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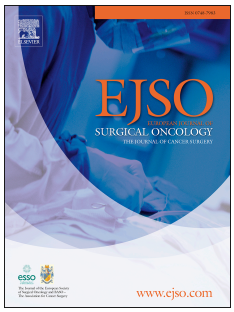

Survival Outcomes and Interval Between Lymphoscintigraphy and SLNB in Cutaneous Melanoma- Findings of a Large Prospective Cohort Study

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Survival Outcomes and Interval Between Lymphoscintigraphy and SLNB in Cutaneous Melanoma- Findings of a Large Prospective Cohort Study

Running head: Timing of lymphoscintigraphy in melanoma

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Abbreviations

AJCC	American Joint Committee on Cancer
CT	Computed Tomography
DSS	Disease Specific Survival
IRAS	Integrated Research Application System
MRI	Magnetic Resonance Imaging
NS	Not significant
OS	Overall Survival
PET CT	Positron Emission Tomography ComputedTomography
PFS	Progression Free Survival PFS
SD	Standard Deviation
SLNB	Sentinel lymph node biopsy
Tc99m	Technetium 99
WLE	Wide Local excision

Introduction: Sentinel lymph node biopsy (SLNB) in cutaneous melanoma (CM) is performed to identify patient at risk of regional and distant relapse. We hypothesized that timing of lymphoscintigraphy may influence the accuracy of SLNB and patient outcomes.

Methods: We reviewed prospective data on patients undergoing SLNB for CM at a large university cancer-center between 2008-2015, examining patient and tumor demographics and time between lymphoscintigraphy (LS) and SLNB. Kaplan-Meier survival analysis assessed disease-specific (DSS) and overall-survival (OS), stratified by timing of LS. Cox multivariate regression analysis assessed independent risk factors for survival.

Results: We identified 1015 patients. Median follow-up was 45 months (IQR 26-68 months). Univariate analysis showed a 6.8% absolute DSS (HR 1.6 [1.03-2.48], p= 0.04) benefit and a 10.7% absolute OS (HR 1.64 [1.13-2.38], p=0.01) benefit for patients whose SLNB was performed < 12 hrs of LS (n= 363) compared to those performed >12 hours (n=652). Multivariate analysis identified timing of LS as an independent predictor of OS (p=0.007) and DSS (p=0.016) when competing with age, sex, Breslow thickness (BT) and SLN status. No difference in nodal relapse rates (5.2% v 4.6%; p=0.67) was seen. Both groups were matched for age, sex, BT and SLN status.

Conclusion: These data have significant implications for SLNB services, suggesting delaying SLNB >12 hours after LS using a Tc99-labelled nanocolloid has a significant negative survival impact for patients and should be avoided. We

hypothesise that temporal tracer migration is the underlying cause and advocate further trials investigating alternative, 'stable' tracer-agents.

Introduction

Sentinel lymph node biopsy (SLNB) was established by Morton *et al.* [1] as a means of accurately locating and staging lymphatic fields draining a specific melanoma primary tumor site in patients with clinical stage I and II disease. This technique has evolved from surgical wide local excision and elective lymphadenectomy [2] in all patients to selective combined multidisciplinary-delivered pre-operative nodal basin identification by lymphoscintigraphy, and intra-operative dual-localization using radio-labelled tracers [3] and blue dye to specifically isolate the nodes for removal and histopathological staging, thereby reducing surgical morbidity in 80% of patients who would otherwise not develop regional nodal disease[4].

In keeping with this, radio-tracers have been developed with the goal of creating a stable, specific colloid that is taken up and retained by antigen-presenting cells within a lymph node without the potential to migrate proximally to higher second echelon nodes [5]. Technetium-99m labelled nanocolloid is the preferred agent in our unit, with lymphoscintigraphy undertaken on same day, or day before surgery, and early and delayed images taken to ensure accuracy of nodal identification and exclusion of higher echelon nodes. Studies by Kalady and Oldan et al [6,7] using Tc 99 labeled sulphur-colloid and blue dye demonstrated accurate sentinel node identification

without missing metastatic disease in patients injected up to 24 hours prior to surgery[6]. We observed a small but significant number of patients who presented during follow up with nodal or distant melanoma metastasis following negative SLNB. We hypothesized that timing of lymphoscintigraphy was potentially causing a negative impact on patients, and undertook a retrospective review of our prospective melanoma database, examining outcomes of all patients with melanoma post SLNB.

