Original Research

Timing of sentinel node biopsy independently predicts disease-free and overall survival in clinical stage I-II melanoma patients: A multicentre study of the Italian Melanoma Intergroup (IMI)

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Abstract *Background:* Sentinel lymph node biopsy (SNB) still remains a key procedure to appropriately stage melanoma patients and to select those who are candidate to novel treatments with immunotherapy and targeted therapy in the adjuvant setting. The impact of timing of SNB on disease-free survival (DFS) and overall survival (OS) is still unclear.

Material and methods: The study was conducted at 6 Italian Melanoma Intergroup (IMI) centres and included 8953 consecutive clinical stage I-II melanoma patients who were diagnosed, treated, and followed up between November 1997 and March 2018. All patients were prospectively included in dedicated IMI database. Multivariable Cox regression analyses were performed to investigate how baseline characteristics and time interval until SNB are related to DFS and OS.

Results: Considering the whole population, at multivariable analysis, after adjusting for age, gender, Breslow thickness, site, ulceration, and the SNB status, a delay in the timing of SNB was associated with a better DFS (adjusted hazard ratio [aHR, delayed versus early SNB] 0.98, 95% confidence interval [CI] 0.97–0.99, $p < 0.001$) and OS (aHR 0.98, 95% CI 0.97–0.99, $p = 0.001$). Specifically, in patients with a negative SNB status, a beneficial impact of delayed SNB (i.e. at least 32 days after primary excision) was confirmed for DFS (aHR 0.70, 95%CI 0.63–0.79, $p < 0.001$) and OS (aHR 0.69, 95%CI 0.61–0.78, $p < 0.001$), whereas in those with a positive SNB status, DFS (aHR 0.96, 95%CI 0.84–1.09, $p = 0.534$) and OS (aHR 0.94 95% CI 0.81–1.08, $p = 0.374$) were not significantly different in patients with early or delayed SNB.

Conclusions: Our study does not support a strict time interval for SNB. These results may be useful for national guidelines, for counselling patients and reducing the number of high urgency referrals.

1. Introduction

Primary cutaneous melanoma (PCM) accounts for only 4% of all skin cancers, but it causes the greatest number of skin cancer-related deaths worldwide [1]. As for other tumoural histotypes, it is important to appropriately predict PCM prognosis through reliable, validated prognostic biomarkers for patients' counseling, tailoring appropriate postoperative treatment, and stratification in prospective clinical trials [2].

The American Joint Committee on Cancer (AJCC) staging system is the most widely accepted and used approach to melanoma staging [3]. Patients with early, locoregional disease are classified into distinct stages based on Breslow thickness (BT), ulceration, and the sentinel lymph node (SN) status, which, in turn, includes the number of positive lymph nodes after completion lymph node dissection (CLND) in the case of a positive sentinel node biopsy (SNB).

Recently two clinical trials, the Multicenter Selective Lymphadenectomy Trial-II and the German Dermatologic Cooperative Oncology Group study (DeCOG-SLT) challenged the need to perform lymphadenectomy, because this procedure does not impact on outcome and is not informative for staging the vast majority of patients [4,5].

Nevertheless, SNB still remains a key procedure to appropriately stage patients and to select those who are candidate to novel treatments with immunotherapy and targeted therapy in the adjuvant setting [6]. As a consequence, it is likely that the number of performed SNB will increase, and the surgical waiting lists will lengthen.

Currently, there are conflicting data on the maximum allowable time interval between PCM resection and the subsequent wide local excision (WLE) and SNB. Several experts in the field advocate performing the SNB as soon as possible, but this inevitably negatively affects the routine surgical activity. The surgeon waiting lists are long particularly for the small surgical interventions, and this could potentially affect the way these interventions are performed. In universal health-care systems covered by the national healthcare insurance, the urgency to perform as soon as possible the SNB can potentially push towards privately executed procedures and introducing disparities.

The Italian Melanoma Intergroup (IMI) core centres have prospectively collected database with specific information on diagnosis, histopathological characteristics, timing of surgical procedures, and melanoma-specific outcome.

The aim of this study was to investigate if time interval between the PCM primary excision (PE) and SNB is associated with disease-free (DFS) and overall survival (OS), in the largest cohort of PCM patients so far reported.

2. Materials and methods

The approval to conduct the study was obtained from the local Ethical Committees of the participating centres. The study included consecutive patients with PCM diagnosed, treated, and followed-up prospectively in 6 IMI centres (Istituto Nazionale Tumori, Milan, Papa Giovanni XXIII Cancer Center, Bergamo, Dermatologic Clinic of the University of Florence, Veneto Institute of Oncology of Padua, Department of Dermatology of the University of Turin and Istituto Nazionale Tumori, Naples). Since before 1998, SNB was not routinely performed, and patients with PCM diagnosed before 1997 were not considered eligible. The clinical and pathological parameters extracted from the database included gender, date of birth, date of diagnosis of PCM, date of SNB, BT, ulceration, SN status, surgical procedures, systemic therapies, and follow-up, including date of relapse and death.

2.1. Surgical procedures

Diagnosis of the primary melanoma was based on the excisional biopsy and histopathological examination in all cases. Excisional biopsy was performed with total thickness excision and a narrow margin, according to the Italian guidelines (www.aiom.it).

