Prognostic factors in distinct melanoma types

Ipenburg, N.A.

CHAPTER 8

Summary and discussion

INTRODUCTION

Although clinical aspects of melanoma have been extensively studied, the literature largely concerns relatively healthy 20-70 years old patients.[1,2] Special populations, such as the elderly, children, patients with multiple primary melanoma (MPM) and those with familial melanoma, are frequently excluded from clinical studies. The studies presented in this thesis were aimed to assess prognostic factors and management of patients with clinically localized melanoma, in particular among the aforementioned special populations.

But how do these special populations differ from the frequently studied middle-aged patient with sporadic melanoma? Is tumor mitotic rate also an important prognostic factor in children and adolescents? Should SNB be performed in all patients with clinically-localized melanoma? And, is it possible to predict survival of patients with sentinel node (SN)-negative melanoma more accurately?

Chapter two and **chapter three** concerns lymphatic mapping combined with focused ultrasound (US) follow-up as an alternative to sentinel node biopsy (SNB). **Chapter four** reports the prognostic significance of tumor mitotic rate in children and adolescent melanoma patients. In **chapter five** we compared the survival of germline cyclin-dependent kinase inhibitor 4 *(CDKN2A)* mutation carriers with sporadic melanoma patients and in **chapter six** we assessed the occurrence and prognostic value of SN-positivity in these *CDKN2A*-positive melanoma patients. We externally validated a prognostic model for SN-negative melanoma patients in **chapter seven**. In this last chapter the results of these studies, together with those in recent literature, are summarized and discussed.

ELDERY PATIENTS

SNB was introduced to identify node-positive patients who were then to undergo early treatment by completion lymph-node dissection (CLND).[3,12] The prognostic significance of the tumor-status of the SN and the survival benefit from early treatment of lymph node metastases have been well established. Results of the Multicenter Selective Lymphadenectomy Trial II (MSLT-II) and the German Dermatologic Cooperative Oncology Group Selective Lymphadenectomy Trial (DeCOG-SLT) demonstrated that CLND was not required to achieve the survival benefit.[13,14] As a result, patients with an involved SN are now rarely managed with CLND.[15–17] The emergence of effective adjuvant systemic therapy further increased the importance of SNB. In the past few years, adjuvant immunotherapy and targeted therapy have been shown to improve prognosis of patients with nodal involvement,

including SN-positive patients.[4–11] Patients with clinically-localized melanoma do not receive adjuvant systemic therapy without SNB showing metastatic disease. Still, these benefits do not always justify the potential morbidity from SNB. The extent of the surgery, need for general anesthesia and risk of morbidity are drawbacks. SNB may be considered excessive in elderly or frail patients, if the surgical procedure is too complex. At Melanoma Institute Australia (MIA), SNB was sometimes purposely avoided in elderly or patients with significant comorbidity (**chapter two**). In other patients, the planned procedure was canceled after lymphoscintigraphy (**chapter three**). These patients underwent preoperative lymphoscintigraphy to determine the number of SNs and their location followed by focused US of these nodes at each follow-up visit. This approach was not practiced elsewhere on a regular basis. To determine its merits, we carried out two retrospective cohort studies in order to compare characteristics and survival of 2945 patients who underwent SNB (SNB group) with 160 patients who were conservatively managed due to advanced age and/or comorbidities (observed group) (**chapter two**). In the second study, we compared the 2945 SNB-patients with 203 patients in whom the procedure was canceled after lymphoscintigraphy (canceled group) (**chapter three**). In both studies, SNB-patients were younger than their counterparts. A recent analysis of the National Cancer Database showed that nodal surgery was least common among elderly patients. Only 35% of eligible patients aged 80 years or older underwent SNB.[18] However, in our study SNB was still performed in 75% of those aged ≥ 75 years and in 47% of patients ≥ 85 years. This is in line with previous research that demonstrated that SNB can reliably be performed in elderly patients.[19–24] Recent research also shows that immunotherapy is effective in the elderly.[25–27]

A heterogeneous group of conditions, ranging from cardiovascular conditions to psychiatric disorders, were the reason for omitting SNB in 14 patients (9%) < 65 years of age. Analyses of the Surveillance, Epidemiology, and End Results (SEER) cancer registry lately showed that the overwhelming majority (85%) of patients older than 65 with stage III and IV melanoma had multimorbidity.[28] Another study of the same groupsshowed that healthcare expenditure of elderly and comorbid melanoma patients was associated with increased healthcare costs compared to younger patients and patients without multimorbidity.[29]

