Prognostic factors in distinct melanoma types

N.A. Ipenburg

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Norbert Ipenburg

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ISBN: 978-94-6458-061-7
Cover design: Publiss | www.publiss.nl
Lay-out: Publiss | www.publiss.nl
Print: Ridderprint | www.ridderprint.nl

Financial support for the printing of this thesis was kindly provided by: Huidstichting Chanfleury van IJsselsteijn

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Proefschrift

ter verkrijging van
de graad van doctor aan de Universiteit Leiden,
op gezag van rector magnificus prof. dr. ir. H. Bijl,

volgens besluit van het college voor promoties
te verdedigen op woensdag 2 maart 2022 klokke 11.15 uur

door

Norbertus Anton Ipenburg

geboren te Kampen

in 1988

Promotores

Prof. dr. M.H. Vermeer

Prof. dr. O.E. Nieweg (University of Sydney)

Co-promotor

Dr. R. van Doorn

Leden promotiecommissie

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Prof. dr. M.W. Bekkenk (Amsterdam Universitair Medische Centra)

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CHAPTER 1

General introduction

EPIDEMIOLOGY

Cutaneous melanoma is a malignant tumor of melanocytes, the pigment producing cells residing in the skin. Melanomas also occur in other sites, such as the eye, meninges and mucosa. Over two hundred years ago, the first case of cutaneous melanoma was described.[1,2] In recent decades, the incidence of melanoma has increased across the globe.[3–5] In 2018, 6709 individuals were diagnosed with melanoma in the Netherlands and almost 800 died of the disease.[6]

Individual risk factors for developing melanoma encompass host factors and environmental factors. The most important environmental cause of melanoma is sun exposure. Ultraviolet radiation causes DNA damage. More than 90% of melanomas are attributed to sun exposure.[7–9] Especially intermittent sun exposure, such as sunbathing, is associated with an increased melanoma risk.[10,11] Individuals with large congenital nevi, dysplastic nevi or a high number of melanocytic nevi are at increased risk of developing melanoma.[10,12–15] Other host factors that are associated with an increased melanoma risk are fair skin, red hair, old age, history of skin cancer, and a family history of melanoma.[16–19]

CLINICAL DIAGNOSIS AND DIAGNOSTIC EXCISIONAL BIOPSY

Melanoma most often presents as a new or changing pigmented skin lesion (Figure 1). Several aspects of the lesion are assessed by the dermatologist. Lesions that are different from the other pigmented lesions in the patient, also called ugly duckling sign, should raise suspicion for melanoma.[20] The ABCDE criteria (**A**symmetry, **B**order irregularity, **C**olor variation, **D**iameter > 6mm, **E**volving) are frequently used to evaluate suspicious pigmented lesions with the naked eye.[21] Dermoscopy is essential in the clinical diagnosis of melanoma (Figure 2). It is more accurate than visual inspection alone.[22] The clinical and dermoscopic appearance varies between melanoma subtypes. Superficial spreading melanoma, nodular melanoma, lentigo maligna melanoma, and acral lentiginous melanoma are the four main histological subtypes of melanoma.[21] Nodular melanomas are in general more difficult to detect and have more aggressive characteristics.[21,23]

Excisional biopsy with narrow (1-3mm) margins is the recommended initial management for suspicious pigmented skin lesions.[24–26] However, melanomas are frequently diagnosed by partial biopsy, such as punch, shave or incisional biopsy.[27] In Australia, more than 25% of all melanomas is diagnosed by partial biopsy.[28]

Figure 1. Clinical picture of cutaneous melanoma.

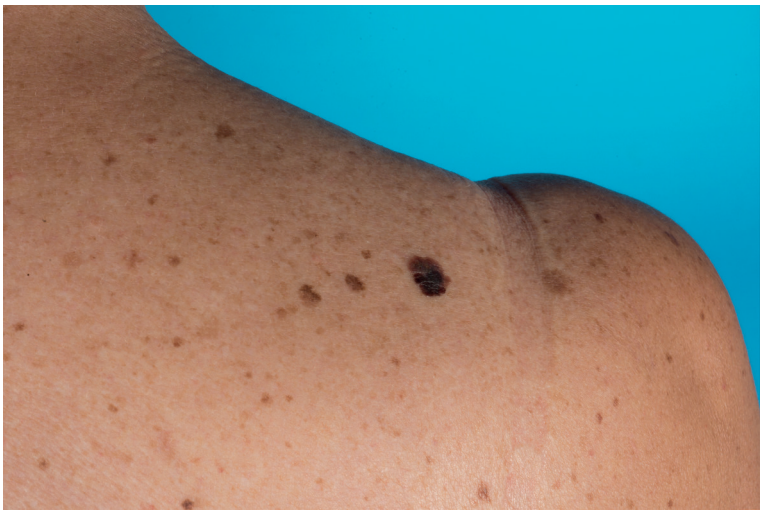
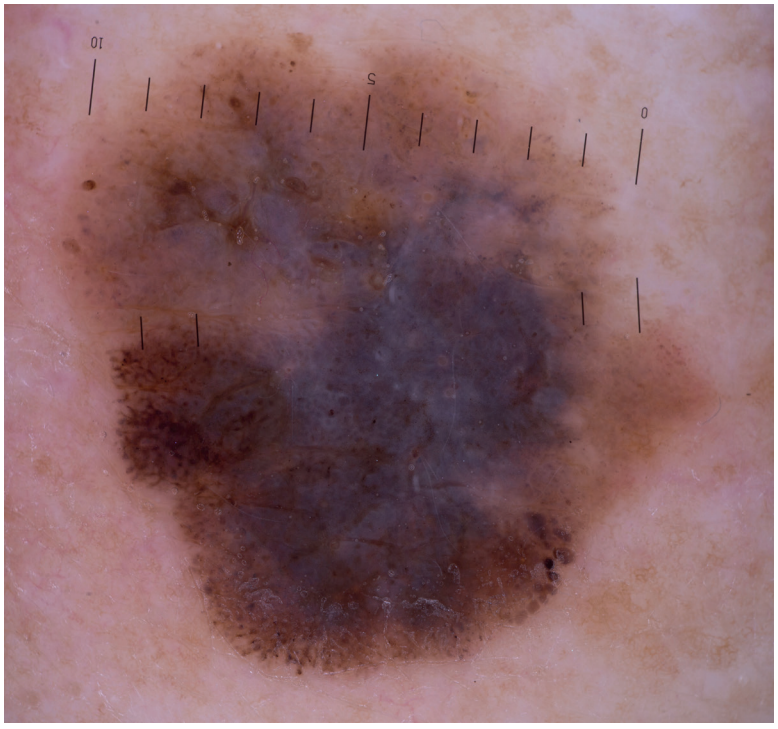


Figure 2. Dermoscopy of cutaneous melanoma.



Histopathological misdiagnosis is more common for melanocytic lesions assessed with partial than with excisional biopsy.[29] Partial biopsies are associated with several pitfalls. Sampling of only the benign part of the lesion might result in misdiagnosis. Partial biopsy of a melanocytic nevus may result in regenerative changes that overlap with the histological features of melanoma. This can lead to overdiagnosis of melanoma. Tumor implantation and inaccurate assessment of important pathological features, such as Breslow thickness, are other potential problems.[28,29]

HISTOPATHOLOGY AND STAGING

Histopathological tumor characteristics are assessed by the (dermato)pathologist on the excisional biopsy specimen. These characteristics are essential in the staging process. All patients are staged using the American Joint Committee on Cancer/Union Internationale Contre le Cancer (AJCC/UICC) melanoma staging classification (Table 1 and 2).[30] Management decisions and prognostic information are derived from this classification.

The thickness of the primary melanoma is an important prognostic feature of a clinically localized melanoma.[31–33] It was first described by Alexander Breslow in 1970 and is therefore also known as Breslow thickness.[34] Tumor thickness is measured from the top of the granular layer of the epidermis to the deepest malignant cells invading the dermis. Initially a cut off thickness of ≤ 0.75mm was used to define thin melanomas with a good prognosis. In the 6th and 7th editions of the AJCC melanoma staging classification, melanomas with a tumor thickness of ≤ 1.0mm were classified as thin.[35,36] A recent study showed that 0.8mm is a clinically important cut-off.[37] This is reflected in the most recent 8th edition of the AJCC/UICC melanoma staging classification.[30]

Ulceration has been part of the melanoma staging system for decades.[30,35,36] Ulcerated tumors have a higher risk of disease recurrence and melanoma-related death.[31,33,38] Although not part of the staging classification, the extent of ulceration is of prognostic significance. Extensively ulcerated melanomas have a worse outcome than minimally ulcerated tumors.[39]

Allen and Spitz were the first to describe the poorer survival of patients having a primary melanoma with many mitoses.[40] Tumor mitotic rate has since been validated as an independent prognostic factor in numerous studies.[41–46] It was incorporated in the 7th edition of the AJCC staging classification but has been removed as a staging parameter in the most recent staging system.[30,36]

Table 1. TNM staging categories.[30]

Tumor (T)	**Tumor thickness**	**Ulceration**
T1a	< 0.8mm	Without ulceration
T1b	< 0.8mm	With ulceration
	0.8 – 1.0mm	With or without ulceration
T2a	>1.0 – 2.0mm	Without ulceration
T2b	>1.0 – 2.0mm	With ulceration
T3a	>2.0 – 4.0mm	Without ulceration
T3b	>2.0 – 4.0mm	With ulceration
T4a	> 4.0mm	Without ulceration
T4b	> 4.0mm	With ulceration
Node (N)	**No. of tumor involved regional lymph nodes**	**Type of metastasis***
N0	0	
N1a	1	Clinically occult
N1b	1	Clinically detected
N1c	0	In-transit, satellite and/or microsatellite metastasis
N2a	2-3	Clinically occult
N2b	2-3	Clinically detected
N2c	1	In-transit, satellite and/or microsatellite metastasis
N3a	≥ 4	Clinically occult
N3b	≥ 4	Clinically detected
N3c	≥ 2	In-transit, satellite and/or microsatellite metastasis
Metastasis (M)	**Site**	
M0	No distant metastasis	
M1a	Skin, soft tissue and/or nonregional lymph node	
M1b	Lung	
M1c	Non-CNS visceral sites	
M1d	CNS	

*Clinically occult lymph node metastases are detected by sentinel node biopsy and without clinical or radiographic evidence of regional lymph node metastasis. Clinically detected nodal metastases are identified by clinical, radiographic or ultrasound examination.

Table 2. AJCC clinical and pathological prognostic stage groups (8th edition).[30]

	Clinical stage				Pathological stage		
	T	**N**	**M**		**T**	**N**	**M**
IA	T1a	N0	M0	IA	T1a	N0	M0
					T1b	N0	M0
IB	T1b	N0	M0	IB	T2a	N0	M0
	T2a	N0	M0				
IIA	T2b	N0	M0	IIA	T2b	N0	M0
	T3a	N0	M0		T3a	N0	M0
IIB	T3b	N0	M0	IIB	T3b	N0	M0
	T4a	N0	M0		T4a	N0	M0
IIC	T4b	N0	M0	IIC	T4b	N0	M0
III	Any T	≥ N1	M0	IIIA	T1-T2a	N1a or N2a	M0
				IIIB	T1-T2a	N1b/c or N2b	M0
					T2b/T3a	N1a-N2b	M0
				IIIC	T1a-T3a	N2c or N3	M0
					T3b/T4a	≥ N1	M0
					T4b	N1a-N2c	M0
				IIID	T4b	N3	M0
IV	Any T	Any N	M1	IV	Any T	Any N	M1

WIDE LOCAL EXCISION AND SENTINEL NODE BIOPSY

If an invasive primary cutaneous melanoma is diagnosed, wide local excision (WLE) of the lesion or biopsy site is indicated to reduce the risk of local recurrence. WLE surgical margins depend on tumor thickness.[24–26] The recommended safety margins are 1cm for melanomas with tumor thickness < 2mm and 2cm for thicker melanomas.[24]

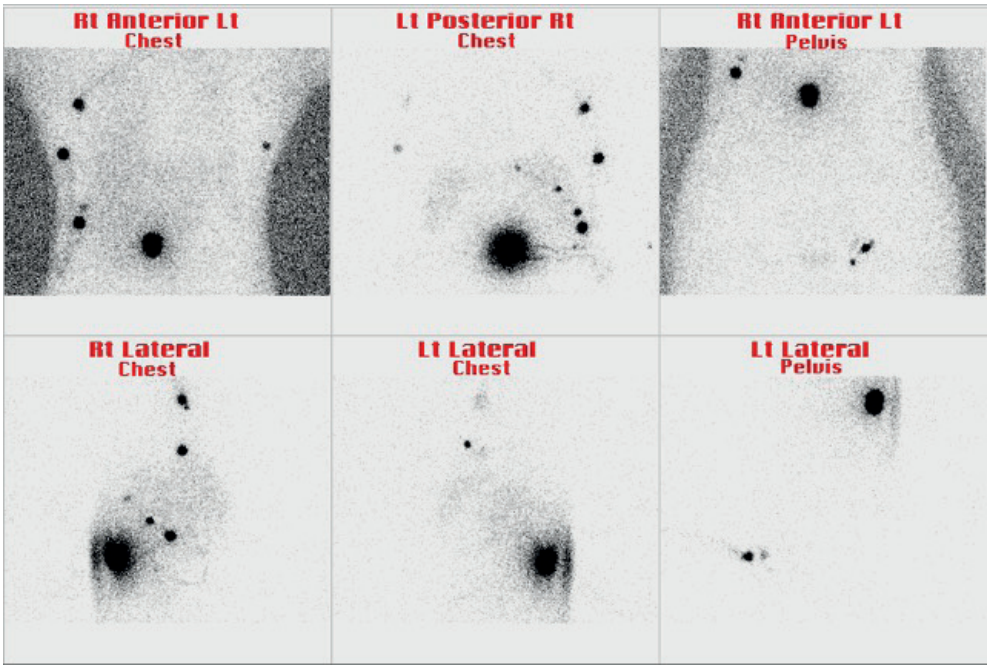
For many years, excision of the primary tumor was often combined with prophylactic regional lymph node dissection.[47] Since only 20% of the clinically localized melanoma patients has involved lymph nodes, many patients could not have any benefit from such a procedure. Prophylactic lymph node dissections were abandoned after studies showed that routine use of this procedure did not improve survival.[48–51]

In 1992, the sentinel node (SN) concept was introduced by Morton and Cochran.[52] A SN is defined as any node on a direct lymphatic drainage pathway from the primary tumor.[53] Multiple drainage pathways and thus multiple SNs can be present in one patient.[54–57] Sentinel node biopsy (SNB) can establish the tumor-status of the entire regional lymph node field.[58,59] Only patients with an involved SN underwent immediate removal of the remaining regional

lymph nodes, the so-called completion lymph-node dissection (CLND). Before the introduction of this procedure in melanoma, the term *sentinel node* was already mentioned in studies of penile cancer, parotid cancer, testis and omentum.[60–64]

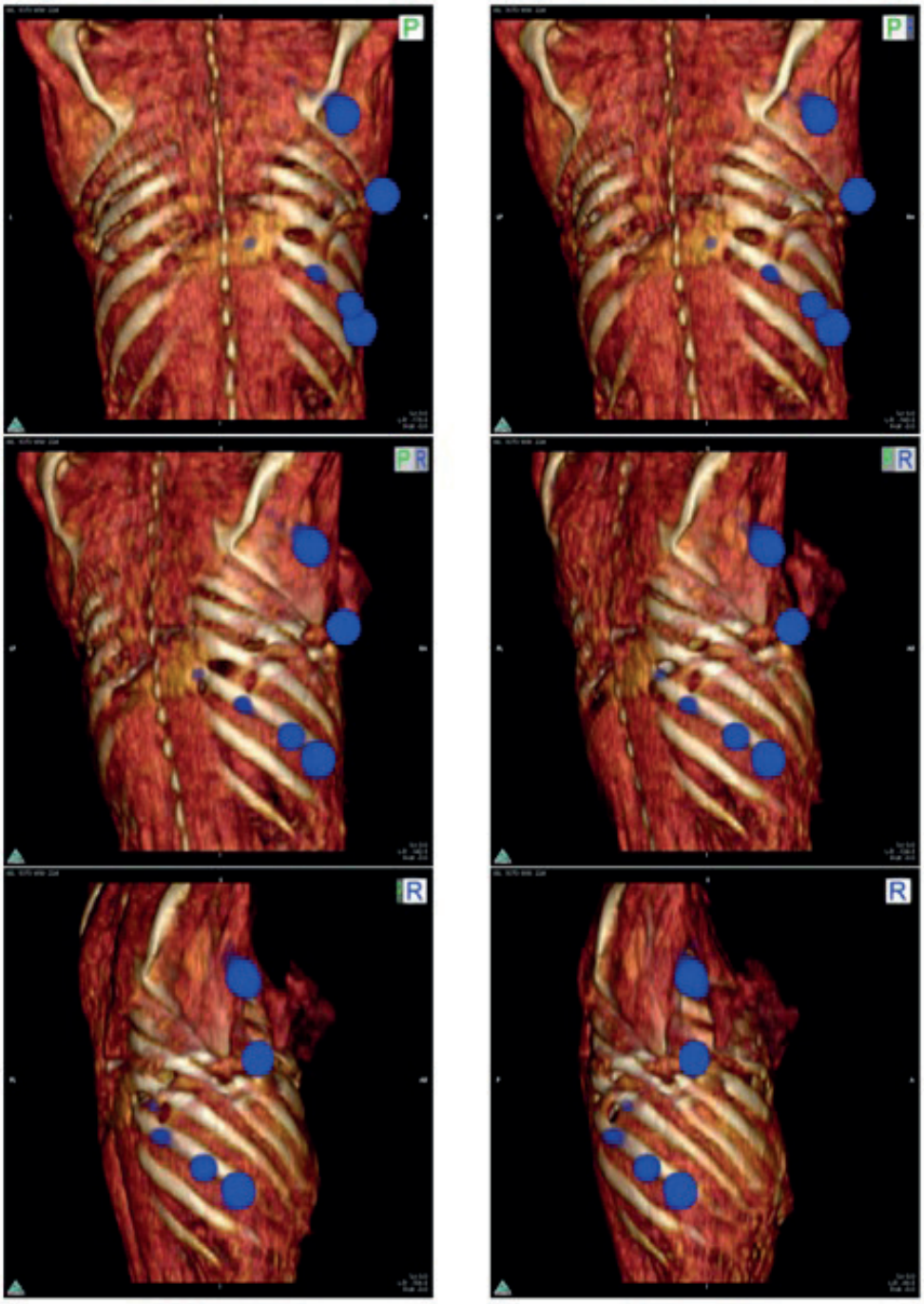
The nuclear medicine physician is of great importance in the identification of these SNs. Technetium-99m colloid is injected at the primary melanoma site. The tracer flows from the primary tumor through the afferent lymph vessel to the lymph nodes. Dynamic and static lymphoscintigraphy visualize the SNs (Figure 3).[65] Single photon emission computed tomography with integrated computerized tomography (SPECT/CT) is added to show the SNs exact anatomical location (Figure 4).[66–69] Non-palpable metastases can be detected by ultrasound (US) after which fine needle biopsy is performed.

Figure 3. Lymphoscintigrams in a patient with a melanoma on the mid back show sentinel nodes in the axillae, on the right chest wall and in the left groin.



The surgeon uses a gamma ray detection probe to locate the SNs. Intra-operatively, patent blue dye is injected intradermally at the primary tumor site. The blue travels the same route as the radiopharmaceutical. The blue-stained afferent lymph vessel can also guide the surgeon to the SNs. Only these lymph nodes are removed and assessed for the presence or absence of metastases.

Figure 4. Single photon emission computed tomography with integrated computerized tomography (SPECT/CT) displays sentinel nodes of melanoma located on the chest.