Methods

Patients

Patients with AJCC clinical stage I-IIC primary melanoma [8] were prospectively selected for SLNB following review of primary histology and radiological imaging results by the specialist multidisciplinary skin cancer team at a University Hospital tertiary referral center in East Anglia, United Kingdom. A retrospective review of prospectively collected computerized patient data from September 2009 until December 2015, specifically examining patients with a histologically confirmed diagnosis of melanoma was undertaken.

Sentinel Node Localization and Identification

Standardized intradermal injections of 20-40MBq Technetium 99 labelled nanocolloid (GE Healthcare, Milan, Italy) to the primary tumor site was performed in Nuclear Medicine, followed by early dynamic imaging at ten minutes and delayed planar imaging at sixty minutes to localize the sentinel node on the preceding day, or day of surgery. Surgery was performed by one of two authors (MDM and MJH). Intra-operatively, patent bleu (Guebert) injections to the primary tumor bed were undertaken and the sentinel node was identified by dual-localization, with intra-operative hand-held gamma probe to confirm accuracy of node removal via radiation counts >10% of the primary site and objective visualization of blue staining of the node and its afferent lymphatic channels. Incision time was recorded in addition to time taken to identification of node. Nodes were preserved in formalin and examined histologically using haematoxylin and eosin and with immunostaining for S100, Melan-A and HMB-45.

Follow up and identification of disease recurrence

Patients with a negative SLNB and no residual disease on wide local excision were followed up in a dedicated skin cancer clinic at three and six-monthly intervals. Patients with high risk melanoma (defined by Breslow thickness >4mm or SLN positivity) were offered whole body CT scans at 6 monthly intervals. Metastatic disease was investigated on basis of clinical history and examination, with ultrasound-guided biopsy used to histologically confirm nodal disease and cross-sectional imaging including MRI brain and whole-body PET-CT to identify distant metastatic disease. Histological confirmation was obtained where feasible to confirm disease-specific recurrence.

Statistical analysis

Statistical analysis using GraphPad Prism 7.0 included Chi squared tests to compare variables, Cox multivariate regression analysis to examine independent factors for outcomes, and Kaplan-Meier survival curves to review nodal recurrence, progression free survival (PFS), disease specific survival (DSS) and overall survival (OS).

Ethical approval

Ethical & HRA approval was obtained (IRAS Ref: 234471) for undertaking a retrospective review of prospectively gathered data from the melanoma database.

Results

Patient characteristics

1015 patients were identified for inclusion in the study. Demographic data and tumor characteristics are shown in Table 1. Mean age was 52 years (range: 18-86) and median Breslow thickness was 2.33mm (range: 0.5mm-24mm). The primary site was not recorded in 4 cases and ulceration status was missing in 45 cases. The overall sentinel node positivity rate was 17.0% (173 patients). Median follow up was 45 months (IQR 26-68 months). Groups were analysed according to early (<12 hours from lymphoscintigraphy to SLNB) versus late (>12 hours) lymphoscintigraphy. There was no significant difference between the cohorts in age, gender, Breslow thickness and sentinel node status.

Sentinel node identification

We observed an increased tendency towards late lymphoscintigraphy to SLNB in the second half of the study time- period. Dividing the study into two cohorts (one treated 2008-2011 and the other 2012-2015) showed a decrease in early lymphoscintigraphy rate from 40.8% to 31.6% (p=0.002 Chi squared test, Figure 1). Lymphoscintograms were reported by a radiologist on the same day, and nodal basin site and numbers of sentinel nodes or second echelon nodes information available to the operating surgeon. Lymphoscintigraphy failed to localize a sentinel node basin in two patients pre-operatively. Histology of tissue removed at surgery demonstrated fibrofatty tissue only. Neither of the patients had recurrence of disease in the follow up period of the study. The average number of lymph nodes identified at lymphoscintigraphy was 1.99, compared to numbers removed at surgery 2.36 (p<0.0001).