In all IMI centres, SNB was performed according to international guidelines criteria. For patients operated up to 2009, according to AJCC staging 6th edition [7], SNB was performed in PCM with BT > 1.0 mm or in presence of risk factors as ulceration, Clark level IV or V, regression or mitosis >1/mm^2. For patients resected from 2009 up to 2013, SNB was considered, according to the AJCC staging 7th edition [3], in PCM patients with BT > 1.0 mm or in presence of risk factors such as ulceration, Clark level IV or V or mitosis >1/mm^2. For all patients, the WLE, with a margin of 1—2 cm depending on the BT, and the SNB were performed in the same setting. SNB was performed according to the triple technique and histopathological analysis of the SN was conducted according to the EORTC Melanoma Group Pathology Protocol [8]. In the event of SNB positivity, a CLND was performed according to the international guidelines before the publication of MSLT2 and DeCOG-SLT trials [4,5].

2.2. Statistical methods

DFS was defined as the time between SNB and disease relapse or death from any cause. OS was defined as the time interval between SNB and death from any cause. Patients who had not relapsed/died or died were censored at the date of the last follow-up visit. Continuous variables were described using mean and standard deviation (SD), the median with the first and third quartile (Q1—Q3; interquartile range, IQR) and minimum and maximum values, whereas categorical variables were described using frequencies and percentages. Chi-square test (or Fisher's exact test as appropriate) and t-test (or analysis of variance as appropriate) were performed to compare the distributions of categorical and continuous variable, respectively. SNB timing was defined as the time between PE and SNB. According to the routine activity in IMI centres, patients who underwent SNB before 1998 or more than 4 months after the PE were excluded from the analysis. SNB timing was analysed according three modalities: as continuous variable accounting for a weekly increase, as categorical variable defined according to the number of months from surgery and as dichotomous variable according to the best cut-off discriminating the patients based on DFS, identified by a CART analysis. The effect of the SNB timing on DFS and OS was explored by Cox proportional hazard models, stratified by centre, and adjusted for the demographical and clinical prognostic characteristics. Results of the analysis were expressed as hazard ratios (HRs), adjusted HRs (aHRs) and 95% confidence intervals (95%CIs). The proportionality of hazards (PH) was assessed by means of the Kolmogorov-type supremum test and evaluating the statistical significance of the interaction of each covariate with time. In case of evidence of no PH for one or more variables, Cox model including also the interaction with time of these variables was developed, and HRs at 6 months, 1 and 5 years were provided.

Moreover, a sensitivity analysis according to the propensity score (PS) approach was performed. The PS was defined for each patient as the probability to undergo a delayed SNB (after the 32nd day from PE) given a set of observed characteristics (age, gender, BT, site of PCM and ulceration), which could have affected the decision of SNB timing. The estimate of PS was obtained by means of a logistic model having SNB timing as dependent variable. The Cox models exploring the SNB timing were adjusted for the PS and for the SN status.

Survival curves were estimated with the Kaplan—Meier (KM) method and compared using the log-rank test.

Statistical significance was set at P < 0.05 for a bilateral test. Analysis was carried out using the SAS (Statistical Analysis System, SAS Institute, version 9.4) software and the R (The CRAN Project, Version 3.6.1) software.

3. Results

Between January 1997 and March 2018, 12,112 consecutive patients with PCM were diagnosed in six IMI centres. Among them, 8953 patients were eligible for this analysis. eFigure S1 summarises the flow diagram of the study.

Table 1
Demographics and clinical characteristics according to best cut-off of SNB timing.

Variable	Early SNB N = 2706	Delayed SNB N = 6247	Overall N = 8953	p-value
Age at PE				0.2117
Mean (SD)	54.1 (15.9)	54.6 (15.5)	54.4 (15.6)	
Median (Q1−Q3)	54.9 (41.7−66.6)	55.1 (42.7−67.0)	55.0 (42.5−66.9)	
Min − Max	0.4−93.0	0.0−93.3	0.0−93.3	
Missing	0	1	1	
Woman	1234 (45.6)	2925 (46.8)	4159 (46.5)	0.2878
Site				<0.0001
Trunk	1179 (43.6)	3035 (48.6)	4214 (47.1)	
Lower limb	951 (35.2)	1682 (26.9)	2633 (29.4)	
Upper limb	312 (11.5)	795 (12.7)	1107 (12.4)	
Head/neck	217 (8.0)	680 (10.9)	897 (10.0)	
Other	46 (1.7)	51 (0.8)	97 (1.1)	
Missing	1	4	5	
Breslow thickness				<0.0001
Mean (SD)	2.8 (2.7)	2.4 (2.6)	2.5 (2.6)	
Median (Q1−Q3)	2.0 (1.1−3.5)	1.6 (1.0−3.0)	1.7 (1.0−3.2)	
Min − Max	0.0−43.0	0.0−65.0	0.0−65.0	
Missing	3	3	6	
T stage				<0.0001
T1	548 (20.3)	1665 (26.7)	2213 (24.7)	
T2	867 (32.1)	2138 (34.2)	3005 (33.6)	
T3	749 (27.7)	1522 (24.4)	2271 (25.4)	
T4	539 (19.9)	919 (14.7)	1458 (16.3)	
Missing	3	3	6	
Ulceration	994 (39.8)	1822 (30.7)	2816 (33.4)	<0.0001
Missing	206	321	527	
SNB timing				−
Mean (SD)	15.6 (11.7)	62.1 (20.6)	48.1 (28.2)	
Median (Q1−Q3)	19.0 (0.0−27.0)	60.0 (45.0−76.0)	47.0 (28.0−68.0)	
Min − Max	0.0−31.0	32.0−121.0	0.0−121.0	
SNB timing − monthly categorisation				−
One month from PE	2557 (94.5)	0 (0.0)	2557 (28.6)	
Two months from PE	149 (5.5)	3206 (51.3)	3355 (37.5)	
Three/four months from PE	0 (0.0)	3041 (48.7)	3041 (34.0)	
SN status				<0.0001*
Negative	1875 (69.3)	4732 (75.7)	6607 (73.8)	
Positive	826 (30.5)	1503 (24.1)	2329 (26.0)	
Not found	4 (0.1)	12 (0.2)	16 (0.2)	
Missing	1	0	1	
CLND in positive SN patients				0.0245
Yes	790 (97.8)	1414 (96.0)	2204	
No	18 (2.2)	59 (4.0)	77	
Missing	18	30	48	

