Sentinel lymph node biopsy in melanoma: The Oxford ten year clinical experience

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Summary Sentinel lymph node biopsy (SLNB) has become an established investigation for assessing microscopic nodal metastasis in melanoma. The American Joint Committee on Cancer (AJCC) incorporates the sentinel node status in its staging criteria for melanoma.

We present our clinical evaluation of performing SLNB in a single UK centre between 1998 and 2008. There were 697 patients with a mean age 53 years (range 13–92). We were able to surgically harvest at least one sentinel node in 694 patients of which 532 (76%) were negative. Of the 162 positive patients, 129 underwent further completion lymphadenectomy with 29 showing further pathologically positive nodes. At median follow up of 46 months, mortality from melanoma for SLN positive and negative patients was 32% and 4%, respectively. Disease recurrence was noted in 10% of the SLN negative group. Survival curves showed significant difference \( (p < 0.001) \) in outcomes for patients grouped by Breslow thickness. Postoperative complications were noted in 6% of patients. No life-threatening complications were noted.

Our results are comparable to other national and international studies. We await the outcomes of ongoing trials to assess the therapeutic value of SLNB for melanoma.

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Introduction

In the developed world the incidence of melanoma is increasing.\(^1\) In the UK, melanoma rates have risen more than any other cancer and now affects over 10,000 people every year.\(^2\) Melanoma now represents the most common cancer in young adults and the mainstay of treatment is surgery.\(^3\) Despite the heightened surgical interventions, the outcome from widespread disease remains poor and is reflected by the increases in morbidity and mortality.\(^4\) Recent evidence for patients with regional (stage III) and distant (stage IV) disease demonstrates a 5-year survival of 39% and 33%, respectively.\(^5\) Pathological features of the primary tumour such as Breslow thickness and ulceration form the basis of the staging of the disease. The American Joint Committee on Cancer (AJCC) classification for melanoma now also incorporates the status of the Sentinel Lymph Node (SLN) in the staging system.\(^6\)

Since its introduction in 1992, Sentinel Lymph Node Biopsy (SLNB) has become an established investigation in melanoma.\(^7\) The premise of this technique is based on the microscopic spread of tumour cells from a primary melanoma to a primary or sentinel node(s). Surgical removal and subsequent detailed pathological analysis of this node determines the presence of microscopic nodal metastases. This result influences further surgery such as Completion Lymph Node Dissection (CLND) as well as eligibility into adjuvant therapy protocols.

Worldwide there have been two foremost randomised controlled trials investigating the role of SLNB in melanoma.\(^8\) The authors have demonstrated results supporting the use of SLNB as an accurate, safe tool for investigating melanoma. Commentators have debated the influence of SLNB on long term patient survival and its therapeutic role.\(^9\) In the UK two major centres have published data regarding their experiences of SLNB.\(^10\) As the procedure gains popularity in the UK there is a need to clarify its role in managing melanoma and re-evaluate the clinical aspects of the technique to provide a better understanding for all involved.

We present our ten-year consecutive clinical experience of performing SLNB for melanoma. To date, it is the largest cohort of patients undergoing the technique in a single UK centre. Based on clinical and pathological features of the primary tumour we evaluated prognostic factors that might predict sentinel node positivity. To assess SLNB as a surgical technique we provide a comprehensive assessment of postoperative complications and challenges encountered while learning this technique. Based on the sentinel node result we evaluated the outcome of patient groups in respect of disease progression and mortality.

