1

2 DR MAY P. CHAN (Orcid ID : 0000-0002-0650-1266)

3

4

6

7

8

9 Comprehensive Histopathologic Comparison of Epidermotropic/Dermal Metastatic

10 Melanoma and Primary Nodular Melanoma

11 (Running Title: Comparison of Metastatic and Primary Melanoma)

12

13 Stephanie L. Skala[1], David P. Arps[2], Lili Zhao[3], Kelly B. Cha[4], Min Wang[1], Paul W. Harms[1,4],

14 Aleodor A. Andea[1,4], Douglas R. Fullen[1,4], May P. Chan[1,4]

15 [1]Department of Pathology, University of Michigan, Ann Arbor, MI

16 [2]Consolidated Pathology Consultants, Libertyville, IL

17 [3]Department of Biostatistics, University of Michigan, Ann Arbor, MI

18 [4]Department of Dermatology, University of Michigan, Ann Arbor, MI

19

20 Corresponding Author:

21 May P. Chan, M.D.

22 Department of Pathology

23 University of Michigan

24 1301 Catherine Street

25 Medical Science I, M3261

26   Ann Arbor, MI 48109, USA

27   Phone: +001 (734) 764-4460

28   Fax: +001 (734) 764-4690

29   E-mail: mpchan@med.umich.edu

30

31   The authors report no conflict of interest.

32   Abstract: 250 words

33   Manuscript: 2557 words

34   Figures: 3   Tables: 3   Supplemental Table: 1

35   **ABSTRACT**

36   **Aims:** Metastatic melanoma involving the epidermis and/or upper dermis may show significant

37   histologic overlap with primary cutaneous melanoma, especially the nodular subtype. Proper

38   histopathologic classification is crucial to appropriate staging and management, yet often

39   challenging. This study aims to identify helpful histopathologic features in differentiating

40   epidermotropic/dermal metastatic melanoma (EDMM) and primary nodular melanoma (PNM).

41   **Methods and Results:** A cohort of EDMM (n=74) and PNM (n=75) was retrospectively

42   reviewed for various histopathologic features, and the data were compared between groups by

43   univariate analysis. Features significantly associated with EDMM included tumor size of <0.2

44   cm, absence of tumor-infiltrating lymphocytes and plasma cells, monomorphism, and

45   involvement of adnexal epithelium. Features associated with PNM included polypoid

46   (exophytic) configuration, prominent tumor-infiltrating plasma cells (TIPs), tumor size of >1 cm,

47   ulceration, epidermal collarette, higher mitotic rate, necrosis, multiple phenotypes, significant

48   pleomorphism, and lichenoid inflammation. By multivariate analysis, a logistic regression model

49   including large tumor size, ulceration, prominent TIPs, lichenoid inflammation, and epidermal

50   collarette was highly predictive of PNM. Six (8%) EDMM cases from three patients

51   demonstrated an "epidermal-only" or "epidermal-predominant" pattern closely simulating in-situ

52   or microinvasive melanoma. Two of these cases were tested by fluorescence in situ hybridization

53   which confirmed clonal relationship with their corresponding primary melanomas.

54   **Conclusions:** This is the first comprehensive histopathologic comparison of EDMM and PNM.

55   Recognition of the above histopathologic associations should aid in correct classification and

56   staging of cutaneous melanoma. Epidermotropic metastatic melanomas may occasionally display

57 an epidermal-only/predominant pattern; accurate diagnosis requires prudent clinical correlation

58 and, when necessary, ancillary molecular tests.

59 **Keywords:** epidermotropic; metastatic melanoma; nodular melanoma; staging

60

61

62

63

64

65

66

67

68

69

70

71

72

73

74

75

76

77

78

79

80 **INTRODUCTION**

81 Distinction between metastatic and primary melanomas is crucial, as therapy and prognosis

82 differ widely. While primary melanoma is treated with wide excision, metastatic melanoma is

83 often treated with systemic therapy.[1] While a patient with multiple primary melanomas is staged

84 based on the lesion with the worst prognostic parameters, patients with satellite and in-transit

85 metastases are considered to have stage IIIB disease, and those with distant cutaneous metastases

86 are considered to have stage IV disease with 10% five-year survival.[2]

87   Because the skin is a common site for melanoma metastasis, histologic differentiation between

88   primary and metastatic melanomas presents a rather common diagnostic dilemma. Primary

89   nodular melanoma (PNM) typically only minimally involves the epidermis, frequently raising

90   consideration for metastatic melanoma even in patients without a prior history of melanoma, as

91   metastatic melanoma may develop following complete regression of the primary tumor.[3-5]

92   Conversely, an epidermotropic/dermal metastatic melanoma (EDMM) may closely mimic a

93   primary melanoma and be misdiagnosed as such, particularly when a prior history of melanoma

94   is not known. Clinical correlation is often imperative in rendering the correct diagnosis.

95   Epidermotropic/dermal metastatic melanoma usually occurs near the site of a primary melanoma,

96   sometimes presenting in crops.[6] In contrast, it is rare for a patient to present with multiple

97   synchronous primary melanomas.[7,8]

98   Historically, epidermal involvement was considered pathognomonic for primary melanoma,[9] but

99   this belief was subsequently disputed.[10] Microscopic features frequently reported in cutaneous

100   metastatic melanoma included thinning of the epidermis, widening of papillary dermis by

101   aggregates of atypical melanocytes, flanking epidermal collarette, lack of inflammation,

102   monomorphism, and fibrotic stroma.[10,11] Lymphovascular invasion was also thought to favor

103   EDMM, however more recent studies have demonstrated this feature in up to 30% of primary

104   melanomas.[12] Similarly, extension of intraepidermal melanocytes beyond dermal melanocytes

105   (architectural "shoulder") was once considered a unique feature of primary melanoma, but was

106   later also reported in EDMM.[8,13,14] The absence of an architectural "shoulder" in PNM further

107   blurs their distinction.