Early SNB, sentinel node biopsy performed within 31 days of primary excision; Delayed SNB, sentinel node biopsy performed at least 31 days after the primary excision; SNB, sentinel node biopsy; SN, sentinel node; PE, primary excision; CLND, complete lymph node dissection; SD, standard deviation; Q1−Q3, first quartile−third quartile.
 * Comparison performed excluding patients with sentinel node not found.

A comparison among centres in terms of demographic and clinical characteristics at diagnosis is reported in eTable 1. The mean timing of SNB ranged from 22.6 days (SD 16.8) to 53.4 days (SD 29.1). Table 1 shows the demographic and clinical characteristics according to the best cut-off of SNB timing identified by the CART analysis (i.e. 31 days). Overall, 2706 (30.2%) and 6247 (69.8%) patients underwent SNB within (early SNB) or after (delayed SNB) 31 days from the PE, respectively. The mean SNB timing was 15.6 days (SD 11.7) in the early SNB group and 62.1 days (SD 20.6) in the delayed SNB group. The proportion of patients with a positive SN was significantly higher in the early SNB group (30.5% and 24.1% for early and delayed SNB respectively, $p < 0.0001$).

The median follow-up was 95.9 months (IQR 52.4−132.1). In the early SNB group, 788 (29.1%) patients relapsed, 854 (31.6%) died and 1045 (38.6%) relapsed or died without relapse (i.e. DFS events). In the delayed SNB group, 1096 (17.5%) patients relapsed,

Table 2
Effect of the SNB timing on relapse free survival. Univariate and multivariable Cox proportional hazard model stratified by centre.

Variable	Univariate models		Multivariable model 1		Multivariable model 2		Multivariable model 3	
	HR [95%CI]	p-value	HR [95%CI]	p-value	HR [95%CI]	p-value	HR [95%CI]	p-value
SNB timing (1 week increase)	0.95 [0.94–0.96]	<0.0001	0.98 [0.97–0.99]	<0.0001	–	–	–	–
SNB timing* (ref. Month 1 after PE)								
Month 2 after PE	0.74 [0.68–0.81]	<0.0001	–	–	0.83 [0.75–0.91]	0.0001	–	–
Month 3–4 after PE	0.68 [0.61–0.75]	<0.0001	–	–	0.82 [0.74–0.92]	0.0004	–	–
SNB timing > 31 days	0.70 [0.64–0.76]	<0.0001	–	–	–	–	0.81 [0.74–0.88]	<0.0001
Breslow thickness (1 mm increase)	1.08 [1.08–1.09]	<0.0001	1.06 [1.05–1.07]	<0.0001	1.06 [1.05–1.07]	<0.0001	1.06 [1.05–1.07]	<0.0001
Age at surgery* (1 year increase)	1.03 [1.03–1.03]	<0.0001	1.03 [1.02–1.03]	<0.0001	1.03 [1.02–1.03]	<0.0001	1.03 [1.02–1.03]	<0.0001
Female sex	0.72 [0.66–0.78]	<0.0001	0.84 [0.77–0.91]	<0.0001	0.84 [0.77–0.91]	<0.0001	0.84 [0.77–0.91]	<0.0001
Site* (ref. Trunk)								
Lower limb	1.02 [0.93–1.12]	0.6131	0.98 [0.89–1.08]	0.6684	0.98 [0.89–1.09]	0.7338	0.98 [0.89–1.08]	0.6871
Upper limb	0.89 [0.78–1.02]	0.0833	0.86 [0.75–0.99]	0.0389	0.87 [0.76–1.00]	0.0468	0.87 [0.76–1.00]	0.0463
Head/neck	1.63 [1.44–1.84]	<0.0001	1.60 [1.41–1.81]	<0.0001	1.60 [1.41–1.82]	<0.0001	1.61 [1.41–1.82]	<0.0001
Other	3.41 [2.56–4.55]	<0.0001	1.88 [1.40–2.54]	<0.0001	1.88 [1.40–2.54]	<0.0001	1.87 [1.39–2.53]	<0.0001
Ulceration*	2.97 [2.73–3.22]	<0.0001	2.05 [1.88–2.23]	<0.0001	2.06 [1.90–2.25]	<0.0001	2.06 [1.89–2.24]	<0.0001
SNB outcome* (ref. Absent)								
Positive	2.57 [2.37–2.78]	<0.0001	2.14 [1.96–2.33]	<0.0001	2.15 [1.97–2.34]	<0.0001	2.14 [1.96–2.34]	<0.0001
Not found	1.83 [0.82–4.08]	0.1403	1.17 [0.44–3.13]	0.7532	1.18 [0.44–3.16]	0.7390	1.19 [0.44–3.17]	0.7349

SNB, sentinel node biopsy; PE, primary excision; CI, confidence interval; HR, hazards ratio.
Effect of 1 week increase of SNB timing adjusted for also the interactions with time of the variables with * is HR [1 week increase] 0.98 [95%CI 0.97–0.99], p = 0.0001.
Effect of SNB timing >31 days adjusted for also the interactions with time of the variables with * is HR 0.81 [95%CI 0.75–0.89], p < 0.0001.
  * Evidences of non-proportionality of hazards.

