Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
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)

Mario Mandalà a,*, Francesca Galli b, Roberto Patuzzo c, Andrea Maurichi c, Simone Mocellin d, Carlo R. Rossi e, Eliana Rulli b, Maria Montesco f, Pietro Quaglino g, Virginia Caliendo h, Vincenzo De Giorgi i, Barbara Merelli j, Corrado Caracò j, Dario Piazzalunga k, Alice Labianca a, Simone Ribero g, Rebecca Senetta l, Andrea Gianatti m, Barbara Valeri n, Daniela Massi o, Paolo A. Ascierto p, Mario Santinami c, for The Italian Melanoma Intergroup (IMI)

a Unit of Medical Oncology, Papa Giovanni XXIII Hospital, Bergamo, Italy
b Methodology for Clinical Research Laboratory, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy
c Melanoma and Sarcoma Unit, Department of Surgery, IRCCS Fondazione Istituto Nazionale dei Tumori, Milan, Italy
d Unit of Surgery, Veneto Institute of Oncology - IOV

e University of Padua, Italy
f Pathological Anatomy and Histology, Veneto Institute of Oncology - IOV, Istituto di Ricovero e Cura a Carattere Scientifico, Padua, Italy
g Dermatologic Clinic, Department of Medical Sciences, University of Turin Medical School, Turin, Italy
h Department of Surgery, University of Turin Medical School
i Department of Dermatology, University of Turin, Italy
j Istituto Nazionale Tumori IRCCS Fondazione G. Pascale, Naples, Italy
k Unit of Surgery, Papa Giovanni XXIII Hospital, Bergamo, Italy
l Pathology Division, ‘Città della Salute e della Scienza di Torino’ University Hospital, Turin, Italy
m Unit of Pathology, Papa Giovanni XXIII Hospital, Bergamo, Italy
n Department of Pathology and Laboratory Medicine, IRCCS Fondazione Istituto Nazionale dei Tumori di Milano, Milan, Italy
o Histopathology and Molecular Diagnostics, Careggi University Hospital, Florence, Italy
p Unit Melanoma, Cancer Immunotherapy and Innovative Therapies, Istituto Nazionale Tumori IRCCS Fondazione G. Pascale’, Naples, Italy

Received 28 April 2020; received in revised form 26 June 2020; accepted 1 July 2020
Available online 30 July 2020

* Corresponding author. Unit of Medical Oncology, Department of Oncology and Haematology, Papa Giovanni XXIII Cancer Center Hospital, Piazza OMS 1, 24100, Bergamo, Italy. Fax: +39 035 267 4985.
E-mail address: mmandala@asst-pg23.it (M. Mandalà).

https://doi.org/10.1016/j.ejca.2020.07.001
0959-8049/© 2020 Elsevier Ltd. All rights reserved.
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. 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/mm². 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/mm². 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.
A comparison among centres in terms of demographic and clinical characteristics at diagnosis is reported in Table 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,
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 endpoints, evidence of no PH was found for age, site of PC, 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 DFS (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.

Tables 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)
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 SN] 0.96 [95%CI 0.84–1.09], p = 0.5339) and OS (aHR [delayed versus early SN] 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,
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 naive T cells by antigen presenting cells in the draining lymph nodes [20]. When a specific antigen is presented by antigen presenting cells, the naive 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
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
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 melan-
oma, processing, maturation of antigen presenting
cells, antigen presentation and priming require time.
Indeed, an immune response requires precise coordina-
tion of molecular and cellular signaling, tightly regu-
lated 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 diag-
nostic biopsy is likely to be greater and more variable
than the interval between biopsy and SNB. This inter-
val, 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 cate-
gories (months after primary resection), iii) and two
different groups according to the CART analysis.
Importantly, these results were confirmed by a sensi-
tivity 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 in-
terval until 4 months may be not detrimental for pa-
tients 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.