Histopathological examination is performed on multiple sections stained with hematoxylin and eosin and immunohistochemical markers, such as S100, HMB45, MelanA and SOX10.[70,71]

The SN status is the most important prognostic factor in patients with a clinically localized melanoma.[58,72–75] Patients with a positive SN have a worse prognosis than SN-negative patients. The first Multicenter Selective Lymphadenectomy Trial (MSLT-I) proved the importance of this staging procedure and showed that patients who underwent SNB had fewer recurrences than patients who underwent WLE and nodal observation.[58] SNB combined with CLND also improved melanoma-specific survival (MSS) of patients with an intermediate-thickness melanoma (1.2 – 3.5mm) who had occult nodal metastases.[58] SN-positivity is rare (<5%) in melanomas < 0.8mm in thickness. Melanomas with a tumor thickness of 0.8 – 1.0 mm have a 8 to 12% change of having spread to a SN.[76–79] Therefore, SNB is recommended for patients with a clinically localized melanoma that has a thickness ≥0.8mm or if ulceration is present (T1b or higher).[24,25] SNB seems reliable when performed after WLE, but concomitant WLE and SNB is preferred.[80]

SPECIAL POPULATIONS

Elderly

Elderly people have the highest melanoma incidence and mortality.[81–83] Between 1989 and 2015, the incidence in Dutch men aged ≥ 70 years has increased with more than 500%.[84] Compared to younger patients, primary melanomas of older patients are on average thicker, more often ulcerated and have more dermal mitoses.[38,85,86] Nodular melanomas are also more frequent.[86,87] While their melanomas are more aggressive, the SN-positivity rate is lower in these patients.[38,88,89] Age-related lymphatic dysfunction might be an explanation for this inverse correlation.[90]

Melanoma guidelines are also applicable to elderly patients.[24,25] However, studies show substandard surgical treatment in this group of patients.[87,91] Incisional biopsies and suboptimal excision margins are common.[86,87,91] SNB is less frequently performed in older patients with clinically localized melanoma.[86,91,92] Clinical decision-making in the elderly is complicated by several factors, of which frailty, medical comorbidities and reduced life-expectancy are examples.

Children and adolescents

Pediatric melanoma is arbitrarily defined as melanoma diagnosed below the age of 20 years.[21] It is the most common type of skin cancer in children and adolescents.[93] Pediatric melanoma is frequently associated with pre-existing conditions such as large congenital melanocytic nevi and xeroderma pigmentosum.[94] While within most age groups melanoma incidence has increased, a declining incidence of pediatric melanoma is observed.[3,95–97]

Only 0.1% of the melanoma cases occur in children and adolescents. Due to the rarity, melanoma is often not considered in this age group.[93] The clinical features are also frequently atypical and do not follow the conventional ABCDE criteria.[98,99] Modified ABCD criteria (**A**melanotic, **B**leeding or **B**ump, **C**olor uniformity, **D**e novo and any **D**iameter) have therefore been proposed.[99] Children and adolescents have been excluded from randomized controlled trials studying different aspects of melanoma management.[33,58,100] Currently, adult melanoma guidelines are applied to pediatric melanoma patients. SNB is also performed in pediatric melanoma patients. Paradoxically, pediatric patients have a higher incidence of SN-metastasis but a more favorable survival rate than adults.[38,101,102]

Familial melanoma

Approximately 10% of patients diagnosed with melanoma have a positive family history.[103,104] Genes implicated in familial melanoma include cyclin-dependent kinase inhibitor 2A (*CDKN2A*), cyclin-dependent kinase inhibitor 4 (*CDK4*), BRCA1-associated protein-1 (*BAP1*), protection of telomeres 1 (*POT1*), telomerase reverse transcriptase (*TERT*), ACD shelterin complex subunit and telomerase recruitment factor (*ACD*), telomeric repeat-binding factor 2-interacting protein (*TERF2IP)* and microphthalmia-associated transcription factor (*MITF*).[104–106] Genetic testing is recommended for patients who meet the criteria for familial melanoma, which are defined as the occurrence of three or more melanomas in multiple members of a family, at least two of which are diagnosed in first-degree relatives.[107] Clinical genetic consultation is also advised when two-first degree relatives are diagnosed with melanoma, families in which melanoma and pancreatic cancer are diagnosed, patients with three or more melanomas, patients with melanoma diagnosed before the age of 18 years, patients with multiple BAP1-deficient melanocytic nevi and patients with a combination of melanoma and pancreatic cancer or uveal melanoma.[107] Patients with hereditary breast and ovarian cancer syndrome (*BRCA1* and *BRCA2*), Li-Fraumeni syndrome (*TP53*), xeroderma pigmentosum, and PTEN hamartoma tumor syndromes (*PTEN)* are also at increased risk of developing melanoma.[105] Germline mutations in *CDKN2A* are found in about 20-40% of

melanoma families.[108,109] In the Netherlands, the most prevalent *CDKN2A* germline mutation is the p16-Leiden mutation (c.225-243del19). This specific founder mutation probably originated from an endogamous population.[110,111] The high penetrance gene *CDKN2A* encodes two different tumor suppressor proteins: p16INK4A (p16) and p14ARF (p14). These patients have a life-time melanoma risk of about 70% and frequently at a young age.[104,105,112] *CDKN2A* mutation carriers also have an increased risk of developing pancreatic cancer, head and neck tumors, and lung cancer.[113] Recent studies on survival of *CDKN2A* germline mutation carriers with melanoma showed conflicting results.[114–116] In a Swedish cohort, these melanoma patients had a worse survival than sporadic melanoma patients.[114,115] However, an Italian group found no survival difference.[116]

AREAS OF UNCERTAINTY

Clinically localized melanoma has been extensively studied. However, several clinical questions are still unanswered.

SNB has become a routine staging procedure in patients with clinically localized melanoma. However, SNB may be less attractive in some categories. SNB is sometimes omitted in patients with advanced age, substantial comorbidities or if SNB is likely to be technically challenging. Instead of SNB, preoperative lymphoscintigraphy followed by focused US of the identified SNs is performed at each follow-up visits. It is unknown whether focused US of the lymph nodes is an acceptable alternative for SNB in these special populations.

Due to the rarity of melanoma in children and adolescents, little is known on prognostic factors in these young patients. In adult melanoma patients, tumor mitotic rate is one of the strongest predictors of survival. Previous studies showed that tumor mitotic rate is lower in pediatric melanomas than in other age groups. However, the prognostic significance of mitotic rate in clinically localized pediatric melanoma is uncertain.

The biology of melanoma in familial melanoma patients carrying the *CDKN2A* germline mutation seems to be more aggressive. As mentioned, previous studies showed conflicting results regarding a survival difference between *CDKN2A* mutation carriers and sporadic melanoma patients. The frequency of SN-positivity and its prognostic significance are also uncertain.

Individual prognostic factors can be combined into a prognostic model enabling personalized follow-up and treatment of individual patients. The European Organisation for Research

and Treatment of Cancer (EORTC) built a prognostic model and nomogram for recurrence and melanoma-specific mortality in SN-negative melanoma patients. Currently, it is not known how applicable and accurate this prognostic model is to other populations. External validation is essential to ensure the applicability to other melanoma populations.

AIM AND OUTLINE OF THIS THESIS

This thesis describes prognostic factors and management of special melanoma populations.

Chapter two describes patients who underwent lymphoscintigraphy but did not undergo SNB because of advanced age and/or comorbidities. Instead, they were monitored with focused US of their SNs at each follow-up visit. Survival outcomes of this group were compared to patients who did undergo SNB. The aim of this study was to assess whether lymphoscintigraphy with focused US follow-up of SNs is a reasonable management alternative to SNB in patients who are elderly and/or have substantial comorbidities.

Chapter three concerns a cohort study of patients with clinically localized melanoma in whom the intended SNB was canceled after preoperative lymphoscintigraphy. Demographics and melanoma characteristics of this group were compared to patients in whom SNB was performed. The study in chapter three sought to determine if lymphoscintigraphy with focused US follow-up of SNs is an acceptable alternative for patients in whom a SNB procedure is likely to be challenging.

Chapter four describes children and adolescents diagnosed with melanoma. The aim of the study was to assess the prognostic value of tumor mitotic rate in these young patients.

Chapter five compares the characteristics and survival of *CDKN2A* mutation carriers with sporadic melanoma patients. This study aimed to assess whether presence of a pathogenic *CDKN2A* germline mutation was associated with survival in melanoma patients.

Chapter six reports the characteristics and outcome of hereditary melanoma patients carrying germline *CDKN2A* mutations who underwent SNB. The goal of this study was to assess the frequency and predictive value of SN-positivity in *CDKN2A* mutation carriers.

Chapter seven describes the external validation of a prognostic model, including Breslow thickness, ulceration and primary tumor site, to predict survival of patients with SN-negative melanoma. The secondary aim of the study was to assess whether the prognostic model could be improved by adding other prognostic factors.

REFERENCES

1. Bodenham DC. A study of 650 observed malignant melanomas in the South-West region. *Ann R Coll Surg Engl*. 1968;43(4):218-239.
2. Home E. *Observations on Cancer, Case VIII*.; 1805.
3. Paulson KG, Gupta D, Kim TS, et al. Age-specific incidence of melanoma in the United States. *JAMA Dermatol*. 2020;156(1):57.
4. Arnold M, Holterhues C, Hollestein LM, et al. Trends in incidence and predictions of cutaneous melanoma across Europe up to 2015. *J Eur Acad Dermatol Venereol*. 2014;28(9):1170-1178.
5. Whiteman DC, Green AC, Olsen CM. The growing burden of invasive melanoma: Projections of incidence rates and numbers of new cases in six susceptible populations through 2031. *J Invest Dermatol*. 2016;136(6):1161-1171.
6. Integraal Kankercentrum Nederland. Cijfers over kanker. Accessed March 18, 2020. https://www.cijfersoverkanker.nl.
7. Berwick M, Buller DB, Cust A, et al. Melanoma epidemiology and prevention. *Cancer Treat Res*. 2016;167(6):17-49.
8. Armstrong BK, Kricker A. How much melanoma is caused by sun exposure? *Melanoma Res*. 1993;3(6):395-401.
9. Parkin DM, Mesher D, Sasieni P. 13. Cancers attributable to solar (ultraviolet) radiation exposure in the UK in 2010. *Br J Cancer*. 2011;105 Suppl:S66-9.
10. Gandini S, Sera F, Cattaruzza MS, et al. Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. *Eur J Cancer*. 2005;41(1):45-60.
11. Nelemans PJ, Rampen FHJ, Ruiter DJ, Verbeek ALM. An addition to the controversy on sunlight exposure and melanoma risk: A meta-analytical approach. *J Clin Epidemiol*. 1995;48(11):1331-1342.
12. Bataille V, de Vries E. Melanoma-part 1: epidemiology, risk factors, and prevention. *BMJ*. 2008;337:a2249-a2249.
13. Bauer J, Garbe C. Acquired melanocytic nevi as risk factor for melanoma development. A comprehensive review of epidemiological data. *Pigment Cell Res*. 2003;16(3):297-306.
14. Garbe C, Krüger S, Orfanos CE, et al. Associated factors in the prevalence of more than 50 common melanocytic nevi, atypical melanocytic nevi, and actinic lentigines: Multicenter case-control study of the central malignant melanoma registry of the German dermatological society. *J Invest Dermatol*. 1994;102(5):700-705.
15. Grob JJ, Gouvernet J, Aymar D, et al. Count of benign melanocytic nevi as a major indicator of risk for nonfamilial nodular and superficial spreading melanoma. *Cancer*. 1990;66(2):387-395.
16. Tucker MA, Goldstein AM. Melanoma etiology: Where are we? *Oncogene*. 2003;22(20):3042-3052.
17. Bliss JM, Ford D, Swerdlow AJ, et al. Risk of cutaneous melanoma associated with pigmentation characteristics and freckling: systematic overview of 10 case-control studies. The International Melanoma Analysis Group (IMAGE). *Int J Cancer*. 1995;62(4):367-376.
18. van der Leest RJT, Flohil SC, Arends LR, de Vries E, Nijsten T. Risk of subsequent cutaneous malignancy in patients with prior melanoma: A systematic review and meta-analysis. *J Eur Acad Dermatol Venereol*. 2015;29(6):1053-1062.
19. van der Leest RJT, Hollestein LM, Liu L, Nijsten T, de Vries E. Risks of different skin tumour combinations after a first melanoma, squamous cell carcinoma and basal cell carcinoma in Dutch population-based cohorts: 1989-2009. *J Eur Acad Dermatol Venereol*. 2018;32(3):382-389.

20. Grob JJ. The "ugly duckling" sign: Identification of the common characteristics of nevi in an individual as a basis for melanoma screening. *Arch Dermatol*. 1998;134(1):103-a-104.

21. Garbe C, Amaral T, Peris K, et al. European consensus-based interdisciplinary guideline for melanoma. Part 1: Diagnostics – Update 2019. *Eur J Cancer*. 2020;126:141-158.

22. Dinnes J, Deeks JJ, Chuchu N, et al. Dermoscopy, with and without visual inspection, for diagnosing melanoma in adults. *Cochrane Database Syst Rev*. 2018;12:CD011902.

23. Dessinioti C, Dimou N, Geller AC, et al. Distinct clinicopathological and prognostic features of thin nodular primary melanomas: An international study from 17 centers. *J Natl Cancer Inst*. 2019;111(12):1314-1322.

24. Garbe C, Amaral T, Peris K, et al. European consensus-based interdisciplinary guideline for melanoma. Part 2: Treatment – Update 2019. *Eur J Cancer*. 2020;126:159-177.

25. Swetter SM, Tsao H, Bichakjian CK, et al. Guidelines of care for the management of primary cutaneous melanoma. *J Am Acad Dermatol*. 2019;80(1):208-250.

26. Veerbeek L, Kruit WHJ, de Wilt JHW, Mooi WJ, Bergman W, Multidisciplinaire richtlijnwerkgroep melanoom. [Revision of the national guideline 'Melanoma']. *Ned Tijdschr Geneeskd*. 2013;157(12):A6136.

27. Kelly JW, Henderson M a, Thursfield VJ, Slavin J, Ainslie J, Giles GG. The management of primary cutaneous melanoma in Victoria in 1996 and 2000. *Med J Aust*. 2007;187(9):511-514.

28. Luk PP, Vilain R, Crainic O, McCarthy SW, Thompson JF, Scolyer RA. Punch biopsy of melanoma causing tumour cell implantation: Another peril of utilising partial biopsies for melanocytic tumours. *Australas J Dermatol*. 2015;(November 2014):227-231.

29. Ng JC, Swain S, Dowling JP, Wolfe R, Simpson P, Kelly JW. The impact of partial biopsy on histopathologic diagnosis of cutaneous melanoma: Experience of an Australian tertiary referral service. *Arch Dermatol*. 2010;146(3):234-239.

30. 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.

31. Balch CM, Soong SJ, Gershenwald JE, et al. Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. *J Clin Oncol*. 2001;19(16):3622-3634.

32. Balch CM, Soong S, Ross MI, et al. Long-term results of a multi-institutional randomized trial comparing prognostic factors and surgical results for intermediate thickness melanomas (1.0 to 4.0 mm). Intergroup Melanoma Surgical Trial. *Ann Surg Oncol*. 2000;7(2):87-97.

33. 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.

34. Breslow A. Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. *Ann Surg*. 1970;172(5):902-908.

35. Balch CM, Buzaid AC, Soong S, et al. Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. *J Clin Oncol*. 2001;19(16):3635-3648.

36. Balch CM, Gershenwald JE, Soong SJ, et al. Final version of 2009 AJCC melanoma staging and classification. *J Clin Oncol*. 2009;27(36):6199-6206.

37. Lo SN, Scolyer RA, Thompson JF. Long-term survival of patients with thin (T1) cutaneous melanomas: A Breslow thickness cut point of 0.8 mm separates higher-risk and lower-risk tumors. *Ann Surg Oncol*. 2018;25(4):894-902.

38. 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.
39. In 't Hout FEM, Haydu LE, Murali R, Bonenkamp JJ, Thompson JF, Scolyer RA. Prognostic importance of the extent of ulceration in patients with clinically localized cutaneous melanoma. *Ann Surg*. 2012;255(6):1165-1170.
40. Allen AC, Spitz S. Malignant melanoma. A clinicopathological analysis of the criteria for diagnosis and prognosis. *Cancer*. 1953;6(1):1-45.
41. 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.
42. 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.
43. 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.
44. 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.
45. 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.
46. 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.
47. Neuhaus SJ, Clark MA, Thomas JM. Dr. Herbert Lumley Snow, MD, MRCS (1847-1930): The original champion of elective lymph node dissection in melanoma. *Ann Surg Oncol*. 2004;11(9):875-878.
48. Veronesi U, Adamus J, Bandiera DC, et al. Delayed regional lymph node dissection in stage I melanoma of the skin of the lower extremities. *Cancer*. 1982;49(11):2420-2430.
49. Cascinelli N, Morabito A, Santinami M, MacKie R, Belli F. Immediate or delayed dissection of regional nodes in patients with melanoma of the trunk: A randomised trial. *The Lancet*. 1998;351(9105):793-796.
50. Balch CM, Soong S-J, Bartolucci AA, et al. Efficacy of an elective regional lymph node dissection of 1 to 4 mm thick melanomas for patients 60 years of age and younger. *Ann Surg*. 1996;224(3):255-266.
51. Sim FH, Taylor WF, Pritchard DJ, Soule EH. Lymphadenectomy in the management of stage I malignant melanoma: A prospective randomized study. *Mayo Clin Proc*. 1986;61(9):697-705.
52. Morton DL. Technical details of intraoperative lymphatic mapping for early stage melanoma. *Arch Surg*. 1992;127(4):392.
53. Nieweg OE, Tanis PJ, Kroon BB. The definition of a sentinel node. *Ann Surg Oncol*. 2001;8(6):538-541.
54. Ribero S, Osella-Abate S, Pasquali S, et al. Prognostic role of multiple lymphatic basin drainage in sentinel lymph node-negative trunk melanoma patients: A multicenter study from the Italian melanoma intergroup. *Ann Surg Oncol*. 2016;23(5):1708-1715.

55. Porter GA, Ross MI, Berman RS, Lee JE, Mansfield PF, Gershenwald JE. Significance of multiple nodal basin drainage in truncal melanoma patients undergoing sentinel lymph node biopsy. *Ann Surg Oncol*. 2000;7(4):256-261.
56. Federico AC, Chagpar AB, Ross MI, et al. Effect of multiple-nodal basin drainage on cutaneous melanoma. *Arch Surg*. 2008;143(7):632-637.
57. Ahmadzadehfar H, Hinz T, Wierzbicki A, et al. Significance of multiple nodal basin drainage in patients with truncal melanoma. *Q J Nucl Med Mol Imaging* 2016;60(3):274-279.
58. 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.
59. 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.
60. Cabanas RM. An approach for the treatment of penile carcinoma. *Cancer*. 1977;39(2):456-466.
61. Sayegh E, Brooks T, Sacher E, Busch F. Lymphangiography of the retroperitoneal lymph nodes through the inguinal route. *J Urol*. 1966;95(1):102-107.
62. Gould EA, Winship T, Philbin PH, Kerr HH. Observations on a "sentinel node" in cancer of the parotid. *Cancer*. 1960;13(1):77-78.
63. Braithwaite LR. The flow of lymph from the ileocæcal angle, and its possible bearing on the cause of duodenal and gastric ulcer. *Br J Surg*. 1923;11(41):7-26.
64. Nieweg OE, Uren RF, Thompson JF. The history of sentinel lymph node biopsy. *Cancer J*. 2015;21(1):3-6.
65. Uren RF, Howman-Giles R, Chung D, Thompson JF. Guidelines for lymphoscintigraphy and F18 FDG PET scans in melanoma. *J Surg Oncol*. 2011;104(4):405-419.
66. Vermeeren L, Valdés Olmos RA, Klop WMC, et al. SPECT/CT for sentinel lymph node mapping in head and neck melanoma. *Head Neck*. 2011;33(1):1-6.
67. Vermeeren L, van der Ploeg IMC, Olmos RAV, et al. SPECT/CT for preoperative sentinel node localization. *J Surg Oncol*. 2010;101(2):184-190.
68. van der Ploeg IMC, Valdés Olmos RA, Kroon BBR, et al. The yield of SPECT/CT for anatomical lymphatic mapping in patients with melanoma. *Ann Surg Oncol*. 2009;16(6):1537-1542.
69. van der Ploeg IMC, Nieweg OE, Kroon BBR, et al. The yield of SPECT/CT for anatomical lymphatic mapping in patients with breast cancer. *Eur J Nucl Med Mol Imaging*. 2009;36(6):903-909.
70. Rawson R V., Scolyer RA. From Breslow to BRAF and immunotherapy: Evolving concepts in melanoma pathogenesis and disease progression and their implications for changing management over the last 50 years. *Hum Pathol*. 2020;95:149-160.
71. Scolyer RA, Murali R, McCarthy SW, Thompson JF. Pathologic examination of sentinel lymph nodes from melanoma patients. *Semin Diagn Pathol*. 2008;25(2):100-111.
72. Cascinelli N, Belli F, Santinami M, et al. Sentinel lymph node biopsy in cutaneous melanoma: the WHO melanoma program experience. *Ann Surg Oncol*. 2000;7(6):469-474.
73. Thompson JF, Scolyer RA, Kefford RF. Cutaneous melanoma. *The Lancet*. 2005;365(9460):687-701.
74. Morton DL, Thompson JF, Essner R, et al. Validation of the accuracy of intraoperative lymphatic mapping and sentinel lymphadenectomy for early-stage melanoma. *Ann Surg*. 1999;230(4):453.
75. Gershenwald JE, Thompson W, Mansfield PF, et al. Multi-institutional melanoma lymphatic mapping experience: The prognostic value of sentinel lymph node status in 612 stage I or II melanoma patients. *J Clin Oncol*. 1999;17(3):976-976.