Length of time between lymphoscintigraphy and SLNB influences survival outcomes

Survival analysis was performed, stratifying by timing of Tc99m nanocolloid injections and lymphoscintigraphy to time of SLNB. We divided it into 6, 9, 12 and 18 hours from surgery. We observed a significant difference in overall survival (OS) and disease-specific survival (DSS), with a cut-off at 12 hours (Figure 2A+B). Univariate analysis demonstrated a 10.7% OS benefit at 96 months (Hazard ratio 1.64 [1.13-2.38], p=0.010) (Figure 2A) and a DSS benefit of 6.8% at 96 months (Figure 2B, Hazard ratio 1.60 [1.03-2.48], p=0.035) for patients who underwent lymphoscintigraphy less than 12 hours prior to SLNB compared to those who underwent it more than 12 hours afterwards. There was no significant difference between nodal relapse rates (p=0.67) as shown in Figure 2C or progression-free survival (p=0.16, data not shown). Subgroup analysis was performed to compare optimal timing of lymphoscintigraphy, dividing groups into patients undergoing lymphoscintigraphy <6 hours, <9 hours, <12 hours and >18 hours prior to SLNB. This showed a clear dichotomy in OS with threshold at patients treated under versus over 12 hours, p< 0.003 (Figure 3).

Lymphoscintigraphy timing is an independent predictor of OS and DSS

Cox Multivariate analysis confirmed timing of lymphoscintigraphy as an independent predictor of OS (p<0.007) and DSS (p<0.016) when competing with age, sex, Breslow thickness and sentinel node status. This data is shown in Table 4. Female gender, age and tumor-free sentinel node all demonstrated improved overall DSS and OS.

Discussion

Running a sentinel node service requires multidisciplinary coordination between nuclear medicine, the operating team and histopathology department. According to our local protocol, patients routinely spend up to 120 minutes undergoing early and delayed imaging in nuclear medicine prior to surgery. With access to nuclear medicine facilities limited to day-time working hours and performing of surgery on planned elective lists, undertaking lymphoscintigraphy the day prior to surgery confers several economic advantages, specifically coordination of pre-operative planning, dedicated SLNB operating sessions and having single operator performing the surgery, maintaining efficiency and consistency of the service. Other groups have investigated feasibility of delayed SLNB between 18-24 hours after injections in both breast [9,10] and melanoma [6,7,11,12] patients, and have reported this is a safe and acceptable means for node identification; with delayed SLNB resulting in reduction in background radioactivity and enhancing accuracy in node detection. Moreover, repeat imaging did not demonstrate migration of tracer to higher echelon nodes. None of the patients in the studies developed nodal recurrence, and concordance with blue dye of 69% was reported [11] On this basis, our unit policy has been to perform SLNB up to 24 hours after radio-tracer injections and lymphoscintigraphy, without repeating planar imaging on the day of surgery in the late group. We observed in our study that at surgery significantly more lymph nodes were removed compared to the numbers of nodes identified on imaging ($p<0.0001$), hence making the possibility of inadequate sampling of lymph nodes less likely.

There was a slight change in rate of early versus late sentinel node biopsies post radio-tracer injection over the study time frame. There was a higher proportion of patients undergoing delayed lymphoscintigraphy in the second cohort of the study. It is unlikely therefore that this

in itself has introduced a bias as it is likely that survival is improved in the second half of the study period.

Technetium 99m- Nanocolloid is eliminated primarily by the kidneys, and to a lesser extent by gastrointestinal tract, and has*in vivo* half-life of 32 hours, with particle size between 3-16nm [13]. From the findings of our study, we postulate, that the radio-tracer may have migrated more proximally to higher echelon nodes, hence remaining detectable at time of surgery using the gamma probe however not necessarily representing the true sentinel node. We were unable to directly examine the concordance of the blue dye staining of the sentinel node with the nanocolloid, which is a potential limitation of this study. Other groups [14] have reported that accuracy of blue dye alone in sentinel node identification is between 52-95%, while use of dye with radio-tracer allows node localization rates of 98-99%. In terms of identification of tumor positive nodes, radio-tracers have reported accuracy of 100% [15] compared to 80% by blue dye. Hu et al [16]report that blue dye does not improve tumor detection in SLNB and is not retained by the lymph nodes unlike the colloids.