108   Further complicating this issue are rare reports of purely intraepidermal epidermotropic

109   metastatic melanoma.[11,14,15] Despite their striking resemblance to melanoma in situ, the clinical

110   presentation of numerous lesions and their small size, circumscription and symmetry supported

111   the interpretation of epidermotropic metastatic melanomas with an "epidermal-only" pattern.

112   In this study, we performed a comprehensive histopathologic analysis of EDMM and PNM in

113   order to identify the most helpful features in differentiating the two.

114

115

116

117

118

119

120

121

122

123

124

125 **METHODS**

126 This study has been approved by the Institutional Review Board at the University of Michigan.

127 Our pathology database was searched for EDMM and PNM using word searches for

128 "nodular"+"melanoma", "epidermotropic"+"melanoma", and "metastatic melanoma" from 2000-

129 2016. Final classification as EDMM or PNM was based on careful clinicopathologic correlation.

130 Patients with prior history of melanoma were excluded from the PNM group, whereas patients

131 without a previously diagnosed primary melanoma were excluded from the EDMM group.

132 Metastases distant from the previous primary melanoma site were also excluded from the

133 EDMM group; only locoregional metastases (e.g., from the same extremity) were included. To

134 qualify as EDMM, a metastatic melanoma should involve the epidermis/adnexal epithelium

135 and/or the upper dermis (papillary dermis or superficial reticular dermis). Hematoxylin and eosin

136 (H&E) stained slides of the final cohort were reviewed by two dermatopathologists (D.P.A. and

137 M.P.C.) and a pathology resident (S.L.S.) for various histopathologic features as listed in Tables

138 1 and 2. Selected features are defined below.

139 A tumor was polypoid (exophytic) if its epicenter was above the skin surface. An architectural

140 "shoulder" was defined as an intraepidermal component extending at least three rete ridges

141 beyond the dermal component. Pleomorphism was scored as: 0 = mostly monomorphic cells, 1 =

142 pleomorphism appreciated at medium to high magnification, 2 = pleomorphism readily

143 appreciated at low magnification, often with bizarre looking cells. Tumors consisting of two or

144 more morphologically distinct cell populations were considered to have multiple phenotypes.

145 Mitotic rate was the number of dermal mitotic figures in a 1 mm^2 "hot spot" (i.e., most

146 mitotically active area). As per the AJCC guidelines for melanoma,[2] tumor-infiltrating

147 lymphocytes (TILs) were either absent (0), non-brisk (1), or brisk (2). Tumor-infiltrating plasma

148 cells (TIPs) were scored as: 0 = no intratumoral plasma cells, 1 = rare aggregates of intratumoral

149 plasma cells only appreciable upon close inspection, 2 = prominent, readily appreciable

150 aggregates of intratumoral plasma cells. Regression was defined as a discrete fibrotic area with

151 melanophages, lymphocytes, and increased vascularity. Tumoral melanosis referred to large

152 aggregates of melanophages replacing a portion of the melanoma. Unlike lymphovascular

153 invasion in which melanoma cells are present within a vascular lumen, angiotropism referred to

154 extravascular melanoma cells bulging into the vascular lumen but covered by endothelial cells.

155 In univariate analysis (performed by M.P.C.), comparison of each feature between EDMM and

156 PNM was performed using Chi-square (categorical) or two-sample t test (continuous).

157 Multivariate analysis was conducted by L.Z. using SAS (version 9.4, SAS Institute, Cary, NC).

158 A logistic regression model was obtained by a stepwise variable selection procedure based on a

159 significance level of 0.15. The parameter estimates, p-values from Wald chi-square tests, and the

160 area under the receiver operating characteristic (ROC) curve were reported. A p-value of <0.05

161 was considered significant.

162 Fluorescence in situ hybridization (FISH) was performed by A.A.A., P.W.H., and M.W. on

163 selected EDMM cases with an unusual "epidermal-only" or "epidermal-predominant" pattern

164 and their corresponding primary tumors to confirm clonal relationship. Four-micron sections

165 were prepared from each selected formalin-fixed paraffin-embedded tissue block; one was

166 stained with H&E, and two were hybridized with Vysis Melanoma FISH probe kit including

167 probes 6p25 (*RREB1*), 6q23 (*MYB*), CEP6, and 11q13 (*CCND1*), and Vysis LSI probes 8q24

168 (*MYC*) and 9p21 (*CDKN2A*)/CEP9 according to manufacturer's instructions. Thirty cells were

169 evaluated and the percentage of nuclei with copy number changes, including gain of 6p25, loss

170 of 6q23, gain of 11q13, gain of 8q24, and homozygous loss of 9p21 was recorded and considered

171 as positive or negative based upon pre-determined thresholds.[16]

172

173

174

175

176

177

178

179

180

181

182

183

184

185

186

187

188

189

190

191

192

193 **RESULTS**

194 The final cohort consisted of 74 EDMM specimens obtained from 44 patients, and 75 PNM

195 specimens from 75 patients. There was no overlap of the patient pools of the two groups.

196 Epidermal involvement was observed in 62 (84%) EDMM cases and 59 (79%) PNM cases.

197 Histopathologic features demonstrating statistically significant differences between EDMM and

198 PNM by univariate analysis are listed in Table 1. Features associated with EDMM included

199 greatest dimension of <0.2 cm, TIPs score of 0, pleomorphism score of 0, TILs score of 0, and

200 involvement of adnexal epithelium (Figure 1). Features associated with PNM included polypoid

201 (exophytic) configuration, TIPs score of 2, greatest dimension of >1 cm, ulceration, epidermal

202 collarette, higher mitotic rate, tumor necrosis, multiple phenotypes, pleomorphism score of 2,

203 and lichenoid inflammation (Figure 2). Ulceration was seen much more frequently in lesions >1

204 cm (24/33; 73%) compared to lesions <1 cm (16/116; 14%) (p<0.0001). Other examined features

205 did not show significant differences between groups (Table 2). Multivariate analysis resulted in a

206 predictive model for PNM which included large tumor size, ulceration, prominent TIPs,

207   lichenoid inflammation, and epidermal collarette (Table 3). The area under the ROC curve was

208   0.90, indicating a high predictive accuracy of this model.