Melanomas of patients in the observed group and the canceled group were more frequently located in the head and neck region, drained to more nodes and regions than melanomas of SNB-patients. Lymphatic drainage of head and neck melanomas is often to multiple sites and less predictable than for melanomas on limbs.[30–32] The procedure can be further complicated by the presence of a SN in the parotid gland, which occurs in 35% of the head and neck melanoma patients.[33] In these patients there is a risk of permanent facial nerve damage. A

recent Dutch study also showed that higher age and melanoma located on the head and neck were associated with non-enactment of SNB.[34]

At the end of follow-up, 21 observed patients (13%) and 27 canceled patients (13%) had developed a regional nodal recurrence. A previous meta-analysis revealed that US is able to detect metastatic nodes that are two to three times smaller than can be detected by physical examination.[35] In both of our studies, US detected the recurrence in one third of the patients before they became clinically apparent. In the majority of patients focused US could not have had an influence on the outcome. The median number of metastatic nodes in these groups was higher than in the patients who underwent immediate CLND because of an involved SN. A comparable prospective study from the United Kingdom showed that, although the median number of involved nodes was again higher in the US group, melanoma-specific survival (MSS) rates were similar.[36] As expected, regional lymph node-free survival was worse in observed and canceled patients. Canceled patients also had worse recurrence-free survival (RFS) than SNB patients. Lymphatic mapping with focused US follow-up of SNs appears to be an acceptable management strategy to avoid SNB in elderly or frail melanoma patients or for patients in whom a SNB procedure is likely to be challenging.

Since CLND has largely become obsolete after publication of the MSLT-II and DeCOG-SLT trials, the importance of SNB has become even more important because of its value in the selection of patients for adjuvant therapy.[13,14]

PEDIATRIC MELANOMA

Tumor mitotic rate is a strong and important predictor of survival in adults with primary cutaneous melanoma.[37–42] Due to the rarity of pediatric melanoma, it was unknown if tumor mitotic rate was also of clinical importance for children and adolescents with melanoma. Large pediatric melanoma studies generally use data from the SEER database or National Cancer Database.[43–45] Key tumor characteristics such as tumor mitotic rate and Breslow thickness are frequently missing in these databases. We conducted a cohort study of 156 patients aged < 20 years with clinically localized melanoma to assess the prognostic value of tumor mitotic rate in this age group (**chapter four**). In our study, a higher tumor mitotic rate was independently associated with worse RFS. Breslow thickness did not correlate independently with RFS or MSS. Prior studies showed conflicting results regarding the prognostic impact of Breslow thickness in pediatric melanoma. In two studies, Breslow thickness was an independent predictor of recurrence.[46,47] However, in a National Cancer Database study and

a large multicenter study Breslow thickness was not associated with MSS.[45,48] A multicenter retrospective case series of 38 fatal pediatric melanoma patients showed that adolescent melanoma had a more aggressive disease course compared to childhood melanoma. Mitoses were present in all their reported patients.[4249] In our study, children had more advanced melanomas than adolescents but survival was similar for the two groups. The first studies on the use of immunotherapy and targeted therapy in pediatric melanoma patients showed promising results.[49,50]

Even though mitotic rate was removed from the 8th edition of the AJCC/UICC melanoma staging classification, **chapter four** shows that it is essential to assess and report this parameter in all young melanoma patients. The AJCC melanoma expert panel also emphasized the importance of this tumor characteristics for clinical tool development.[51–53] More research is needed to determine if the prognostic value of tumor mitotic rate and Breslow thickness are really different between children and adults.

MELANOMA IN GERMLINE *CDKN2A* MUTATION CARRIERS

While SNB was introduced almost 30 years ago, no studies have been published on its applicability to patients with hereditary melanoma due to germline *CDKN2A* mutations (FAMMM syndrome).[3] Over 40% of *CDKN2A* mutation carriers have multiple primary melanomas, excluding them from previous clinical trials of SNB.[54–56]

There is ongoing debate regarding the prognostic impact of germline *CDKN2A* mutation status on survival of melanoma patients. Therefore, we compared survival, patient and tumor characteristics of 89 *CDKN2A* mutation carriers with 56,929 sporadic melanoma patients (**chapter five**). As expected, *CDKN2A* mutation carriers were on average younger and more often developed MPM.[55–58] Sporadic melanoma patients had more often nodular melanomas. In a recent multicenter study from the United States, Italy and Spain, histologic slides were evaluated for melanomas diagnosed in *CDKN2A, CDK4* and *POT1* mutation carriers. While spitzoid morphology was associated with *POT1* mutations, melanomas from *CDKN2A* carriers were histologically similar to sporadic cases.[59] In our study, *CDKN2A* carriers had less advanced melanomas than their sporadic counterparts. Previous studies showed conflicting results on this matter. Some researchers found no difference, while others also discovered that *CDKN2A* mutation carriers had less advanced melanomas at diagnosis.[55–60] After controlling for known confounders, no significant difference in overall survival (OS) and RFS was seen between *CDKN2A* mutation carriers and sporadic melanoma patients.