Patients and methods

Over a ten year period, 697 patients underwent SLNB for melanoma at the Oxford Radcliffe NHS Trust, Oxford, UK. We employed a standard technique for harvesting the sentinel node. This involved a combination of pre-operative lymphoscintigraphy (using technetium 99m sulphur colloid) and an on-table injection of blue dye. Our centre, like other European centres, use’s Patent Blue V dye (Laboratoire Guerbet, Aulnay-Sous-Bois, France). We inject between 0.5 and 1.0 ml intra-dermally of standard 2.5% Patent Blue V dye around the biopsy scar. Our choice of dye differs from that used in the United States which is Iso sulfan Blue (Tyco Healthcare, Bedford, MA, USA). The sentinel lymph node was identified intra-operatively using both a gamma probe and direct visualisation. The node was harvested using ligacips to seal the afferent and efferent lymphatics, and the radioactive count was measured ex vivo using the gamma probe. Echelon nodes were harvested if they had a count $\geq 10\%$ of the sentinel node regardless of whether they were blue or not.\(^11\) The background count of the lymph node basin was then measured to ensure that no further ‘hot’ nodes remain.

Patients were offered an SLNB based on initial pathological findings which included a primary tumour with Breslow thickness $\geq 1.0$ mm. SLNB was offered to patients with Breslow thickness $< 1.0$ mm in the presence of certain high risk pathological features such as Clarks level IV, V or ulceration. Patients who had clinical or radiological detectable distant disease or were not suitable for further therapy were not offered an SLNB. Patients were followed up in the outpatient setting one week postoperatively and then on a three monthly basis for the first three years. Thereafter follow up was six monthly for two years, in total completing five years of standard follow up. In certain cases, follow up consultations were shared by a range of doctors from plastic surgery, oncology and dermatology.

Patients were consented pre-operatively regarding the investigative nature of the SLNB, the potential benefits of the technique and made aware of subsequent surgical implications if a positive result was detected. Clinical data regarding the primary site and relevant pathological details were collected prospectively by means of a proforma. These data, in combination with pathological reports, clinical case notes and correspondence were incorporated on a specialist melanoma database and maintained prospectively. Statistical analysis was based on chi squared analysis, Kaplan–Meier survival curves and log rank analysis. These were performed using Stata software (Version 11.0, StataCorp LP, Texas, USA).

Pathological analysis of sentinel nodes involved an initial bisection of the node along its hilum enabling processing of all nodal tissue. An initial slide was stained with haematoxylin and eosin (H&E) and 10 further unstained slides were produced (discarding 20-30 micron of tissue between each section). All slides were examined histologically, if melanoma cells were detected then immunohistochemistry (S100, HMB-45 and MelanA) was applied to the slides for confirmation. If no tumour was evident from the initial slide then alternate slides were stained. If no melanoma cells were detected in this series then a further 10 close step sections were cut and stained as before. Our method for calculating the number of mitosis for primary tumours is based on identifying a hotspot area within a High Power Field (HPF). Our technique has changed over the ten year period and is now compliant with the current AJCC recommendations. We first identify a field that contains a mitosis or number of mitoses. The count is started here and in an adjacent non-overlapping field until one mm\(^2\) of tissue is covered.
Results

From June 1998 to December 2008, 354 males and 343 females underwent SLNB for melanoma in our centre. The mean age at diagnosis for males and females were 54 years (SD ± 15.9) and 68 years (SD ± 15.9), respectively.

In males the anatomical distribution of the primary melanoma was predominately the trunk (47%), followed by the upper limbs (19%), lower limbs (19%) and head and neck (14%). In females the most common anatomical site for primary tumours were the lower limbs (43%). Followed by upper limbs (27%), trunk (20%) and head and neck (10%). For both genders, the mean Breslow thickness of the primary melanoma was 2.44 mm (SD ± 1.92). The most common subtypes of primary melanoma were superficial spreading (41%) followed by nodular (29%).

In total 1303 sentinel lymph nodes were harvested from 710 nodal basins. The median number of sentinel lymph nodes harvested was two (range one to seven). Nodal basins included axilla (313 cases), groin (257 cases), neck (76 cases), bilateral axillae (31 cases), parotid (19 nodes), bilateral groins (six cases), popliteal fossa (five cases) epiglottic (two cases) and bilateral neck (one case).