1198 (19.2%) died and 1553 (24.9%) relapsed or died without relapse. Tables 2 and 3 report the univariable and multivariable analyses on DFS and OS, respectively. For both end-points, evidence of no PH was found for age, site of PCM, ulceration and SN status. Moreover, no PH was detected for BT in the analysis on DFS and for SNB timing with monthly categorisation in the analysis on OS. At multivariable analysis, after adjusting for age, gender, BT, site, ulceration and the SN status, a delay in the timing of SNB was associated with a better DFS (aHR [1 week increase] 0.98, 95% confidence interval [CI] 0.97–0.99, p < 0.0001) and OS (aHR [1 week increase] 0.98, 95%CI 0.97–0.99, p = 0.0006). Similar results were observed after adjusting for the interaction with time of variables with no PH.

A positive impact on DFS was found in patients who underwent SNB at the second or third/fourth month after the PE (aHR [second versus first month] 0.83 95% CI 0.75–0.91, p = 0.0001; aHR [third/fourth versus first month] 0.82 95%CI 0.74–0.92, p = 0.0004). Most importantly, a longer OS was observed in patients who underwent SNB at the second and third/fourth month after the PE (aHR [second versus first month] 0.76 95% CI 0.69–0.85, p < 0.0001; aHR [third/fourth versus first month] 0.85 95%CI 0.75–0.95, p = 0.0062). Similar

results on OS were observed after adjusting for the interaction with time of variables with no PH. Fig. 1A and B shows the KM curves according to the SNB timing for DFS and OS, respectively.

Considering the cut-off defined according to the CART analysis, a SNB performed at least 32 days after the PE was associated with both a better DSF (aHR 0.81, 95%CI 0.74–0.88, p < 0.0001) and OS (aHR 0.78, 95%CI 0.71–0.85, p < 0.0001). Similar results were observed after adjusting for the interaction with time of variables with no PH or adjusting for the propensity score (DFS: aHR [delayed versus early SNB] 0.81, 95% CI 0.75–0.88, p < 0.0001; OS: aHR [delayed versus early SNB] 0.80, 95%CI 0.73–0.88, p < 0.0001). Fig. 1C and D show the KM curves for DFS and OS according to the cut-off determined by CART analysis.

eTables 2 and 3 report the HRs at 6 months, 1 and 5 years of variables with evidence of no PH. Although evidence of no PH, the variations of the HRs at 6 months, 1 and 5 years seem to be negligible.

Given the above results, we performed a subgroup analysis in patients with negative and positive SN, respectively. Fig. 2 summarises the multivariable Cox analysis according to this subgroup analysis.

In patients with a negative SN status, a beneficial impact of delayed SNB (i.e. at least 32 days after PE)

Table 3
Effect of the time to SNB on overall survival. Univariate and multivariable Cox proportional hazard model stratified by centre.

Variable	Univariate models		Multivariable model 1		Multivariable model 2		Multivariable model 3	
	HR [95%CI]	p-value	HR [95%CI]	p-value	HR [95%CI]	p-value	HR [95%CI]	p-value
SNB timing (1 week increase)	0.96 [0.95–0.97]	<0.0001	0.98 [0.97–0.99]	0.0006	–	–	–	–
SNB timing[*] (ref. Month 1 after PE)								
Month 2 after PE	0.73 [0.66–0.81]	<0.0001	–	–	0.76 [0.69–0.85]	<0.0001	–	–
Month 3–4 after PE	0.74 [0.66–0.83]	<0.0001	–	–	0.85 [0.75–0.95]	0.0062	–	–
SNB timing > 31 days	0.72 [0.65–0.78]	<0.0001	–	–	–	–	0.78 [0.71–0.85]	<0.0001
Breslow thickness[*] (1 mm increase)	1.09 [1.09–1.10]	<0.0001	1.07 [1.06–1.08]	<0.0001	1.07 [1.06–1.08]	<0.0001	1.07 [1.06–1.08]	<0.0001
Age at surgery[*] (1 year increase)	1.04 [1.04–1.04]	<0.0001	1.04 [1.03–1.04]	<0.0001	1.04 [1.03–1.04]	<0.0001	1.04 [1.03–1.04]	<0.0001
Female sex	0.63 [0.58–0.69]	<0.0001	0.77 [0.70–0.85]	<0.0001	0.78 [0.70–0.86]	<0.0001	0.78 [0.71–0.86]	<0.0001
Site[*] (ref. Trunk)								
Lower limb	0.88 [0.80–0.98]	0.0202	0.85 [0.75–0.95]	0.0038	0.85 [0.76–0.95]	0.0037	0.84 [0.75–0.94]	0.0025
Upper limb	0.88 [0.76–1.02]	0.1004	0.84 [0.72–0.98]	0.0290	0.85 [0.73–0.99]	0.0373	0.85 [0.72–0.99]	0.0322
Head/neck	1.45 [1.27–1.67]	<0.0001	1.36 [1.18–1.57]	<0.0001	1.36 [1.18–1.58]	<0.0001	1.37 [1.18–1.58]	<0.0001
Other	3.31 [2.43–4.51]	<0.0001	1.83 [1.33–2.52]	0.0002	1.83 [1.33–2.52]	0.0002	1.81 [1.32–2.50]	0.0003
Ulceration[*]	3.12 [2.85–3.42]	<0.0001	2.16 [1.96–2.38]	<0.0001	2.18 [1.98–2.40]	<0.0001	2.17 [1.97–2.38]	<0.0001
SNB outcome[*] (ref. Absent)								
Positive	2.51 [2.30–2.75]	<0.0001	2.03 [1.84–2.24]	<0.0001	2.07 [1.87–2.28]	<0.0001	2.03 [1.84–2.24]	<0.0001
Not found	1.61 [0.60–4.30]	0.3432	0.67 [0.17–2.69]	0.5731	0.67 [0.17–2.67]	0.5655	0.66 [0.16–2.66]	0.5604