Fig. 2. Subgroup analysis on disease-free survival and overall sur-
vival according to SN status. SN, sentinel lymph node; SNB,
sentinel node biopsy; PE, primary excision; CI, confidence inter-
val; HR, hazards ratio.
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           | 30                                                  | Not specified    | Not reported                                                  | SN−: HR [Early versus delayed] 1.77, 95%CI 0.97−3.26          |
|                 | SN+: 141           |                                                     |                  |                                                               | SN+: HR [Early versus delayed] 0.29, 95%CI 0.11−0.77 [MSS]     |
| Carpenter [10]  | SN−: 412           | 28 (A) and 56 (B)                                   | 2.8 years        | A) HR [Early versus delayed] 1.01, 95%CI 0.64−1.58            |
|                 | SN+: 61            |                                                     |                  | B) HR [Early versus delayed] 0.80, 95%CI 0.37−1.73            |
| 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          | 43                                                  | 50 months        | Not reported                                                   | HR [1 day increase] 1.0, 95%CI 0.99−1.01                         |
|                 | SN+: 705           |                                                     |                  |                                                               | SN−: HR [Early versus delayed] 2.6, 95%CI 1.5−4.6              |
|                 | SN−: 1498          | 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+: 464           |                                                     |                  |                                                               | SN+: Not significant (data not shown) [OS calculated from the excision of the primary melanoma plus 120 days. MSS] |
| Tejera-Vaquerizo [13]| SN−: 2051 | 30                                                  | 95.7 months      | HR [Early versus delayed] 0.98, 95%CI 0.81−1.18               |
|                 | SN+: 432           |                                                     |                  |                                                               | HR [Early versus delayed] 1.05, 95%CI 0.83−1.34 [MSS]          |
|                 | SN−: 274           | 40                                                  | 103 months       | HR [Early versus delayed] 1.68, 95%CI 1.07−2.65 [DFS calculated from primary excision. Propensity score matching] |
|                 | SN+: 66            |                                                     |                  | HR [Early versus delayed] 1.77, 95%CI 1.11−2.83 [OS calculated from primary excision. Propensity score matching] |
|                 | SN not found: 10   |                                                     |                  | Not reported                                                   | HR [1 day increase] 1.0, 95%CI 0.99−1.01                         |
| Richtig [16]   | SN+: 121           | 43                                                  | 42 months        | Not reported                                                   | Not reported                                                   |
| Gambichler [17] | SN−: 667           | 7                                                   | Not specified    | Not specified                                                 | Not specified                                                 |
| Oude Ophuis [18]| SN+: 1015          | 47                                                  | 36 months        | Not reported                                                   | HR [Delayed versus early] 0.91, 95%CI 0.64−1.28 [MSS]           |
| Parrett [19]    | SN−: 414           | 40                                                  | 11.7 years       | HR [Delayed versus early] 0.91, 95%CI 0.64−1.28 [MSS]         |
|                 | SN+: 78            |                                                     |                  |                                                               |                                                               |

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.

Funding

This work was supported by Italian Melanoma Inter-group (IMI, grant 3/2015), the Italian network for melanoma treatment and research (www.melanomaimi.it).

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.
References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016;66:7–30.

[2] Mandalà M, Massi D. Tissue prognostic biomarkers in primary cutaneous melanoma. Virchows Arch 2014;464:265–81.

[3] Balch CM, Gershenwald JE, Soong SJ, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol 2009;27:6199–206.

[4] Leiter U, Stadler R, Mauch C, et al. Complete lymph node dissection versus no dissection in patients with sentinel lymph node biopsy positive melanoma (DeCOG-SLT): a multicentre, randomised, phase 3 trial. Lancet Oncol 2016;17:757–67.

[5] Faries MB, Thompson JF, Cochran AJ, et al. Completion dissection or observation for sentinel-node metastasis in melanoma. N Engl J Med 2017;376:2211–22.

[6] van Akkooi AC, Nowecki ZI, Voit C, et al. Sentinel node tumor burden according to the Rotterdam criteria is the most important prognostic factor for survival in melanoma patients: a multicenter study in 388 patients with positive sentinel nodes. Ann Surg 2008;248:949–55.