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

210   microscopically indistinguishable from melanoma in situ and microinvasive melanoma (Figure

211   3). In two of these patients, multiple new pigmented lesions developed around the site of a

212   previously resected primary scalp melanoma. Another patient presented with multiple small

213   pigmented lesions on the right leg after excision of a primary right heel melanoma. Two

214   epidermal-only/predominant EDMM lesions from the two patients with scalp melanoma were

215   studied by FISH, which showed identical copy number changes to their corresponding primary

216   melanomas (Supplemental Table 1), confirming a clonal relationship.

217

218

219

220

221

222

223

224

225

226

227

228

229

230

231

232

233

234

235

236

237

DISCUSSION

Histologic distinction between cutaneous metastatic melanoma and primary melanoma carries
important diagnostic, prognostic, and treatment implications, yet to our knowledge none of the
previous studies have systematically compared the histopathologic features of the two. We
selected 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 histopathologic features in differentiating EDMM and PNM. Of
these, tumor size is one of the most objective discriminating factors. Nodules >1 cm are more
likely to be primary, whereas lesions <0.2 cm are much more likely 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 are smaller than 0.2 cm, one should be aware
that primary "micromelanomas" do exist, and may be detected more readily with the use of
dermoscopy.[17,18] Hence, tumor size should be viewed as a strong but not absolute discriminator
of metastatic and primary melanomas.

In addition to tumor size, tumor silhouette also provides useful information. Polypoid/exophytic
lesions are much more likely to be PNM, as only one EDMM in our series displays a polypoid
silhouette. The rarity of polypoid EDMM is also reflected in the literature.[11,19] Epidermal
collarette has been previously reported in both EDMM and primary melanomas.[20] In our cohort,
epidermal collarette is significantly associated with PNM. This association is probably related to
the larger tumor size in this group, as epidermal collarette is a consequence of expansile dermal
growth.

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

265   Although architectural "shoulder" was traditionally thought to be specific for primary melanoma,

266   later studies have also shown this feature in EDMM.[8,13,14] Our findings support the latter by

267   showing architectural shoulder in 17% of EDMM. Notably, six EDMM lesions from three

268   patients were purely or predominantly intraepidermal, microscopically indistinguishable from

269   melanoma in situ and microinvasive melanoma. The metastatic nature of these lesions is

270   supported by the identical copy number abnormalities to their corresponding primary

271   melanomas. The occurrence of multiple lesions in the vicinity of the primary melanoma site also

272   provides compelling clinical evidence for metastases. However, when clinicopathologic

273   correlation fails to elucidate the primary versus metastatic nature of a lesion, molecular studies

274   may be needed to facilitate more accurate staging.[15,21]

275   A number of cytomorphologic features aid in the distinction between PNM and EDMM. In

276   general, significant pleomorphism favors a diagnosis of PNM, whereas monomorphism favors

277   EDMM. Furthermore, primary melanomas are more likely to comprise multiple morphologically

278   distinct subpopulations secondary to genetic divergence within the tumor. In contrast, metastatic

279   melanomas more commonly show one phenotype only, probably reflective of a selected tumor

280   subclone harboring increased metastatic potential.[22] Pseudomaturation, referring to partial

281   diminution of cell size with dermal descent, has been described in the majority of nevoid

282   melanomas[23] but also in other melanoma subtypes as well as EDMM. This feature is not helpful

283   in their distinction.

284   While a coexisting nevus tends to be found more frequently in PNM than EDMM, the difference

285   falls short of statistical significance (p=0.0578). It is well known that primary melanoma may

286   arise from a preexisting nevus via malignant transformation. A recent study showed that the

287   incidence of associated nevus was highest among superficial spreading melanomas (37%) and

288   lowest among nodular melanomas (16%).[24] This relatively low frequency in PNM may have

289   accounted for the lack of a significant difference when compared with EDMM.

290   Although mitotic figures can be brisk in metastatic melanoma,[25] our study found significantly

291   higher mitotic rate in PNM. Nevertheless, given the wide range and significant overlap of mitotic

292   rates in the two groups, the observed difference is of limited practical value. As rapidly

293   proliferating tumors often outgrow their blood supply, tumor necrosis may result and is again

294   more commonly found in PNM. Previous studies have found tumor necrosis to be significantly

295   associated with increased tumor thickness, increased Ki-67 proliferation index, and

296   ulceration.[26,27] It is therefore possible that the lower incidence of tumor necrosis in EDMM may

297   be in part attributable to smaller tumor size and infrequent ulceration in this group.

298   Melanoma can metastasize via lymphatic, hematogenous, or angiotropic route.[28-33] Both

299   angiotropism and lymphovascular invasion are fair predictors of metastatic potential in

300   melanoma.[34] In our study, both lymphovascular invasion and angiotropism were seen in small

301   subsets of PNM and EDMM cases, and failed to distinguish between groups. Perineural invasion

302   is believed to be another means by which satellite metastases form in melanoma. This, too, does

303   not serve as a useful discriminator.

304   Our data indicate that prominent TIPs are strongly suggestive of PNM, observed in 29% of these

305   cases compared to 1% of EDMM. Assessment of TIPs is fairly straightforward, rendering it a

306   powerful and practical discriminator. Mascaro et al. reported clusters of plasma cells in 22% of

307   primary melanomas, a feature that was associated with increased Breslow thickness, ulceration,

308   and poor survival.[35] A recent study confirmed these associations as well as higher

309   mitoticactivity.[36] Interestingly, when found in metastatic melanomas, TIPs were reported as a

310   favorable prognosticator.[37] Further investigation is needed to elucidate the immunologic

311   relationship between TIPs and melanoma cells.

312   Because absence of TILs is associated with sentinel lymph node metastasis, whereas brisk TILs

313   are associated with prolonged recurrence-free survival,[38,39] one may expect fewer TILs in

314   metastatic melanomas. A study reported a higher percentage of primary melanomas (67%)

315   contained lymphoid infiltrate compared to metastatic foci (9%) in the same patients.[40] Another

316   study of nodal metastatic melanomas found that TILs were absent in 46% of cases.[41] In the skin,

317   we showed that absence of TILs is significantly associated with EDMM, suggesting enhanced

318   capability in evading immune detection.[42] Similarly, lichenoid inflammation is less common in

319   EDMM than PNM, despite the essentially identical frequency of epidermal involvement in these

320   groups.