76. Murali R, Haydu LE, Quinn MJ, et al. Sentinel lymph node biopsy in patients with thin primary cutaneous melanoma. *Ann Surg*. 2012;255(1):128-133.
77. Andtbacka RHI, Gershenwald JE. Role of sentinel lymph node biopsy in patients with thin melanoma. *J Natl Compr Canc Netw*. 2009;7(3):308-317.
78. Cordeiro E, Gervais M-K, Shah PS, Look Hong NJ, Wright FC. Sentinel lymph node biopsy in thin cutaneous melanoma: A systematic review and meta-analysis. *Ann Surg Oncol*. 2016;23(13):4178-4188.
79. Han D, Zager JS, Shyr Y, et al. Clinicopathologic predictors of sentinel lymph node metastasis in thin melanoma. *J Clin Oncol*. 2013;31(35):4387-4393.
80. Gannon CJ, Rousseau DL, Ross MI, et al. Accuracy of lymphatic mapping and sentinel lymph node biopsy after previous wide local excision in patients with primary melanoma. *Cancer*. 2006;107(11):2647-2652.
81. Tsai S, Balch C, Lange J. Epidemiology and treatment of melanoma in elderly patients. *Nat Rev Clin Oncol*. 2010;7(3):148-152.
82. 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.
83. Kruijff S, Bastiaannet E, Francken AB, Schaapveld M, van der Aa M, Hoekstra HJ. Breslow thickness in the Netherlands: A population-based study of 40 880 patients comparing young and elderly patients. *Br J Cancer*. 2012;107(3):570-574.
84. Schuurman MS, Hollestein LM, Bastiaannet E, et al. Melanoma in older patients: Declining gap in survival between younger and older patients with melanoma. *Acta Oncol*. 2020;59(1):4-12.
85. Lasithiotakis K, Leiter U, Meier F, et al. Age and gender are significant independent predictors of survival in primary cutaneous melanoma. *Cancer*. 2008;112(8):1795-1804.
86. 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.
87. 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.
88. Chao C, Martin RCG, Ross MI, et al. Correlation between prognostic factors and increasing age in melanoma. *Ann Surg Oncol*. 2004;11(3):259-264.
89. 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.
90. Conway WC, Faries MB, Nicholl MB, et al. Age-related lymphatic dysfunction in melanoma patients. *Ann Surg Oncol*. 2009;16(6):1548-1552.
91. 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):1359-1366.
92. 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.
93. de Vries E, Steliarova-Foucher E, Spatz A, Ardanaz E, Eggermont AMM, Coebergh JWW. Skin cancer incidence and survival in European children and adolescents (1978-1997). Report from the Automated Childhood Cancer Information System project. *Eur J Cancer*. 2006;42(13):2170-2182.
94. Pappo AS. Melanoma in children and adolescents. *Eur J Cancer*. 2003;39(18):2651-2661.
95. Campbell LB, Kreicher KL, Gittleman HR, Strodtbeck K, Barnholtz-Sloan J, Bordeaux JS. Melanoma incidence in children and adolescents: Decreasing trends in the united states. *J Pediatr*. 2015;166(6):1505-1513.

96. Barr RD, Ries LAG, Lewis DR, et al. Incidence and incidence trends of the most frequent cancers in adolescent and young adult Americans, including "nonmalignant/noninvasive" tumors. *Cancer*. 2016;122(7):1000-1008.

97. Siegel DA, King J, Tai E, Buchanan N, Ajani UA, Li J. Cancer incidence rates and trends among children and adolescents in the United States, 2001-2009. *Pediatrics*. 2014;134(4):e945-55.

98. Ferrari A, Bono A, Baldi M, et al. Does melanoma behave differently in younger children than in adults? A retrospective study of 33 cases of childhood melanoma from a single institution. *Pediatrics*. 2005;115(3):649-654.

99. 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.

100. Thomas JM, Newton-Bishop J, A'Hern R, et al. Excision margins in high-risk malignant melanoma. *N Engl J Med*. 2004;350(8):757-766.

101. 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.

102. Livestro DP, Kaine EM, Michaelson JS, et al. Melanoma in the young: Differences and similarities with adult melanoma: A case-matched controlled analysis. *Cancer*. 2007;110(3):614-624.

103. Kibbi N, Kluger H, Choi JN. Melanoma: Clinical Presentations. In: *Cancer Treatment and Research*. Vol 167. Kluwer Academic Publishers; 2016:107-129.

104. Read J, Wadt KAW, Hayward NK. Melanoma genetics. *J Med Genet*. 2016;53(1):1-14.

105. Ransohoff KJ, Jaju PD, Tang JY, Carbone M, Leachman S, Sarin KY. Familial skin cancer syndromes Increased melanoma risk. *J Am Acad Dermatol*. 2016;74(3):423-434.

106. Potjer TP, Bollen S, Grimbergen AJEM, et al. Multigene panel sequencing of established and candidate melanoma susceptibility genes in a large cohort of Dutch non-CDKN2A/CDK4 melanoma families. *Int J Cancer*. 2019;144(10):2453-2464.

107. Halk AB, Potjer TP, Kukutsch NA, Vasen HFA, Hes FJ, van Doorn R. Surveillance for familial melanoma: Recommendations from a national centre of expertise. *Br J Dermatol*. 2019;181(3):594-596.

108. Goldstein AM, Chan M, Harland M, et al. Features associated with germline CDKN2A mutations: a GenoMEL study of melanoma-prone families from three continents. *J Med Genet*. 2006;44(2):99-106.

109. Kefford RF, Newton Bishop JA, Bergman W, Tucker MA. Counseling and DNA testing for individuals perceived to be genetically predisposed to melanoma: a consensus statement of the Melanoma Genetics Consortium. *J Clin Oncol*. 1999;17(10):3245-3251.

110. Bergman W, Gruis NA, Frants RR. The Dutch FAMMM family material: Clinical and genetic data. *Cytogenet Cell Genet*. 1992;59(2-3):161-164.

111. Gruis NA, van der Velden PA, Sandkuijl LA, et al. Homozygotes for CDKN2 (p16) germline mutation in Dutch familial melanoma kindreds. *Nat Genet*. 1995;10(3):351-353.

112. 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).

113. Potjer TP, Kranenburg HE, Bergman W, et al. Prospective risk of cancer and the influence of tobacco use in carriers of the p16-Leiden germline variant. *Eur J Hum Genet*. 2015;23(5):711-714.

114. 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.

115. 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.

116. 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.

CHAPTER 2

Focused ultrasound surveillance of lymph nodes following lymphoscintigraphy without sentinel node biopsy; a useful and safe strategy in elderly or frail melanoma patients

Norbertus A. Ipenburg
John F. Thompson
Roger F. Uren
David Chung
Omgo E. Nieweg

Annals of Surgical Oncology. 2019 Sep;26(9):2855-2863

ABSTRACT

Background. Sentinel node (SN) biopsy (SNB) has become standard of care in clinically localized melanoma patients. Although it is minimally invasive, advanced age and/or comorbidities may render SNB inadvisable in some patients. Focused ultrasound follow-up of SNs identified by preoperative lymphoscintigraphy may be an alternative in these patients. This study examines the outcomes in patients managed in this way at a major melanoma treatment center.

Methods. All patients with clinically localized cutaneous melanoma who underwent lymphoscintigraphy and in whom SNB was intentionally not performed due to advanced age and/or comorbidities were included.

Results. Between 2000 and 2009, 160 patients (5.2% of the total) underwent lymphoscintigraphy without SNB because of advanced age and/or comorbidities. Compared with the 2945 patients who had a SNB, the 160 patients were older, had thicker melanomas that were more often located in the head and neck region, and had more SNs in more nodal regions. Of the 160 patients, 150 (94%) were followed with ultrasound examination of their SNs at each follow-up visit; this identified 33% of the nodal recurrences before they became clinically apparent. Compared with SN-positive patients who were treated by completion lymph node dissection, observed patients who developed nodal recurrence had more involved nodes when a delayed lymphadenectomy was performed. Melanoma-specific survival, recurrence-free survival, and distant recurrence- free survival rates were similar, while regional lymph node-free survival was worse.

Conclusions. Lymphoscintigraphy with focused ultrasound follow-up of SNs is a reasonable management alternative to SNB in patients who are elderly and/or have substantial comorbidities.

INTRODUCTION

Sentinel node (SN) biopsy (SNB) is a routine procedure in patients with clinically localized primary cutaneous melanoma. It offers prognostic and staging information and prolongs survival in SN-positive patients with intermedi- ate-thickness melanomas.[1,2] Recent studies have demonstrated that adjuvant immunotherapy and targeted therapy improve survival of stage III patients, including those with minimal nodal involvement. These findings further increase the significance of SNB.[3–5] However, the procedure may be considered inappropriate in some patients, for various reasons. Elderly patients, for example, have a significantly reduced risk of nodal involvement and a higher risk of complications.[6–8] The drawbacks may also outweigh the benefits in patients with substantial comorbidities. At Melanoma Institute Australia (MIA), SNB is sometimes intentionally avoided in such patients. Lymphoscintigraphy is still performed and the location of each SN is marked on the overlying skin with a minute tattoo spot. These nodes are then examined and followed with focused high-resolution ultrasonography (US). This strategy is not known to be practiced elsewhere on a regular basis. The aim of this study was to assess our experience with this approach. Specific matters to be assessed were the prevalence of omitting the SNB, the reason(s), characteristics of these patients, the follow-up strategy, the stage of the disease at the time of a regional node field recurrence, and the ways in which these metastases were detected and managed. Survival was compared with that of patients who did undergo SNB.

PATIENTS AND METHODS

Patients

The database of MIA, which contains prospectively collected information, was queried for all patients with clinically localized cutaneous melanoma who underwent SNB between November 2000 and December 2009 (SNB patients) and all patients in whom lymphoscintigraphy and US were performed but in whom SNB was intentionally not scheduled due to advanced age and/or comorbidities (observed patients). Patients were excluded if they had melanoma in situ, multiple primary melanomas (micro)satellites or in-transit metastases, if preoperative ultrasound revealed nodal metastasis, if no SN was identified intraoperatively, if wide local excision had been performed before lymphoscintigraphy, or if SNB had been performed elsewhere. The study was approved by the MIA Research Committee. Written informed consent was obtained from all patients.

Lymphoscintigraphy and sentinel node biopsy

A SN was defined as a node on a direct lymphatic drainage pathway from the primary tumor.[9] SNB was offered to patients with clinically localized melanoma with a Breslow thickness ≥1 mm, or for melanomas <1 mm if adverse features were present, such as young age, ulceration of the primary tumor, Clark level IV or V invasion, or a tumor mitotic rate ≥1. Details of the lymphatic mapping and SNB techniques used at MIA have been described previously.[10] In short, preoperative dynamic and static lymphoscintigraphy were performed. Since 2008, single photon emission computed tomography with integrated computerized tomography (SPECT/CT) has been routinely performed. The location of each SN was marked on the skin with a pin-point permanent tattoo. Patent blue dye and a gamma ray detection probe were used for intraoperative detection of the identified SNs. SNs were serially sectioned and were examined using S100 and HMB-45 immunohistochemistry[11] Completion lymph node dissection (CLND) was typically performed in patients with an involved SN, unless they participated in a study (MSLT-II) and were randomized to observation of the nodal region.[12]

Follow-up

In patients who were observed, focused high-resolution US of the marked SN basin was performed at each follow-up visit. Lymph nodes were considered to be abnormal if focal low-level internal echoes were present in the cortex of the node or the node had become rounded in shape with the hilum displaced to the side or completely obliterated by low-level internal echoes.[13] Subcapsular thickening of > 2.5 mm over a section of the node was also considered abnormal. Fine needle aspiration biopsy was performed in patients with nodes that were considered to be suspicious for metastasis on US assessment. Follow-up was every 4 months for the first 2 years, every 6 months for the next 3 years, and annually thereafter.

Statistical analysis

Baseline characteristics of patients in the observation and SNB groups were compared. Comparison of continuous variables was performed using the Mann–Whitney *U* test, and values of categorical variables were compared using the Pearson's Chi-square test or Fisher's exact test, as appropriate. Melanoma-specific survival (MSS) was calculated from the date of diagnosis to the date of melanoma-related death. Censoring for MSS occurred at the date of death from non-melanoma cause or at the end of follow-up, whichever came first. The event of interest was first recurrence for recurrence-free survival (RFS), first distant recurrence for distant RFS (DRFS), and first regional node recurrence for regional lymph

node-free survival (RLNFS). Kaplan–Meier curves were created and covariates were compared using the log-rank test. Type of management was the variable of interest in this study. To adjust for potential confounders, known prognostic factors (sex, age, primary tumor site, Breslow thickness, tumor mitotic rate, and ulceration) were added to the multivariable Cox proportional hazards models.[14–18] To increase the validity of the predictions outside the studied cohort, stepwise methods were not used and full models were built.[19] The proportional hazards assumption was checked for all included variables. P-values were two-sided and were considered statistically significant if <0.05. Statistical analyses were performed using SPSS 25.0 software for Mac (IBM Corporation, Armonk, NY, USA).

RESULTS

Cohort characteristics

Between 2000 and 2009, 2945 patients with clinically localized cutaneous melanoma underwent SNB and 160 patients (5.2% of the total) underwent lymphoscintigraphy and US, but not SNB because of advanced age and/or comorbidities. Table 1 shows the clinical and pathology characteristics of all patients. Observed patients were older (median 81 vs. 58 years, $P < 0.001$) than SNB patients. The youngest observed patient was 26 years of age and the oldest 95 years. Fourteen patients (9%) were < 65 years of age. Morbid obesity, cardiovascular disease, pulmonary embolism, schizophrenia, aplastic anemia with thrombocytopenia, penile malignancy with radiotherapy to both groins, pregnancy, rheumatoid arthritis, and wheelchair-bound multiple sclerosis were the reasons for not scheduling the SNB in these patients. Compared with SNB patients, melanomas of observed patients were significantly thicker (median 2.5 vs. 1.8 mm; $P < 0.001$), had a higher tumor mitotic rate (median 4 vs. 3/mm^2; $P = 0.002$), and were more frequently located in the head and neck region (34% vs. 16%; $P < 0.001$). In observed patients, lymphoscintigraphy revealed drainage to more nodal regions ($P = 0.004$) and more SNs ($P = 0.04$).

Table 1. Clinicopathologic characteristics of patients in this study.

Characteristic	**Observation (n=160)**	**SNB (n=2945)**	**P-value**
Gender			0.82#
Male	97 (60.6)	1758 (59.7)	
Female	63 (39.4)	1187 (40.3)	
Age (years)			<0.001§
< 65	14 (8.8)	1967 (66.8)	
65 – 74	18 (11.3)	585 (19.9)	
75 – 84	81 (50.6)	351 (11.9)	
≥ 85	47 (29.4)	42 (1.4)	
Median (interquartile range)	81 (76-86)	58 (46.5-69.5)	
Melanoma location			<0.001#
Head and neck	55 (34.4)	465 (15.8)	
Upper limb	48 (30.0)	773 (26.2)	
Lower limb	20 (12.5)	740 (25.1)	
Trunk	37 (23.1)	967 (32.8)	
Breslow thickness (mm)			<0.001§
0 – 1	9 (5.6)	424 (14.4)	
1.01 – 2	55 (34.4)	1283 (43.6)	
2.01 – 4	51 (31.9)	836 (28.4)	
> 4	45 (28.1)	394 (13.4)	
Missing	0 (0)	8 (0.3)	
Median (interquartile range)	2.5 (1.1-3.9)	1.8 (0.95-2.65)	
Tumor mitotic rate/mm²			0.002§
0	10 (6.3)	290 (9.8)	
≥1	141 (88.1)	2519 (85.5)	
Missing	9 (5.6)	136 (4.6)	
Median (interquartile range)	4 (0.5-7.5)	3 (1-5)	
Ulceration			0.060#
Absent	98 (61.3)	2047 (69.5)	
Present	49 (30.6)	730 (24.8)	
Missing	13 (8.1)	168 (5.7)	
Tumor type			<0.001*
Superficial spreading melanoma	39 (24.4)	1264 (42.9)	
Nodular melanoma	64 (40.0)	935 (31.7)	
Acral lentiginous melanoma	3 (1.9)	48 (1.6)	
Lentigo maligna melanoma	13 (8.1)	49 (1.7)	
Desmoplastic melanoma	22 (13.8)	268 (9.1)	
Other	0 (0)	12 (0.4)	
Missing	19 (11.9)	369 (12.5)	

Clark level			<0.001*
II	5 (3.1)	49 (1.7)	
III	32 (20.0)	784 (26.6)	
IV	87 (54.4)	1847 (62.7)	
V	28 (17.5)	223 (7.6)	
Missing	8 (5.0)	42 (1.4)	
No. of drainage sites			0.004*
0	1 (0.6)	0 (0.0)	
1	110 (68.8)	2281 (77.5)	
2	46 (28.7)	565 (19.2)	
3	3 (1.9)	82 (2.8)	
4	0 (0)	14 (0.5)	
Missing	0 (0)	3 (0.1)	
Drainage site of identified SNs			<0.001*
Axilla	69 (43.1)	1453 (49.3)	
Groin	20 (12.5)	789 (26.8)	
Neck	62 (38.8)	618 (21.0)	
Popliteal	1 (0.6)	16 (0.5)	
Other	7 (4.4)	66 (2.2)	
Missing	1 (0.6)	3 (0.1)	
No. of SNs identified on lymphoscintigram			0.04*
0	1 (0.6)	1 (0)	
1	34 (21.3)	809 (27.5)	
2	56 (35.0)	984 (33.4)	
≥3	69 (43.1)	1131 (38.4)	
Missing	0 (0)	20 (0.7)	

Data are expressed as n (%) unless otherwise specified
Pearson's Chi-square
* Fisher's exact test
§ Mann-Whitney *U* test

Survival

The median follow-up duration was 42 months (interquartile range 15 – 96 months). Of the 160 observed patients, 150 (94%) were followed with high-resolution US of their SNs at each follow-up visit. Of the remaining 10 patients, four were followed with only periodic physical examination of their lymph node fields, and six were lost to follow-up. The site of first recurrence differed between the observed and SNB patients (P = 0.03), with regional nodal recurrence being more common in the observed group (11% vs. 4%), while distant metastasis was more frequently seen in the SNB group (6% vs. 4%) (Table 2). SNB patients had significantly better RFS and RLNFS on univariable analysis (Table 3). MSS and DRFS

were similar in the two groups (Figure 1). After adjusting for all major prognostic factors, the multivariable analyses showed a superior RLNFS [observation group hazard ratio (HR) = 2.0; 95% confidence interval (CI) 1.2 - 3.3]. MSS (observation group HR = 0.9, 95% CI 0.6 – 1.6), RFS (observation group HR = 1.1, 95% CI 0.7 - 1.5), and DRFS (observation group HR = 0.9, 95% CI 0.5 – 1.5) were not significantly different between the two groups (Appendix 1 and 2).