These results are in line with a recent Italian publication in which no survival difference was established.[57] However, two Swedish studies found that germline *CDKN2A* carriers had worse survival.[55,61] In a recent Australian study, pathogenic germline mutations, including *CDKN2A*, were associated with poor OS in stage III/IV melanoma patients with completely resected tumors.[62] Since all cancer predisposition genes were combined, the independent prognostic value of a germline *CDKN2A* mutation could not be assessed in this study. Comparison of these studies is complicated by differences in type and location of the *CDKN2A* germline mutation, inclusion of single primary melanoma (SPM) patients, control group, outcome and statistical analyses.[55,57,61] Further studies are needed to clarify the uncertainty regarding the prognostic importance of a germline *CDKN2A* mutation for the survival of melanoma patients.

In **chapter six** we described a multicenter, retrospective case series of 23 *CDKN2A* mutation carriers with clinically localized melanoma who underwent SNB. In our study, the SN-positivity rate of 22% was in line with what has been reported for sporadic melanoma patients.[54,55] Due to small numbers, we were not able to draw conclusions regarding the prognostic value of SNB. Based on this study, we conclude that there should be no reluctance to perform SNB in this particular patient group who frequently develop multiple primary melanomas at a young age.

PROGNOSTIC MODELS

Prognostic models and nomograms can aid clinicians in tailoring treatment to the individual patient's situation. The European Organisation for Research and Treatment of Cancer (EORTC) built a prediction model for RFS and MSS using data of 3180 European SN-negative melanoma patients.[63] Ulceration, anatomical location and Breslow thickness were included in their final model. The EORTC model was able to correctly predict recurrence in 74% of the patients (c-index of 0.74) and melanoma-specific mortality in 76% of the patients (c-index of 0.76).

To ensure the accuracy and applicability of prognostic models in other populations, external validation is essential.[64] In **chapter seven**, we described the validation of the EORTC model in a cohort of 4235 Australian SN-negative melanoma patients. As expected, the model performance was not as good as in the original dataset.[64] The EORTC model could correctly predict melanoma-specific mortality and recurrence in 69% of the MIA patients (c-index 0.69 for RFS and MSS). Differences in baseline characteristics, e.g. more men, more head and neck

melanomas, and drainage to more SNs in the MIA cohort, were probably the most important reasons for the small discrepancy in predictive value. We tried to further improve the accuracy of the model by adding other known prognostic factors. Eight potential prognostic factors were added to the EORTC model: sex, age, melanoma subtype, Clark level, tumor mitotic rate, regression, total number of SNs removed and number of SN fields. Sex, age, melanoma subtype and tumor mitotic rate improved the predictive ability of the models by 2% (c-index 0.71 for RFS and MSS). Since simplicity is essential for clinicians, this small improvement does not justify changing the easy-to-use EORTC model. Recently, a Dutch population-based validation study confirmed the value of the EORTC nomogram in predicting RFS in SN-negative melanoma patients.[65] Unfortunately, MSS was not investigated in this study. In conclusion, **chapter seven** demonstrated the value of the EORTC nomogram in predicting survival in SN-negative melanoma patients.[63,65] The EORTC nomogram makes it possible to identify specific populations of SN-negative melanoma patients with a high risk of recurrence or melanoma-specific mortality. Patients with a thick, ulcerated melanoma located in the head and neck region have the highest risk of an unfavorable outcome.[63] The EORTC nomogram could be used in clinical practice to personalize follow-up and to select high-risk SN-negative patients for trials of adjuvant systemic therapy.

GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

In this thesis, we showed the differences and similarities between several distinct melanoma populations. Management of patients of high age needs to be different due to frailty, comorbidity and a reduced risk of SN involvement. The results described in this thesis combined with those from recently published studies demonstrate that melanoma in this patient group has a distinct biological behavior. This necessitates a different approach to sentinel node biopsy and interpretation of its results. More specifically, lymphatic mapping combined with focused US of the SNs should be considered more often in frail patients.

The same holds true for pediatric melanoma patients. Melanoma behaves differently in these young patients and the prognostic value of known predictors of survival is also different from the well-studied adult population. These differences show the need for specific guidelines for the diagnosis, treatment and follow-up of children and adolescents with melanoma. Melanomas in *CDKN2A* mutation carriers are different from the ones in sporadic patients. While the results from this thesis and previous literature show that melanomas in the two populations present differently, uncertainty regarding survival differences will remain. However, this thesis does prove the reliability of performing SNB in this special population.

Sometimes two groups are actually the same but differently managed. High-risk stage II and stage IIIB/C melanoma patients have an equally poor prognosis but do not receive the same kind of treatment. Results from this thesis facilitate the use of an easy-to-use nomogram in clinical practice to personalize follow-up and to select high-risk SN-negative patients for trials of adjuvant systemic therapy.

