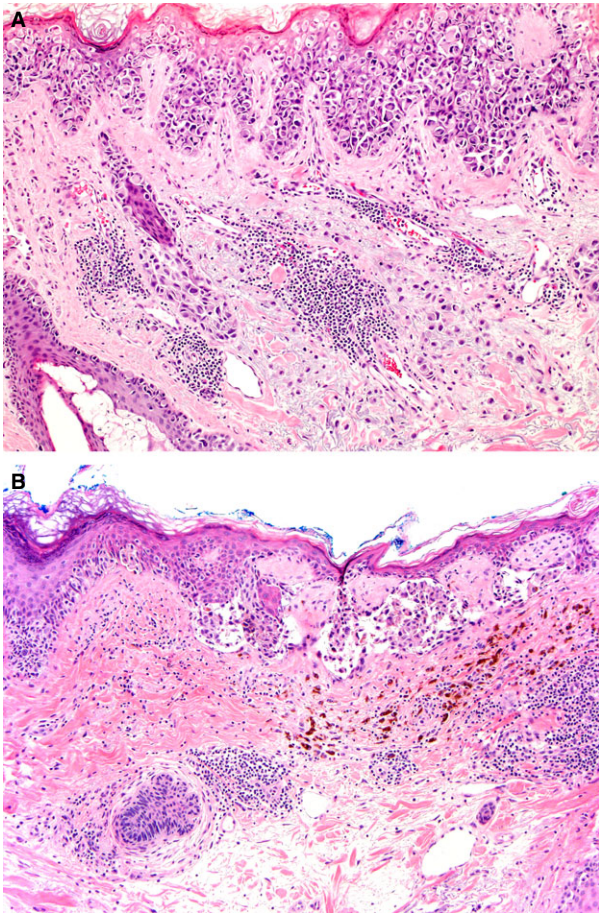


Figure 3. Epidermotropic metastatic melanomas with epidermal-predominant and epidermal-only patterns. **A**, This scalp lesion was taken from near the site of a previously resected primary melanoma. Most of the melanoma cells are present within the epidermis, giving rise to an architectural 'shoulder' and the appearance of a microinvasive melanoma. Adnexal involvement is also present [haematoxylin and eosin (H&E)]. **B**, Another patient presented with a purely intraepidermal metastatic melanoma on the scalp, closely resembling melanoma *in situ* (H&E). [Colour figure can be viewed at wileyonlinelibrary.com].

only metastatic melanoma involving the epidermis and/or upper dermis, because these cases most closely simulate primary melanoma. Likewise, we confined our cohort of primary melanoma to the nodular subtype, as it is most likely to generate diagnostic confusion with EDMM.

We identified multiple useful histopathological features for differentiating EDMM and PNM. Of these, tumour size is one of the most objective discriminating factors. Nodules >10 mm are more likely to be primary, whereas lesions <2 mm are much more likely to be metastatic. Because large melanomas are more likely to ulcerate, we also found PNM to be associated with ulceration. Although none of the

PNM lesions in our series was smaller than 2 mm, one should be aware that primary 'micromelanomas' do exist, and may be detected more readily with the use of dermoscopy.^{17,18} Hence, tumour size should be viewed as a strong but not absolute discriminator of metastatic and primary melanomas.

In addition to tumour size, tumour silhouette also provides useful information. Polypoid/exophytic lesions are much more likely to be PNM, as only one EDMM in our series showed a polypoid silhouette. The rarity of polypoid EDMM is also reflected in the literature.^{11,19} Epidermal collarettes have been previously reported in both EDMM and primary melanomas.²⁰ In our cohort, epidermal collarettes were significantly associated with PNM. This association is probably related to the larger tumour size in this group, as epidermal collarettes result from expansile dermal growth.

None of the intraepidermal features (lentiginous growth, junctional nests, pagetoid spread, and involvement of preserved rete ridges) are associated with either group. Interestingly, involvement of the adnexal epithelium is significantly associated with EDMM, indicating that, in addition to epidermotropism, adnexotropism is also common in metastatic melanoma.

Although architectural shoulder was traditionally thought to be specific for primary melanoma, later studies have also shown this feature in EDMM.^{8,13,14} Our findings support the latter by showing architectural shoulder in 19% of EDMMs. Notably, six EDMM lesions from three patients were purely or predominantly intraepidermal, and were microscopically indistinguishable from melanoma *in situ* and microinvasive melanoma. The metastatic nature of these lesions is supported by the identical copy number abnormalities to those in their corresponding primary melanomas. The occurrence of multiple lesions in the vicinity of the primary melanoma site also provides compelling clinical evidence for metastases. However, when clinicopathological correlation fails to elucidate the primary versus metastatic nature of a lesion, molecular studies may be needed to facilitate more accurate staging.^{15,21}

A number of cytomorphological features aid in the distinction between PNM and EDMM. In general, significant pleomorphism favours a diagnosis of PNM, whereas monomorphism favours a diagnosis of EDMM. Furthermore, primary melanomas are more likely to comprise multiple morphologically distinct subpopulations secondary to genetic divergence within the tumour. In contrast, metastatic melanomas more commonly show one phenotype only,

probably reflective of a selected tumour subclone harbouring increased metastatic potential.²² Pseudomaturation, referring to partial diminution of cell size with dermal descent, has been described in the majority of naevoid melanomas²³ but also in other melanoma subtypes, as well as EDMM. This feature is not helpful in their distinction.