Table 2. Characteristics regarding recurrence and treatment of patients

Characteristic	**Observation (n=160)**	**SNB (n=2945)**	**P-value**
SN status			
Negative	NA	2531 (85.9)	NA
Positive	NA	404 (13.7)	
Missing	NA	10 (0.3)	
CLND			
Performed	NA	316 (10.7)	NA
Not performed	NA	2629 (89.3)	
Site of first recurrence			0.03*
Local	7 (4.4)	103 (3.5)	
In-transit	3 (1.9)	94 (3.2)	
Regional nodal	17 (10.6)	131 (4.4)	
Distant	6 (3.8)	163 (5.5)	
Multiple sites	4 (2.5)	110 (3.7)	
No. of metastatic nodes			
Mean (SD)	2.9 (2.7)	1.7 (1.7)	0.02§

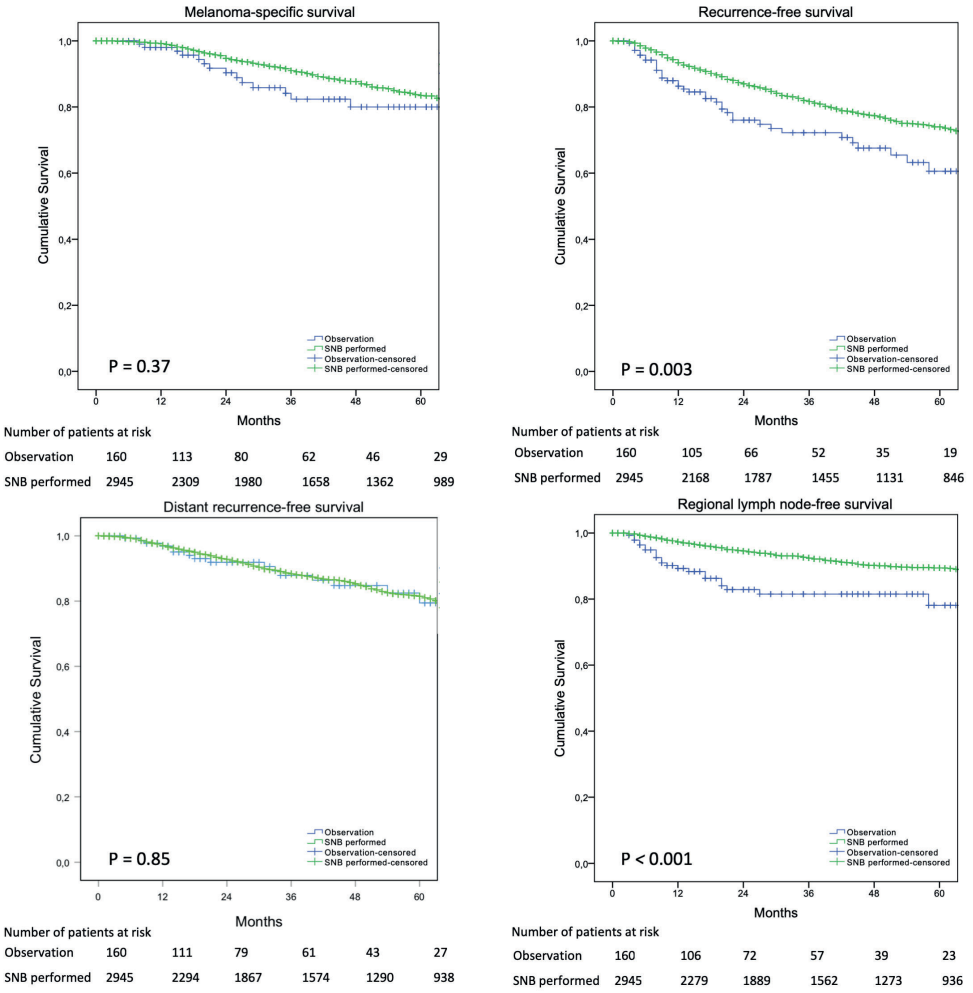
Data are expressed as n (%) unless otherwise specified
NA not applicable, SD standard deviation

* Fisher's exact test
§ Mann-Whitney *U* test

Table 3. Results of univariable survival analysis.

Variable	**5-year melanoma-specific survival**	**5-year recurrence-free survival**	**5-year regional lymph node-free survival**	**5-year distant recurrence-free survival**
Management				
Observation (%)	80	61	79	79
SNB (%)	84	74	90	82
P-value	0.37	0.003	<0.001	0.85

Figure 1. Melanoma-specific, recurrence-free, distant recurrence-free and regional lymph node-free survival according to type of management.



Immediate lymphadenectomy versus delayed lymphadenectomy

Twenty-one patients (13%) developed a recurrence in a node field that was being observed. US detected these nodal recurrences in seven patients (33%), CT in three (14%), four (19%) were detected at physical examination by a doctor and the remaining seven patients (33%) noticed the recurrence themselves. The nodal recurrence was directly underneath the tattoo in seven patients (33%). Two of the seven patients in whom the nodal recurrence was detected by US were found to have synchronous distant metastasis.

Fourteen of the 21 patients (66%) underwent therapeutic CLND. Limited local node excision with adjuvant radiotherapy was performed in one patient with cervical lymph node metastases. Widespread distant metastatic disease was the reason for not performing nodal surgery in two patients, two patients declined an operation, one patient died within 1 month after diagnosis of the regional nodal recurrence, and in one elderly patient with rapidly progressing disease and a recent deep venous thrombosis, surgery was considered inappropriate. The mean number of metastatic nodes in those patients who underwent therapeutic CLND was higher than in those patients who underwent immediate CLND because of an involved SN (2.9 vs. 1.7; $P = 0.02$).

DISCUSSION

Surgical decision making in elderly and frail patients is often complex and occasionally the risks of a staging procedure outweigh the benefits. Increasing incidence and mortality rates of elderly melanoma patients emphasize the importance of an adequate management strategy for this group of patients.[20,21] The present study shows that focused US follow-up after lymphoscintigraphy proved to be an acceptable approach in elderly or frail patients in whom it has been decided to avoid SNB. It allows early diagnosis of nodal metastases, albeit not as early as with SNB, and does not jeopardize MSS, RFS or DRFS.

Previous research has demonstrated that SNB is readily able to be performed in the older population, and, in the majority of elderly patients, the SN is in fact procured.[7,22–26] In our study, 75% of patients aged 75 years or older underwent the procedure, and it was still performed in 47% of those aged 85 years and over. The emergence of effective adjuvant systemic treatment in node-positive patients makes SNB an even more important staging tool, although the effectiveness of drug therapy in frail patients is currently uncertain since only patients with an Eastern Cooperative Oncology Group (ECOG) performance status <2 were included in the trials that have been performed.[3–5] Not performing SNB impedes access to adjuvant systemic therapy. Still, it is unclear whether adjuvant therapy improves melanoma-specific survival more than systemic therapy after a recurrence is detected. SNB is already therapeutic in a large proportion of node positive patients.[1,12]

While SNB is an important staging tool, only 13% of the observed patients developed a regional nodal recurrence and the other 87% would not have benefited from the procedure. Other reasons to be more restrained when considering SNB in the elderly population are the overall higher risk of operative and postoperative morbidity, the lower rate of nodal involvement, and the higher false negative rate of the procedure.[6–8,27,28] Although SNB is a

minor and fairly superficial procedure away from the vital organs and carries little morbidity, general anesthesia is typically used.[29,30] Performing SNB under local anesthesia may be technically feasible but this is not common practice in most centers.[31] Some earlier studies have shown a correlation between comorbidity or performance status and the decision to perform SNB, while others have not.[22,25,26] In our study, comorbid conditions were the reason for not performing the procedure in all patients < 65 years of age in whom SNB was omitted. A heterogeneous group of conditions was identified, varying from psychiatric ailments to bleeding disorders and cardiovascular conditions.

The current study is unique in that lymphoscintigraphy was performed in all patients, despite the fact that SNB was intentionally not scheduled, followed by focused US of the identified lymph nodes at each visit. The exact location of the SNs was marked on the skin with a permanent tattoo spot, allowing accurate repeated assessment. High-resolution US was performed at each follow-up visit in 94% of the observed patients. Depending on the drainage region, US is able to pick up metastatic nodes that are two to three times smaller than can be detected by physical examination.[32] For the majority of patients, focused US did not add to the follow-up in an impactful way in our study. Most regional lymph node metastases were not detected by focused US. In only one-third of patients was US able to identify nodal metastases before they became otherwise apparent.

Although observed patients were considered unfit for SNB, 66% of the observed patients with a regional nodal recurrence still underwent therapeutic CLND. Recently, we showed that excision of clinically positive metastatic cervical lymph nodes followed by radiotherapy is a reasonable alternative for therapeutic CLND in frail patients.[33] This new approach was used for one of the three observed patients with cervical macrometastasis. As shown previously, observed patients who developed nodal macrometastasis and underwent regional node dissection had significantly more involved nodes compared to SN-positive patients who had CLND.[1] Even though previous research has shown that survival correlates inversely with number of involved nodes, MSS of the observed and SNB group did not differ significantly in the present study, possibly due to small numbers.[12] There are several limitations affecting this study. For instance, ECOG performance status was not formally assessed and recorded for all patients. Other limitations were the retrospective design, selection bias and short follow-up for some patients.

CONCLUSIONS

Omission of SNB due to advanced age and/or comorbidities occurred in 5.2% of patients in whom the procedure would generally have been considered appropriate. In comparison with patients who underwent SNB, these patients were older and had more advanced melanomas that were more often located in the head and neck region. The MSS, RFS, and DRFS rates were similar in the two groups, while RLNFS was worse in observed patients. Lymphoscintigraphy with focused US follow-up of identified SNs thus appears to be a reasonable management strategy to avoid SNB in patients who are elderly or have substantial comorbidities.

REFERENCES

1. 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.
2. Wong SL, Balch CM, Hurley P, et al. Sentinel lymph node biopsy for melanoma: American Society of Clinical Oncology and Society of Surgical Oncology joint clinical practice guideline. *J Clin Oncol*. 2012;30(23):2912-2918.
3. 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.
4. 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.
5. Long G V, 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.
6. Sondak VK, Taylor JMG, Sabel MS, et al. Mitotic rate and younger age are predictors of sentinel lymph node positivity: lessons learned from the generation of a probabilistic model. *Ann Surg Oncol*. 2004;11(3):247-258.
7. 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.
8. Balch CM, Thompson JF, Gershenwald JE, et al. Age as a predictor of sentinel node metastasis among patients with localized melanoma: An inverse correlation of melanoma mortality and incidence of sentinel node metastasis among young and old patients. *Ann Surg Oncol*. 2014;21(4):1075-1081.
9. Nieweg OE, Tanis PJ, Kroon BB. The definition of a sentinel node. *Ann Surg Oncol*. 2001;8(6):538-541.
10. Uren RF, Howman-Giles R, Chung D, Thompson JF. Guidelines for lymphoscintigraphy and F18 FDG PET scans in melanoma. *J Surg Oncol*. 2011;104(4):405-419.
11. Scolyer RA, Murali R, McCarthy SW, Thompson JF. Pathologic examination of sentinel lymph nodes from melanoma patients. *Semin Diagn Pathol*. 2008;25(2):100-111.
12. 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.
13. Uren RF, Howman-Giles R, Thompson JF, et al. High-resolution ultrasound to diagnose melanoma metastases in patients with clinically palpable lymph nodes. *Australas Radiol*. 1999;43(2):148-152.
14. Balch CM, Gershenwald JE, Soong SJ, et al. Final version of 2009 AJCC melanoma staging and classification. *J Clin Oncol*. 2009;27(36):6199-6206.
15. 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.
16. 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.
17. Joosse A, Collette S, Suciu S, et al. Superior outcome of women with stage I/II cutaneous melanoma: pooled analysis of four European Organisation for Research and Treatment of Cancer phase III trials. *J Clin Oncol*. 2012;30(18):2240-2247.
18. Balch CM, Soong SJ, Gershenwald JE, et al. Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. *J Clin Oncol*. 2001;19(16):3622-3634.

19. Steyerberg EW. Selection of main effects. In: *Clinical Prediction Models*. Springer Science+Business Media; 2009:191-210.
20. Jemal A, Saraiya M, Patel P, et al. Recent trends in cutaneous melanoma incidence and death rates in the United States, 1992-2006. *J Am Acad Dermatol*. 2011;65(5 Suppl 1):S17-25.e1-3.
21. Linos E, Swetter SM, Cockburn MG, Colditz GA, Clarke CA. Increasing burden of melanoma in the United States. *J Invest Dermatol*. 2009;129(7):1666-1674.
22. 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.
23. 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.
24. 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.
25. 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.
26. 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.
27. Scoggins CR, Martin RCG, Ross MI, et al. Factors associated with false-negative sentinel lymph node biopsy in melanoma patients. *Ann Surg Oncol*. 2010;17(3):709-717.
28. Sinnamon AJ, Neuwirth MG, Bartlett EK, et al. Predictors of false negative sentinel lymph node biopsy in trunk and extremity melanoma. *J Surg Oncol*. 2017;116(7):848-855.
29. Morton DL, Cochran AJ, Thompson JF, et al. Sentinel node biopsy for early-stage melanoma: Accuracy and morbidity in MSLT-I, an international multicenter trial. *Ann Surg*. 2005;242(3):302-313.
30. Moody JA, Ali RF, Carbone AC, Singh S, Hardwicke JT. Complications of sentinel lymph node biopsy for melanoma – A systematic review of the literature. *Eur J Surg Oncol*. 2017;43(2):270-277.
31. Stoffels I, Dissemond J, Körber A, et al. Reliability and cost-effectiveness of sentinel lymph node excision under local anaesthesia versus general anaesthesia for malignant melanoma: a retrospective analysis in 300 patients with malignant melanoma AJCC Stages I and II. *J Eur Acad Dermatol Venereol*. 2011;25(3):306-310.
32. 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.
33. Kroon HM, van der Bol WD, Tonks KT, Hong AM, Hruby G, Thompson JF. Treatment of clinically positive cervical lymph nodes by limited local node excision and adjuvant radiotherapy in melanoma patients with major comorbidities. *Ann Surg Oncol*. 2018;25(12):3476-3482.

Appendix 1. Cox multivariable analysis of melanoma-specific survival and recurrence-free survival.

		Melanoma-specific survival			**Recurrence-free survival**		
Factor	**Value**	HR	95% CI	P-value	HR	95% CI	P-value
Management*	Observation	0.94	0.56-1.60	0.83	1.07	0.74-1.54	0.73
Gender	Male	1.42	1.12-1.80	0.004	1.25	1.05-1.49	0.01
Age	/year	1.01	0.997-1.01	0.20	1.01	1.00-1.01	0.004
Melanoma location (reference: head and neck)	Upper limb	0.72	0.52-0.99	0.045	0.81	0.64-1.03	0.09
	Lower limb	0.84	0.61-1.15	0.27	0.98	0.78-1.25	0.89
	Trunk	0.94	0.70-1.14	0.65	0.72	0.57-0.91	0.005
Breslow thickness (reference: 0 – 1 mm)	1.01 – 2 mm	1.66	0.88-3.12	0.12	1.42	0.95-2.13	0.09
	2.01 – 4 mm	3.14	1.67-5.89	<0.001	2.46	1.63-3.69	<0.001
	> 4 mm	5.38	2.83-10.24	<0.001	3.69	2.42-5.64	<0.001
Tumor mitotic rate (reference: 0)	≥1	2.91	1.36-6.22	0.006	1.98	1.26-3.13	0.003
Ulceration	Present	1.71	1.37-2.13	<0.001	1.62	1.36-1.92	<0.001

* sentinel node biopsy versus observation

Appendix 2. Cox multivariable analysis of regional lymph node-free survival and distant recurrence-free survival.

		Regional lymph node-free survival			**Distant recurrence-free survival**		
Factor	**Value**	HR	95% CI	P-value	HR	95% CI	P-value
Management*	Observation	1.99	1.21-3.29	0.007	0.88	0.53-1.46	0.61
Gender	Male	1.59	1.19-2.13	0.002	1.31	1.06-1.62	0.01
Age	/year	1.01	1.00-1.02	0.09	1.00	0.99-1.01	0.67
Melanoma location (reference: head and neck)	Upper limb	1.20	0.79-1.80	0.39	0.70	0.52-0.94	0.02
	Lower limb	1.83	1.23-2.71	0.003	0.79	0.59-1.05	0.11
	Trunk	0.79	0.52-1.20	0.27	0.84	0.64-1.09	0.19
Breslow thickness (reference: 0 – 1 mm)	1.01 – 2 mm	1.93	0.92-4.03	0.08	1.59	0.95-2.67	0.08
	2.01 – 4 mm	2.62	1.24-5.50	0.01	3.01	1.80-5.05	<0.001
	> 4 mm	2.99	1.38-6.48	0.006	4.56	2.67-7.79	<0.001
Tumor mitotic rate (reference: 0)	≥1	5.00	1.59-15.77	0.006	1.84	1.07-3.17	0.03
Ulceration	Present	1.87	1.41-2.47	<0.001	1.76	1.44-2.16	<0.001

* sentinel node biopsy versus observation

CHAPTER 3

Outcome of melanoma patients who did not proceed to sentinel node biopsy after preoperative lymphoscintigraphy

Norbertus A. Ipenburg
Omgo E. Nieweg
Roger F. Uren
John F. Thompson

Annals of Surgical Oncology. 2017 Jan;24(1):117-126

ABSTRACT

Background. At our institution, a planned sentinel node biopsy (SNB) procedure is occasionally canceled after preoperative lymphoscintigraphy. This study reports the frequency of this, the reasons, and the management and outcomes of these patients.

Methods. All patients with clinically localized cutaneous melanoma treated at Melanoma Institute Australia between 2000 and 2009 whose planned SNB procedure was not undertaken after lymphoscintigraphy were included in this retrospective study.

Results. Of the 3148 patients in whom the procedure had been planned, 203 patients (6.4 %) did not have a SNB. The main reason for not proceeding with SNB (in 84 % of cases) was the lymphoscintigraphic demonstration of multiple drainage fields and/or multiple sentinel nodes (SNs). Patients who did not proceed to SNB were significantly older than those who did, more often had melanomas of the head or neck, and had more SNs and more nodal drainage fields. Of the 203 patients, 181 (89 %) were followed with high-resolution ultrasound of their SNs, which identified 33 % of the nodal recurrences before they were clinically apparent. Patients whose SNB was canceled had significantly worse recurrence-free survival and regional node disease-free survival, but melanoma- specific survival was similar. Compared to SN-positive patients, node-positive patients without SNB had significantly more involved nodes when a delayed lymphadenectomy was performed, but melanoma-specific survival was not significantly different after a median follow-up of 42 months.

Conclusions. Lymphoscintigraphy with ultrasound follow-up of previously identified SNs is an acceptable management strategy for patients in whom a SNB procedure is likely to be challenging.

INTRODUCTION

In patients with intermediate-thickness melanomas, sentinel node biopsy (SNB) has become a routine procedure that provides staging and prognostic information, reduces the risk of a nodal recurrence and results in improved melanoma-specific survival when combined with completion node dissection in those who are node positive.[1,2] Preoperative lymphoscintigraphy is an essential element of the procedure.[3,4] At Melanoma Institute Australia, a planned SNB is sometimes canceled after preoperative lymphoscintigraphy has been performed. The decision not to proceed with sentinel node biopsy is based on weighing the drawbacks against the benefits. The benefits are improved staging, improved regional control and early treatment of nodal involvement with an improved survival rate. The drawbacks concern the duration and extent of the operation, difficulty of the operation, the risk of morbidity, and the general health of the patient. These patients are then followed with ultrasound (US) of their nodal field. This practice is not known to occur elsewhere on a regular basis.

The purposes of this study were to gather information on these patients and to determine whether this is an accept- able management strategy. Specific aims were to determine the incidence of omitting the intended SNB and the reasons for it in order to document the characteristics of these patients, to investigate the methods of follow-up, to report the stage of the disease at time of regional nodal recurrence, and to describe the ways in which these metastases were detected and managed. Survival was compared to that of patients who did undergo SNB, and melanoma-specific survival of node-positive patients in both populations was also compared.

PATIENTS AND METHODS

Patients

MIA's prospectively collected database was queried to identify patients with clinically localized cutaneous melanoma in whom SNB was performed (SNB group) between November 2000 and December 2009 and for patients in whom the planned biopsy was canceled after lymphoscintigraphy had been performed (SNB-canceled group). Patients were excluded from the study if they had melanoma-in situ, multiple primary melanomas, (micro)satellites, or in-transit metastases; if preoperative US revealed nodal metastasis; if no sentinel node (SN) was identified intraoperatively; if wide local excision was performed before lymphoscintigraphy; or if SNB was performed elsewhere. The protocol of this retrospective cohort study was approved by MIA's research committee.

Methods

Sentinel node biopsy is routinely recommended in patients with a melanoma ≥1 mm Breslow thickness. It is discussed with patients with a melanoma between 0.75 and 1.00 mm Breslow thickness in the presence of adverse histologic features such as ulceration or an elevated tumor mitotic rate. The techniques of lymphoscintigraphy and SNB used at MIA have been described in detail previously.[5,6] Briefly, 30 MBq (0.8 mCi) technetium-99m antimony trisulfide colloid is injected intradermally as close as possible to the melanoma site, followed by dynamic and static imaging. Since 2008, single photon emission computed tomography with integrated computerized tomography (SPECT/CT) has been routinely added. A SN is defined as any node on a direct lymphatic drainage pathway from the primary tumor.[7] Patent blue dye and a handheld gamma ray detection probe are used to guide the intraoperative detection of the SNs.[3] Multiple sections of each removed SN are examined histopathologically using hematoxylin and eosin and immunohistochemical stains (S100 and HMB-45).[8] Completion lymph node dissection has typically been performed in patients with an involved SN, unless they participated in a study (MSLT-II) in which they were randomized to observation of the nodal region.[9] Follow-up intervals are at the discretion of the surgeon.