In conclusion, future melanoma studies focusing on special populations such as children, elderly, and familial melanoma patients are essential to further personalize medicine. Due to the rarity of many of these subgroups, collaborative cross-continental studies are needed to improve the diagnostic process, therapeutic possibilities, and prognosis of these patients.

High-risk clinically localized melanoma patients (stage IIB/IIC) have worse survival than stage IIIA melanoma patients.[51] Adjuvant immunotherapy and targeted therapy improves prognosis of stage III patients but it is unknown if the same holds true for high-risk stage II patients. Currently, the safety and efficacy of adjuvant therapy in these patients is being studied (NCT04309409, NCT03757689, NCT04099251, NCT03553836, NCT03405155). Based on the MSLT-trials, SNB may be assumed to prolong disease-free survival for all patients and prolong melanoma-specific survival for those with nodal metastases from intermediate-thickness melanomas.[13,54] If it can be established that adjuvant systemic therapy can accomplish the same with less morbidity, the role of SNB will diminish substantially. However, until even more reliable prognostic factors are found, SN status remains important for the assessment of an individual's prognosis.[54,66]

Numerous molecular biomarkers have been discovered, but the clinical potential and applicability of mRNA-signatures, methylation markers, circulating tumor cells, gene expression profiles, and microRNAs have to be studied further.[67–72] We were not able to assess the prognostic value of SNB in *CDKN2A* mutation carriers. Due to close surveillance, melanomas of FAMMM syndrome patients are diagnosed at an earlier stage than sporadic melanoma patients.[55,56] A significantly larger, multicenter cohort study is needed to answer this question. Until then, there is no reason to change the threshold of performing SNB in familial melanoma patients.

While immunotherapy has improved survival of advanced melanoma patients, little is known about the effectivity of this treatment for stage IV familial melanoma patients. Most high-risk genes are involved in DNA repair mechanisms, which are also needed for lymphocyte development and T-cell differentiation.[73–75] Immunotherapy might not be ideal for patients

who do not have the ability to generate a proper antitumor immune response. Results of studies on this matter are conflicting.[76–78] In a small Swedish study, patients with *CKDN2A* mutated melanoma had improved immunotherapy responses.[77] A recent study from the Mayo Clinic, showed no survival difference between sporadic melanoma patients and *CDKN2A* carriers who were treated with immune checkpoint inhibitors.[76] In a third collaborative European study, none of the patients with pathogenic or likely pathogenic germline mutations, including *CDKN2A*, responded to combined treatment with nivolumab and ipilimumab. Presence of such a germline variant was also independently associated with worse MSS.[78] Co-deletion of the gene Janus kinase 2 (*JAK2*), also located at chromosome 9, might be one of the reasons for this increased risk of resistance to immotherapy.[79] Since immunotherapy is associated with significant adverse effects, it is of great importance to identify the patients who could benefit from this treatment. More research is needed to assess the effectivity and safety of immunotherapy in familial melanoma patients.

In the last three years, multiple research groups have focused on the development of prognostic models and nomograms for patients with clinically localized melanoma.[63,65,80–83] Regression tree analysis makes it possible to more accurately delineate groups with different survival rate. It produces an easily understandable graph for classification and prediction purposes.[84–87] In a future study, we will develop classification systems for SN-negative and SN-positive melanoma patients, that could be used for personalizing follow-up and selecting patients for adjuvant systemic therapy.