Age was determined to be an independent risk factor in predicting DSS ($p< 0.0048$) and OS ($p<0.001$). Studies by Conway et al [17] and Chao et al [18] demonstrated that lymphatic function declined with age, potentially due to changes in the dermis, alteration in tissue turgor and changes in lymph nodes with fatty infiltration, with significant reduction in count rates with age >60. Use of concurrent blue dye at time of surgery did not affect the gamma counts in this patient population, reducing risk of volumes injected influencing the sentinel node uptake of tracers.

Nodal relapse rate between patients undergoing early versus late SLNB did not differ at follow up, with recurrence occurring in 19 patients (5.5%) in the early group (n=344) and 30 patients (4.8%) of late group (n=622, p=0.16 ns). Our findings concur with other studies examining false negative rates for SLNB, which have been reported as between 6-21% [19]. MSLT 1 [20] demonstrated removal of the sentinel node prolonged DSS for all patients with cutaneous melanoma, and in patients with AJCC stage 3 disease prolonged distant metastatic disease melanoma-specific survival. We observed in our patient population, that patients undergoing early SLNB following lymphoscintigraphy had improved overall and DSS compared to patients undergoing late SLNB, suggesting that in itself, removal of the sentinel node has an impact on overall prognosis.

Other groups [21] have reported that late recurrences tend to arise from melanomas on distal sites (i.e limbs), however in our study population, we did not observe any difference in terms of primary disease location and late recurrences, as seen in Table 1 our samples were evenly matched in terms of anatomical site distribution, so this is unlikely to account for the difference in survival.

New advances in radio-tracers have led to development of cell-receptor specific radiopharmaceutical. Tilmanocept is a Tc99m labelled radio-tracer with specific affinity for CD206 receptors expressed on antigen-presenting cells. Phase III trials demonstrated it to have high concordance in sentinel node identification in both melanoma and breast cancer patients when compared with blue dye [15,22]. In a recent trial of its use in SLNB for head and neck cancer, 6% of the study cohort (n=5) patients with cutaneous SCC were included and none were demonstrated to have nodal metastasis following SLNB and elective neck

dissection. [23,24] Advances in development of targeted receptor-specific radio-tracer could lead to improvements in accuracy of SLNB in patients with cutaneous melanoma.

International differences exist between radio-tracers used for the pre-operative identification of the SLN. In the United States micro-filtered Tc99 labelled Sulfur colloid is used with the majority of the particles measuring 0.2 microns or greater in diameter. In comparison, European centers use Tc99m nanocolloid (particle size <0.08 microns) and Australian centers employ Tc99m antimony tri-sulfide (particle size 0.003-0.03 microns)[25-27] thus particle sizes of the colloid particles routinely used in the US are significantly larger. Colloid migration to the nodal basin is determined by both lymphatic flow and particle size [28] Our study is based on the use of nanocolloid as a primary radio-tracer, other large studies examining feasibility of delayed lymphoscintigraphy were based on Tc99m Sulfur colloid. We suggest that the order of magnitude difference in particle sizes in the colloids between the studies is the major underlying reason for the discrepancy in the findings with regards to patient outcomes in delayed lymphoscintigraphy, whereby the difference in survival is produced by temporal migration of the tracer agent from the sentinel node to the non-sentinel node, resulting in a sampling error, with the incorrect node being harvested at SLNB. A proportion of these will be positive meaning they will be allowed to propagate metastases undetected, therefore negatively impacting on survival.

This current study is limited to data from a single center, therefore validation of data from other sites may help to improve our understanding of the observations made from our patient group.

Conclusion

To the best of our knowledge, this is the largest series to examine DSS and OS in melanoma patients undergoing early and delayed SLNB following Tc99 nanocolloid radio-tracer injections. We believe our data may have significant implications for SLNB services using this tracer agent. It suggests that delaying SLNB beyond 12 hours after lymphoscintigraphy using a Tc99-labelled nanocolloid has a significant and large negative survival impact for patients and should be avoided. We hypothesise that temporal tracer migration is the underlying cause and we advocate further trials investigating alternative, 'stable' tracer agents.

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FIGURE LEGENDS

Figure 1

Comparison of distribution of timing of lymphoscintigraphy by year

Patients were divided into two cohorts (2008-2011 and 2012-2015) and the proportion of patients undergoing lymphoscintigraphy early (<12 hours) was compared with late (>12 hours). The results show showed a decrease in early lymphoscintigraphy rate from 40.8% to 31.6% in the second cohort (Chi squared test p=0.002)

Figure 2

Comparison of OS, DSS and nodal relapse rates in early versus late treatment groups

2A Kaplan Meier survival curve, which shows that there is a reduction in OS of 10.6 % (p=0.01, HR 1.64 [1.13-2.38]) in patients undergoing SLNB > 12 hours post Tc-99m nanocolloid injections.