Statistical analysis

Clinicopathologic variables in relation to type of management (i.e. SNB versus SNB canceled) were analyzed and compared. The unpaired *t* test was used for hypothesis testing of normally distributed continuous variables, and the Mann-Whitney *U* test was used for continuous data that were not normally distributed. Calculation of P-values for categorical data was done with Pearson's χ^2 or Fisher's exact test, as appropriate. All test statistics were two tailed, and the significance level was set at $P < 0.05$. Survival rates were calculated by the Kaplan–Meier product-limit method. Covariates (management, gender, age, primary tumor site, Breslow thickness, tumor mitotic rate, ulceration, and tumor histology) were compared with the log-rank test. Patients with an unknown cause of death were excluded from the melanoma-specific survival analyses (n = 102). The mean and median follow-up duration in the group without SNB were both 42 months (interquartile range 18.5–65.5 months). Type of management was the variable of interest in this study. To adjust for potential confounders, known prognostic factors (gender, age, primary tumor site, Breslow thickness, tumor mitotic rate, ulceration, and tumor histology) were added to the multivariable Cox proportional hazards models.[10–17] To increase the validity of the predictions outside the studied cohort, stepwise methods were not used and full models were built.[18] The proportional hazards assumption was checked for all included variables. Stata 12 statistical software (StataCorp, College Station, TX) was

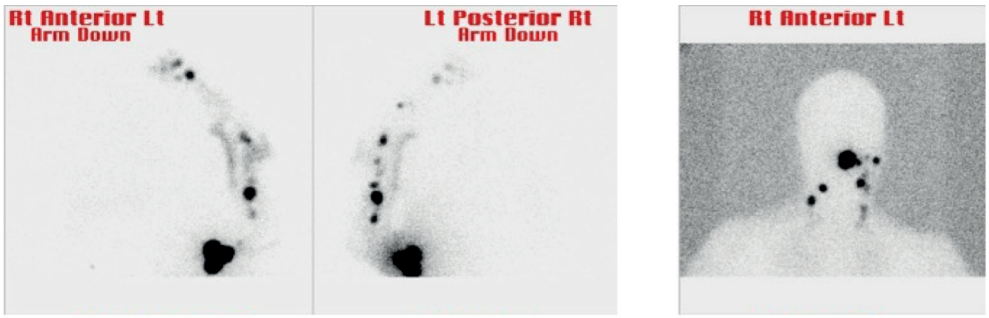
used for the assessment of the proportional hazards assumption. All other analyses were performed by SPSS 22.0 software for Mac (IBM SPSS, Chicago, IL).

RESULTS

Frequency of omitting the intended SNB and reasons

A total of 3667 patients with a clinically localized cutaneous melanoma underwent lymphoscintigraphy before a planned SNB in the selected time period. After imaging, the scheduled SNB was canceled in 203 (6.4%) of the 3148 patients who fulfilled the study entry criteria and was performed in the remainder. The lymphoscintigraphic demonstration of multiple drainage fields and/or multiple SNs were the reasons for refraining from the initially intended SNB in 170 (84%) of these 203 patients. Figure 1 shows two examples of lymphoscintigrams of patients whose procedures were canceled. Other reasons for canceling the scheduled SNB were the lack of SN visualization on the lymphoscintigrams in seven patients (3%), an unusual drainage pattern (SNs close to the umbilicus and in both groins) in one patient (0.5%), and a lymphoscintigram considered not to accurately reflect drainage from the melanoma after reconstruction of the nose in one patient (0.5%). The reason for not performing the SNB could not be identified in the remaining 24 patients (12%).

Figure 1. Examples of lymphoscintigrams that made surgeon decide to cancel the SNB.
A. Four SNs in left upper arm, two infraclavicular SNs, and two supraclavicular SNs.
B. SNs in left preauricular region, three SNs in left submandibular region, one SN in right submandibular, and one SN in right mid neck region.



Characteristics of patients, melanomas and lymphoscintigrams

Table 1 shows the clinical and pathologic characteristics of all patients who had or did not have a SNB procedure. Compared to patients who had a SNB, patients in the SNB-canceled group were more often male (69% versus 60%; P = 0.01), were older (mean 62 years versus 57; P < 0.001), more often had a primary melanoma in the head and neck region (38% versus 16%; P < 0.001), had a lower tumor mitotic rate (median of 2 and 3; P = 0.01) and had a different distribution of melanoma types (P < 0.001). SNB was significantly more often omitted in patients with a melanoma that was <1mm in Breslow thickness (P = 0.03). SNB-canceled patients more often had a superficial spreading melanoma and less frequently a nodular melanoma. Clark level and incidence of ulceration were similar in the two populations. Lymphoscintigraphy revealed drainage to significantly more nodal regions (mean 1.7 and 1.3) and to more SNs (mean 3.7 and 2.4) in SNB-canceled patients. Their SNs were most often located in the neck (47%), while the most frequent SN region in the others was the axilla (49%).

Table 1. Clinicopathologic characteristics of patients in whom the SNB was canceled and patients in whom SNB was performed.

Characteristic	**SNB canceled (n=203)**	**SNB performed (n=2945)**	**P-value**
Gender			0.01#
Male	139 (68.5)	1758 (59.7)	
Female	64 (31.5)	1187 (40.3)	
Age (years)			
Mean (SD)	62 (16.9)	57 (15.3)	<0.001$
Primary tumor site			<0.001#
Head and neck	77 (37.9)	465 (15.8)	
Upper limb	15 (7.4)	773 (26.2)	
Lower limb	45 (22.2)	740 (25.1)	
Trunk	66 (32.5)	967 (32.8)	
Breslow thickness			0.12#
0 – 1 mm	40 (19.7)	424 (14.4)	
1.01 – 2 mm	78 (38.4)	1283 (43.6)	
2.01 – 4 mm	60 (29.6)	836 (28.4)	
> 4 mm	22 (10.8)	394 (13.4)	
Missing	3 (1.5)	8 (0.3)	
Median (IQR)	1.65 (0.85-2.45)	1.80 (0.95-2.65)	0.10§
Tumor mitotic rate/mm^2			0.002#
0	34 (16.7)	290 (9.8)	
≥1	156 (76.8)	2519 (85.5)	
Missing	13 (6.4)	136 (4.6)	
Median (IQR)	2 (0-4)	3 (1-5)	0.01§

Ulceration			0.49#
Absent	138 (68.0)	2047 (69.5)	
Present	43 (21.2)	730 (24.8)	
Missing	22 (10.8)	168 (5.7)	
Histology			<0.001#
Superficial spreading melanoma	96 (47.3)	1264 (42.9)	
Nodular melanoma	35 (17.2)	935 (31.8)	
Other	40 (19.7)	377 (12.8)	
Missing	32 (15.8)	368 (12.5)	
Clark level			0.06*
II	9 (4.4)	49 (1.7)	
III	48 (23.6)	784 (26.6)	
IV	125 (61.6)	1847 (62.7)	
V	16 (7.9)	223 (7.6)	
Missing	5 (2.5)	42 (1.4)	
No. of SNs identified on lymphoscintigram			<0.001*
0	7 (3.4)	1 (0)	
1	6 (3.0)	809 (27.5)	
2	31 (15.3)	984 (33.4)	
≥3	153 (75.4)	1131 (38.4)	
Missing	6 (3.0)	20 (0.7)	
Mean (SD)	3.7 (1.7)	2.4 (1.2)	<0.001§
Drainage site of identified SNs			<0.001*
Axilla	42 (20.7)	1453 (49.3)	
Groin	47 (23.2)	789 (26.8)	
Neck	95 (46.8)	618 (21.0)	
Popliteal	2 (1.0)	16 (0.5)	
Other	9 (4.4)	66 (2.2)	
Missing	8 (3.9)	3 (0.1)	
No. of drainage sites			<0.001*
0	7 (3.4)	0 (0.0)	
1	102 (50.2)	2281 (77.5)	
2	46 (22.7)	565 (19.2)	
3	36 (17.7)	82 (2.8)	
4	11 (5.4)	14 (0.5)	
Missing	1 (0.5)	3 (0.1)	
Mean (SD)	1.7 (1.1)	1.3 (0.6)	<0.001§

Data are expressed as n (%) unless otherwise specified

# Pearson's chi square
$ unpaired *t* test
§ Mann-Whitney *U* test

* Fisher's exact test

Recurrence and survival

Of the 203 SNB-canceled patients, 181 (89%) were followed with high-resolution US of their lymph node fields at each follow-up visit. The other patients were followed with physical examination of their node fields, while one patient was followed with CT scans. Regional lymph node recurrence was more common in the SNB-canceled group (12% versus 4% in the SNB group), whereas a distant metastasis was the more frequent first recurrence in the SNB group (6% versus 3% in SNB-canceled group; P < 0.001) (Table 2).

Table 2. Characteristics regarding treatment and recurrence of patients in whom the SNB was canceled and patients in whom SNB was performed.

Characteristic	**SNB canceled (n=203)**	**SNB performed (n=2945)**	**P-value**
Site of first recurrence			<0.001*
Local	17 (8.4)	103 (3.5)	
In-transit	3 (1.5)	94 (3.2)	
Regional nodal	25 (12.3)	131 (4.4)	
Distant	7 (3.4)	163 (5.5)	
Multiple sites	5 (2.5)	110 (3.7)	
SN status			
Negative	NA	2531 (85.9)	NA
Positive	NA	404 (13.7)	
Missing	NA	10 (0.3)	
CLND			
Performed	NA	316 (10.7)	NA
Not performed	NA	2629 (89.3)	
No. of metastatic nodes			
Mean (SD)	2.4 (2.2)	1.7 (1.7)	0.02§

Data are expressed as n (%) unless otherwise specified
NA not applicable, *CLND* completion lymph node dissection

$ unpaired *t*-test
* Fisher's exact test

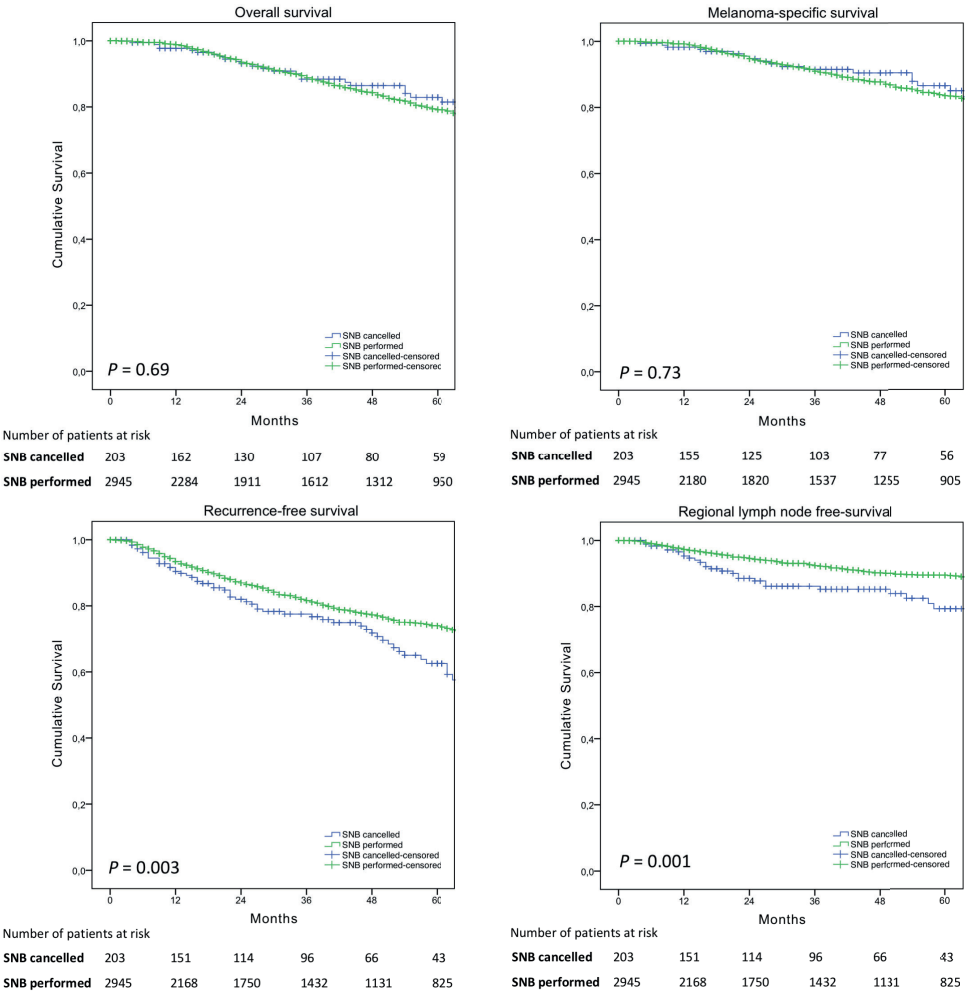
Univariable analysis showed that SNB patients had significantly improved recurrence-free and regional lymph node disease-free survival (Table 3). Melanoma-specific and overall survival were similar in the two groups (see also Figure 2). After adjusting for all major prognostic factors, the multivariable analyses showed the same associations as the univariable analyses with respect to melanoma-specific survival [hazard ratio (HR) SNB-canceled group = 0.88;

95% confidence interval (CI) 0.5 - 1.5] and overall survival (SNB-canceled group HR = 0.84; 95% CI 0.6 - 1.3).

Table 3. Results of univariable survival analyses of categorical variables.

	5-year recurrence-free survival	5-year regional lymph node-free survival	5-year melanoma-specific survival	5-year overall survival
Management				
SNB canceled	63%	79%	87%	83%
SNB performed	74%	90%	84%	79%
P-value	0.003	0.001	0.73	0.69

Figure 2. Melanoma-specific, overall, recurrence-free and regional lymph node-free survival according to type of management.



Concordant with univariable analysis, recurrence-free survival (HR =1.59; 95% CI 1.2 - 2.2) and regional lymph node-free survival (HR = 2.24; 95% CI 1.4 - 3.5) were significantly worse without SNB in multivariable analyses.Two of the 21 patients who were followed with clinical examination of their nodal regions developed a recurrence. One patient was found to have in-transit metastases after 4 months and died of melanoma 16 months after the primary melanoma was diagnosed. Another patient developed liver and spleen metastases and died 9 months after the primary melanoma was diagnosed. None of these 21 patients developed a nodal recurrence.

Immediate lymphadenectomy versus delayed lymphadenectomy

In the SNB-canceled group, 27 patients (13%) developed a recurrence in the identified regional node field, and all but one received a delayed regional node dissection. The regional node recurrence was found at physical examination by a doctor in 10 patients (37%) and by US in nine (33%); the other eight patients (30%) noticed the recurrence themselves. The mean number of metastatic nodes in these patients was higher than in the patients who underwent a completion node dissection because of an involved SN (2.4 versus 1.7; P = 0.02). Five patients in the former group had distant metastases when regional nodal recurrence was diagnosed (18.5%). Melanoma-specific survival was similar in the two groups in the univariable and multivariable analysis (SNB-canceled group HR = 0.49; 95% CI 0.2 – 1.2; P = 0.13).

DISCUSSION

SNB remains the standard, but the new approach of follow-up with focused US after lymphoscintigraphy identifies metastases early and avoids complex surgery when the surgeon thinks that the risks exceed the potential benefits. In addition to the complex or unusual lymph drainage pattern, lack of SN visualization on the lymphoscintigrams, and an unreliable lymphoscintigram, there may have been other factors that led to the decision to omit SNB. These patients tended to be male and somewhat older, and their melanomas were more often in the head and neck region. Their lesions were generally thinner and had a lower tumor mitotic rate compared to patients in whom the surgeon proceeded with the planned SNB. Lymphoscintigraphy identified more SNs and drainage fields in patients in whom SNB was canceled than those in whom the procedure was carried out.

SNB for trunk, head, and neck melanomas was most frequently canceled, as lymphatic drainage of these primary lesions is less predictable than for melanomas on limbs, and drainage

is often to multiple sites.[19–21] There is conflicting evidence on whether drainage to multiple nodal regions is an independent prognostic factor for SN metastasis.[22–24] In truncal melanoma patients, multiple nodal region drainage was independently related to an increased risk of nodal metastases in one study, while another found no association between the number of drainage regions and disease progression.[22,23] Drainage to a single nodal region was associated with a greater risk of locoregional recurrence in the Sunbelt Melanoma Trial.[24] In 35% of the patients with a melanoma in the head and neck region, a SN is located in the parotid gland.[4] Identifying and removing such a SN can be challenging, particularly for surgeons who do not regularly undertake head and neck surgery, and there is always a risk of facial nerve injury.

There may also be a temptation to omit the procedure in patients who have a melanoma with a Breslow thickness outside the intermediate thickness range for which a survival benefit has been shown in case of metastasis.[2] SNB was significantly more often omitted in patients with a melanoma that had a Breslow thickness of <1 mm. Patients with thick primary tumors often have distant disease to begin with, and their prognosis is poor whether or not a node dissection is performed. However, our data do not suggest a reluctance to perform SNB in these patients, probably because of its value to improve regional control. This is relevant because operations for a palpable nodal recurrence are often more extensive; require a longer hospital admission; are associated with more morbidity, higher costs, and reduced quality of life; and may be followed by radiotherapy.[25] In addition to differences in lymphoscintigraphy findings and primary tumor factors, the population in whom the SNB was canceled was significantly older than the other group. Although increasing age is known to be associated with a reduced risk of nodal metastases, the patients' charts did not mention age as a reason to refrain from SNB.[26,27]

The majority (89 %) of the patients without SNB were followed with high-resolution US of their lymph node regions at each follow-up visit. Guided by lymphoscintigrams including SPECT/CT since 2008, the nuclear medicine physicians at our institution mark the location of the SN or SNs with a small tattoo on the skin. This facilitates the relocalization of these SNs so that the nodes directly at risk of containing metastasis can be scrutinized using focused US. The minimum size for a lymph node metastasis to be detectable with US is commonly reported to be around 3 mm in the neck, 4 mm in the groin, and 5 mm in the axilla, whereas physical examination picks up a metastatic node only when it is at least 1 cm in size under favorable conditions.[28,29]

There is evidence that routine US improves detection of nodal recurrence in patients who have not had a SNB.[30] The sensitivity of US ranges from 92 to 99% with a specificity of 98%,

while the sensitivity of physical examination is between 25 and 51% with a specificity of 91-98%.[31–39] A meta-analysis confirmed that US of lymph nodes for the detection of metastases is superior to physical examination.[40] In the present study, a third of the nodal recurrences were detected by focused US, confirming the usefulness of this technique in the follow-up of these patients.

Patients without SNB understandably had worse regional lymph node–free survival and a worse recurrence-free survival compared to patients who did undergo the procedure. If recurrence occurs in SNB-positive patients who have had a completion lymph node dissection, it will most likely be distant nodal or visceral metastasis. When regional node dissection was performed because of nodal recurrence, significantly more nodes were found to be involved compared to SN-positive patients who had an immediate lymphadenectomy. Although the number of metastatic nodes is known to be inversely correlated with survival, melanoma-specific survival was found to be similar in the two groups when multivariable analysis was performed. [41] One may contemplate that the number of patients in the study may have been too small to establish an existing survival difference.

A number of studies have compared SNB with nodal observation, but none of these mentioned patients in whom SNB was canceled after lymphoscintigraphy and in whom the nodes were followed with focused US.[2,42–48] To our knowledge, ours is the first such study. Refraining from SNB is not only the subject of this study but also might introduce ascertainment bias when comparing outcomes to those of patients who did undergo the procedure. The short follow-up for some patients and the incomplete pathology data (mainly on tumor mitotic rate and ulceration) are other limitations of the present study.

CONCLUSION

Omission of SNB after lymphoscintigraphy occurred in 6.4 % of the patients and was mainly due to the presence of multiple SNs and/or drainage sites. These patients are generally older and tend to have a melanoma in the head and neck region or on the trunk. Although associated with a worse regional lymph node–free survival and more involved nodes when a regional nodal metastasis occurs, overall and melanoma-specific survival are not impeded. As a result, US follow-up of SNs identified on lymphoscintigraphy is an acceptable management strategy when facing a challenging SNB.