Of the 694 patients in whom a sentinel node was indentified 162 (23%) were positive. From this group CLND was undertaken in 126 patients and of these, 29 patients had further pathologically positive lymph nodes ( occult disease) from the nodal basin pathology. The number of patients with positive and negative sentinel nodes based on the primary tumour factors are shown in Table 1. Chi squared analysis was undertaken to assess the prognostic impact of primary tumour factors on positive SLN result. There was a significant correlation (p < 0.001) between a positive SLN result and Breslow thickness and ulceration. Clinical factors such as male gender and lower limb anatomical site were also significantly associated with a positive result (p < 0.05).

Median follow up for all patients was 46 months (range 1–138 months). Disease progression was defined as local recurrence, regional recurrence or distant metastasis. Mortality was specific for melanoma. Survival curves for sentinel node positive and negative patients are shown in Figure 1. At median follow up survival probability for SLN negative and SLN positive patients was 98% and 68%, respectively, showing a significant difference (p < 0.001). Long term follow up of positive SLNB patients demonstrated disease progression in 40% and overall 32% died from melanoma (mean survival of 29 months). In contrast, of the 532 SLN negative patients only 21 (4%) died of melanoma. Long term follow up showed disease progression in 10% of the SLN negative group. In this group of 53 patients, the first site of recurrence noted was distant (19 patients), nodal (17 patients), local (11 patients) and in transit (6 patients). Of the 17 patients who presented with nodal recurrence all but one were in the same nodal basin sampled at the SLNB and therefore false negative results. In these 16 patients, all SLN pathology slides were reviewed and did not contradict the initial report. The median time for recurrence for this false negative group was 39 months (range 4–76 months). At maximum follow up of 110 months, 3 of these 16 patients, died from melanoma.

Survival curves for the entire group based on positive and negative SLN results stratified for primary Breslow thickness are shown in Figure 2. SLNB conducted on patients with less than 1 mm primary tumours were excluded from analysis due to the low numbers involved. The curves represent survival probability for each thickness group and show highly significant differences based on log rank analysis.

Twenty-six patients (3.8%), consisting of 15 females and 11 males, underwent an SLNB with a primary melanoma Breslow thickness of less than 1.0 mm. The median Breslow thickness of this subgroup was 0.83 mm (range 0.30 mm–0.95 mm). In this subgroup, 16 patients had a Clarks level of IV or V, two patients showed ulceration and eight patients had a mitotic rate of greater than 1 per mm². One out of these twenty-six patients was found to have a positive SLNB and underwent CLND of the left axilla, which showed no further disease. Pathological review of this patient’s primary melanoma demonstrated presence of regression, microsatellites and a mitotic rate of greater than 1 per mm². At five year follow up this patient had no evidence of disease progression.

In all but two patients (0.3%) a sentinel node was identified intra-operatively. In a further patient, at the time of SLNB, regional disease was evident and a clinical decision was made to perform a complete nodal dissection simultaneously. Of the remaining 694 patients, postoperative surgical complications were noted in 39 (5.6%) patients. These included seroma (20 patients), lymphoedema (seven patients), haematoma (five patients), wound breakdown (four patients), cellulitis (two patients) and adverse reaction to patent blue V dye (one patient). Of the 20 patients who had seromas, nine did not require any intervention, ten had percutaneous aspirations (mean volume drained 25 ml) in the outpatient department, which resolved the seroma. One patient had recalcitrant seroma and required operative intervention. Of the five patients with haematomas, three were managed conservatively and two underwent surgical drainage. Both cases of cellulitis resolved readily, with one patient requiring a five day course of oral antibiotics. Seven patients had signs of lymphoedema but this resolved in all cases and patients were asymptomatic at long term follow up. Four cases of wound dehiscence were managed by dressings. One patient was noted to have an adverse reaction, in the form of urticaria, following an intra-dermal injection of 0.5 ml of 2.5% Patent Blue V to her primary biopsy scar. The patient was haemodynamically stable and was treated with intravenous corticosteroid and antihistamines. The patient made a complete recovery and the procedure was completed. No anaphylactic reactions were noted in our study group.