2B Kaplan Meier survival curve, which shows a significant reduction (p=0.04) in DSS in patients undergoing SLNB > 12 hours post Tc-99m nanocolloid injections.

2C Kaplan Meier survival curve which does not show any significant difference (p=0.67) in nodal relapse rates between early versus late treatment groups

Figure 3

Comparison of OS in treatment groups according to time course

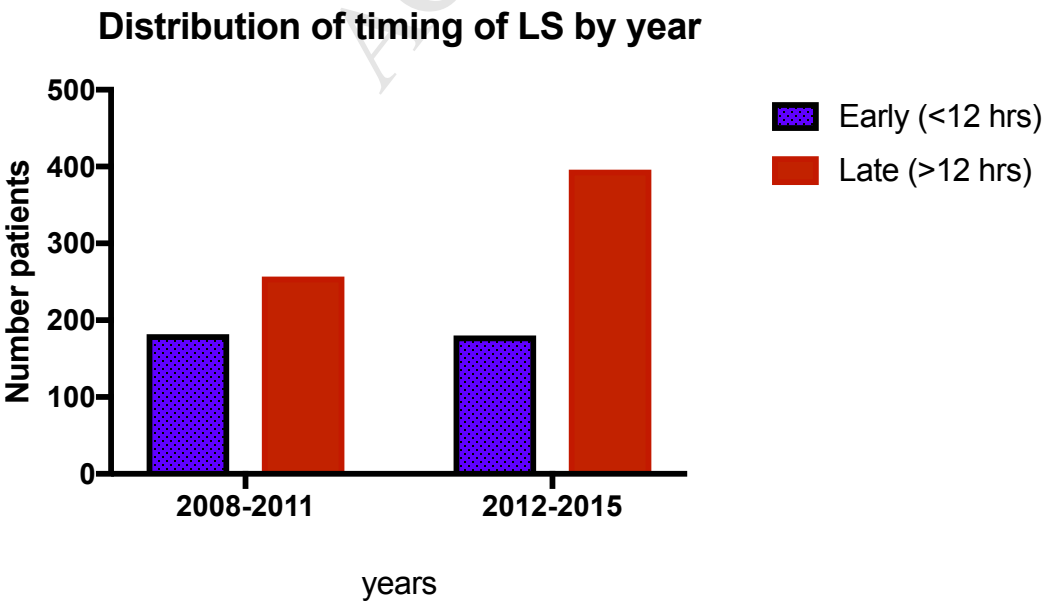
OS is compared between groups patients undergoing Tc99m-nanocolloid injections 6,9, 12 and 18 hours prior to sentinel lymph node biopsy using Kaplan Meier curves. The results show a significant difference (p<0.003) between patients undergoing early versus late lymphoscintigraphy, with the greatest difference observed at 12 hours

Table 1
Comparison of demographics and tumor characteristics between early (<12 hours) and late (>12 hours) post lymphoscintigraphy. There was no significant difference (ns) between groups for sex, age, tumor characteristics and sentinel node status

