BJD
British Journal of Dermatology

Prospective study of formalin-fixed Mohs surgery and haematoxylin and eosin stains with control contralateral biopsies for lentigo maligna: 5-year follow-up results

C.M. Lawrence,[1] R. Rahim,[1] F. Charlton[2] and A. Husain[2]

Departments of [1]Dermatology and [2]Histopathology, Royal Victoria Infirmary, Newcastle NE1 4LP, U.K

Correspondence
Clifford Lawrence.
E-mail: clifford.lawrence@nuth.nhs.uk

Accepted for publication
16 January 2014

Funding sources
None.

Conflicts of interest
None declared.

DOI 10.1111/bjd.12841

Summary

Background There is little consensus on the optimum form of surgical management for lentigo maligna (LM). Currently, because malignant melanocytes spread down adnexal structures, full-thickness skin removal is the only surgical option. Interpretation of Mohs histological specimens is difficult because of the presence of abnormal melanocytes in otherwise normal sun-damaged skin.

Objectives To investigate Slow Mohs for surgical excision of LM, to see whether the use of control contralateral skin biopsies would enable the end point of excision to be more easily interpreted and to investigate factors that influence the subclinical amelanotic extensions of LM.

Methods The Slow Mohs technique for formalin-fixed tissue was used in 74 patients with LM. Before surgery LMs were classified as well defined, poorly defined, incompletely excised or recurrent. Control biopsies were taken from healthy skin of the contralateral side. Specimens were processed in formalin, stained with haematoxylin and eosin (H&E) and the results read at 24–48 h. The excision margin required for complete excision was measured and patients were followed for a minimum of 5 years to exclude recurrence.

Results On average the final excision margin required was 6·7 mm. Margins were significantly greater for ill-defined, recurrent and incompletely excised LM compared with well-defined LM. The presence of depigmented patches preoperatively did not correlate with the excision margin, but LMs showing nesting required significantly wider excision margins. There were seven (12%) recurrences at a mean 4·4 years after surgery in the group with 5-year follow-up. Recurrence occurred only in recurrent and ill-defined primary LM.

Conclusions The use of Slow Mohs formalin-fixed tissue and H&E section staining, even with comparator biopsies, does not provide sufficient discrimination to identify residual disease confidently.

What's already known about this topic?

- There is no established optimal surgical management for lentigo maligna (LM).
- LM may spread beyond the visible pigmented border.

What does this study add?

- Some 62% of LMs contain amelanotic extensions.
- Excision margins of 6 mm were sufficient for 96% of well-defined LMs but insufficient for ill-defined, recurrent or incompletely excised lesions.
- LMs showing nesting require significantly wider excision margins than those without melanocyte nesting.
- Contralateral control biopsies do not help to distinguish between involved and uninvolved tissue.

Lentigo maligna (LM) is a slow-growing subtype of melanoma *in situ* arising on sun-exposed sites. It is estimated to occur in 1·17% of white Americans and to carry a 1–3% lifetime risk of clinical malignant transformation.[1] The 10–50% incidence of invasive disease present in excised LM specimens suggests that in many cases invasive disease is not clinically apparent,[2–5] but presumably would become evident if the patient lived long enough.[6]

There is little consensus on the optimum form of management. Complete surgical excision is generally preferred, although other therapies and watchful waiting may be pragmatic in frail, elderly patients.[7] Nonsurgical treatment options such as Grenz ray therapy[8] offer 88% long-term cure rates, and topical imiquimod remains a promising but as yet unproven therapy. Cryotherapy[9] is advocated despite reports of malignant melanoma arising after apparent clearance following LM cryotherapy.[10] The presence of malignant melanocyte migration deep into follicles invalidates techniques such as laser destruction and dermabrasion that involve superficial skin removal followed by re-epithelialization. In effect, surgical full-thickness skin removal is the only logical surgical option because of the need to remove skin adnexal structures.

Surgical options include wide local excision, staged surgical excision (SSE),[11,12] Mohs micrographic surgery (MMS)[13] and three-dimensional histology of formalin-fixed tissue[14] for LM melanoma. The recommended 5-mm margin for LM is based on a 1992 consensus opinion.[15] This approach may result in the unnecessary excision of uninvolved skin or result in incomplete excision due to the presence of amelanotic tumour extensions,[16] resulting in recurrence rates of up to 20%.[17] By contrast SSE and MMS have the advantage of histopathology confirmation of lesion clearance prior to defect repair.