321   Perhaps contrary to common belief,[11] our study did not identify any significant difference in the

322   frequency of tumor regression. Late regression, evidenced by fibrosis, increased vascularity,

323   epidermal atrophy, and melanophages, has been reported in 10-35% of primary melanomas.[43]

324   Although a review article cited a much lower incidence of spontaneous regression in metastatic

325   melanomas (0.25%), this figure is likely skewed by the exceedingly low incidence of regression

326   in extracutaneous metastases.[44] Our cohort demonstrates regression in 17% and 9% of PNM and

327   EDMM cases, respectively. Several factors may explain the lack of significant difference. First,

328   regression has been reported to occur less frequently in nodular melanoma (13%) compared to

329   other subtypes.[45] Second, the histopathologic features of regression are not specific and may be

330   indistinguishable from changes of chronic friction or prior trauma. Lastly, one of our EDMM

331   cases with regression was excised after immunotherapy (pembrolizumab), which may have

332   triggered regression of the metastatic melanoma.[46] Tumoral melanosis—a histologic variant of

333   regression[47]—similarly fails to differentiate between PNM and EDMM.

334   In conclusion, we conducted the first comprehensive histopathologic comparison of EDMM and

335   PNM, and identified multiple useful discriminators with significant associations not previously

336   reported. Of these, the collective findings of large tumor size, ulceration, prominent TIPs,

337   epidermal collarette, and lichenoid inflammation strongly support a diagnosis of PNM based on a

338   logistic regression model. Other highly useful characteristics based on univariate analysis include

339   small tumor size and absence of TILs which favor EDMM, and polypoid configuration, tumor

340   necrosis, and multiple phenotypes which favor PNM. While the importance of clinical

341   correlation cannot be overemphasized, recognition of the above constellation of histopathologic

342   features, especially when clinical history is limited, should allow for more accurate

343   classification, hence more precise tumor staging and prognostication for melanoma patients.

344

345

351

352 **CONFLICTS OF INTEREST**

353 The authors declare no conflicts of interest.

354

355 **REFERENCES**

356

357 1. PDQ Adult Treatment Editorial Board. Melanoma Treatment (PDQ): Health Professional

358 Version. PDQ Cancer Information Summaries [Internet]. Bethesda (MD): National Cancer

359 Institute (US); 2002-2015 Aug 25.

360

361 2. Balch CM, Gershenwald JE, Soong S-J, et al. Final version of 2009 AJCC melanoma staging

362 and classification. J Clin Oncol. 2009;27(36):6199-6206.

363

364 3. High WA, Stewart D, Wilbers CR, et al. Completely regressed primary cutaneous malignant

365 melanoma with nodal and/or visceral metastases: a report of 5 cases and assessment of the

366 literature and diagnostic criteria. J Am Acad Dermatol. 2005;53(1):89-100.

367

368 4. Emanuel PO, Mannion M, Phelps RG. Complete regression of primary malignant melanoma.

369 Am J Dermatopathol. 2008;30(2):178-181.

370

371 5. Mărgăritescu I, Chiriţă AD, Vasilescu F. Completely regressed primary cutaneous melanoma -

372 difficulties in diagnosis and classification. Rom J Morphol Embryol. 2014;55(2 Suppl):635-642.

373

374 6. Ariel IM. Disseminated melanoma with unique unilateral distribution. Cancer.

375 1975;36(6):2143-2146.

376

377 7. Warner TF, Gilbert EF, Ramirez G. Epidermotropism in melanoma. J Cutan Pathol.

378 1980;7(1):50-54.

379

380 8. Bengoechea-Beeby MP, Velasco-Oses A, Mourino Fernandez F, et al. Epidermotropic

381 metastatic melanoma: are the current histologic criteria adequate to differentiate primary from

382 metastatic melanoma? Cancer. 1993;72(6):1909-1913.

383

384   9. Allen AC, Spitz S. Malignant melanoma: A clinicopathological analysis of the criteria for

385   diagnosis and prognosis. Cancer. 1953;6(1):1-45.

386

387   10. Kornberg R, Harris M, Ackerman AB. Epidermotropically metastatic malignant melanoma.

388   Arch Dermatol. 1978;114(1):67-69.

389

390   11. Plaza JA, Torres-Cabala C, Evans H, et al. Am J Dermatopathol 2010;32(2):129-136.

391

392   12. Storr SJ, Safuan S, Mitra A, et al. Objective assessment of blood and lymphatic vessel

393   invasion and association with macrophage infiltration in cutaneous melanoma. Mod Pathol.

394   2011;25(4):493-504.

395

396   13. Heenan PJ and Clay CD. Epidermotropic metastatic melanoma simulating multiple primary

397   melanomas. Am J Dermatopathol. 1991;13(4):396-402.

398

399   14. Abernethy JL, Soyer HP, Kerl H, et al. Epidermotropic metastatic malignant melanoma

400   simulating melanoma in situ: a report of 10 examples from two patients. Am J Surg Pathol.

401   1994;18(11):1140-1149.

402

403   15. Lestre S, João A, Ponte P, et al. Intraepidermal epidermotropic metastatic melanoma: a

404   clinical and histopathological mimicker of melanoma in situ occurring in multiplicity. J Cutan

405   Pathol. 2011;38(6):514-520.

406

407   16. Gerami P, Jewell SS, Morrison LE, et al. Fluorescence in situ hybridization (FISH) as an

408   ancillary diagnostic tool in the diagnosis of melanoma. Am J Surg Pathol. 2009;33(8):1146-

409   1156.

410

411   17. Bono A, Bartoli C, Baldi M, et al. Micro-melanoma detection. A clinical study on 22 cases of

412   melanoma with a diameter equal to or less than 3 mm. Tumori. 2004;90(1):128-131.