Discussion

The use of SLNB in Oxford has increased over the last ten years, with 120 procedures being performed in 2008. To date, this paper presents the largest number of SLNB conducted in a single UK centre. Our patient demographics with mean age at diagnosis of 54 years (range 13–92 years) are consistent with findings in other centres. The anatomical
distribution of the primary melanoma affecting the trunk in males (47%) and lower limb in females (43%) confirms common disease patterns based on anatomical sites for gender.10 Of the 694 cases, that underwent a successful SLNB 23% yielded a positive result. This is comparable to other UK and international studies where the rates of detecting positive sentinel node ranged from 10% to 30%.9,11 This broad range has been attributed to variations in mean Breslow thickness, proportion of ulcerated tumours and the protocols implemented to conduct pathological analysis.12 Reported rates of positive SLNB from intermediate thickness primary tumours range between 15% and 20%.12 In our intermediate thickness group (Breslow >1.0 mm and <3.9 mm) the percentage of positive SLNB was 17%.

In our series 26 patients with a primary tumour Breslow thickness of less than 1 mm underwent SLNB. Conventionally, SLNB is offered to patients with primary tumours with Breslow thickness ≥1 mm. Recent guidelines refer to specific pathological features which should be considered when offering SLNB to patients with thin melanomas (less than 1 mm).4 Studies have shown that up to 16% of patients with thin melanomas will develop further disease progression.13 Of note 36 patients (22%) from the SLNB positive group did not undergo further completion surgery. Four patients declined further surgery after discussion of their sentinel node result. Eight patients had progressed to distant metastases. The remaining 24 patients were discussed at a skin cancer multi-disciplinary team meeting and

### Table 1: Numbers of patients with positive and negative sentinel lymph node biopsies based on associated disease factors.

<table>
<thead>
<tr>
<th>Factors</th>
<th>All Cases</th>
<th>SLN Positive</th>
<th>SLN Negative</th>
<th>P value</th>
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<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>351 (51)</td>
<td>93</td>
<td>258</td>
<td>p = 0.05</td>
</tr>
<tr>
<td>Female</td>
<td>343 (49)</td>
<td>69</td>
<td>274</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20 years</td>
<td>13 (2)</td>
<td>7</td>
<td>6</td>
<td>p = 0.46</td>
</tr>
<tr>
<td>20-39 years</td>
<td>136 (20)</td>
<td>34</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>40-59 years</td>
<td>275 (40)</td>
<td>62</td>
<td>213</td>
<td></td>
</tr>
<tr>
<td>60-79 years</td>
<td>246 (35)</td>
<td>53</td>
<td>193</td>
<td></td>
</tr>
<tr>
<td>&gt;79 years</td>
<td>24 (3)</td>
<td>6</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Primary tumour thickness</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>0-0.99 mm</td>
<td>26 (4)</td>
<td>1</td>
<td>25</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>1.0-1.99 mm</td>
<td>343 (49)</td>
<td>48</td>
<td>295</td>
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<td>2.0-3.99 mm</td>
<td>221 (32)</td>
<td>65</td>
<td>156</td>
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<td>≥ 4.0 mm</td>
<td>98 (14)</td>
<td>47</td>
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<td>Not obtainable</td>
<td>6 (1)</td>
<td>1</td>
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<td>Clark's level</td>
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<td>II</td>
<td>15 (2)</td>
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</tr>
<tr>
<td>III</td>
<td>152 (22)</td>
<td>30</td>
<td>122</td>
<td></td>
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<tr>
<td>IV</td>
<td>402 (58)</td>
<td>96</td>
<td>306</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>34 (5)</td>
<td>13</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Not obtainable</td>
<td>91 (13)</td>
<td>20</td>
<td>71</td>
<td></td>
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<tr>
<td>Ulceration</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>180 (26)</td>
<td>69</td>
<td>111</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>No</td>
<td>458 (66)</td>
<td>84</td>
<td>374</td>
<td></td>
</tr>
<tr>
<td>Not obtainable</td>
<td>56 (8)</td>
<td>13</td>
<td>43</td>
<td></td>
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<td>Anatomical site</td>
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<td></td>
</tr>
<tr>
<td>Head &amp; neck</td>
<td>81 (12)</td>
<td>13</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Trunk</td>
<td>235 (34)</td>
<td>61</td>
<td>174</td>
<td></td>
</tr>
<tr>
<td>Upper limb</td>
<td>157 (23)</td>
<td>23</td>
<td>134</td>
<td></td>
</tr>
<tr>
<td>Lower limb</td>
<td>216 (31)</td>
<td>63</td>
<td>153</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Other</td>
<td>5 (1)</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Percentages of total patient group are shown in brackets. Sentinel lymph node biopsy procedure was not completed in three patients and these are excluded from analysis. P values are based on chi squared analysis.