REFERENCES

1. Wong SL, Balch CM, Hurley P, et al. Sentinel lymph node biopsy for melanoma: American Society of Clinical Oncology and Society of Surgical Oncology joint clinical practice guideline. *J Clin Oncol*. 2012;30(23):2912-2918.
2. 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.
3. Nieweg OE, Jansen L, Kroon BB. Technique of lymphatic mapping and sentinel node biopsy for melanoma. *Eur J Surg Oncol*. 1998;24(6):520-524.
4. Thompson JF, Uren RF. Lymphatic mapping in management of patients with primary cutaneous melanoma. *Lancet Oncol*. 2005;6(11):877-885.
5. Uren RF, Howman-Giles R, Chung D, Thompson JF. Guidelines for lymphoscintigraphy and F18 FDG PET scans in melanoma. *J Surg Oncol*. 2011;104(4):405-419.
6. Verwer N, Scolyer RA, Uren RF, et al. Treatment and prognostic significance of positive interval sentinel nodes in patients with primary cutaneous melanoma. *Ann Surg Oncol*. 2011;18(12):3292-3299.
7. Nieweg OE, Tanis PJ, Kroon BB. The definition of a sentinel node. *Ann Surg Oncol*. 2001;8(6):538-541.
8. Scolyer RA, Murali R, McCarthy SW, Thompson JF. Pathologic examination of sentinel lymph nodes from melanoma patients. *Semin Diagn Pathol*. 2008;25(2):100-111.
9. Nieweg OE. Current status of sentinel lymph node biopsy in patients with melanoma. *Rozhl Chir*. 2014;93(10):485-490.
10. Balch CM, Gershenwald JE, Soong SJ, et al. Final version of 2009 AJCC melanoma staging and classification. *J Clin Oncol*. 2009;27(36):6199-6206.
11. Breslow A. Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. *Ann Surg*. 1970;172(5):902-908.
12. 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.
13. 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.
14. Joosse A, Collette S, Suciu S, et al. Superior outcome of women with stage I/II cutaneous melanoma: pooled analysis of four European Organisation for Research and Treatment of Cancer phase III trials. *J Clin Oncol*. 2012;30(18):2240-2247.
15. Scoggins CR, Ross MI, Reintgen DS, et al. Gender-related differences in outcome for melanoma patients. *Ann Surg*. 2006;243(5):693-698; discussion 698-700.
16. Balch CM, Soong SJ, Gershenwald JE, et al. Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. *J Clin Oncol*. 2001;19(16):3622-3634.
17. Callender GG, Egger ME, Burton AL, et al. Prognostic implications of anatomic location of primary cutaneous melanoma of 1 mm or thicker. *Am J Surg*. 2011;202(6):659-664; discussion 664-5.
18. Steyerberg EW. Selection of main effects. In: *Clinical Prediction Models*. Springer Science+Business Media; 2009:191-210.

19. 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.
20. 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.
21. 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.
22. Porter GA, Ross MI, Berman RS, Lee JE, Mansfield PF, Gershenwald JE. Significance of multiple nodal basin drainage in truncal melanoma patients undergoing sentinel lymph node biopsy. *Ann Surg Oncol*. 2000;7(4):256-261.
23. Ahmadzadehfar H, Hinz T, Wierzbicki A, et al. Significance of multiple nodal basin drainage in patients with truncal melanoma. *Q J Nucl Med Mol Imaging*. 2016;60(3):274-279.
24. Federico AC, Chagpar AB, Ross MI, et al. Effect of multiple-nodal basin drainage on cutaneous melanoma. *Arch Surg*. 2008;143(7):632-637.
25. Faries MB, Thompson JF, Cochran A, et al. The impact on morbidity and length of stay of early versus delayed complete lymphadenectomy in melanoma: Results of the multicenter selective lymphadenectomy trial (I). *Ann Surg Oncol*. 2010;17(12):3324-3329.
26. Balch CM, Thompson JF, Gershenwald JE, et al. Age as a predictor of sentinel node metastasis among patients with localized melanoma: An inverse correlation of melanoma mortality and incidence of sentinel node metastasis among young and old patients. *Ann Surg Oncol*. 2014;21(4):1075-1081.
27. White RL, Ayers GD, Stell VH, et al. Factors predictive of the status of sentinel lymph nodes in melanoma patients from a large multicenter database. *Ann Surg Oncol*. 2011;18(13):3593-3600.
28. Voit CA, van Akkooi ACJ, Schäfer-Hesterberg G, et al. Rotterdam Criteria for sentinel node (SN) tumor burden and the accuracy of ultrasound (US)-guided fine-needle aspiration cytology (FNAC): can US-guided FNAC replace SN staging in patients with melanoma? *J Clin Oncol*. 2009;27(30):4994-5000.
29. Voit CA, van Akkooi ACJ, Eggermont AMM, et al. Fine needle aspiration cytology of palpable and nonpalpable lymph nodes to detect metastatic melanoma. *J Natl Cancer Inst*. 2011;103(23):1771-1777.
30. Krüger U, Kretschmer L, Thoms K-M, et al. Lymph node ultrasound during melanoma follow-up significantly improves metastasis detection compared with clinical examination alone: a study on 433 patients. *Melanoma Res*. 2011;21(5):457-463.
31. Prayer L, Winkelbauer H, Gritzmann N, Winkelbauer F, Helmer M, Pehamberger H. Sonography versus palpation in the detection of regional lymph-node metastases in patients with malignant melanoma. *Eur J Cancer*. 1990;26(7):827-830.
32. Binder M, Kittler H, Steiner A, Dorffner R, Wolff K, Pehamberger H. Lymph node sonography versus palpation for detecting recurrent disease in patients with malignant melanoma. *Eur J Cancer*. 1997;33(11):1805-1808.
33. Rossi CR, Seno A, Vecchiato A, et al. The impact of ultrasound scanning in the staging and follow-up of patients with clinical stage I cutaneous melanoma. *Eur J Cancer*. 1997;33(2):200-203.

34. Blum A, Schlagenhauff B, Stroebel W, Breuninger H, Rassner G, Garbe C. Ultrasound examination of regional lymph nodes significantly improves early detection of locoregional metastases during the follow-up of patients with cutaneous melanoma: results of a prospective study of 1288 patients. *Cancer*. 2000;88(11):2534-2539.

35. Voit C, Mayer T, Kron M, et al. Efficacy of ultrasound B-scan compared with physical examination in follow-up of melanoma patients. *Cancer*. 2001;91(12):2409-2416.

36. Garbe C, Paul A, Kohler-Späth H, et al. Prospective evaluation of a follow-up schedule in cutaneous melanoma patients: recommendations for an effective follow-up strategy. *J Clin Oncol*. 2003;21(3):520-529.

37. Schmid-Wendtner M-H, Paerschke G, Baumert J, Plewig G, Volkenandt M. Value of ultrasonography compared with physical examination for the detection of locoregional metastases in patients with cutaneous melanoma. *Melanoma Res*. 2003;13(2):183-188.

38. Hofmann U, Szedlak M, Rittgen W, Jung EG, Schadendorf D. Primary staging and follow-up in melanoma patients--monocenter evaluation of methods, costs and patient survival. *Br J Cancer*. 2002;87(2):151-157.

39. Leiter U, Marghoob AA, Lasithiotakis K, et al. Costs of the detection of metastases and follow-up examinations in cutaneous melanoma. *Melanoma Res*. 2009;19(1):50-57.

40. 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.

41. Balch CM, Gershenwald JE, Soong SJ, et al. Multivariate analysis of prognostic factors among 2,313 patients with stage III melanoma: Comparison of nodal micrometastases versus macrometastases. *J Clin Oncol*. 2010;28(14):2452-2459.

42. Möhrle M, Schippert W, Rassner G, Garbe C, Breuninger H. Is sentinel lymph node biopsy of therapeutic relevance for melanoma? *Dermatology*. 2004;209(1):5-13.

43. Gutzmer R, Al Ghazal M, Geerlings H, Kapp A. Sentinel node biopsy in melanoma delays recurrence but does not change melanoma-related survival: a retrospective analysis of 673 patients. *Br J Dermatol*. 2005;153(6):1137-1141.

44. Starz H, Balda B-R. Benefit of sentinel lymphadenectomy for patients with nonulcerated cutaneous melanomas in the Breslow range between 0.76 and 1 mm: a follow-up study of 148 patients. *Int J Cancer*. 2007;121(3):689-693.

45. Koskivuo I, Talve L, Vihinen P, Mäki M, Vahlberg T, Suominen E. Sentinel lymph node biopsy in cutaneous melanoma: a case-control study. *Ann Surg Oncol*. 2007;14(12):3566-3574.

46. Leiter U, Buettner PG, Bohnenberger K, et al. Sentinel lymph node dissection in primary melanoma reduces subsequent regional lymph node metastasis as well as distant metastasis after nodal involvement. *Ann Surg Oncol*. 2010;17(1):129-137.

47. Satzger I, Meier A, Hoy L, et al. Sentinel node dissection delays recurrence and prolongs melanoma-related survival: an analysis of 673 patients from a single center with long-term follow-up. *Ann Surg Oncol*. 2011;18(2):514-520.

48. van der Ploeg APT, Haydu LE, Spillane AJ, et al. Outcome following sentinel node biopsy plus wide local excision versus wide local excision only for primary cutaneous melanoma: analysis of 5840 patients treated at a single institution. *Ann Surg*. 2014;260(1):149-157.

CHAPTER 4

The prognostic value of tumor mitotic rate in children and adolescents with cutaneous melanoma: a retrospective cohort study

Norbertus A. Ipenburg
Serigne N. Lo
Ricardo E. Vilain
Lodewijka H.J. Holtkamp
James S. Wilmott
Omgo E. Nieweg
John F. Thompson
Richard A. Scolyer

Journal of the American Academy of Dermatology. 2020 Apr;82(4):910-919

ABSTRACT

Background. Mitotic rate is a strong predictor of outcome in adult patients with primary cutaneous melanoma, but for children and adolescent patients this is unknown.

Objective: We sought to assess the prognostic value of primary tumor mitotic rate in children and adolescents with primary melanoma.

Methods. This was a cohort study of 156 patients who were <20 years of age and who had clinically localized cutaneous melanoma. Patients <12 years of age were classified as children and those 12 to 19 years of age as adolescents. Clinicopathologic and outcome data were collected. Recurrence-free and melanoma-specific survival were calculated. Univariable and multivariable analyses were performed using Cox proportional hazard models.

Results. Thirteen of 156 patients (8%) were children. Mitotic rate was $\geq 1/mm^2$ in 104 patients (67%) and correlated with increasing Breslow thickness. A positive sentinel node was found in 23 of 61 patients (38%) who underwent sentinel node biopsy. The median follow-up was 61 months. Five-year melanoma-specific and recurrence-free survival were 91% and 84%, respectively. Mitotic rate was a stronger predictor of outcome than tumor thickness, and was the only factor independently associated with recurrence-free survival.

Limitations. This research was conducted at a single institution and the sample size was small.

Conclusion. Mitotic rate is an independent predictor of recurrence-free survival in children and adolescents with clinically localized melanoma.

INTRODUCTION

Melanoma is the most common skin cancer in children and adolescents.[1] Still, <1% of all melanomas occur in patients < 20 years of age.[2] Because of its rarity, the published literature on melanoma in children and adolescents is sparse and treatment is primarily based on adult guidelines.

Tumor mitotic rate is one of the strongest predictors of survival in adults with clinically localized primary cutaneous melanoma.[3–7] Evidence suggests that the mitotic rate is lower in melanomas occurring in children and adolescents than in other age groups.[8] Few studies have assessed the prognostic value of mitotic rate in childhood and adolescent melanoma.[8–12] Most reports including > 100 children and adolescents with melanoma did not evaluate the effect of mitotic rate on prognosis or had many missing values.[2,13–20]

The purpose of this study was to assess the prognostic significance of mitotic rate in clinically localized primary cutaneous melanoma in children and adolescents. Secondary aims were to report the clinicopathologic features in a large cohort of melanoma patients <20 years of age, to compare children with adolescent patients, and to assess the relationship between mitotic rate and tumor thickness in this age group.

PATIENTS AND METHODS

Patients

The prospectively collected database of Melanoma Institute Australia (MIA) was queried for this retrospective cohort study. Between 1993 and 2013, 259 melanoma patients <20 years of age were managed at MIA. To be included in the current study, a diagnosis of primary cutaneous melanoma had to have been confirmed by ≥1 MIA-affiliated pathologists. Borderline lesions, such as atypical Spitz nevi/tumors, melanocytomas or atypical melanocytic proliferations, were excluded after pathology review (n=27). Patients were also excluded if they had melanoma in situ (n=34), a metastasis from an unknown primary melanoma (n=5), multiple primary melanomas (n=5), mucosal melanoma (n=1), macrometastasis at diagnosis (n=4), or if an MIA-affiliated pathologist could not review the pathology slides (n=27). One hundred fifty-six patients fulfilled the inclusion criteria. Institutional Review Board approval was obtained (Sydney South West Area Health Service institutional ethics review committee protocol no. X15-0454).

Data collection

Patients who present to MIA for management of their melanoma after a diagnosis has been established have their pathology slides reviewed by ≥1 MIA-affiliated pathologists at the Royal Prince Alfred Hospital, Sydney, Australia. The primary tumor pathological characteristics are assessed and recorded in a second pathology report (the "MIA pathology report") and the histopathology slides are returned to the source pathology laboratory. The data used in this study were extracted from MIA pathology reports. In cases with missing data and when the histopathology slides were still available, the cases were rereviewed and missing data were recorded. Data on demographics, primary tumor characteristics, sentinel node (SN) status, recurrence, treatment, and follow-up were obtained. Patients were stratified by age into 2 groups: <12 years of age (children) and 12–19 years of age (adolescents). Twelve years of age was selected to represent the onset of puberty.[21]

Mitotic rate

Tumor mitotic rate was measured according to the recommendations of the 1982 International Pathology Workshop.[22] Mitoses were recognized by the presence of extensions of chromatin extending from a condensed chromatin mass. The number of mitoses was counted in a 1-mm^2 area (approximately 5 high power fields). The count started in the dermal area of the tumor with the greatest density of mitoses (the 'hot spot') and continued in immediately adjacent, nonoverlapping fields.[22,23]

Statistical analysis

Baseline characteristics were summarized using median (interquartile range) for continuous variables and proportions for categorical variables. Characteristics of childhood and adolescent patients were compared using the Pearson's χ^2 or Fisher's exact test for categorical features and the Mann-Whitney *U* test for continuous variables. Melanoma-specific survival (MSS) was calculated as the time from initial diagnosis until melanoma-related death. Patients who died from nonmelanoma causes or those still alive at last follow-up were censored. Recurrence-free survival (RFS) was defined as the time from diagnosis until recurrence or death. Censoring occurred at the end of follow-up. Univariable and multivariable analyses using Cox proportional hazard models were used to assess the prognostic value of covariates for RFS and MSS. Mitotic rate was the variable of interest in this study. Other known prognostic factors in adult melanoma, such as gender, age, primary tumor site, Breslow thickness, ulceration and SN status were investigated in a univariable analysis.[5,8,24,25] Given

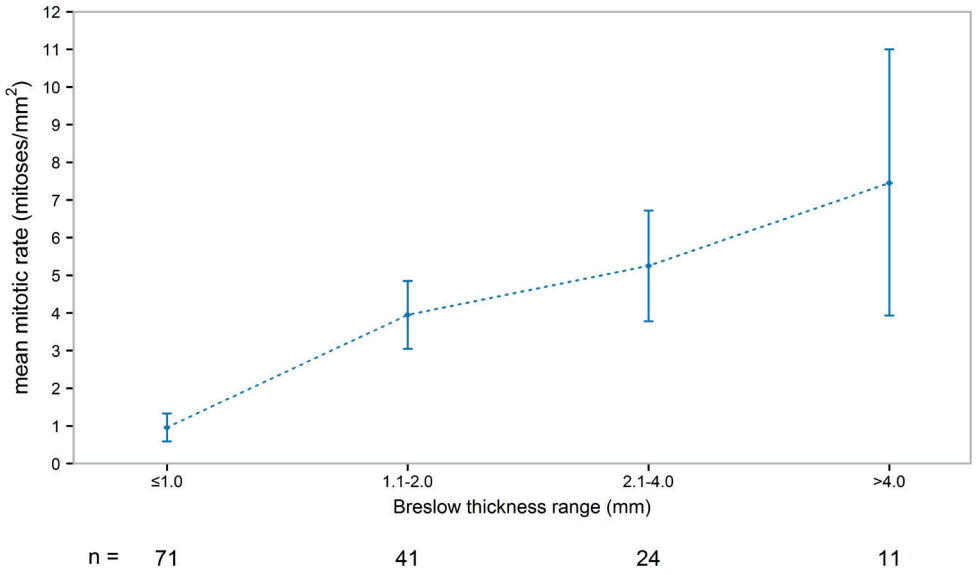
the number of patients who developed recurrence (n=28), only the two covariates with P-value <0.20 from the univariable analysis and with <10% missing values were included in the multivariable model. The proportional hazards assumption was checked for the included variables. P-values were two-sided and P<0.05 was considered statistically significant. Statistical analyses were performed with SPSS 25.0 software for Mac (IBM SPSS, Chicago, IL).

RESULTS

Patient and tumor characteristics

Baseline characteristics of the 156 patients are shown in Table 1. The median age was 17.5 years (range 1–19 years). Thirteen patients (8%) were children at the time of diagnosis, while 143 (92%) were adolescents. Melanomas were most often thin (median Breslow thickness 1.0 mm), nonulcerated (65%) and located on the trunk (34%). The mitotic rate was ≥1/mm^2 in 104 patients (67%) and correlated with increasing Breslow thickness (Figure 1).

Figure 1. Mitotic rates versus Breslow thickness of primary melanomas.



Sentinel node biopsy (SNB) was performed in 61 patients, with 23 (38%) having a positive SN. Of the 77 patients with tumors >1 mm thick, 48 (62%) underwent SNB. Nineteen SN-

positive patients (83%) underwent completion lymph node dissection. Additional nodal metastases were found in 4 of these patients (21%). None of the 4 SN-positive patients who did not have a completion lymph node dissection developed a recurrence.

Childhood versus adolescent patients

Substantial differences in characteristics were observed between the childhood and adolescent patients (Table 1). Childhood melanomas (n=13) were thicker (median 2.7 mm vs. 1.0 mm; P=0.002) and were more often located in the head and neck region (n=5; 38%); adolescent melanomas (n=143) were most frequently located on the trunk (n=51; 36%). Melanoma subtype was also different between the 2 groups, with Spitzoid melanoma (n=8; 62%) being the most common subtype in children and superficial spreading melanoma (n=59; 41%) the most common in adolescent patients (P=0.007). Ulceration (n=4 (31%) in children vs. n=22 (15%) in adolescents; P=0.12) and mitotic rate ≥ 1 (n=10 (77%) in children vs. n=94 (66%) in adolescents; P=0.15) were not significantly different. There was no significant difference (P=0.26) in the frequency with which SNB was performed between children (n=7; 54%) and adolescent patients (n=54; 38%). Prepubertal patients had more often a positive SN than adolescent patients but this difference was not statistically significant (n=5 (71%) vs. n=18 (33%); P=0.09).

Table 1. Clinicopathological characteristics.

Characteristic	**All patients (*n* = 156)**	**Childhood patients (*n* = 13)**	**Adolescent patients (*n* = 143)**	**P-value***
Gender				
Male	82 (53)	4 (31)	78 (55)	0.15
Female	74 (47)	9 (69)	65 (45)	
Primary tumor site				
Head and neck	37 (24)	5 (38)	32 (22)	0.30
Upper limb	35 (22)	4 (31)	31 (22)	
Lower limb	31 (20)	2 (15)	29 (20)	
Trunk	53 (34)	2 (15)	51 (36)	
Breslow thickness				
0 – 1 mm	79 (51)	3 (23)	76 (53)	0.003
1.01 – 2 mm	41 (26)	2 (15)	39 (27)	
2.01 – 4 mm	25 (16)	4 (31)	21 (15)	
>4 mm	11 (7)	4 (31)	7 (5)	
Median (interquartile range)	1.0 (1.3)	2.7 (3.8)	1.0 (1.1)	0.002
Mitotic rate (per mm2)				
<1	43 (28)	2 (15)	41 (29)	0.51
≥1	104 (67)	10 (77)	94 (66)	

Missing	9 (6)	1 (8)	8 (6)	
Median (interquartile range)	2 (5)	3 (5)	2 (4)	0.15
Ulceration				
Absent	102 (65)	6 (46)	96 (67)	0.12
Present	26 (17)	4 (31)	22 (15)	
Missing	28 (18)	3 (23)	25 (17)	
Tumor type				
Superficial spreading melanoma	61 (39)	2 (15)	59 (41)	0.007
Nodular melanoma	23 (15)	2 (15)	21 (15)	
Spitzoid melanoma	29 (19)	8 (62)	21 (15)	
Other	2 (1)	0 (0)	2 (1)	
Missing	41 (26)	1 (8)	40 (28)	
Clark level				
II	41 (26)	3 (23)	38 (27)	0.001
III	49 (31)	0 (0)	49 (34)	
IV	61 (39)	8 (62)	53 (37)	
V	3 (2)	2 (15)	1(1)	
Missing	2 (1)	0 (0)	2 (1)	
Sentinel node biopsy				
Performed	61 (39)	7 (54)	54 (38)	0.26
Not performed	95 (61)	6 (46)	89 (62)	
Sentinel node status				
Negative	38 (62)	2 (29)	36 (67)	0.09
Positive	23 (38)	5 (71)	18 (33)	
Total no. of sentinel nodes - median (interquartile range)	3 (3)	1 (2)	3 (2)	0.05
Recurrence				
Yes	28 (18)	1 (8)	28 (20)	0.46
No	128 (82)	12 (92)	115 (80)	
Site of first recurrence				
Local	1 (4)	1 (100)	0	0.04
In-transit	3 (11)	0 (0)	3 (11)	
Regional nodal	19 (68)	0 (0)	19 (70)	
Distant	5 (18)	0 (0)	5 (19)	
Last follow-up status				
No evidence of disease	135 (87)	12 (92)	123 (86)	1.0
Alive with disease	2 (1)	0 (0)	2 (1)	
Died from disease	16 (10)	1 (8)	15 (10)	
Died from unknown cause	2 (1)	0 (0)	2 (1)	
Missing	1 (1)	0 (0)	1 (1)	

Values in parentheses are percentages unless indicated otherwise; * comparison of children and adolescent patients.