Frozen-section MMS has the benefit of a rapid section turnaround but is limited by poor cytological definition due to a cytoplasmic retraction artefact, making melanocyte identification difficult.[18] To ensure optimal haematoxylin and eosin (H&E) melanocyte morphology, we have used a formalin-fixed Mohs surgical technique, or Slow Mohs,[19] to map the extent of malignant cells beyond the visible pigmented border.

Because chronically sun-damaged skin contains melanocyte changes that can be similar to those seen in LM,[20,21] we have used a control biopsy from a contralateral skin site to aid histological evaluation of completeness of excision. Here we report the 5-year follow-up results in 74 patients with LM.

Materials and methods

All procedures were performed under local anaesthetic. Using a magnifying loop and under good lighting, the pigmented tumour margin was identified and marked before local anaesthetic injection. Wood's light examination was not used to help identify tumour margins as our previous attempts had proven technically difficult and unhelpful. In 68 patients the LM debulk excision was a 2-mm margin of normal skin around the clinically visible pigmented border; in one patient a 1-mm margin was taken. This excision was followed by a 2-mm Mohs surgical excision margin, so that 68 previously untreated LMs had a minimum 4-mm margin and one a 3-mm margin. No margin of normal skin was taken around the debulk excision in five patients in an attempt to preserve normal tissue. A control biopsy from apparently normal skin, on the contralateral site, was taken from 38 patients. The Mohs sections were orientated using tissue-edge dye and a map drawn. The specimen was processed in formalin through a routine laboratory, stained with H&E and the results were available within 24–48 h as described previously.[19] The slides were read by a pathologist and the Mohs surgeon. Repeat-measured margin excisions were performed until a tumour-free level was achieved. Positive margins were defined as the presence of nested or confluent single melanocytes with significantly cytological atypia.

Data were collected prospectively from all patients undergoing Slow Mohs surgery for surgically resectable new, incompletely excised or recurrent-after-excision LM from 1998 to 2006. Data collected included patient age and sex; tumour duration, site, size and previous treatment; presence of pigment loss within or around the pigmented macule and indication for Mohs. The indication for Mohs was classified clinically into four groups, as well-defined primary LM, ill-defined primary LM, incompletely excised LM and recurrent LM. The debulk specimens were examined within 1 week to confirm the diagnosis and to look for the presence of nesting and evidence of invasion. Mohs procedure details including number of stages and blocks, defect diameter, excision margin beyond the visible edge and closure technique were recorded.

The outcome at follow-up was determined by clinical review. In four cases, followed as outpatients for 2·8, 4·6, 4·6 and 4·7 years, 5-year follow-up could be achieved only by telephone interview.

Comparison of the presence or absence of melanocyte nesting and depigmented patches, and the effect of the indication for Mohs on the required excision margin were tested using the Mann–Whitney test.

Results

Patients and tumour characteristics

In total 74 patients (38 female, 36 male), mean age 68 years (range 45–93) were treated (Table 1). The mean maximum visible diameter was 17 mm (range 2–45) and tumours arose on the following sites: cheek (n = 41), periocular (n = 10), nose (n = 7), other head and neck sites (n = 11) and trunk and limbs (n = 5). Patients reported a mean LM duration of 7·2 years (range 0·1–60).

Surgery performed

Clearance was attempted and achieved in all cases. A mean of 13 blocks (range 3–67) were generated following an average 2·1 (range 1–7) excision stages to reach clear excision margins. Overall, 28 patients were cleared after one Mohs stage, 30 after two, eight after three, four after four, one after five, two after six, and one after seven stages. In the 46 patients