REFERENCES

1. Garcovich S, Colloca G, Sollena P, et al. Skin cancer epidemics in the elderly as an emerging issue in geriatric oncology. *Aging Dis*. 2017;8(5):643-661.
2. Rogiers A, van den Oord JJ, Garmyn M, et al. Novel therapies for metastatic melanoma: An update on their use in older patients. *Drugs Aging*. 2015;32(10):821-834.
3. Morton DL. Technical details of intraoperative lymphatic mapping for early stage melanoma. *Arch Surg*. 1992;127(4):392.
4. Tarhini AA, Lee SJ, Hodi FS, et al. Phase III study of adjuvant ipilimumab (3 or 10 mg/kg) versus high-dose interferon alfa-2b for resected high-risk melanoma: North American Intergroup E1609. *J Clin Oncol*. 2020;38(6):567-575.
5. Dummer R, Hauschild A, Santinami M, et al. Five-year analysis of adjuvant dabrafenib plus trametinib in stage III melanoma. *N Engl J Med*. 2020;383(12):1139-1148.
6. Ascierto PA, Del Vecchio M, Mandalá M, et al. Adjuvant nivolumab versus ipilimumab in resected stage IIIB-C and stage IV melanoma (CheckMate 238): 4-year results from a multicentre, double-blind, randomised, controlled, phase 3 trial. *Lancet Oncol*. 2020;21(11):1465-1477.
7. Eggermont AMM, Blank CU, Mandala M, et al. Adjuvant pembrolizumab versus placebo in resected stage III melanoma. *N Engl J Med*. 2018;378(19):1789-1801.
8. Weber J, Mandala M, Del Vecchio M, et al. Adjuvant nivolumab versus ipilimumab in resected stage III or IV melanoma. *N Engl J Med*. 2017;377(19):1824-1835.
9. Long GV, Hauschild A, Santinami M, et al. Adjuvant dabrafenib plus trametinib in stage III BRAF-mutated melanoma. *N Engl J Med*. 2017;377(19):1813-1823.
10. Eggermont AMM, Chiarion-Sileni V, Grob J-J, et al. Prolonged survival in stage III melanoma with ipilimumab adjuvant therapy. *N Engl J Med*. 2016;375(19):1845-1855.
11. Eggermont AMM, Chiarion-Sileni V, Grob J-J, et al. Adjuvant ipilimumab versus placebo after complete resection of high-risk stage III melanoma (EORTC 18071): a randomised, double-blind, phase 3 trial. *Lancet Oncol*. 2015;16(5):522-530.
12. Thompson JF, McCarthy WH, Bosch CMJ, et al. Sentinel lymph node status as an indicator of the presence of metastatic melanoma in regional lymph nodes. *Melanoma Res*. 1995;5(4):255-260.
13. Faries MB, Thompson JF, Cochran AJ, et al. Completion dissection or observation for sentinel-node metastasis in melanoma. *N Engl J Med*. 2017;376(23):2211-2222.
14. Leiter U, Stadler R, Mauch C, et al. Final analysis of DeCOG-SLT trial: No survival benefit for complete lymph node dissection in patients with melanoma with positive sentinel node. *J Clin Oncol*. 2019;37(32):3000-3008.
15. Nijhuis AAG, Spillane AJ, Stretch JR, et al. Current management of patients with melanoma who are found to be sentinel node-positive. *ANZ J Surg*. 2020;90(4):491-496.
16. Hui JYC, Burke E, Broman KK, et al. Surgeon decision-making for management of positive sentinel lymph nodes in the post-Multicenter Selective Lymphadenectomy Trial II era: A survey study. *J Surg Oncol*. 2021;123(2):646-653
17. Bredbeck BC, Mubarak E, Zubieta DG, et al. Management of the positive sentinel lymph node in the post-MSLT-II era. *J Surg Oncol*. 2020;122(8):1778-1784.
18. Bateni SB, Johns AJ, Gingrich AA, et al. Elderly age is associated with more conservative treatment of invasive melanoma. *Anticancer Res*. 2020;40(5):2895-2903.

19. Cavanaugh-Hussey MW, Mu EW, Kang S, Balch CM, Wang T. Older age is associated with a higher incidence of melanoma death but a lower incidence of sentinel lymph node metastasis in the SEER databases (2003-2011). *Ann Surg Oncol*. 2015;22(7):2120-2126.

20. Grotz TE, Puig CA, Perkins S, Ballman K, Hieken TJ. Management of regional lymph nodes in the elderly melanoma patient: Patient selection, accuracy and prognostic implications. *Eur J Surg Oncol*. 2015;41(1):157-164.

21. Ciocan D, Barbe C, Aubin F, et al. Distinctive features of melanoma and its management in elderly patients: a population-based study in France. *JAMA Dermatol*. 2013;149(10):1150-1157.

22. Rees MJ, Liao H, Spillane J, et al. Melanoma in the very elderly, management in patients 85 years of age and over. *J Geriatr Oncol*. 2018;9(5):488-493.

23. Rees MJ, Liao H, Spillane J, et al. Localized melanoma in older patients, the impact of increasing age and comorbid medical conditions. *Eur J Surg Oncol*. 2016;42(9):1359-66

24. Sabel MS, Kozminski D, Griffith K, Chang AE, Johnson TM, Wong S. Sentinel lymph node biopsy use among melanoma patients 75 years of age and older. *Ann Surg Oncol*. 2015;22(7):2112-2119.

25. Howell AV, Gebregziabher M, Thiers BH, et al. Immune checkpoint inhibitors retain effectiveness in older patients with cutaneous metastatic melanoma. *J Geriatr Oncol*. 2021;12(3):394-401.

26. De Luca R, Meraviglia S, Blasi L, Maiorana A, Cicero G. Nivolumab in metastatic melanoma: Good efficacy and tolerability in elderly patients. *Curr Oncol*. 2020;27(2):e75-e80.