SNB, sentinel node biopsy; PE, primary excision; CI, confidence interval; HR, hazards ratio.
Effect of 1 week increase of SNB timing adjusted for also the interactions with time of the variables with * is HR [1 week increase] 0.98 [95%CI 0.97–0.99], p = 0.0016.
Effect of doing the SLNB during the second month from PE adjusted for also the interactions with time of the variables with * is HR 0.77 [95%CI 0.69–0.86], p < 0.0001.
Effect of doing the SLNB during the third/fourth month from PE adjusted for also the interactions with time of the variables with * is HR 0.86 [95%CI 0.76–0.97], p = 0.0125.
Effect of SNB timing >31 days adjusted for also the interactions with time of the variables with * is HR 0.79 [95%CI 0.72–0.86], p < 0.0001.
 * Evidences of non-proportionality of hazards.

was confirmed for DFS (aHR 0.70 95%CI 0.63–0.79, p < 0.0001) and OS (aHR 0.69 95%CI 0.61–0.78, p < 0.0001). In patients with a positive SN status, DFS (aHR [delayed versus early SNB] 0.96 95%CI 0.84–1.09, p = 0.5339) and OS (aHR [delayed versus early SNB] 0.94 95%CI 0.81–1.08, p = 0.3738) were not significantly different between patients with early or delayed SNB.

4. Discussion

The most striking result of our study is that the interval between excision of a PCM and the SNB could have a prognostic impact in patients with a negative SN, being DFS and OS worse in patients who undergo early SNB, whereas no effect was found in patients with positive SN. In patients with negative SN, the delayed SNB procedure was associated with a 30% risk reduction of recurrence and/or death.

The results of our study could have some important clinical implications. From a clinical standpoint, our results do not support a strict time interval for WLE and SNB, and this notion could be important for national guidelines and to counsel patients and reduce the number of high urgency referrals.

In our series, a higher proportion of positive SN was found in early versus delayed SNB subgroup of patients (30.5% versus 24.1%). Moreover, patients who underwent an early SNB had a higher median BT (2.0 mm versus 1.6 mm) and more ulcerated melanomas (39.8% versus 30.7%). A positive SN was associated to well-known unfavourable prognostic factors, and we cannot exclude that physicians may have selected patients to get an early SNB owing to negative prognostic factors.

The results of the present study should be considered in the context of the current literature. To date, the impact of a longer time interval until SNB on DFS and OS has been reported in 11 studies [9–19], which included patients with negative and/or positive SN (Table 4). The results so far reported are conflicting because of heterogeneity in patients' characteristics,
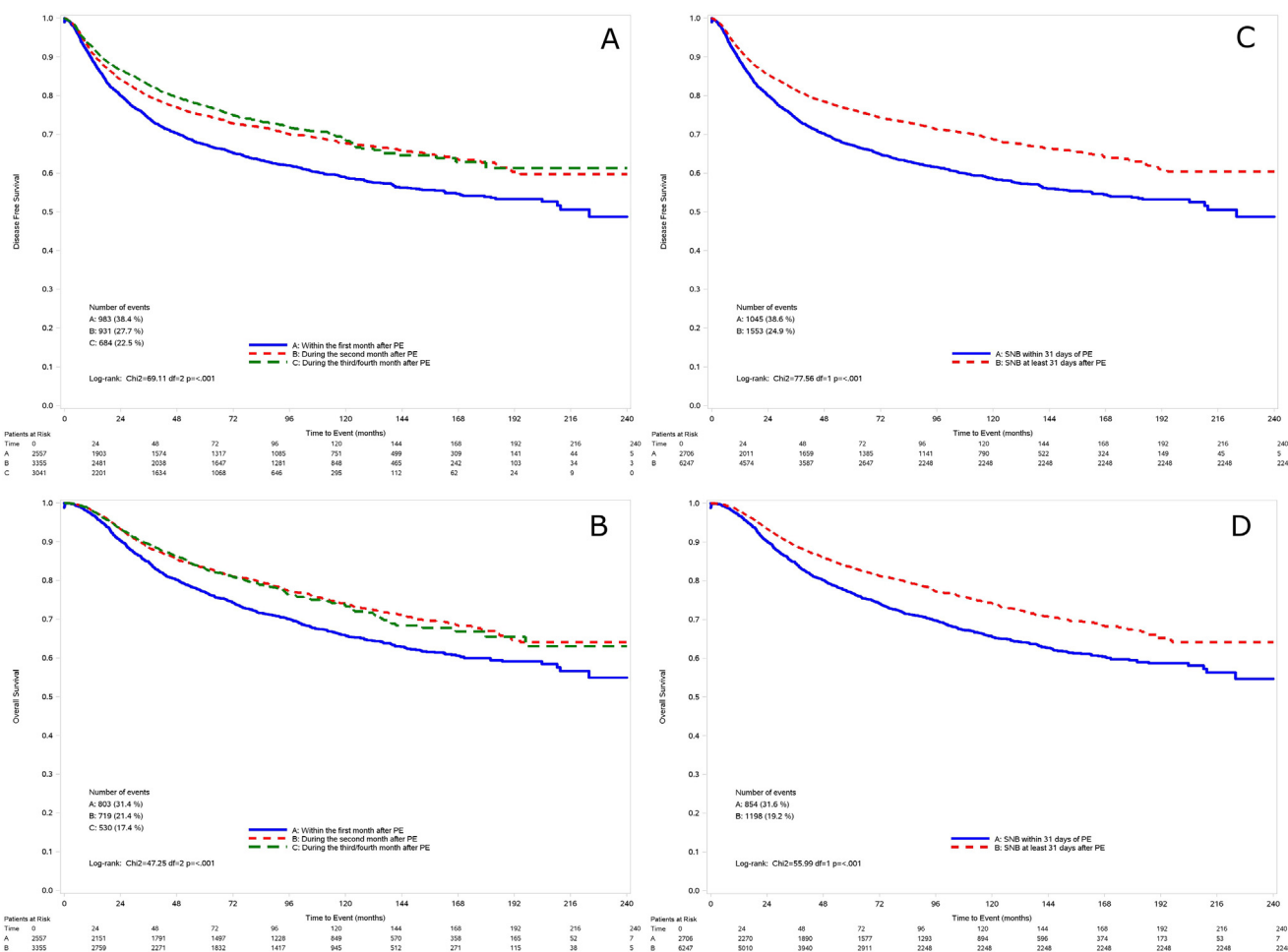
Fig. 1. Kaplan-Meier curves showing disease-free survival and overall survival according to SNB timing categorised monthly (A and B) and according to CART analysis (C and D). SNB, sentinel node biopsy; PE, primary excision.