Table 1 Patients with lentigo maligna (LM) treated by Slow Mohs

	Well-defined LM	Ill-defined LM	Incompletely excised LM	Recurrent LM	Overall
Number of patients	25	36	5	8	74
Age (years), mean	65	70	68	69	68 (range 45–93)
Mean largest visible diameter (mm)	14	20	7	22	17
Presence of invasion on debulk specimen	0	3	0	0	3
Number of patients showing LM persistence after first Mohs	8/25	28/36	4/5	6/8	46/74 (62%)
Mean number of Mohs stages	1·3	2·1	2·6	3·9	2·1
Margin for clearance (mm), mean (range)	4·6 (2–8)	6·2 (2–16)	8·4 (4–12)	15 (4–30)	6·7 (2–30)
Number needing > 6-mm margin for clearance	1/25 (4%)	8/36 (22%)	3/5 (60%)	5/8 (58%)	17/74 (23%)
Number who died disease free with < 5-year FU	3 (after 3·2–4·3 years' FU)	13 (after 0·4–4·7 years' FU)	0	1 (after 3·2 years' FU)	17 (after 0·4–4·7 years' FU)
Number lost to FU	0	1	0	0	1
Number with minimum 5-year FU	22	22	5	7	56
Number with clinical recurrence after minimum 5-year FU	0	3/22 (14%)	0	4/7 (57%)	7/56 (12%)
Interval to recurrence (years), mean (range)	NA	2·4, 5·3 & 7·4	NA	4 (0·6–6·6)	4·4 (0·6–7·4)

FU, follow-up; NA, not available.

still positive after the first Mohs stage, LM was present in 8–100% (mean 34%) of the blocks. The interval between the first Mohs procedure and clearance was 2 days (range 0–11). The mean interval between first Mohs and closure was 4·3 days (range 0–15, median 6). Closure was performed by skin graft in seven patients, direct closure in 39, flap repair in 24 and secondary intention in four.

Mohs debulk histology

In the absence of nesting or continuous runs of abnormal melanocytes, the histological end point was difficult to assess. Sections showing multiple but nonconfluent single melanocytes with significant cytological atypia were difficult to distinguish from LM. The use of comparator biopsies in 38 patients was not considered helpful by the pathologists or Mohs surgeon. These Mohs specimens were reported as being unremarkable (64%) or showing scattered atypical melanocytes (16%), solar elastosis (14%) or normal melanocytes (3%), and one patient (3%) was found to have changes considered indistinguishable from LM in the apparently clinically normal control biopsy.

Excision margin

The final excision margin from the visible pigmented margin or previous scar was a mean 6·7 mm (range 2–30). The overall mean number of Mohs stages required to achieve clear margins was 2·1. There were significant differences in the excision margin required for clearance depending on indication for Mohs (Table 1) as follows: well-defined primary LM (n = 25, mean 4·6 mm, range 2–8), ill-defined primary LM (n = 36, mean 6·2 mm, range 2–16), incompletely excised LM (n = 5, mean 8·4 mm, range 4–12) and recurrent LM (n = 8, mean 15 mm, range 4–30) (Fig. 1).

The eight patients with recurrent LM required more Mohs stages (3·9 vs. 2), wider excision margins [15 mm (range 4–30) vs. 6 mm (range 2–16)] and had a larger pretreatment maximum diameter (22 vs. 17 mm) compared with the 66 patients with nonrecurrent disease (Table 1). There was no difference in patient age (69 vs. 68 years) between the two groups.

White patches indicating areas of pigment loss were present in 20/74 patients. In this group the required excision margin was 8·4 mm compared with 6·1 mm for the 54 without white patches (not significant, Mann–Whitney test). LM with tumour nests (n = 47) required a mean excision margin of 7·3 mm. The 14 LMs without nesting had excision margins of 4·2 mm ($P < 0·01$ Mann–Whitney test). There were no data on 13 patients.

Presence of invasive disease

Preoperatively, three patients with recurrent and two with incompletely excised LM were known to have had a fully

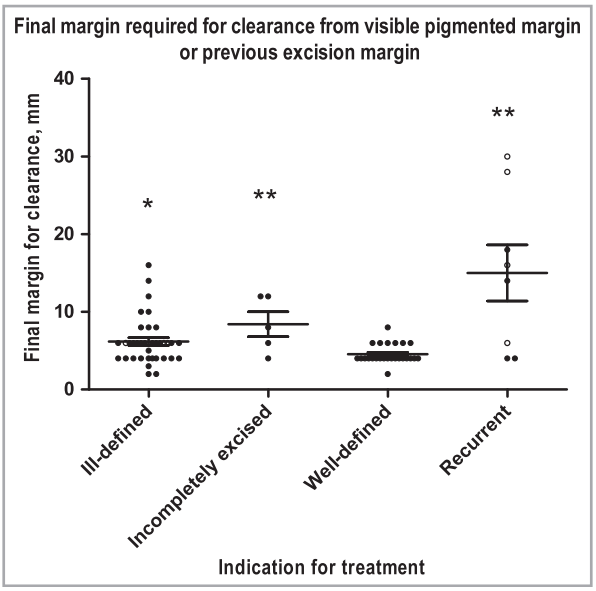

Fig 1. Comparison of excision margins measured from the visible pigmented margin or previous excision scar line, mean + SEM. Comparisons with well-defined lentigo maligna. $^{**}P < 0{\cdot}01$, $^{*}P < 0{\cdot}05$. Open circles (○) indicate clinical recurrence after Mohs surgery.