Figure 1 Kaplan-Meier survival curve for all patients with sentinel node positive and negative biopsies. Log rank analysis p < 0.001.
reviewed by medical oncology. Based on the evidence available at the time further surgery based on their pathological findings was not thought to be of benefit. The pathology of the sentinel node was reviewed for each patient and the focus of disease was noted to be less than 1 mm. At the time controversy existed as to the significance of microscopic nodal disease and its potential for disease progression and we were mindful of the morbidity associated with completion lymphadenectomy. In view of this and following discussion with these patients it was agreed not to proceed with completion surgery. Routine ultrasound surveillance was not offered to these patients. Some authorities have questioned the clinical significance of subcapsular microscopic melanoma deposits. Subsequent studies and the 2009 AJCC guidelines have addressed the issue of microscopic metastases and its effect on a patients disease stage. Currently we consider any microscopic metastases as clinically significant disease for the patient.

In our hands, the technique has not resulted in mortality or life-threatening complications. We were unable to identify a sentinel node intra-operatively in the axillae of two patients (0.3%). In these cases no signal was detectable nor was blue dye visible in the nodal basins and the procedure was not completed. Identifying a sentinel node is a technically challenging operation but the dual approach of pre-operative lymphoscintigraphy and intra-operative blue dye injection has enabled the surgeon to identify the nodal basin more accurately. This is particularly useful when the primary tumour is situated on the trunk and can have multiple nodal basins (groin and axilla). The intra-operative use of a hand held gamma probe allows the surgeon to identify a ‘hot’ node within that nodal basin, enabling an identification rate greater than 96%. Morton and colleagues state that SLNB requires a learning period and recommend a minimum of 55 cases to achieve a 95% accuracy rate in identifying the sentinel node. All surgeons in our centre, who have an interest in melanoma surgery, are compliant with these recommendations. Our study achieved a success rate of 99.7% of surgically harvesting a node. This should not be misinterpreted as the false negative rate. We defined false negative sentinel lymph node patients as those who developed disease in the sampled nodal basin. This definition is in keeping with other studies. There remains debate regarding the method of calculating the false negative rate. It can be underestimated by calculating the number of cases as a percentage of the total study population, which in our series would be 2%. A more accurate representation would be based on the methodology used by Van Akkooi et al.: False negative rate = [false negative patients/(false negative + true positive patients) × 100]. They used this method to calculate the false negative rate from 19 SLNB studies with a total of 18,532 patients and reported higher

Figure 2  Kaplan–Meier survival curves for patients with sentinel node positive and negative biopsies based on primary tumour thickness. Log rank analysis shown in brackets.
rates, ranging from 9 to 21%. Based on this calculation our false negative rate is 9% (16/178).