27. Archibald WJ, Victor AI, Strawderman MS, Maggiore RJ. Immune checkpoint inhibitors in older adults with melanoma or cutaneous malignancies: The Wilmot Cancer Institute experience. *J Geriatr Oncol*. 2020;11(3):496-502.

28. Rai P, Shen C, Kolodney J, Kelly KM, Scott VG, Sambamoorthi U. Prevalence and risk factors for multimorbidity in older US patients with late-stage melanoma. *J Geriatr Oncol*. 2021;12(3):388-393

29. Rai P, Shen C, Kolodney J, Kelly KM, Scott VG, Sambamoorthi U. Immune checkpoint inhibitor use, multimorbidity and healthcare expenditures among older adults with late-stage melanoma. *Immunotherapy*. 2021;13(2):103-112.

30. Uren RF, Howman-Giles RB, Shaw HM, Thompson JF, McCarthy WH. Lymphoscintigraphy in high-risk melanoma of the trunk: Predicting draining node groups, defining lymphatic channels and locating the sentinel node. *J Nucl Med*. 1993;34(9):1435-1440.

31. Thompson JF, Uren RF, Shaw HM, et al. Location of sentinel lymph nodes in patients with cutaneous melanoma: New insights into lymphatic anatomy. *J Am Coll Surg*. 1999;189(2):195-204.

32. Reynolds HM, Dunbar PR, Uren RF, Blackett SA, Thompson JF, Smith NP. Three-dimensional visualisation of lymphatic drainage patterns in patients with cutaneous melanoma. *Lancet Oncol*. 2007;8(9):806-812.

33. Thompson JF, Uren RF. Lymphatic mapping in management of patients with primary cutaneous melanoma. *Lancet Oncol*. 2005;6(11):877-885.

34. El Sharouni M-A, Witkamp AJ, Sigurdsson V, van Diest PJ. Trends in Sentinel Lymph Node Biopsy Enactment for Cutaneous Melanoma. *Ann Surg Oncol*. 2019;26(5):1494-1502.

35. Bafounta ML, Beauchet A, Chagnon S, Saiag P. Ultrasonography or palpation for detection of melanoma nodal invasion: A meta-analysis. *Lancet Oncol*. 2004;5(11):673-680.

36. Hayes AJ, Moskovic E, O'Meara K, et al. Prospective cohort study of ultrasound surveillance of regional lymph nodes in patients with intermediate-risk cutaneous melanoma. *Br J Surg*. 2019;106(6):729-734.

37. Azzola MF, Shaw HM, Thompson JF, et al. Tumor mitotic rate is a more powerful prognostic indicator than ulceration in patients with primary cutaneous melanoma: An analysis of 3661 patients from a single center. *Cancer*. 2003;97(6):1488-1498.

38. Francken AB, Shaw HM, Thompson JF, et al. The prognostic importance of tumor mitotic rate confirmed in 1317 patients with primary cutaneous melanoma and long follow-up. *Ann Surg Oncol*. 2004;11(4):426-433.

39. Thompson JF, Soong S-J, Balch CM, et al. Prognostic significance of mitotic rate in localized primary cutaneous melanoma: An analysis of patients in the multi-institutional American Joint Committee on Cancer melanoma staging database. *J Clin Oncol*. 2011;29(16):2199-2205.

40. Wat H, Senthilselvan A, Salopek TG. A retrospective, multicenter analysis of the predictive value of mitotic rate for sentinel lymph node (SLN) positivity in thin melanomas. *J Am Acad Dermatol*. 2016;74(1):94-101.

41. Mandalà M, Galli F, Cattaneo L, et al. Mitotic rate correlates with sentinel lymph node status and outcome in cutaneous melanoma greater than 1 millimeter in thickness: A multi-institutional study of 1524 cases. *J Am Acad Dermatol*. 2017;76(2):264-273.e2.

42. Barnhill RL, Katzen J, Spatz A, Fine J, Berwick M. The importance of mitotic rate as a prognostic factor for localized cutaneous melanoma. *J Cutan Pathol*. 2005;32(4):268-273.

43. Strouse JJ, Fears TR, Tucker MA, Wayne AS. Pediatric melanoma: Risk factor and survival analysis of the surveillance, epidemiology and end results database. *J Clin Oncol*. 2005;23(21):4735-4741.

44. Lorimer PD, White RL, Walsh K, et al. Pediatric and adolescent melanoma: A National Cancer Data Base update. *Ann Surg Oncol*. 2016;23(12):4058-4066.

45. Lange JR, Palis BE, Chang DC, Soong S-J, Balch CM. Melanoma in children and teenagers: an analysis of patients from the National Cancer Data Base. *J Clin Oncol*. 2007;25(11):1363-1368.

46. Paradela S, Fonseca E, Pita-Fernández S, et al. Prognostic factors for melanoma in children and adolescents: A clinicopathologic, single-center study of 137 Patients. *Cancer*. 2010;116(18):4334-4344.