excised invasive component within the LM at the initial excision; no invasion was found in the debulk excision of these five patients. Within the debulk excision of three patients with ill-defined LM an invasive component with a Breslow thickness of 1 mm, 1·1 mm or 0·15 mm was found. After careful assessment of the excision margin in the debulk specimen plus the final Mohs margin it was considered that in each case the invasive component was adequately excised, and no wider excision was performed in these patients.

Follow-up and recurrence

Seventeen patients died after 0·4–4·7 years of follow-up without evidence of LM recurrence. One patient was lost to follow-up and the remaining 56 patients were followed up for a minimum of 5 years (range 5–9·5 years) or until recurrence. There were seven out of 56 (12%) recurrences at a mean 4·4 years (range 0·6–7·4) after at least 5 years of follow-up in the whole group. Recurrence was associated with the indication for Mohs surgery: four of the seven recurrent LMs and three of the 22 ill-defined primary LMs followed up for at least 5 years recurred. None of the incompletely excised or well-defined primary LMs recurred.

Discussion

Our study shows that the interpretation of formalin-fixed H&E-stained sections for histological control of LM margins is difficult and of limited value, despite the use of contralateral control biopsies for comparison. In our study 6-mm margins, close to the recommended 5-mm margin, were adequate only for patients with well-defined LM, producing clearance in 96% of cases with no recurrences. By contrast, eight out of 36 (22%) ill-defined, five out of eight (62%) recurrent and three out of five (60%) incompletely excised LMs required wider margins. Several studies have demonstrated that 5-mm excision margins are often insufficient for complete LM removal.[22–27] In the present study, 10/74 (14%) lesions required excision margins > 10 mm to achieve clearance. One study reported 30% of cases requiring > 10-mm margins,[22] while another suggested that 86% are completely excised with 6-mm margins and 99% are completely excised using 9-mm margins.[16] Using formalin-fixed tissue and straight-edged, polygonal 5-mm layers, a 5-mm margin was adequate in 42% of cases, whereas 58% required a ≥ 10-mm margin, and a high proportion of recurrent LMs required > 5-mm margins compared with primary LMs.[22]

Incomplete excision of LM is caused by retained, clinically invisible, nonpigmented or amelanotic outgrowths of LM. The observation that more than one Mohs stage was required in 46/74 (62%) of our patients and that 8–100% of the blocks were positive demonstrates that these amelanotic extensions are common and unpredictable, particularly in ill-defined, recurrent and incompletely excised LM. The presence of visible white patches before surgery indicating pigment loss was associated with the need for wider excision margins, although these differences were not significant. LMs showing nesting on the debulk excision did require significantly wider excision margins to obtain histological clearance.

The difficulty in histological assessment of LM excision margins is well recognized.[28] We anticipated that the use of matched site control biopsies would overcome this weakness. However, despite their use we still found it difficult to assess disease extent and to know when healthy tissue had been reached. Sun-damaged skin contains abnormal melanocytes.[20] Cross-sectional excision specimens that include tumour and perilesional skin are easier for the pathologist to assess, as a range of changes from obvious LM to sun-damage changes only are visible on one section. The use of immunocytochemical stains may provide a more reliable method of assessing melanoma and melanoma *in situ* tissue margins.[25,29] Our use of routine formalin-fixed sections involved a 24–48-h delay between stages, resulting in an average 4·3-day delay between first Mohs excision and final closure in our patients. The use of rapid-processing techniques for formalin-fixed tissue including microwave processing could reduce this delay.[30]

Our 5-year recurrence rate of seven out of 56 (12%) is higher than in many other reports following SSE or MMS.[23,24,27,31] However, most of these studies have incomplete 5-year follow-up, a significant weakness given that three of our seven recurrences became apparent only > 5 years after surgery. By contrast, Zitelli *et al.*, using frozen sections and H&E stains, reported cure rates of 99·5% for melanoma and melanoma *in situ* after 5-year follow-up.[31]

Our results show that cure rates depend, as always, on initial disease severity. None of the 25 well-defined or five incompletely excised LMs recurred; the small size of the latter

group means it is unsafe to draw any firm conclusions about the actual recurrence risk in this group. The 5-year recurrence rate for the previously untreated LMs was 7% (three recurrences in 44 patients with well-defined and poorly defined LM), compared with a recurrence rate for previously treated LM of 33% (four recurrences in 12 patients with recurrent or incompletely excised LM).