Sentinel lymph node biopsy was introduced as a minimally invasive procedure to provide important information regarding the regional spread of melanoma. It was initially regarded as a means of avoiding unnecessary elective completion lymph node dissections, which are associated with significant morbidity. Not all publications associated with sentinel node biopsy make reference to complications or morbidity. A previous study by the St George’s Melanoma Unit reported a total complication rate of 16.5% with seroma formation being most common. A study by Cuchet and colleagues evaluating 62 patients undergoing SLNB reported early complications in 8%, again with seroma occurring most commonly. The Multicentre Selective Lymphadenectomy Trial-I (MSLT-I) reported their morbidity associated with SLNB to be 10.1%. Reviewing the MSLT-I data nearly half of these complications were from seroma or haematoma, followed by infection (4.6%) and wound dehiscence (1.2%). The Sunbelt melanoma trial reported a complication rate of 4.6% associated with sentinel node biopsy, with no long-term consequences for the patient. Our overall complication rate of 5.6%, with seroma occurring most frequently reflects the low complication rate of this procedure.

Since its introduction SLNB has established itself as an investigation for melanoma. There remains controversy regarding its role in the treatment of melanoma. The MSLT-I designed by Morton and colleagues aimed to evaluate the benefit of sentinel lymph node mapping over wide local excision alone. The authors did report a significant, albeit small benefit, for disease free survival (DFS) for the SLN arm. Disagreement exists regarding the methods of calculating the DFS by the MSLT-I group and the prognostic false positive nature of the sentinel node. The subsequent MSLT-II aims to evaluate the therapeutic role of SLNB for melanoma. We await the results of this trial. In comparison to these multicentre trials our number of patients are modest. However our evaluation does reflect reported trends. The rate of positive microscopic nodal disease of 23% is similar to previous studies. At long term follow up, our melanoma mortality of SLN positive (32%) and negative (4%) patients is lower than that reported in the prospective observational study from Glasgow. Kettlewell and colleagues reported mortality for SLN positive and negative patients to be 44% and 8%, respectively. Our rate of disease recurrence (10%) for SLN negative patients was less than cited by the Glasgow group (15%) and by Gershenwald et al (13%).

The MSLT and other major trials have focused on the intermediate thickness melanoma group. Controversy exists regarding the role for SLNB in patients with thick (>4 mm) primary tumours. Of the centres in the UK that perform SLNB, not all offer this investigation to patients with thick melanomas. Our data demonstrates that approximately half of the patients with thick melanomas (51/98) have a negative SLN status; at median follow up (46 months) the survival probability for SLN positive and negative patients was 85% and 35% respectively (p < 0.001). Other institutions have also reported this significant difference in thick melanoma groups and found ulceration and increasing Breslow thickness to be related to outcome. In our series of 98 patients with thick primary tumours, ulceration was noted in 51% of SLN negative and 66% of SLN positive patients. Mean Breslow thickness for the SLN negative and positive patients were 5.9 mm and 6.5 mm respectively. Based on our small cohort of patients with thick melanomas, there is survival difference. This supports the use of SLNB in this subset to gain important prognostic information, which may influence further adjuvant therapy.

Sentinel lymph node sampling aims to provide an insight into microscopic melanoma metastasis based on the spread of disease through lymphatics. At present SLNB’s use is limited to that of an investigatory tool. Our clinical experience of performing this technique has enabled us better to quantify disease prognosis for our patients. For our surgeons the SLNB result now influences the decision for further lymph node basin dissection. The presence of nodal disease status enables patient stratification and informing eligibility for clinical adjuvant trials offered in our region. We recognise the value of good communication within the multi-disciplinary setting to improve accuracy of this procedure as SLNB is reliant upon specialist nuclear medicine and pathological support. For all stakeholders, melanoma remains an unpredictable cancer that requires tailored treatment for stratified patient groups. We await the results of ongoing long-term trials to determine if SLNB has therapeutic value in the combined treatment of melanoma.

Conflicts of interest

None.

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