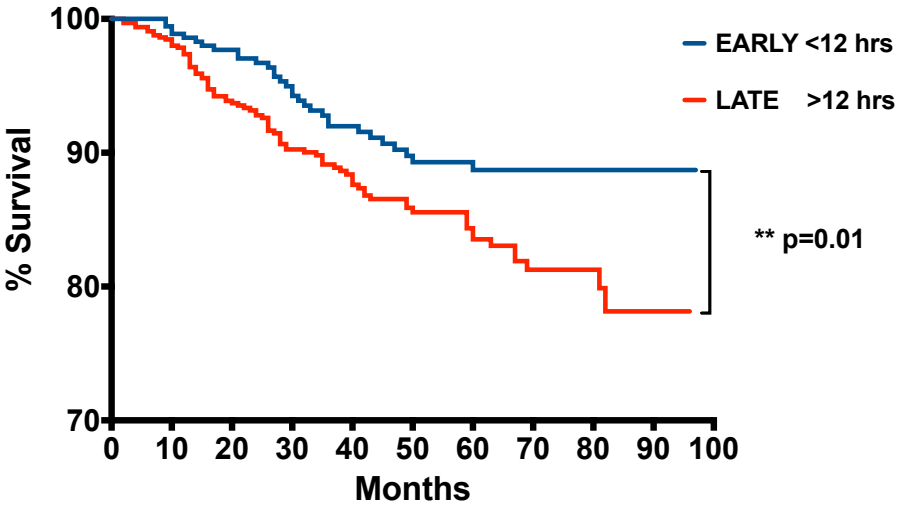
Patient and Tumor Demographics	**EARLY (%) n=362**	**LATE (%) n=653**	p
Male	176 (%)	345 (%)	ns
Female	186 (51.2%)	308 (47.2%)	
Age ± SD (years)	60.1 ± 13.6	60.6 ± 13.7	
Breslow thickness			
Median	1.8	1.7	ns
Mean	2.44 ±2.18	2.32±1.88	
Tumor site			
Limb	162 (44.8%)	292 (44.7%)	ns
Trunk	141 (38.9%)	264 (40.4%)	
Head and neck	57 (15.8%)	95 (14.6%)	
Not recorded	2 (0.6%)	2 (0.3%)	
Ulceration			
Present	73 (20.2%)	168 (25.7%)	
Absent	268 (74%)	461 (70.6%)	
Unknown	21 (5.8%)	24 (3.7%)	
Sentinel node			
Negative	296 (81.8%)	546 (83.6%)	ns
Positive	66 (18.2%)	107 (16.4%)	
Nodal relapses			
Number	19	30	ns
Incidences	5.25%	4.60%	

Table 2:
Cox Multivariate analysis of overall survival and disease specific survival examining independent risk factors. Each of timing of lymphoscintigraphy (early versus late), gender age, Breslow thickness and sentinel node status were independently associated with survival

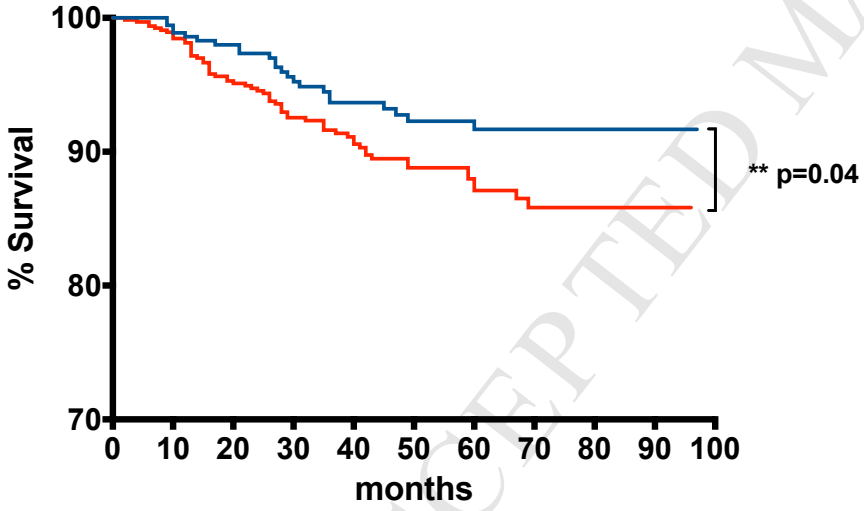
Cox Multivariate Analysis	Overall Survival		Disease Specific Survival	
	Risk Ratio	P	Risk Ratio	P
Timing of lymphoscintigraphy (EARLY VS LATE	1.78 [1.17-2.7]	**0.0067**	1.82 [1.12-2.96]	**0.0162**
Female Sex	0.654 [0.44-0.96]	**0.0302**	0.62 [0.39-0.96]	**0.0361**
Age	1.04 [1.02-1.06]	**<0.001**	1.02 [1.01-1.05]	**0.0048**
Breslow Thickness	1.13 [1.07-1.19]	**<0.001**	1.15 [1.09-1.22]	**<0.001**
Sentinel Node Status	3.01 [2.03-4.47]	**<0.001**	3.83 [2.44-6]	**<0.001**



2A: Overall Survival



2B. Disease-Specific Survival



2C. Nodal Relapse