413

414   18. Bono A, Tolomio E, Trincone S, et al. Micro-melanoma detection: a clinical study on 206

415   consecutive cases of pigmented skin lesions with a diameter ≤3 mm. Br J Dermatol.

416   2006;155(3):570-573.

417

418   19. Hayashi H, Kawashima T, Hosokawa K, et al. Epidermotropic metastatic malignant

419   melanoma with a pedunculated appearance. Clin Exp Dermatol. 2003;28(6):666-668.

420

421   20. Guerriere-Kovach PM, Hunt EL, Patterson JW, et al. Primary melanoma of the skin and

422   cutaneous melanomatous metastases: comparative histologic features and immunophenotypes.

423   Am J Clin Pathol. 2004;122(1):70-77.

424

425   21. Bahrami S, Cheng L, Wang M, et al. Clonal relationships between epidermotropic metastatic

426   melanomas and their primary lesions: a loss of heterozygosity and X-chromosome inactivation-

427   based analysis. Mod Pathol. 2007;20(8):821-827.

428

429   22. Nowell PC. The clonal evolution of tumor cell populations. Science, New Series.

430   1976;194(4260):23-28.

431

432   23. Zembowicz A, McCusker M, Chiarelli C, et al. Morphological analysis of nevoid melanoma:

433   a study of 20 cases with a review of the literature. Am J Dermatopathol. 2001;23(3):167-175.

434

435   24. Lin WM, Luo S, Muzikansky A, et al. Outcome of patients with de novo versus nevus-

436   associated melanoma. J Am Acad Dermatol. 2015;72(1):54-58.

437

438   25. Bulkley GB, Cohen MH, Banks PM, et al. Long-term spontaneous regression of malignant

439   melanoma with visceral metastases: report of a case with immunologic profile. Cancer.

440   1975;36(2):485-494.

441

442   26. Bachmann IM, Ladstein RG, Straume O, et al. Tumor necrosis is associated with increased

443   alpha(v)beta(3) integrin expression and poor prognosis in nodular cutaneous melanomas. BMC

444   Cancer. 2008;8:362.

445

446 27. Ladstein RG, Bachmann IM, Straume O, et al. Tumor necrosis is a prognostic factor in thick

447 cutaneous melanoma. Am J Surg Pathol. 2012;36(10):1477-1482.

448

449 28. Gerami P, Shea C, Stone MS. Angiotropism in epidermotropic metastatic melanoma: another

450 clue to the diagnosis. Am J Dermatopathol. 2006;28(5):429-433.

451

452 29. Barnhill R, Lugassy C. Angiotropic malignant melanoma and extravascular migratory

453 metastasis: description of 36 cases with emphasis on a new mechanism of tumour spread.

454 Pathology. 2004;36(5):485-490.

455

456 30. Johnson C, Ramos-Caro F, Hassanein A. Ultra-late erysipeloid angiotropic metastatic

457 malignant melanoma. Int J Dermatol. 2001;40(7):446-447.

458

459 31. Moreno A, Espanol I, Romagosa V. Angiotropic malignant melanoma. Report of two cases. J

460 Cutan Pathol. 1992;19(4):325-329.

461

462 32. Saluja A, Money N, Zivony D, et al. Angiotropic malignant melanoma: a rare pattern of local

463 metastases. J Am Acad Dermatol. 2001;44(5):829-832.

464

465 33. Shea C, Kline M, Lugo J, et al. Angiotropic metastatic malignant melanoma. Am J

466 Dermatopathol. 1995;17(1):58-62.

467

468 34. Barnhill R, Dy K, Lugassy C. Angiotropism in cutaneous melanoma: a prognostic factor

469 strongly predicting risk for metastasis. J Invest Dermatol. 2002;119(3):705-706.

470

471 35. Mascaro JM, Molgo M, Castel T, et al. Plasma Cells within the infiltrate of primary

472 cutaneous malignant melanoma of the skin: a confirmation of its histoprognostic value. Am J

473 Dermatopathol. 1987;9(6):497-499.

474

475 36. Bosisio FM, Wilmott JS, Volders N, et al. Plasma cells in primary melanoma. Prognostic

476 significance and possible role of IgA. Mod Pathol. 2016;29(4):347-358.

477

478 37. Erdag G, Schaefer JT, Smolkin ME et al. Immunotype and immunohistologic characteristics

479 of tumor infiltrating immune cells are associated with clinical outcome in metastatic melanoma.

480 Cancer Res. 2012;72(5):1070-1080.

481

482 38. Azimi F, Scolyer RA, Rumcheva P, et al. Tumor-infiltrating lymphocyte grade is an

483 independent predictor of sentinel lymph node status and survival in patients with cutaneous

484 melanoma. J Clin Oncol. 2012;30(21):2678-2683.

485

486 39. Fortes C, Mastroeni S, Mannooranparampil TJ, et al. Tumor-infiltrating lymphocytes predict

487 cutaneous melanoma survival. Melanoma Res. 2015;25(4):306-311.

488

489 40. Payan HM, Gilbert EF, and Jacobs WH. Lymphocytic reaction around primary and

490 metastatic melanomas. South Med J. 1970;63(11):1350-1352.

491

492 41. Mihm MC Jr, Clemente CG, Cascinelli N. Tumor-infiltrating lymphocytes in lymph node

493 melanoma metastases: a histopathologic prognostic indicator and an expression of local immune

494 response. Lab Invest. 1996;74(1):43-47.

495

496 42. Osborn JL, Greer SF. Metastatic melanoma cells evade immune detection by silencing

497 STAT1. Int J Mol Sci. 2015;16(2):4343-4361.

498

499 43. Blessing K, McLaren KM. Histological regression in primary cutaneous melanoma:

500 recognition, prevalence and significance. Histopathology. 1992;20(4):315-322.

501

502 44. Kalialis LV, Drzewiecki KT, Klyver H. Spontaneous regression of metastases from

503 melanoma: a review of the literature. Melanoma Res. 2009;19(5):275-282.

504

505 45. Tas F and Erturk K. Presence of histological regression as a prognostic factor in cutaneous
506 melanoma patients. Melanoma Res. 2016;26(5):492-496.