Although wide local excision margins[32] are advocated in head and neck melanoma and melanoma *in situ*, these margins are often reduced to prevent a disfiguring or poorly functioning outcome. This may explain the poor survival outcomes for head and neck melanoma compared with trunk and limb disease of equivalent severity.[33,34] Head and neck LM and LM melanoma are probably best managed by MMS or SSE. Rapid frozen-section immunostaining[35] is likely to be more effective than using formalin-fixed tissue and H&E stains. The discovery of invasive disease in the debulk excision is not a bar to this approach. Others have shown excellent results using Mohs margins for LM and LM melanoma.[31]

In conclusion, recurrent and incompletely excised LMs require large excision margins, and recurrent lesions have a high further recurrence rate. Excision margins required for clearance of ill-defined LM were > 6 mm in 22% of cases, whereas 96% of well-defined LMs were cleared with a 6-mm margin. The use of Slow Mohs formalin-fixed tissue and H&E section staining, even with comparator biopsies, does not provide sufficient discrimination to identify residual disease confidently. If recurrence occurs because of weakness in interpreting LM histology, the technique used here is not reliable. Alternatively, if recurrence is a consequence of field change or skip lesions occurring within LM, no method that depends on indentifying tumour extent by tracing out histological changes will succeed.

References

1 Weinstock MA, Sober AJ. The risk of progression of lentigo maligna to lentigo maligna melanoma. *Br J Dermatol* 1987; **16**:303–10.
2 Wayte DM, Helwig EB. Melanotic freckle of Hutchinson. *Cancer* 1968; **21**:893–911.
3 Somach SC, Taira JW, Pitha JV, Everett MA. Pigmented lesions in actinically damaged skin. Histopathologic comparison of biopsy and excisional specimens. *Arch Dermatol* 1996; **132**:1297–302.
4 Hazan C, Dusza SW, Delgado R *et al.* Staged excision for lentigo maligna and lentigo maligna melanoma: a retrospective analysis of 117 cases. *J Am Acad Dermatol* 2008; **58**:142–8.
5 Bosbous MW, Dzwierzynski WW, Neuburg M. Staged excision of lentigo maligna and lentigo maligna melanoma: a 10-year experience. *Plast Reconstr Surg* 2009; **124**:1947–55.
6 Cohen LM. Lentigo maligna and lentigo maligna melanoma. *J Am Acad Dermatol* 1997; **36**:913.
7 Mahendran R, Newton-Bishop JA. Survey of U.K. current practice in the treatment of lentigo maligna. *Br J Dermatol* 2001; **144**:71–6.
8 Hedblad MA, Mallbris L. Grenz ray treatment of lentigo maligna and early lentigo maligna melanoma. *J Am Acad Dermatol* 2012; **67**:60–8.
9 de Moraes AM, Pavarin LB, Herreros F *et al.* Cryosurgical treatment of lentigo maligna. *J Dtsch Dermatol Ges* 2007; **5**:477–80.
10 McKenna DB, Cooper EJ, Kavanagh GM *et al.* Amelanotic malignant melanoma following cryosurgery for atypical lentigo maligna. *Clin Exp Dermatol* 2000; **25**:600–4.
11 Dhawan SS, Wolf DJ, Rabinovitz HS, Poulos E. Lentigo maligna, the use of rush permanent sections in therapy. *Arch Dermatol* 1990; **126**:928–30.
12 Johnson TM, Headington JT, Baker SR, Lowe L. Usefulness of the staged excision for lentigo maligna and lentigo maligna melanoma: the square procedure. *J Am Acad Dermatol* 1997; **37**:758–64.
13 Mohs FE, Snow SN, Larson PO. Mohs micrographic surgery fixed-tissue technique for melanoma of the nose. *J Dermatol Surg Oncol* 1990; **16**:1111–20.
14 Moehrle M, Dietz K, Garbe C, Breuninger H. Conventional histology vs. three-dimensional histology in lentigo maligna melanoma. *Br J Dermatol* 2006; **154**:453–9.