number of patients included according to the SN status, the time interval to SNB which varies from 7 to 59 days and finally, the median follow-up.

With regards to SN-positive patients, while Fortes *et al.* found a benefit of early SNB [9], three other large studies did not [12,13,18]. Specifically, Tejera-Vaquerizo [13] reported that interval to SNB had no effects on survival in a SN-positive cohort of 464 patients. Similarly, in two large, well-conducted studies by the EORTC melanoma group, including 1015 and 705 patients, respectively, the interval between primary melanoma excision and SNB was not associated with survival in SN-positive patients. Our study confirms these findings [12,18].

With regards to SN-negative cohorts, the results are still conflicting. In seven studies, the interval between primary melanoma excision and SNB was not associated with DFS and/or OS [9−12,14,17,19]. More recently, Tejera-Vaquerizo *et al.* [13], in a retrospective study including 1498 SN-negative patients, did find a

detrimental effect of a short time interval on OS. Our study included 6607 SN-negative patients, and again a strong effect of time interval was found in this relatively low risk melanoma population.

Melanoma is an immunogenic cancer. Melanoma cells display multiple antigens and peptide epitopes that are targetable by the host immune system, and several immunotherapy strategies have been developed in the adjuvant and metastatic setting in the last decade.

Induction of a specific, clonal antitumour T-cell response depends on the priming of specific naïve T cells by antigen presenting cells in the draining lymph nodes [20]. When a specific antigen is presented by antigen presenting cells, the naïve T cells are activated [21]. Priming of helper and cytotoxic antitumour T cells seems to take place in the SN and potentially is associated with an antitumour T-cell response in melanoma.

Nevertheless, several steps are required for an efficient immune response including the transport, processing and presentation of melanoma antigens in the
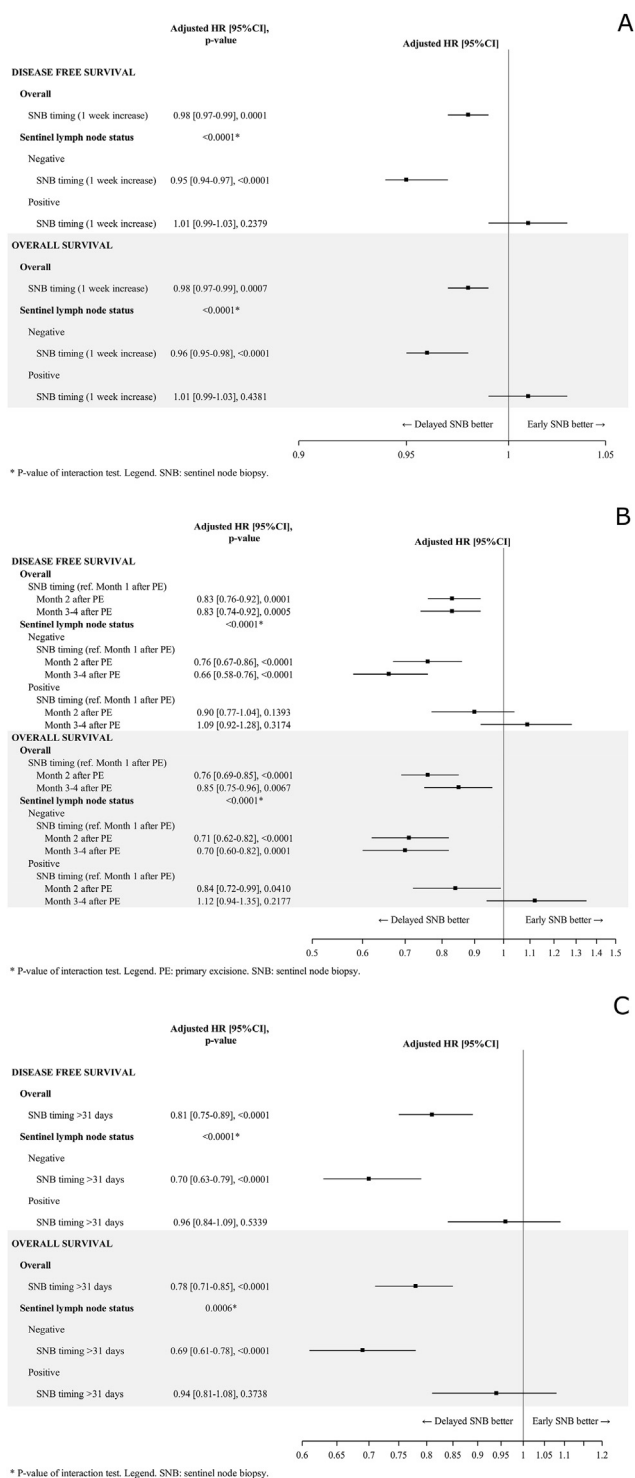
A