Recurrence and survival

Median follow-up time was 61 months (interquartile range 10–111 months). Melanoma recurrence occurred in 28 patients (18%), and 16 patients (10%) died. Regional lymph nodes were the most common site of first recurrence (19 patients), while 5 patients had their first recurrence at a distant site. All patients whose first recurrence was in a regional node had a negative SN. The time between diagnosis of the primary melanoma and first recurrence ranged from 3 months to 13 years. Five patients (31%) had a recurrence after >5 years. MSS at 5 years was 91% (95% confidence interval (CI) 86%-96%) and 10-year MSS was 88% (95% CI 81%–95%). Five-year RFS was 84% (95% CI 77%-90%) and 10-year RFS was 77% (95% CI 67%–86%). Appendix 1 shows the characteristics of the 16 patients who died. One patient was 10 years old when her melanoma was diagnosed, while the other patients were adolescents. MSS and RFS were not significantly different between the two age groups (P=0.83 and P=0.54). Mitoses were present in the primary melanomas of 14 patients (88%) and 2 patients (13%) had melanomas with a Breslow thickness < 1 mm. Ten patients received chemotherapy, while 3 patients received targeted therapy or immunotherapy.

Prognostic factors

On univariable analysis, Breslow thickness (P=0.001), mitotic rate (P<0.001), and melanoma subtype (P=0.04) were found to be significantly associated with RFS. Gender, age, ulceration, primary tumor site, and SN status were not significantly associated with RFS. Figure 2 shows the RFS curves according to mitotic rate. On multivariable analysis including mitotic rate and Breslow thickness, mitotic rate correlated independently with RFS (hazard ratio (HR)=1.2; 95% CI 1.1–1.3), while Breslow thickness did not (HR=1.1; 95% CI 0.9–1.2). The univariable analysis indicated a significantly increased risk of melanoma-related death with increasing mitotic rate (P=0.001). The other covariates were not significantly associated with MSS (Table 2). Multivariable analysis could not be performed for MSS due to an insufficient number of events (16 melanoma-related deaths).

Figure 2. Recurrence-free survival of patients with melanoma according to mitotic rate.

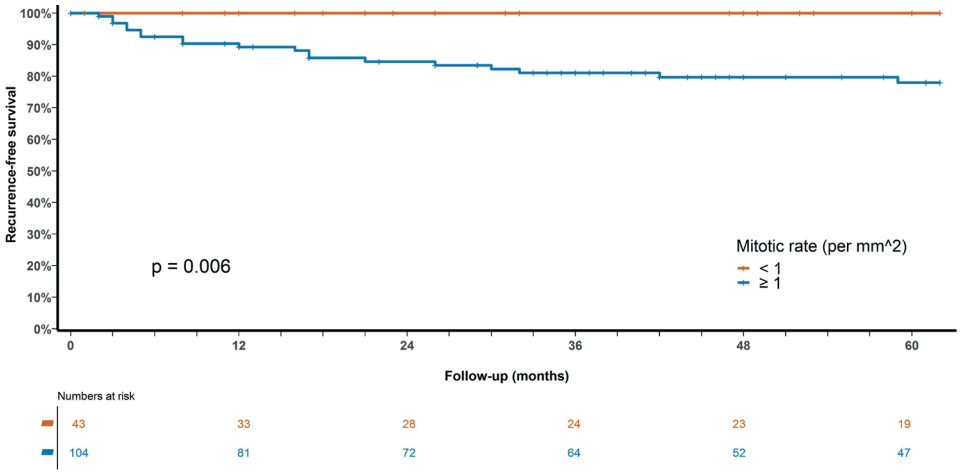


Table 2. Univariable and multivariable analysis of recurrence-free survival and melanoma-specific survival.

		Recurrence-free survival				Melanoma-specific survival	
		Univariable		Multivariable		Univariable	
Variables	**N**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**
Gender	156		0.28				0.31
Male		(reference)				(reference)	
Female		0.7 (0.3 – 1.4)				0.6 (0.2 – 1.6)	
Age (per 1 year increase)	156	1.1 (0.9 – 1.2)	0.28			1.1 (0.9 – 1.3)	0.46
Breslow thickness (per 1 mm increase)	156	1.2 (1.1 – 1.4)	0.001	1.1 (0.9 – 1.2)	0.48	1.1 (0.9 – 1.5)	0.30
Mitotic rate (per mm^2)	147	1.2 (1.1 – 1.3)	<0.001	1.2 (1.1 – 1.3)	0.005	1.3 (1.1 – 1.5)	0.001
Ulceration	132		0.24				0.16
Absent		1.0 (reference)				1.0 (reference)	
Present		1.7 (0.7 – 4.3)				2.3 (0.7 – 7.3)	
Primary tumor site	156		0.35				0.48
Lower Limb		1.0 (reference)				1.0 (reference)	
Trunk		1.0 (0.4 – 2.9)				2.7 (0.6 – 11.1)	
Head and Neck		1.7 (0.6 – 4.6)				1.4 (0.3 – 7.4)	
Upper limb		0.6 (0.2 – 2.0)				1.2 (0.2 – 6.1)	
Tumor type	115		0.04				0.20
Superficial spreading		1.0 (reference)				1.0 (reference)	
Nodular		2.9 (1.2 – 7.1)				2.3 (0.8 – 6.9)	
Spitzoid		0.6 (0.2 – 2.3)				0.3 (0 – 2.5)	
Other		-				-	
Sentinel node status	61		0.24				0.08
Negative		(reference)				(reference)	
Positive		2.8 (0.5 – 15.3)				7.1 (0.8 – 64.2)	

HR: hazard ratio; CI: confidence interval

DISCUSSION

This single institutional cohort study shows that tumor mitotic rate is the most important independent prognostic factor for RFS in children and adolescents with clinically localized melanoma, with a marginally stronger influence than tumor thickness. Having accurate information about the mitotic rate of the primary melanoma could improve prognostic stratification and treatment planning for individual patients in these age groups. It is important that this parameter is evaluated and recorded in all melanoma pathology reports.

In adults, the prognostic importance of mitotic rate has been demonstrated in numerous large independent studies.[3–7] Although mitotic rate was an essential part of the 7th edition of the American Joint Committee on Cancer (AJCC) melanoma staging system, it has been scarcely studied in childhood and adolescent melanoma.[25] The rarity of melanoma in these patients, with an annual incidence rate of around 5 per million, is probably one of the main reasons for the lack of studies.[26] Larger childhood and adolescent melanoma studies generally use data from the National Cancer Database or the Surveillance, Epidemiology, and End Results (SEER) database.[2,13,15] Although valuable, these databases have several limitations. For instance, central pathology review is lacking, recurrence rates are not available, and details of key tumor characteristics such as Breslow thickness, ulceration and mitotic rate are frequently missing.

Breslow thickness is the strongest prognostic feature in primary cutaneous melanoma in adult patients.[27] Interestingly, Breslow thickness was not a significant predictor for melanoma-specific survival in our study of childhood and adolescent patients. A similar finding was also reported in a study based on the National Cancer Database.[15] Another large multicenter study showed that primary tumor site and gender were independent prognostic factors for MSS, while mitotic rate and Breslow thickness were not.[8] However, two previous studies did show that Breslow thickness was an independent predictor of recurrence.[12,28]

On univariable analysis, MSS was significantly worse with increasing mitotic rate. Unfortunately, multivariable analysis could not be performed for MSS due to an insufficient number of events (16 melanoma-related deaths).[29] In line with our results, three previous melanoma studies in young patients showed that the presence of mitoses was associated with an increased risk of metastasis on univariable analysis. However, when adjusted for other prognostic factors, this association was not seen, possibly because of the small sample sizes or the number of missing values in these studies.[10,12,28] No significant effect on overall survival has been found.[9,11]

In line with previous reports, childhood patients had thicker melanomas than adolescent patients in our study.[11–13,18] The primary tumor location was also different for the two groups, with head and neck sites being more in children and the trunk being the most frequent location in adolescents.[13,15] Patients with melanoma who are in their late teens are sometimes inappropriately classified as children. Our results confirm that melanoma behaves differently in children and adolescents, but MSS and RFS were similar. In contrast, a previous study reported better survival for children.[30] This may reflect the fact that cases reported as borderline tumors, such as atypical Spitz tumors, were specifically excluded in our study, whereas these may have been classified as melanoma in other studies.[31]

Metastatic disease was identified in 38% of the patients who underwent SNB in our study. Previous studies had reported SN positivity rates of between 18 and 50% in children and adolescents with melanoma.[11,12,20,28,32–34] Contrary to previous studies, RFS and MSS were not significantly different for SN-positive and SN-negative patients in our study.[14,18,20] Paradoxically, young patients have a higher incidence of SN metastasis but a more favorable survival than adults.[8,13,32] The reasons for this remain unclear but superior function of the immune system in younger patients has been proposed as a possible explanation.[33] In childhood and adolescence, melanomas frequently resemble benign lesions, which makes them hard to diagnose both clinically and pathologically.[34] Almost 50% of the melanomas in young adults do not fulfill the classic melanoma ABCD criteria.[35] Recent genomic analysis showed that melanomas in adolescents and young adults harbor mutation patterns that differ from those in older patients.[36]

Five-year MSS was 91% in our study and 5-year RFS was 84%. Several prior studies reported comparable survival rates with 5-year MSS ranging from 89% to 97 and 5-year RFS ranging from 68 to 90%.[9,11,18,37,38] Of the 15 patients who died of melanoma and in whom mitotic rate was assessed, 10 had a tumor mitotic rate of $<6/mm^2$. Five of 28 patients with recurrence (31%) experienced that recurrence after >5 years. As in adults, children and adolescents remain at risk of recurrence even after ≥10 years.[20,39] Childhood and adolescent patients are also twice as likely to develop a subsequent melanoma compared with adult patients.[40] This emphasizes the importance of continuing follow-up of patients who developed melanoma when they are young for more than the usual 5-year period recommended in the melanoma management guidelines of some countries.[41]

The strengths of our study include the relatively large cohort of patients. In addition, pathology slides of all patients were reviewed by experienced pathologists, increasing the reliability of the diagnosis and of histologic and staging data. There are also several limitations

affecting the study. Because of the moderate number of events, multivariable analysis could not be performed for MSS and only mitotic rate and Breslow thickness could be included in the multivariable analysis for RFS. Supplementary Table 2 shows the unstable multivariable analysis of RFS and MSS including Breslow thickness, mitotic rate, and ulceration. Although all cases were reviewed by an MIA-affiliated pathologist, some histological parameters were missing. The pathology slides of some patients were not available for reassessment. Other limitations are the retrospective design, the arbitrary age cut-off that was used to separate children and adolescents, referral bias, and the short follow-up of some patients.

CONCLUSION

Our study indicates that mitotic rate is an important prognostic feature for RFS in children and adolescents who develop melanoma, and it is therefore essential that this parameter be assessed and reported in the primary tumors of all young melanoma patients. Although mitotic rate was the only independent predictor of RFS, a larger study numbers is required to confirm these results. By extrapolating the number of recurrences in our study, approximately 500 children and adolescent patients would be needed to assess the prognostic value of the other prognostic factors that are common in adults. A collaborative study involving multiple melanoma centers would be needed.

REFERENCES

1. de Vries E, Steliarova-Foucher E, Spatz A, Ardanaz E, Eggermont AMM, Coebergh JWW. Skin cancer incidence and survival in European children and adolescents (1978-1997). Report from the Automated Childhood Cancer Information System project. *Eur J Cancer*. 2006;42(13):2170-2182.
2. 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.
3. 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.
4. 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.
5. 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.
6. 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.
7. 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.
8. 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.
9. Freemyer B, Hamilton E, Warneke CL, et al. Treatment outcomes in pediatric melanoma-Are there benefits to specialized care? *J Pediatr Surg*. 2016;51(12):2063-2067.
10. Paradela S, Fonseca E, Pita-Fernández S, Prieto VG. Spitzoid and non-spitzoid melanoma in children: a prognostic comparative study. *J Eur Acad Dermatol Venereol JEADV*. 2013;27(10):1214-1221.
11. Averbook BJ, Lee SJ, Delman KA, et al. Pediatric melanoma: Analysis of an international registry. *Cancer*. 2013;119(22):4012-4019.
12. 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.
13. 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.
14. Mu E, Lange JR, Strouse JJ. Comparison of the use and results of sentinel lymph node biopsy in children and young adults with melanoma. *Cancer*. 2012;118(10):2700-2707.
15. 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.
16. Brecht IB, Garbe C, Gefeller O, et al. 443 paediatric cases of malignant melanoma registered with the German Central Malignant Melanoma Registry between 1983 and 2011. *Eur J Cancer*. 2015;51(7):861-868.
17. Brecht IB, De Paoli A, Bisogno G, et al. Pediatric patients with cutaneous melanoma: A European study. *Pediatr Blood Cancer*. 2018;65(6):e26974.

18. Moore-Olufemi S, Herzog C, Warneke C, et al. Outcomes in Pediatric Melanoma. *Ann Surg*. 2011;253(6):1211-1215.
19. Aldrink JH, Selim MA, Diesen DL, et al. Pediatric melanoma: a single-institution experience of 150 patients. *J Pediatr Surg*. 2009;44(8):1514-1521.
20. Han D, Zager JS, Han G, et al. The unique clinical characteristics of melanoma diagnosed in children. *Ann Surg Oncol*. 2012;19(12):3888-3895.
21. Euling SY, Herman-Giddens ME, Lee PA, et al. Examination of US puberty-timing data from 1940 to 1994 for secular trends: panel findings. *Pediatrics*. 2008;121 Suppl:S172-91.
22. McGovern VJ, Cochran AJ, Van der Esch EP, Little JH, MacLennan R. The classification of malignant melanoma, its histological reporting and registration: a revision of the 1972 Sydney classification. *Pathology*. 1986;18(1):12-21.
23. Scolyer RA, Shaw HM, Thompson JF, et al. Interobserver reproducibility of histopathologic prognostic variables in primary cutaneous melanomas. *Am J Surg Pathol*. 2003;27(12):1571-1576.
24. Joosse A, Collette S, Suciu S, et al. Superior outcome of women with stage I/II cutaneous melanoma: pooled analysis of four European Organisation for Research and Treatment of Cancer phase III trials. *J Clin Oncol*. 2012;30(18):2240-2247.
25. Balch CM, Gershenwald JE, Soong SJ, et al. Final version of 2009 AJCC melanoma staging and classification. *J Clin Oncol*. 2009;27(36):6199-6206.
26. Austin MT, Xing Y, Hayes-Jordan AA, Lally KP, Cormier JN. Melanoma incidence rises for children and adolescents: an epidemiologic review of pediatric melanoma in the United States. *J Pediatr Surg*. 2013;48(11):2207-2213.
27. 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.
28. 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.
29. Peduzzi P, Concato J, Feinstein AR, Holford TR. Importance of events per independent variable in proportional hazards regression analysis II. Accuracy and precision of regression estimates. *J Clin Epidemiol*. 1995;48(12):1503-1510.
30. Bartenstein DW, Kelleher CM, Friedmann AM, et al. Contrasting features of childhood and adolescent melanomas. *Pediatr Dermatol*. 2018;35(3):354-360.
31. Elder DE, Massi D, Scolyer RA, Willemze R. *WHO Classification of Skin Tumours*. 4th ed.; 2018.
32. Livestro DP, Kaine EM, Michaelson JS, et al. Melanoma in the young: Differences and similarities with adult melanoma: A case-matched controlled analysis. *Cancer*. 2007;110(3):614-624.
33. Howman-Giles R, Shaw HM, Scolyer R a, et al. Sentinel lymph node biopsy in pediatric and adolescent cutaneous melanoma patients. *Ann Surg Oncol*. 2010;17(1):138-143.
34. Mitkov M, Chrest M, Diehl NN, Heckman MG, Tollefson M, Jambusaria-Pahlajani A. Pediatric melanomas often mimic benign skin lesions: A retrospective study. *J Am Acad Dermatol*. 2016;75(4):706-711.e4.
35. Carrera C, Scope A, Dusza SW, et al. Clinical and dermoscopic characterization of pediatric and adolescent melanomas: Multicenter study of 52 cases. *J Am Acad Dermatol*. 2018;78(2):278-288.
36. Wilmott JS, Johansson PA, Newell F, et al. Whole genome sequencing of melanomas in adolescent and young adults reveals distinct mutation landscapes and the potential role of germline variants in disease susceptibility. *Int J Cancer*. 2019;144(5):1049-1060.

37. Le Q, Norris D, McClean CA, et al. Single institution experience of paediatric melanoma in Victoria, Australia. *Australas J Dermatol.* 2017;58(2):117-121.

38. Réguerre Y, Vittaz M, Orbach D, et al. Cutaneous malignant melanoma in children and adolescents treated in pediatric oncology units. *Pediatr Blood Cancer.* 2016;63(11):1922-1927.

39. Stanelle EJ, Busam KJ, Rich BS, et al. Early-stage non-Spitzoid cutaneous melanoma in patients younger than 22 years of age at diagnosis: Long-term follow-up and survival analysis. *J Pediatr Surg.* 2015;50(6):1019-1023.

40. Jung GW, Weinstock MA. Clinicopathological comparisons of index and second primary melanomas in paediatric and adult populations. *Br J Dermatol.* 2012;167(4):882-887.

41. Trotter SC, Sroa N, Winkelmann RR, Olencki T, Bechtel M. A global eeview of melanoma follow-up guidelines. *J Clin Aesthetic Dermatol.* 2013;6(9):18-26.

Appendix 1. Characteristics of patients who died of melanoma.

Patient	**Age**	**Gender**	**Site**	**Tumor type**	**Breslow thickness** (mm)	**Mitotic rate** (/mm2)	**Ulceration**	**SNB**	**CLND**	**Site of first recurrence**	**Time until recurrence** (months)	**Time between recurrence and death** (months)	**Treatment after recurrence**
1	17	Male	Lower limb	NM	2.7	5	Absent	Positive	Negative	In transit	145	40	Isolated limb infusion
													Radiotherapy
													Local surgery
													Neurosurgery
													Chemotherapy
2	18	Male	Upper limb	SSM	1.6	14	Absent	Positive	Negative	Distant	17	4	Whole brain radiotherapy
													Chemotherapy
3	16	Male	Trunk	NM	4.2	13	Present	Positive	Positive (1 node)	In transit	5	14	Local surgery
													Radiotherapy
													Chemotherapy
4	19	Male	Trunk	Unknown	1.5	4	Absent	Positive	Negative	Distant	102	16	Radiotherapy
													Targeted therapy (dabrafenib)
													Immunotherapy (ipilimumab)

5	19	Female	Head and neck	SSM	1.0	1	Absent	Negative	NA	Regional node	8	4	Neck dissection Adjuvant radiotherapy Chemotherapy
6	15	Female	Lower limb	Spitzoid	1.0	Unknown	Unknown	Not performed	NA	Regional node	130	35	Inguinal lymph node dissection Radiotherapy Immunotherapy (ipilimumab) Targeted therapy (dabrafenib)
7	16	Male	Trunk	NM	1.6	5	Present	Not performed	NA	Regional node	26	24	Axillary lymph node dissection Chemotherapy
8	18	Female	Lower limb	NM	2.4	7	Absent	Not performed	NA	Regional node	17	20	Inguinal lymph node dissection Local surgery Chemotherapy Targeted therapy
9	18	Male	Upper limb	NM	3.3	13	Present	Not performed	NA	Regional node	8	13	Axillary lymph node dissection Local surgery Radiotherapy Chemotherapy
10	19	Male	Trunk	NM	4.0	10	Present	Not performed	NA	Regional node	5	31	Axillary lymph node dissection Adjuvant radiotherapy

11	19	Male	Upper limb	SSM	1.1	1	Present	Not performed	NA	Regional node	107	14	Axillary lymph node dissection Adjuvant radiotherapy Chemotherapy
12	19	Female	Trunk	SSM	1.7	2	Absent	Not performed	NA	Regional node	3	31	Axillary lymph node dissection Further treatment is unknown
13	18	Male	Trunk	Unknown	1.8	4	Unknown	Not performed	NA	Regional node	16	20	Axillary lymph node dissection Adjuvant radiotherapy Metastasectomy No chemotherapy
14	10	Female	Head and neck	NM	1.8	5	Unknown	Not performed	NA	Local	4	18	Neck dissection Adjuvant radiotherapy Chemotherapy
15	18	Male	Head and neck	SSM	0.4	0	Unknown	Not performed	NA	Distant	156	59	Metastasectomy Further treatment is unknown
16	18	Female	Trunk	SSM	0.9	3	Absent	Not performed	NA	Distant	42	20	Metastasectomy Radiotherapy Chemotherapy

SNB: sentinel node biopsy; CLND: completion lymph node dissection; SSM: superficial spreading melanoma; NM: nodular melanoma; NA: not applicable

CHAPTER 5

The influence of *CDKN2A* germline mutations on survival of melanoma patients: a retrospective cohort study

Norbertus A Ipenburg
Mary-Ann El Sharouni
Remco van Doorn
Paul J van Diest
Monique E van Leerdam
Jasper I van der Rhee
Jelle Goeman
Nicole A Kukutsch
and the Netherlands Foundation for Detection
of Hereditary Tumors collaborative investigators[#]

* Both authors contributed equally to this article.