Although a coexisting naevus tends to be found more frequently in PNM than in EDMM, the difference falls short of statistical significance ($P = 0.0578$). It is well known that primary melanoma may arise from a pre-existing naevus via malignant transformation. A recent study showed that the incidence of associated naevus was highest among superficial spreading melanomas (37%) and lowest among nodular melanomas (16%).²⁴ This relatively low frequency in PNM may have accounted for the lack of a significant difference from EDMM.

Although mitotic figures can be brisk in metastatic melanoma,²⁵ our study showed a significantly higher mitotic rate in PNM. Nevertheless, given the wide range and significant overlap of mitotic rates in the two groups, the observed difference is of limited practical value. As rapidly proliferating tumours often outgrow their blood supply, tumour necrosis may result, and is again more commonly found in PNM. Previous studies have found tumour necrosis to be significantly associated with increased tumour thickness, an increased Ki67 proliferation index, and ulceration.^{26,27} It is therefore possible that the lower incidence of tumour necrosis in EDMM may be, in part, attributable to smaller tumour size and infrequent ulceration in this group.

Melanoma can metastasise via lymphatic, haematogenous or angiotropic routes.^{28–33} Both angiotropism and lymphovascular invasion are fair predictors of metastatic potential in melanoma.³⁴ In our study, both lymphovascular invasion and angiotropism were seen in small subsets of PNM and EDMM cases, and failed to distinguish between groups. Perineural invasion is believed to be another means by which satellite metastases form in melanoma. This, too, does not serve as a useful discriminator.

Our data indicate that prominent TIPs are strongly suggestive of PNM, being observed in 29% of these cases as compared with 1% of EDMMs. Assessment of TIPs is fairly straightforward, making it a powerful and practical discriminator. Mascaro *et al.* reported clusters of plasma cells in 22% of primary melanomas, a feature that was associated with increased Breslow thickness, ulceration, and poor survival.³⁵ A recent study confirmed these associations, as well as

higher mitotic activity.³⁶ Interestingly, when found in metastatic melanomas, TIPs were reported to constitute a favourable prognosticator.³⁷ Further investigation is needed to elucidate the immunological relationship between TIPs and melanoma cells.

Because absence of TILs is associated with sentinel lymph node metastasis, whereas brisk TILs are associated with prolonged recurrence-free survival,^{38,39} one may expect fewer TILs in metastatic melanomas. A study reported that a higher percentage of primary melanomas (67%) contained lymphoid infiltrate than metastatic foci (9%) in the same patients.⁴⁰ Another study of nodal metastatic melanomas found that TILs were absent in 46% of cases.⁴¹ In the skin, we showed that absence of TILs is significantly associated with EDMM, suggesting an enhanced ability to evade immune detection.⁴² Similarly, lichenoid inflammation is less common in EDMM than in PNM, despite the essentially identical frequency of epidermal involvement in these groups.

Perhaps contrary to common belief,¹¹ our study did not identify any significant difference in the frequency of tumour regression. Late regression, as evidenced by fibrosis, increased vascularity, epidermal atrophy, and melanophages, has been reported in 10–35% of primary melanomas.⁴³ Although a review article cited a much lower incidence of spontaneous regression in metastatic melanomas (0.25%), this figure is probably skewed by the exceedingly low incidence of regression in extracutaneous metastases.⁴⁴ Our cohort showed regression in 17% and 9% of PNM and EDMM cases, respectively. Several factors may explain the lack of a significant difference. First, regression has been reported to occur less frequently in nodular melanoma (13%) than in other subtypes.⁴⁵ Second, the histopathological features of regression are not specific, and may be indistinguishable from changes of chronic friction or prior trauma. Finally, one of our EDMM cases with regression was excised after immunotherapy (pembrolizumab), which may have triggered regression of the metastatic melanoma.⁴⁶ Tumoral melanosis—a histological variant of regression⁴⁷—similarly fails to differentiate between PNM and EDMM.

In conclusion, we conducted the first comprehensive histopathological comparison of EDMM and PNM, and identified multiple useful discriminators with significant associations that have not been previously reported. Of these, the collective findings of large tumour size, ulceration, prominent TIPs, epidermal collarettes and lichenoid inflammation strongly support a diagnosis of PNM on the basis of a logistic regression model. Other highly useful characteristics

based on univariate analysis include small tumour size, and an absence of TILs, which favour EDMM, and a polypoid configuration, tumour necrosis, and multiple phenotypes, which favour PNM. Although the importance of clinical correlation cannot be overemphasised, recognition of the above constellation of histopathological features, especially when the clinical history is limited, should allow for more accurate classification, and hence more precise tumour staging and prognostication for melanoma patients.

Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

S. L. Skala, D. P. Arps, D. R. Fullen and M. P. Chan designed the research study. S. L. Skala, D. P. Arps, K. B. Cha, M. Wang, P. W. Harms, A. A. Andea and M. P. Chan obtained the data. S. L. Skala, L. Zhao, M. Wang, P. W. Harms, A. A. Andea and M. P. Chan analysed the data. S. L. Skala and M. P. Chan wrote the paper. All authors performed critical review of the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Comparison of copy number changes detected by fluorescence *in-situ* hybridisation in two cases of epidermal-only/predominant metastatic melanoma with their corresponding primary tumours.