Adjusted HR [95%CI], p-value | **Adjusted HR [95%CI]**

DISEASE FREE SURVIVAL
Overall
  SNB timing (1 week increase) 0.98 [0.97-0.99], 0.0001
Sentinel lymph node status <0.0001*
  Negative
    SNB timing (1 week increase) 0.95 [0.94-0.97], <0.0001
  Positive
    SNB timing (1 week increase) 1.01 [0.99-1.03], 0.2379
OVERALL SURVIVAL
Overall
  SNB timing (1 week increase) 0.98 [0.97-0.99], 0.0007
Sentinel lymph node status <0.0001*
  Negative
    SNB timing (1 week increase) 0.96 [0.95-0.98], <0.0001
  Positive
    SNB timing (1 week increase) 1.01 [0.99-1.03], 0.4381

← Delayed SNB better   Early SNB better →
0.9   0.95   1   1.05

* P-value of interaction test. Legend. SNB: sentinel node biopsy.

B

Adjusted HR [95%CI], p-value | **Adjusted HR [95%CI]**

DISEASE FREE SURVIVAL
Overall
  SNB timing (ref. Month 1 after PE)
    Month 2 after PE 0.83 [0.76-0.92], 0.0001
    Month 3-4 after PE 0.83 [0.74-0.92], 0.0005
Sentinel lymph node status <0.0001*
  Negative
    SNB timing (ref. Month 1 after PE)
      Month 2 after PE 0.76 [0.67-0.86], <0.0001
      Month 3-4 after PE 0.66 [0.58-0.76], <0.0001
  Positive
    SNB timing (ref. Month 1 after PE)
      Month 2 after PE 0.90 [0.77-1.04], 0.1393
      Month 3-4 after PE 1.09 [0.92-1.28], 0.3174
OVERALL SURVIVAL
Overall
  SNB timing (ref. Month 1 after PE)
    Month 2 after PE 0.76 [0.69-0.85], <0.0001
    Month 3-4 after PE 0.85 [0.75-0.96], 0.0067
Sentinel lymph node status <0.0001*
  Negative
    SNB timing (ref. Month 1 after PE)
      Month 2 after PE 0.71 [0.62-0.82], <0.0001
      Month 3-4 after PE 0.70 [0.60-0.82], 0.0001
  Positive
    SNB timing (ref. Month 1 after PE)
      Month 2 after PE 0.84 [0.72-0.99], 0.0410
      Month 3-4 after PE 1.12 [0.94-1.35], 0.2177

← Delayed SNB better   Early SNB better →
0.5 0.6 0.7 0.8 0.9 1 1.1 1.2 1.3 1.4 1.5

* P-value of interaction test. Legend. PE: primary excisione. SNB: sentinel node biopsy.

C

Adjusted HR [95%CI], p-value | **Adjusted HR [95%CI]**

DISEASE FREE SURVIVAL
Overall
  SNB timing >31 days 0.81 [0.75-0.89], <0.0001
Sentinel lymph node status <0.0001*
  Negative
    SNB timing >31 days 0.70 [0.63-0.79], <0.0001
  Positive
    SNB timing >31 days 0.96 [0.84-1.09], 0.5339
OVERALL SURVIVAL
Overall
  SNB timing >31 days 0.78 [0.71-0.85], <0.0001
Sentinel lymph node status 0.0006*
  Negative
    SNB timing >31 days 0.69 [0.61-0.78], <0.0001
  Positive
    SNB timing >31 days 0.94 [0.81-1.08], 0.3738

← Delayed SNB better   Early SNB better →
0.6 0.65 0.7 0.75 0.8 0.85 0.9 0.95 1 1.05 1.1 1.2

* P-value of interaction test. Legend. SNB: sentinel node biopsy.

Fig. 2. **Subgroup analysis on disease-free survival and overall survival according to SN status**. SN, sentinel lymph node; SNB, sentinel node biopsy; PE, primary excision; CI, confidence interval; HR, hazards ratio.

the lymphatic drainage. SNs are thought to be more closely associated with antitumour immunity than non-SNs [22]. However, the presence of melanoma inhibits an immune response by releasing immunosuppressive cytokines and creating an immunosuppressive micro-environment [23,24]. After excision of primary melanoma, processing, maturation of antigen presenting cells, antigen presentation and priming require time. Indeed, an immune response requires precise coordination of molecular and cellular signaling, tightly regulated with multistep cascades, which occur over multiple time and length scales [25]. Our results suggest that early excision of negative SN may, after removal of primary melanoma, impair and stop this process and is therefore associated with a worse DFS and OS. Nevertheless, the interval between melanoma development and the diagnostic biopsy is likely to be greater and more variable than the interval between biopsy and SNB. This interval, which is more difficult to measure, should be considered as well, and this represent an area of future translational and preclinical investigations.