47. Cordoro KM, Gupta D, Frieden IJ, McCalmont T, Kashani-Sabet M. Pediatric melanoma: Results of a large cohort study and proposal for modified ABCD detection criteria for children. *J Am Acad Dermatol*. 2013;68(6):913-925.

48. Balch CM, Soong S, Gershenwald JE, et al. Age as a prognostic factor in patients with localized melanoma and regional metastases. *Ann Surg Oncol*. 2013;20(12):3961-3968.

49. Hawryluk EB, Moustafa D, Bartenstein D, et al. A retrospective multicenter study of fatal pediatric melanoma. *J Am Acad Dermatol*. 2020;83(5):1274-1281.

50. Geoerger B, Kang HJ, Yalon-Oren M, et al. Pembrolizumab in paediatric patients with advanced melanoma or a PD-L1-positive, advanced, relapsed, or refractory solid tumour or lymphoma (KEYNOTE-051): Interim analysis of an open-label, single-arm, phase 1-2 trial. *Lancet Oncol*. 2020;21(1):121-133.

51. Gershenwald JE, Scolyer RA, Hess KR, et al. Melanoma staging: Evidence-based changes in the American Joint Committee on Cancer eighth edition cancer staging manual. *CA Cancer J Clin*. 2017;67(6):472-492.

52. Gershenwald JE, Scolyer RA. Melanoma Staging: American Joint Committee on Cancer (AJCC) 8th edition and beyond. *Ann Surg Oncol*. 2018;25(8):2105-2110.

53. Sreeraman Kumar R, Thapa R, Kim Y, Khushalani NI, Sondak VK, Reed DR. Higher than reported adolescent and young adult clinical trial enrollment during the "Golden Age" of melanoma clinical trials. *Cancer Med*. 2018;7(4):991-996.

54. Morton DL, Thompson JF, Cochran AJ, et al. Final trial report of sentinel-node biopsy versus nodal observation in melanoma. *N Engl J Med*. 2014;370(7):599-609.
55. Helgadottir H, Höiom V, Tuominen R, et al. Germline CDKN2A mutation status and survival in familial melanoma cases. *J Natl Cancer Inst*. 2016;108(11):djw135.
56. van der Rhee JI, Krijnen P, Gruis NA, et al. Clinical and histologic characteristics of malignant melanoma in families with a germline mutation in CDKN2A. *J Am Acad Dermatol*. 2011;65(2):281-288.
57. Dalmasso B, Pastorino L, Ciccarese G, et al. CDKN2A germline mutations are not associated with poor survival in an Italian cohort of melanoma patients. *J Am Acad Dermatol*. 2019;80(5):1263-1271.
58. Staaf J, Harbst K, Lauss M, et al. Primary melanoma tumors from CDKN2A mutation carriers do not belong to a distinct molecular subclass. *J Invest Dermatol*. 2014;134(12):3000-3003.
59. Sargen MR, Calista D, Elder DE, et al. Histologic features of melanoma associated with germline mutations of CDKN2A, CDK4, and POT1 in melanoma-prone families from the United States, Italy, and Spain. *J Am Acad Dermatol*. 2020;83(3):860-869.
60. Zebary A, Omholt K, van Doorn R, et al. Somatic BRAF and NRAS mutations in familial melanomas with known germline CDKN2A status: A GenoMEL study. *J Invest Dermatol*. 2014;134(1):287-290.
61. Helgadottir H, Tuominen R, Olsson H, Hansson J, Höiom V. Cancer risks and survival in patients with multiple primary melanomas: Association with family history of melanoma and germline CDKN2A mutation status. *J Am Acad Dermatol*. 2017;77(5):893-901.
62. Aoude LG, Bonazzi VF, Brosda S, et al. Pathogenic germline variants are associated with poor survival in stage III/IV melanoma patients. *Sci Rep*. 2020;10(1):17687.
63. Verver D, van Klaveren D, Franke V, et al. Development and validation of a nomogram to predict recurrence and melanoma-specific mortality in patients with negative sentinel lymph nodes. *Br J Surg*. 2019;106(3):217-225.
64. Altman DG, Royston P. What do we mean by validating a prognostic model? *Stat Med*. 2000;19(4):453-473.
65. El Sharouni MA, Ahmed T, Witkamp AJ, et al. Predicting recurrence in patients with sentinel node-negative melanoma: Validation of the EORTC nomogram using population-based data. *Br J Surg*. 2021;108(5):550-553
66. Fonseca IB, Lindote MVN, Monteiro MR, et al. Sentinel node status is the most important prognostic information for Clinical Stage IIB and IIC melanoma patients. *Ann Surg Oncol*. 2020;27(11):4133-4140.
67. Mann GJ, Pupo GM, Campain AE, et al. BRAF mutation, NRAS mutation, and the absence of an immune-related expressed gene profile predict poor outcome in patients with stage III melanoma. *J Invest Dermatol*. 2013;133(2):509-517.
68. Rozeman EA, Hoefsmit EP, Reijers ILM, et al. Survival and biomarker analyses from the OpACIN-neo and OpACIN neoadjuvant immunotherapy trials in stage III melanoma. *Nat Med*. 2021;27(2):256-263.
69. Tonella L, Pala V, Ponti R, et al. Prognostic and predictive biomarkers in stage III melanoma: Current insights and clinical implications. *Int J Mol Sci*. 2021;22(9).
70. Huber V, Vallacchi V, Fleming V, et al. Tumor-derived microRNAs induce myeloid suppressor cells and predict immunotherapy resistance in melanoma. *J Clin Invest*. 2018;128(12):5505-5516.
71. Lucci A, Hall CS, Patel SP, et al. Circulating tumor cells and early relapse in node-positive melanoma. *Clin Cancer Res*. 2020;26(8):1886-1895.