507

508 46. Brizio M, Fava P, Astrua C, et al. Complete regression of melanoma skin metastases after
509 electrochemotherapy plus ipilimumab treatment: an unusual clinical presentation. Eur J
510 Dermatol. 2015;25(3):271-272.

511

512 47. Ng SH, Chave TA. Tumoral melanosis as a manifestation of a completely regressed primary
513 melanoma with metastasis. Br J Dermatopathol. 2006;155(3):627-628.

514

515

516

517

518

519

520

521

522

523

524

525

526

527

528

529

530

531

532

533

534 **TABLES**

535 Table 1. Histopathologic features showing statistically significant differences between

536 epidermotropic/dermal metastatic melanoma (EDMM) and primary nodular melanoma (PNM)

537 by univariate analysis.

Histopathologic features	EDMM (n=74)	PNM (n=75)	p-value	Specificity (%)
Greatest diameter <0.2 cm	**19 (26%)**	0 (0%)	<0.0001	100
Absence of tumor-infiltrating plasma cells	**67 (91%)**	38 (51%)	0.0036	49
Monomorphism	**41 (55%)**	24 (32%)	0.0309	68
Absence of tumor-infiltrating lymphocytes	**9 (12%)**	2 (3%)	0.0348	97
Involvement of adnexal epithelium	**32 (43%)**	18 (24%)	0.0417	76
Polypoid (exophytic) configuration	0 (0%)	**16 (21%)**	<0.0001	100
Prominent tumor-infiltrating plasma cells	1 (1%)	**22 (29%)**	<0.0001	99
Greatest diameter >1 cm	2 (3%)	**31 (41%)**	<0.0001	97
Ulceration	4 (5%)	**36 (48%)**	<0.0001	95
Epidermal collarette	18 (24%)	**53 (71%)**	<0.0001	76
Mean mitotic rate (median, range)	4 (2, 0-23)	**13 (11, 1-53)**	<0.0001	--
Tumor necrosis	2 (3%)	**19 (25%)**	0.0002	97
Multiple phenotypes	6 (8%)	**24 (32%)**	0.0012	92
Significant pleomorphism	13 (18%)	**31 (41%)**	0.0073	82
Lichenoid inflammation	3 (4%)	**14 (19%)**	0.0088	96

538

539

540 Table 2. Histopathologic features without statistically significant differences between primary

541 nodular melanoma (PNM) and epidermotropic/dermal metastatic melanoma (EDMM) by

542 univariate analysis.

Histopathologic features	PNM (n=75)	EDMM (n=74)	p-value
Associated nevus	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 tumor-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

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

544

545

546

547

548

549   Table 3. Logistic regression model by multivariate analysis.

Histopathologic features	Estimates with PNM*	p-value
Greatest diameter of >1 cm (vs < 0.2 cm)	2.2024	0.0023
Greatest diameter of 0.2-1 cm (vs <0.2 cm)	0.5340	0.3695
Presence of ulceration	0.8014	0.0166

Histopathologic features	Estimates with PNM*	p-value
Presence of prominent tumor-infiltrating plasma cells	0.9775	0.0487
Presence of lichenoid inflammation	0.7993	0.0675
Presence of epidermal collarette	0.6903	0.0028

550   PNM, primary nodular melanoma.

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

552

553

554

555

556

557

558

559

560

561

562

563

564

565

566

567   **FIGURE LEGENDS**

568

569   Figure 1. Representative features associated with epidermotropic/dermal metastatic melanoma.

570   (A) A small lesion in the upper dermis measuring less than 0.2 cm in greatest dimension (H&E,

571   40x). (B) Another lesion demonstrates a dermal nodule with epidermotropism, cytologic

572   monomorphism, and lack of tumor-infiltrating lymphocytes and plasma cells (H&E, 100x).

573

574   Figure 2. Representative features associated with primary nodular melanoma. (A) A large,

575   ulcerated nodular melanoma with an exophytic polypoid configuration. (H&E, 10x). (B) This

576   tumor is flanked by epidermal collarette (H&E, 20x). (C) Zonal tumor necrosis is noted in the

577   deeper portion of this biopsy (H&E, 20x). (D) Clusters of tumor-infiltrating plasma cells are

578   present in this example (H&E, 400x). (E) This nodular melanoma exhibits multiple distinct

579   phenotypes and significant pleomorphism. One population of cells contain highly pleomorphic

580   and bizarre looking nuclei (top), whereas the other population consists of nests of relatively

581   uniform cells (bottom) (H&E, 200x).

582

583   Figure 3. Epidermotropic metastatic melanomas with epidermal-predominant and epidermal-only

584   patterns. (A) This scalp lesion was taken from near the site of a previously resected primary

585   melanoma. Most of the melanoma cells are present within the epidermis, giving rise to an

586   architectural "shoulder" and the appearance of a microinvasive melanoma. Adnexal involvement

587   is also present (H&E, 100x). (B) Another patient presented with a purely intraepidermal