This study adds novel information to the current literature for several reasons: 1) it is the largest analysis to date on the effect of timing of SNB on survival; 2) all included patients were treated in the context of IMI centres with homogeneous surgical procedures and similar schedule of follow-up; 3) a robust statistical analysis allowed us to evaluate the impact of timing to SNB through different models: i) timing to SNB as a continuous variable, ii) timing to SNB in discrete categories (months after primary resection), iii) and two different groups according to the CART analysis. Importantly, these results were confirmed by a sensitivity analysis according to the PS approach; 4) we provided a comprehensive overview of all studies to date published; 5) the median follow-up is one of the longest so far reported; and 6) our data suggest that a time interval until 4 months may be not detrimental for patients with both positive and negative SN.

We are also aware of some limitations, including 1) the retrospective nature of our analysis, which cannot exclude patient enrollment bias, 2) the histopathological review was not centralised among participating centres, which can increase heterogeneity in the characterization of tumour variables, 3) the lack of a validation cohort.

Our study, in the context of the current literature, has clinical implications considering that the number of performed SNBs will increase, as it is now a gateway to effective adjuvant therapy in stage III. Furthermore, the current COVID-19 pandemic may raise issues in the time schedule of surgical procedures.

In conclusion, our data do not support a strict time interval for SNB, and considering our results and those of previous studies, this notion should be incorporated in current guidelines and to counsel patients to reduce the number of high urgency referrals.

lymph nodes by antigen presenting cells, as well as the subsequent priming of tumour antigen–specific T cells. The SN is the first lymphoid organ that tumour antigens meet after being released from a primary tumour into

Table 4
Clinical studies investigating the impact of timing of sentinel lymph node biopsy on DFS and/or overall survival in melanoma patients.

Author	Number of patients	Cut-off for early/ delayed time interval to SNB (days)	Median follow-up	Multivariable analysis on DFS (Cox proportional hazard model)	Multivariable analysis on OS (Cox proportional hazard model)
Fortes [9]	SN−: 607 SN+:141	30	Not specified	Not reported	SN−: HR [Early versus delayed] 1.77, 95%CI 0.97—3.26 SN+: HR [Early versus delayed] 0.29, 95%CI 0.11—0.77 [MSS]
Carpenter [10]	SN−: 412 SN+: 61	28 (A) and 56 (B)	2.8 years	A) HR [Early versus delayed] 1.01, 95%CI 0.64—1.58 B) HR [Early versus delayed] 0.80, 95%CI 0.37—1.73	A) HR [Early versus delayed] 1.05, 95%CI 0.61 −1.82 B) HR [Early versus delayed] 0.89, 95%CI 0.34 −2.34
Crawford [11]	723 (SN− and SN+)	Analysed as continuous variable	3.34 years	Not reported	HR [1 day increase] 0.89, 95%CI 0.74—1.07 [OS calculated from the primary melanoma excision]
Oude Ophuis [12]	SN−: 2841 SN+: 705	43	50 months	Not reported	HR [1 day increase] 1.0, 95%CI 0.99—1.01
Tejera-Vaquerizo [13]	SN−: 1498 SN+: 464	40	46 months	Not significant for SN − and SN+ (data not shown) [DFS calculated from the excision of the primary melanoma plus 120 days]	SN−: HR [Early versus delayed] 2.6, 95%CI 1.5 −4.6 SN+: Not significant (data not shown) [OS calculated from the excision of the primary melanoma plus 120 days. MSS]
Nelson [14]	SN−: 2051 SN+: 432	30	95.7 months	HR [Early versus delayed] 0.98, 95%CI 0.81—1.18	HR [Early versus delayed] 1.05, 95%CI 0.83—1.34 [MSS]
Tejera-Vaquerizo [15]	SN−: 274 SN+: 66 SN not found: 10	40	103 months	HR [Early versus delayed] 1.68, 95%CI 1.07—2.65 [DFS calculated from primary excision. Propensity score matching]	HR [Early versus delayed] 1.77, 95%CI 1.11—2.83 [OS calculated from primary excision. Propensity score matching]
Richtig [16]	SN+: 121	43	42 months	Not reported	Not reported
Gambichler [17]	SN−: 667 SN+: 229	7	Not specified	Not reported]	Not reported
Oude Ophuis [18]	SN+:1015	47	36 months	Not reported	HR [1 day increase] 1.0, 95%CI 0.99—1.01
Parrett [19]	SN−: 414 SN+: 78	40	11.7 years	HR [Delayed versus early] 0.91, 95%CI 0.64—1.28	HR [Delayed versus early] 0.91, 95%CI 0.67—1.23 [MSS]

MSS, melanoma-specific survival; SN, sentinel lymph node; SN−, negative sentinel lymph node; SN+, positive sentinel lymph node; SNB, sentinel lymph node biopsy; DFS, disease-free survival; OS, overall survival; HR, hazard ratio; 95%CI, 95% confidence interval.

Ethical approval

Approval to conduct this study was obtained from IMI Institutional Review Board and Local Ethical Committees.

Conflict of interest statement

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2020.07.001.

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