72. Tanemura A, Terando AM, Sim M-S, et al. CpG island methylator phenotype predicts progression of malignant melanoma. *Clin Cancer Res*. 2009;15(5):1801-1807.

73. Caddle LB, Hasham MG, Schott WH, Shirley B-J, Mills KD. Homologous recombination is necessary for normal lymphocyte development. *Mol Cell Biol*. 2008;28(7):2295-2303.

74. Bednarski JJ, Sleckman BP. At the intersection of DNA damage and immune responses. *Nat Rev Immunol*. 2019;19(4):231-242.

75. Bredemeyer AL, Helmink BA, Innes CL, et al. DNA double-strand breaks activate a multi-functional genetic program in developing lymphocytes. *Nature*. 2008;456(7223):819-823.

76. DeLeon TT, Almquist DR, Kipp BR, et al. Assessment of clinical outcomes with immune checkpoint inhibitor therapy in melanoma patients with CDKN2A and TP53 pathogenic mutations. *PloS One*. 2020;15(3):e0230306.

77. Helgadottir H, Ghiorzo P, van Doorn R, et al. Efficacy of novel immunotherapy regimens in patients with metastatic melanoma with germline CDKN2A mutations. *J Med Genet*. 2020;57(5):316-321.

78. Amaral T, Schulze M, Sinnberg T, et al. Are pathogenic germline variants in metastatic melanoma associated with resistance to combined immunotherapy? *Cancers*. 2020;12(5).

79. Horn S, Leonardelli S, Sucker A, Schadendorf D, Griewank KG, Paschen A. Tumor CDKN2A-associated JAK2 loss and susceptibility to immunotherapy resistance. *J Natl Cancer Inst*. 2018;110(6):677-681.

80. Friedman C, Lyon M, Torphy RJ, et al. A nomogram to predict node positivity in patients with thin melanomas helps inform shared patient decision making. *J Surg Oncol*. 2019;120(7):1276-1283.

81. Bertolli E, de Macedo MP, Calsavara VF, Pinto CAL, Duprat Neto JP. A nomogram to identify high-risk melanoma patients with a negative sentinel lymph node biopsy. *J Am Acad Dermatol*. 2019;80(3):722-726.

82. Lo SN, Ma J, Scolyer RA, et al. Improved risk prediction calculator for sentinel node positivity in patients with melanoma: The Melanoma Institute Australia nomogram. *J Clin Oncol*. 2020;38(24):2719-2727.

83. Maurichi A, Miceli R, Eriksson H, et al. Factors affecting sentinel node metastasis in thin (T1) cutaneous melanomas: development and external validation of a predictive nomogram. *J Clin Oncol*. 2020;38(14):1591-1601.

84. Hanna AN, Sinnamon AJ, Roses RE, et al. Relationship between age and likelihood of lymph node metastases in patients with intermediate thickness melanoma (1.01-4.00 mm): A National Cancer Database study. *J Am Acad Dermatol*. 2019;80(2):433-440.

85. Kai AC, Richards T, Coleman A, Mallipeddi R, Barlow R, Craythorne EE. Five-year recurrence rate of lentigo maligna after treatment with imiquimod. *Br J Dermatol*. 2016;174(1):165-168.

86. Sinnamon AJ, Neuwirth MG, Yalamanchi P, et al. Association between patient age and lymph node positivity in thin melanoma. *JAMA Dermatol*. 2017;153(9):866.

87. Wiener M, Acland KM, Shaw HM, et al. Sentinel node positive melanoma patients: prediction and prognostic significance of nonsentinel node metastases and development of a survival tree model. *Ann Surg Oncol*. 2010;17(8):1995-2005.

8