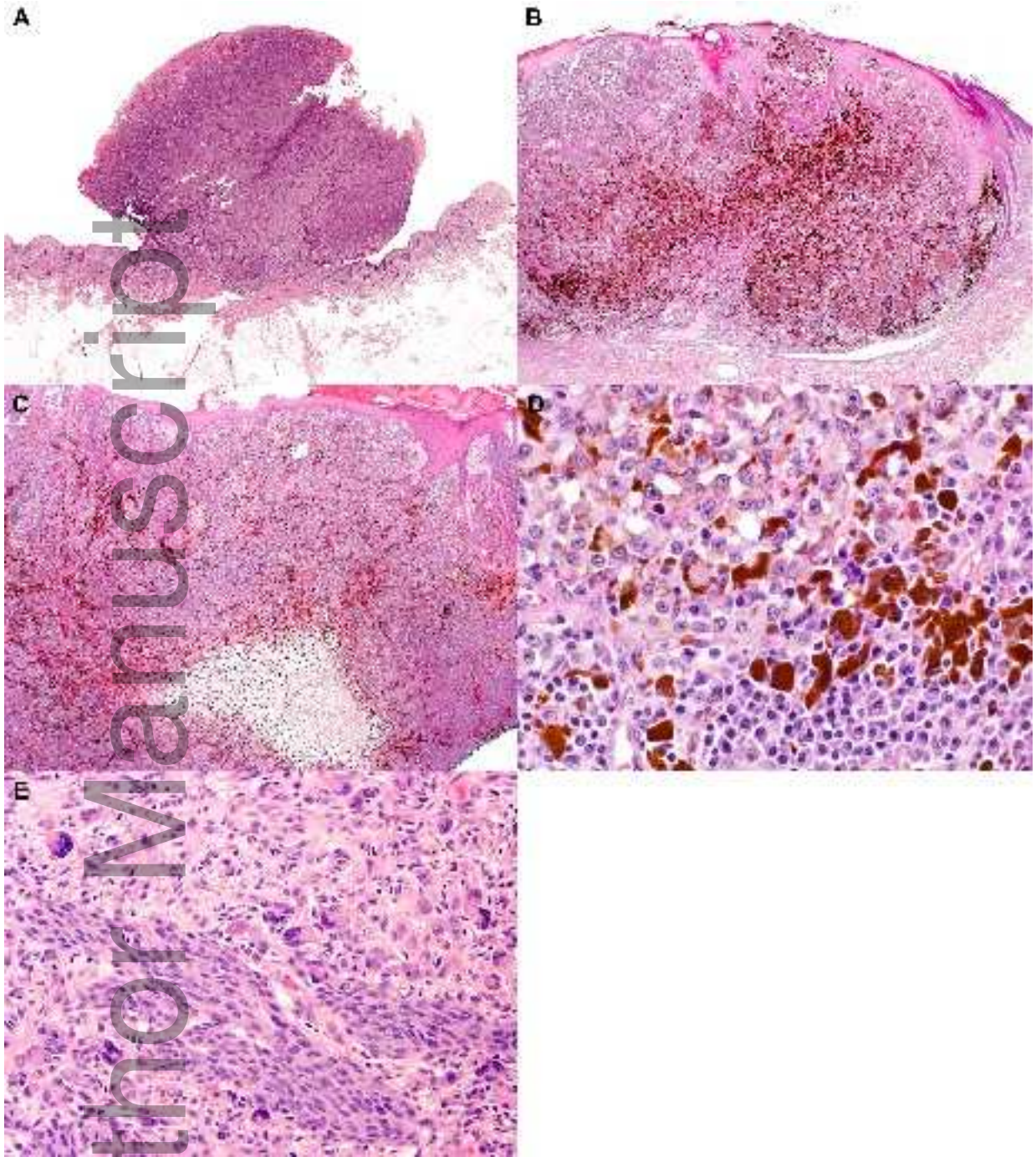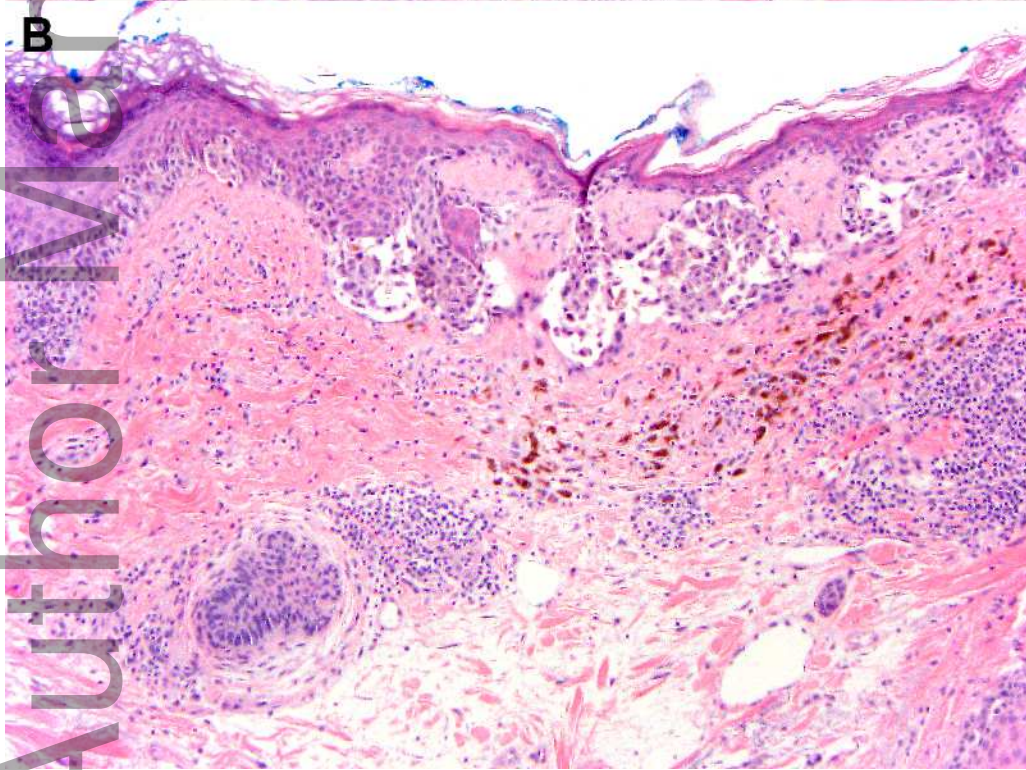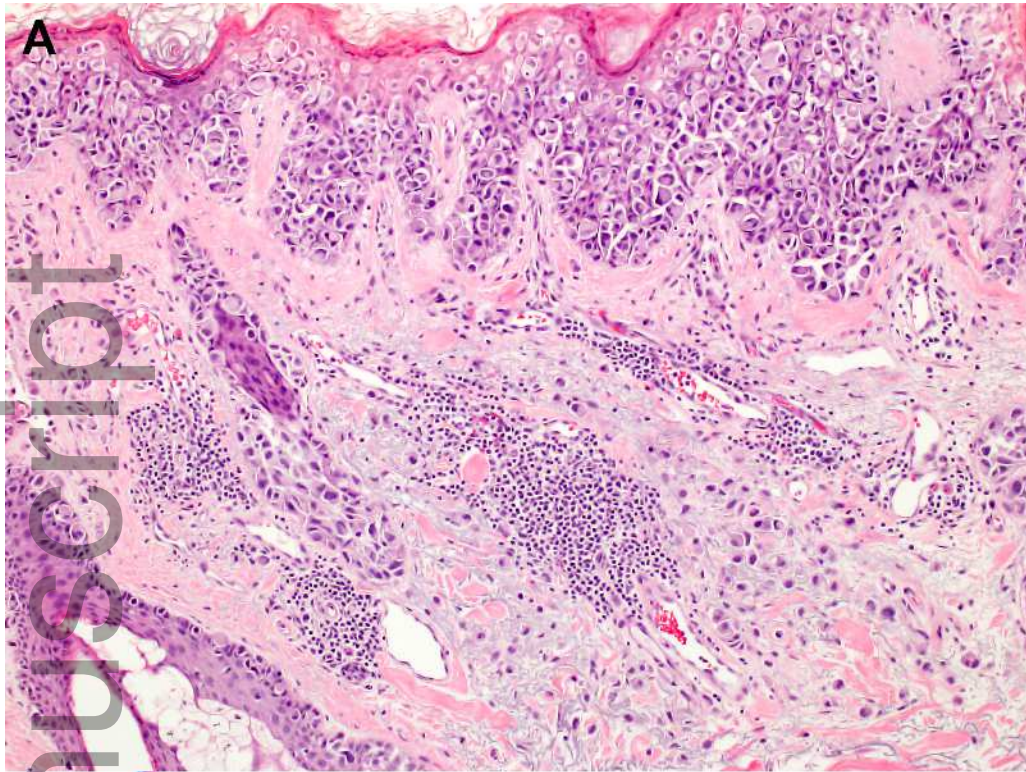588   metastatic melanoma on the scalp, closely resembling melanoma in situ (H&E, 100x).