15 Diagnosis and treatment of early melanoma. NIH Consensus Development Conference. January 27–29, 1992. *Consens Statement* 1992; **10**:1–25.
16 Kunishige JH, Brodland DG, Zitelli JA. Surgical margins for melanoma *in situ*. *J Am Acad Dermatol* 2012; **66**:438–44.
17 Osborne JE, Hutchinson PE. A follow-up study to investigate the efficacy of initial treatment of lentigo maligna with surgical excision. *Br J Plast Surg* 2002; **55**:611–15.
18 Barlow RJ, White CR, Swanson NA. Mohs' micrographic surgery using frozen sections alone may be unsuitable for detecting single atypical melanocytes at the margins of melanoma *in situ*. *Br J Dermatol* 2002; **146**:290–4.
19 Lawrence CM, Haniffa MA, Dahl MG. Formalin-fixed tissue Mohs surgery for basal cell carcinoma: 5-year follow-up data. *Br J Dermatol* 2009; **160**:573–80.
20 Mitchell RE. The effect of prolonged solar radiation on melanocytes of the human epidermis. *J Invest Dermatol* 1963; **41**:199–212.
21 Cohen LM. The starburst cell is useful for distinguishing lentigo maligna from photodamaged skin. *J Am Acad Dermatol* 1996; **35**:962–8.
22 Agarwal-Antal N, Bowen G, Gerwels J. Histologic evaluation of lentigo maligna with permanent sections: implications regarding current guidelines. *J Am Acad Dermatol* 2002; **47**:743–8.
23 Clayton BD, Leshin B, Hitchcock MG *et al.* Utility of rush paraffin-embedded tangential sections in the management of cutaneous neoplasms. *Dermatol Surg* 2000; **26**:671–8.
24 Huilgol SC, Selva D, Chen C *et al.* Surgical margins for lentigo maligna and lentigo maligna melanoma: the technique of mapped serial excision. *Arch Dermatol* 2004; **140**:1087–92.
25 Zalla MJ, Lim KK, Dicaudo DJ, Gagnot MM. Mohs micrographic excision of melanoma using immunostains. *Dermatol Surg* 2000; **26**:771–84.
26 Albertini JG, Elston DM, Libow LF *et al.* Mohs micrographic surgery for melanoma: a case series, a comparative study of immunostains, an informative case report, and a unique mapping technique. *Dermatol Surg* 2002; **28**:656–65.
27 Bub JL, Berg D, Slee A, Odland PB. Management of lentigo maligna and lentigo maligna melanoma with staged excision: a 5-year follow-up. *Arch Dermatol* 2004; **140**:552–8.
28 Florell SR, Boucher KM, Leachman SA *et al.* Histopathologic recognition of involved margins of lentigo maligna excised by staged excision: an interobserver comparison study. *Arch Dermatol* 2003; **139**:595–604.
29 Bhardwaj SS, Tope WD, Lee PK. Mohs micrographic surgery for lentigo maligna and lentigo maligna melanoma using Mel-5 immunostaining. *Dermatol Surg* 2006; **32**:690–6.
30 Mallipeddi R, Stark J, Xie XJ *et al.* A novel 2-hour method for rapid preparation of permanent paraffin sections when treating

melanoma *in situ* with Mohs micrographic surgery. *Dermatol Surg* 2008; **34**:1520–6.
31 Zitelli J, Brown C, Hanusa B. Mohs micrographic surgery for the treatment of primary cutaneous melanoma. *J Am Acad Dermatol* 1997; **37**:236–45.
32 NIH Consensus Conference. Diagnosis and treatment of early melanoma. *JAMA* 1992; **268**:1314–19.
33 Fadaki N, Li R, Parrett B *et al.* Is head and neck melanoma different from trunk and extremity melanomas with respect to sentinel lymph node status and clinical outcome? *Ann Surg Oncol* 2013; **20**:3089–97.
34 Jones EL, Jones TS, Pearlman NW *et al.* Long-term follow-up and survival of patients following a recurrence of melanoma after a negative sentinel lymph node biopsy result. *JAMA Surg* 2013; **148**:456–61.
35 Glass LF, Raziano RM, Clark GS *et al.* Rapid frozen section immunostaining of melanocytes by microphthalmia-associated transcription factor. *Am J Dermatopathol* 2010; **32**:319–25.