[7] Balch CM, Buzaid AC, Soong SJ, et al. Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. J Clin Oncol 2001;19:3635–48.

[8] van Akkooi AC, de Wilt JH, Verhoef C, et al. High positive sentinel node identification rate by EORTC melanoma group protocol. Prognostic indicators of metastatic patterns after sentinel node biopsy in melanoma. Eur J Canc 2006;42:372–80.

[9] Fortes C, Mastroeni S, Caggiati A, et al. The effect of time to sentinel lymph node biopsy on cutaneous melanoma survival. Am J Surg 2016;212:935–40.

[10] Carpenter S, Pockaj B, Dueck A, et al. Factors influencing time between biopsy and definitive surgery for malignant melanoma: do they impact clinical outcome? Am J Surg 2008;196:834–43.

[11] Crawford AB, Nessim C, Weaver J, van Walraven C. Wait times for melanoma surgery: is there an association with overall survival? Ann Surg Oncol 2018;25:265–70.

[12] Oude Ophuis CM, van Akkooi AC, Rutkowski P, et al. Effects of time interval between primary melanoma excision and sentinel node biopsy on positivity rate and survival. Eur J Canc 2016;67:164–73.

[13] Tejera-Vaquerizo A, Nagore E, Puig S, et al. Effect of time to sentinel-node biopsy on the prognosis of cutaneous melanoma. Eur J Canc 2015;51:1780–93.

[14] Nelson DW, Stern S, Elashoff DE, et al. Impact of time between diagnosis and SLNB on outcomes in cutaneous melanoma. J Am Coll Surg 2017;225:302–11.

[15] Tejera-Vaquerizo A, Descalzo-Gallego MA, Traves V, et al. The intriguing effect of delay time to sentinel lymph node biopsy on survival: a propensity score matching study on a cohort of melanoma patients. Eur J Dermatol 2017;27:487–95.

[16] Richtig G, Richtig E, Neiss AN, et al. Does the time interval between sentinel lymph node biopsy and completion lymph node dissection affect outcome in malignant melanoma? A retrospective cohort study. Int J Surg 2020;75:160–4.

[17] Gambichler T, Bünnehm H, Scheel CH, et al. Does early timing of lymph node surgery after resection of the primary tumour improve the clinical outcome of melanoma patients? Clin Exp Derm Epub 2020. https://doi.org/10.1111/ced.14291.

[18] Oude Ophuis CM, Verhoef C, Rutkowski P, et al. The interval between primary melanoma excision and sentinel node biopsy is not associated with survival in sentinel node positive patientsdAn EORTC Melanoma Group study. Eur J Surg Oncol 2016;42:1906–13.

[19] Parrett BM, Accornt NA, Li R, et al. The effect of delay time between primary melanoma biopsy and sentinel lymph node dissection on sentinel node status, recurrence, and survival. Melanoma Res 2012;22:386–91.

[20] Heath WR, Carbone FR. Cross-presentation, dendritic cells, tolerance and immunity. Annu Rev Immunol 2001;19:47–64.

[21] Fridman WH, Pagès F, Sauté-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Canc 2012;12:298–306.

[22] Sakakura K, Chikamatsu K, Sakurai T, et al. Infiltration of dendritic cells and NK cells into the sentinel lymph node in oral cavity cancer. Oral Oncol 2005;41:89–96.

[23] Lelong SP, Peng M, Zhou YM, et al. Cytokine profiles of sentinel lymph nodes draining the primary melanoma. Ann Surg Oncol 2002;9:82–7.

[24] Lee JH, Torisu-Itakara H, Cochran AJ, et al. Quantitative analysis of melanoma-induced cytokine-mediated immunosuppression in melanoma sentinel nodes. Clin Canc Res 2005;11:107–12.

[25] Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoeediting. Annu Rev Immunol 2004;22:329–60.