A
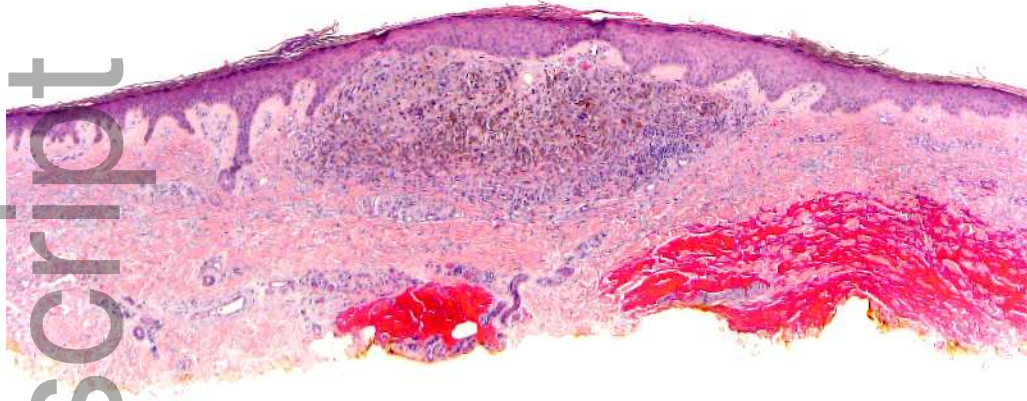
B
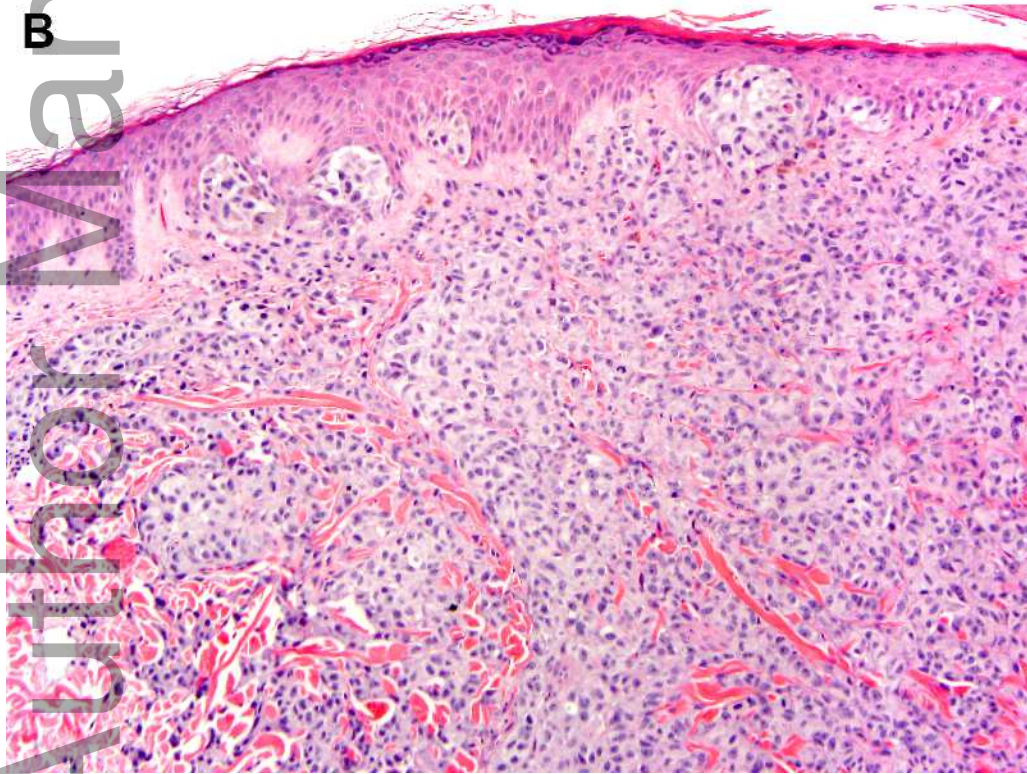
his_13384_f1.tif

his_13384_f2.tif

his_13384_f3.tif