Journal of the American of Academy of Dermatology (accepted)

ABSTRACT

Background. Approximately 10% of patients with cutaneous melanomas have a positive melanoma family history. Germline mutation of the *CDKN2A* gene is the most common cause of familial melanoma. It is uncertain whether carriership affects prognosis.

Objective. To compare survival of *CDKN2A* germline mutation-positive melanoma (*CDKN2A*-mut) patients with sporadic melanoma patients.

Methods. A population-based cohort of sporadic melanoma patients diagnosed between 2000 and 2014 (n=56,929) and a cohort of *CDKN2A*-mut patients (n=89) were analyzed. Recurrence-free survival (RFS) and overall survival (OS) were calculated. Multivariable Cox proportional hazards analyses were performed.

Results. *CDKN2A*-mut patients were significantly younger than sporadic melanoma patients at melanoma diagnosis (median 42 vs. 57 years; $p<0.0001$). Their melanomas were thinner (median Breslow thickness 0.6mm vs. 0.9mm; $p<0.0001$) and less often ulcerated (1% vs. 13%; $p<0.0001$) than sporadic melanoma patients. After correcting for potential confounders, OS and RFS were not significantly different for *CDKN2A*-mut and sporadic patients (OS hazard ratio 1.44; 95% confidence interval 0.9-2.4 and RFS hazard ratio 0.91; 95% confidence interval 0.5-1.8).

Limitations. Retrospective study, cause of death was not available

Conclusion. Presence of a germline *CDKN2A* mutation was not associated with survival in our cohort of melanoma patients.

INTRODUCTION

Approximately 10% of patients with cutaneous melanomas have a positive melanoma family history.[1,2] Pathogenic germline mutation of the *CDKN2A* gene, encoding the p16 and p14 tumor suppressor proteins, is the most common cause of familial melanoma.[1,3] In the Netherlands, the most prevalent inactivating *CDKN2A* mutation is a 19 bp deletion in exon 2 (c.225-243del19), a founder mutation termed the p16-Leiden mutation.[4] Mutation carriers have a life-time risk of melanoma of approximately 70%, and many patients develop melanoma at a younger age.[5] In addition, they are at increased risk of developing solid tumors such as pancreatic cancer and head and neck cancer.[2,4,6–10] Since a subset of patients presents with atypical melanocytic nevi, the condition has been referred to by some as familial atypical multiple mole melanoma (FAMMM) syndrome. For other cancer types, there is evidence that patients with hereditary tumors have different prognoses than patients with sporadic tumors. As an example a number of studies have reported worse survival outcomes for *BRCA1* germline mutation-positive breast cancer patients.[11] Recent studies on survival of *CDKN2A* germline mutation-positive melanoma patients (*CDKN2A*-mut) showed conflicting results.[12–14] Swedish *CDKN2A*-mut patients (n=96) had worse survival than *CDKN2A* germline mutation-negative melanoma patients. In a second study among Swedish *CDKN2A* mutation carriers, 43 *CDKN2A*-mut multiple melanoma patients (MPM) had a worse survival than melanoma patients without this germline mutation.[12,13] However, 106 Italian *CDKN2A*-mut patients had similar survival as a matched cohort of *CDKN2A* germline mutation-negative melanoma patients.[14] Since there is ongoing debate regarding the prognostic impact of germline *CDKN2A* mutation status on survival of melanoma patients, the aim of the current study was to compare the survival of *CDKN2A*-mut with that of patients with sporadic melanoma.

METHODS

This nation-wide retrospective study obtained *CDKN2A*-mut patients from the melanoma database of the Netherlands Foundation for Detection of Hereditary Tumors (NFDHT). The organization and methods of the NFDHT have been described previously.[7,15] Since 1985, Dutch physicians admit patients suspected of familial melanoma to the registry. All reported malignancies are verified by medical records and genealogic studies are performed. The registry collects follow-up data on proven *CDKN2A* mutation carriers and their relatives. In this study, *CDKN2A*-mut patients were carriers of the p16-Leiden variant of *CDKN2A*

(c.225_243del, p.Ala- 76Cysfs*64; RefSeq NM_000077.4) or first-degree relatives of proven carriers of this mutation. Sporadic melanoma patients were extracted from PALGA, the Dutch Nationwide Network and Registry of Histopathology and Cytopathology.[16] Since 1991, PALGA has been collecting data prospectively from all pathology laboratories in the Netherlands. Follow-up data of sporadic melanoma patients were obtained from the Netherlands Cancer Registry, which gathers information about every patient with cancer in the Netherlands. All data were encoded and used anonymously. Ethical approval was granted by the ethical review board of PALGA, Houten, the Netherlands, and Leiden University Medical Center (Protocol number P00.117).

Study population

All adults newly diagnosed with invasive, clinically localized, primary cutaneous melanoma diagnosed between January 1, 2000 and December 31, 2014 were included. Patients were included based on their first primary melanoma diagnosis. Noncutaneous melanoma, melanoma of unknown primary and melanomas occurring among children (<18 years of age) were excluded. Furthermore, patients presenting with clinically detected lymph nodes, in-transit metastases or micro-satellites (stage III) or distant disease (stage IV) at time of diagnosis were excluded. Sentinel node (SN)-positive melanomas were included.

Data collection

Data on patient demographics (gender, age at diagnosis, *CDKN2A* status), primary tumor characteristics (date of diagnosis, primary site, Breslow thickness, melanoma subtype, ulceration status, tumor mitotic rate, sentinel node (SN) status), subsequent melanomas, recurrence (date, site and type), and vital status were recorded. Patients with multiple primary melanomas (MPM) were defined as those with a new primary melanoma on or after the date of first melanoma diagnosis, irrespective of topography.

The outcomes of interest were recurrence-free survival (RFS) and overall survival (OS). In patients with first recurrences at multiple sites, the site with the most unfavorable prognosis was scored as the first site (hierarchal order: local, regional, distant). RFS and OS were calculated from the date of initial melanoma diagnosis to the date of diagnosis of recurrence, or death, respectively. Patients without recurrence were censored at their date of death, the last date known to be alive or January 1st, 2018 (the database cut-off date), whichever occurred first.

Statistical analysis

Categorical variables were summarized as numbers and percentages. Continuous variables were summarized as medians with interquartile range (IQRs). Differences in proportions and medians were analysed using chi-square tests or Mann-Whitney *U* test, respectively. Univariable and multivariable Cox proportional hazards regression analysis were performed to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for RFS and OS. No statistical variable selection procedure was performed, because all clinicopathological factors that were included are basic, readily available in pathological reports. Variables included were *CDKN2A* status, age, gender, year of diagnosis, Breslow thickness, ulceration status, primary site, melanoma subtype and SN status. Because of a relatively large number of missing values for ulceration, a "not known" category was created for this variable.[17] Since it has been suggested that a missing-indicator variable might lead to bias, a sensitivity analysis was performed, categorizing patients with a "not known" status for ulceration as "not present" to assess the impact on HRs of *CDKN2A* status in the multivariable model.[18] Multiple imputation was not considered, given the pathologist involved in this study believes these histopathological variables are not missing at random, but rather because they were not seen during pathological assessment. The missing at random assumption, a condition for multiple imputation, would therefore be too strong.[19] The proportional hazards assumption was examined by plotting a log-minus-log graph for categorical variables. If the lines were parallel, it was assumed that the proportional hazards assumption was not violated. For continuous variables (Breslow thickness and age), Schoenfeld residuals were plotted as a function of time, and a loess curve was fitted. If the curve was horizontal, it was assumed that the proportional hazards assumption was not violated. To assess linearity of continuous variables, Martingale residuals were plotted against time. In case of non-linearity, continuous variables were categorized. All statistical analyses were performed using R version 3·6·1 (R Core Team, Vienna, Austria). Two-sided P-values <0.05 were considered significant.

RESULTS

Patient and tumor characteristics of CDKN2A-mut patients

A total of 89 *CDKN2A*-mut and 56,929 sporadic melanoma patients were eligible for inclusion in this study. The baseline characteristics are shown in Table 1. The majority of *CDKN2A*-mut patients were female (64%) with a median age of 42 years (IQR 31-50 years). Their melanomas were most frequently located on the trunk (39.3%). Melanomas were most often

<0.8mm thick (60.9%). Ulceration was present in 1.1% of the *CDKN2A*-mut patients and mitoses in 32.6%. Eight *CDKN2A*-mut patients underwent SN biopsy of which one had a positive SN (12.5%).

Table 1. Baseline characteristics of CDKN2A germline mutation-positive and sporadic melanoma patients

Characteristics	***CDKN2A*-mut (n=89)**	**Sporadic (n=56929)**	**P-value**
Gender			0.13
Female	57 (64.0)	31916 (56.1)	
Male	32 (36.0)	25013 (43.9)	
Median age at diagnosis in years (IQR)	42 (31-50)	57 (44-68)	<0.0001
Year of diagnosis			<0.0001
2000/2001	15 (16.9)	4928 (8.7)	
2002/2003	17 (19.1)	5459 (9.6)	
2004/2005	13 (14.6)	6396 (11.2)	
2006/2007	15 (16.9)	6979 (12.3)	
2008/2009	10 (11.2)	810 (14.2)	
2010/2011	11 (12.4)	9308 (16.4)	
2012/2013/2014	8 (9.0)	15759 (27.7)	
Primary site			0.04
Head & Neck	5 (5.6)	7127 (12.5)	
Trunk	35 (39.3)	23892 (42.0)	
Upper limb	18 (20.2)	8327 (14.6)	
Lower limb	31 (34.8)	15725 (27.6)	
Not known	0 (0.0)	1858 (3.3)	
Median Breslow thickness in mm (IQR)	0.6 (0.4-0.9)	0.9 (0.5-1.8)	<0.0001
Breslow thickness in mm			<0.0001
<0.8	53 (60.9)	23270 (40.9)	
≤0.8-1.0	16 (18.4)	9311 (16.4)	
1.1-2.0	15 (17.2)	12614 (22.2)	
2.1-4.0	3 (3.4)	7668 (13.5)	
>4.0	0 (0.0)	4066 (7.1)	
Subtype			0.03
Non-nodular	84 (94.4)	49248 (86.5)	
Nodular	5 (5.6)	7679 (13.5)	
Ulceration			<0.0001
No	53 (59.6)	39030 (68.6)	
Yes	1 (1.1)	7587 (13.3)	
Unknown	35 (39.3)	10312 (18.1)	

Characteristics	*CDKN2A*-mut (n=89)	Sporadic (n=56929)	P-value
Mitoses			0.05
No	14 (15.7)	9914 (17.4)	
Yes	29 (32.6)	12522 (22.0)	
Unknown	46 (51.7)	34493 (60.6)	
Multiple melanoma			<0.0001
No (SPM)	51 (57.3)	54645 (96.0)	
Yes (MPM)	38 (42.7)	2284 (4.0)	
SN status			0.50
Negative	7 (87.5)	9162 (77.5)	
Positive	1 (12.5)	2666 (22.5)	
Not performed	81	45099	
Median follow-up in years (IQR)	11.5 (9.4-15.7)	6.3 (3.6-10.3)	<0.0001

Data are expressed as n (%) unless otherwise specified
CDKN2A-mut = *CDKN2A* germline mutation-positive melanoma patients; IQR = interquartile range; SPM = single primary melanoma; MPM = multiple primary melanoma; SN = sentinel node

Differences between CDKN2A-mut and sporadic melanoma patients

CDKN2A-mut patients more often developed MPM than patients with sporadic melanoma (42.7% vs. 4.0%; P<0.0001). The median age at diagnosis of the first melanoma was 15 years lower for *CDKN2A*-mut patients than for patients with sporadic melanoma (42 vs. 57 years; P<0.0001). *CDKN2A*-mut patients had thinner melanomas (median Breslow thickness 0.6mm vs 0.9mm; P<0.0001) and none of the *CDKN2A*-mut patients had a Breslow thickness of more than 4.0 mm (0% vs. 7.1%). Melanomas of sporadic melanoma patients were more often nodular (13.5% vs. 5.6%; P=0.03) and ulcerated (22.0% vs. 1.1%; P<0.0001). Gender and SN status did not differ significantly between the two groups. The median follow-up was 11.5 years for *CDKN2A*-mut patients and 6.3 years for sporadic melanoma patients.

Overall survival according to CDKN2A mutation status

Due to missing data, a total of 51,921 cases were analyzed: 89 (14 deaths) *CDKN2A*-mut patients and 53,589 (10,800 deaths) sporadic melanoma patients. On univariable analysis, the presence of a germline *CDKN2A* mutation was significantly associated with better OS (HR=0.52; 95% CI 0.31-0.88). In multivariable analysis, Breslow thickness per mm, ulceration, SN positivity, and nodular subtype all independently increased the HR with 1.06 (95% CI 1.06-1.07), 2.18 (95% CI 2.08-2.28), 2.42 (95% CI 2.23-2.63), and 1.41 (95% CI

1.34-1.48), respectively. The proportional hazards assumption was not violated for any of the included variables. Due to non-linearity, age and year of diagnosis were categorized. Corrected for all determinants (i.e. gender, Breslow thickness, age, primary site, ulceration, SN status, melanoma subtype, and year of diagnosis), a non-significant HR for *CDKN2A*-mut versus sporadic melanoma patients of 1.44 (95% CI 0.85-2.43) was found (Table 2). Addition of an unknown category as a separate category to ulceration (i.e. "yes" vs. "no" vs. "unknown"), did not change the HR of *CDKN2A*-mut patients (HR 1.43; 95% CI 0.85-2.42).

Recurrence-free survival according to CDKN2A mutation status

On univariable analysis, the presence of a germline *CDKN2A* mutation was associated with a better RFS (HR 0.48; 95% CI 0.24-0.98). After correcting for all aforementioned confounders, RFS was not significantly different for patients with or without a germline *CDKN2A* mutation (HR 0.91; 95% CI 0.45-1.83). Addition of an unknown category to ulceration (i.e. "yes" vs. "no" vs. "unknown") did not change the HR of *CDKN2A*-mut patients (HR 0.91; 95% CI 0.45-1.84).

Table 2. Univariable and multivariable Cox regression for overall survival and recurrence-free survival for all patients (n = 51,921)

Variable	Class	Overall survival (10457 events)				Recurrence free survival (6865 events)			
		Univariable		Multivariable		Univariable		Multivariable	
		HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
CDKN2A	Not mutated	1		1		1		1	
	Mutated	0.52 (0.31-0.88)	0.01	1.44 (0.85-2.43)	0.18	0.48 (0.24-0.98)	0.04	0.91 (0.45-1.83)	0.78
Gender	Male	1		1		1		1	
	Female	0.58 (0.55-0.60)	<0.0001	0.69 (0.66-0.72)	<0.0001	0.59 (0.57-0.63)	<0.0001	0.72 (0.68-0.75)	<0.0001
Breslow thickness	Per mm	1.11 (1.10-1.11)	<0.0001	1.06 (1.06-1.07)	<0.0001	1.11 (1.11-1.11)	<0.0001	1.08 (1.08-1.09)	<0.0001
Age at diagnosis	18-27	1		1		1		1	
	28-37	0.16 (0.94-1.45)	0.17	1.14 (0.91-1.41)	0.26	0.98 (0.82-1.16)	0.80	1.00 (0.84-1.19)	0.99
	38-47	1.67 (1.36-2.05)	<0.0001	1.61 (1.31-1.97)	<0.0001	1.19 (1.01-1.40)	0.04	1.24 (1.05-1.46)	0.01
	48-57	2.54 (2.08-3.09)	<0.0001	2.30 (1.89-2.82)	<0.0001	1.54 (1.31-1.81)	<0.0001	1.43 (1.21-1.68)	<0.0001
	58-67	4.32 (3.55-5.26)	<0.0001	3.66 (3.01-4.47)	<0.0001	1.90 (1.62-2.22)	<0.0001	1.66 (1.42-1.95)	<0.0001
	68-77	8.49 (6.98-10.33)	<0.0001	7.00 (5.75-8.53)	<0.0001	2.30 (1.96-2.69)	<0.0001	1.83 (1.56-2.15)	<0.0001
	78-87	19.55 (16.07-23.79)	<0.0001	14.94 (12.25-18.22)	<0.0001	2.86 (2.42-3.38)	<0.0001	2.02 (1.71-2.40)	<0.0001
	88+	45.53 (37.03-55.97)	<0.0001	29.22 (23.65-36.10)	<0.0001	3.44 (2.74-4.31)	<0.0001	1.49 (1.17-1.91)	0.001
Primary site	Head and neck	1		1		1		1	
	Trunk	0.53 (0.50-0.55)	<0.0001	0.95 (0.90-1.01)	0.09	0.77 (0.71-0.82)	<0.0001	0.88 (0.82-0.95)	0.001
	Upper limb	0.52 (0.48-0.55)	<0.0001	0.78 (0.73-0.83)	<0.0001	0.52 (0.48-.58)	<0.0001	0.61 (0.55-0.67)	<0.0001
	Lower limb	0.46 (0.43-0.48)	<0.0001	0.83 (0.78-0.88)	<0.0001	0.81 (0.76-0.88)	<0.0001	1.01 (0.93-1.09)	0.83

Variable	Class	Overall survival (10457 events)				Recurrence free survival (6865 events)			
		Univariable		Multivariable		Univariable		Multivariable	
		HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Ulceration	No	1		1		1		1	
	Yes	4.25 (4.08-4.42)	<0.0001	2.18 (2.08-2.28)	<0.0001	5.42 (5.16-5.69)	<0.0001	2.97 (2.81-3.14)	<0.0001
SN status	Negative	1		1		1		1	
	Positive	2.87 (2.64-3.11)	<0.0001	2.42 (2.23-2.63)	<0.0001	3.20 (2.95-3.47)	<0.0001	2.39 (2.20-2.60)	<0.0001
	Not performed	1.21 (1.15-1.28)	<0.0001	1.15 (1.09-1.22)	<0.0001	0.71 (0.66-0.75)	<0.0001	0.96 (0.90-1.02)	0.17
Subtype	Non-nodular	1		1		1		1	
	Nodular	2.83 (2.71-2.95)	<0.0001	1.41 (1.34-1.48)	<0.0001	3.61 (3.43-3.80)	<0.0001	1.80 (1.70-1.91)	<0.0001
Year of diagnosis	2000 / 2001	1		1		1		1	
	2002 / 2003	0.94 (0.88-1.01)	0.12	0.87 (0.81-0.94)	<0.0001	0.92 (0.84-1.01)	0.09	0.84 (0.76-0.93)	<0.0001
	2004 / 2005	0.93 (0.86-0.99)	0.03	0.87 (0.81-0.94)	<0.0001	0.93 (0.85-1.02)	0.11	0.89 (0.81-0.98)	0.02
	2006 / 2007	0.99 (0.92-1.06)	0.68	0.93 (0.87-1.01)	0.07	0.98 (0.89-1.07)	0.61	0.98 (0.89-1.07)	0.64
	2008 / 2009	0.93 (0.87-1.01)	0.07	0.82 (0.76-0.88)	<0.0001	0.85 (0.78-0.94)	0.001	0.85 (0.77-0.93)	0.001
	2010 / 2011	0.94 (0.87-1.02)	0.12	0.79 (0.73-0.86)	<0.0001	0.89 (0.81-0.98)	0.01	0.88 (0.80-0.97)	0.007
	2012 / 2013 / 2014	0.91 (0.84-0.98)	0.01	0.71 (0.65-0.76)	<0.0001	0.94 (0.86-1.02)	0.15	0.91 (0.83-0.99)	0.04

HR = hazard ratio; CI = confidence interval; SN = sentinel node

DISCUSSION

In this study, we found no evidence for a survival difference between *CDKN2A* mutation carriers and sporadic melanoma patients. No significant difference in OS and RFS was found between *CDKN2A*-mut and sporadic melanoma patients when controlling for known confounders such as age, gender, Breslow thickness, primary site, year of diagnosis, ulceration, melanoma subtype, and SN status.

The results of the current study are in line with those of Dalmasso et al., who also did not find a significant difference in survival between *CDKN2A*-mut and *CDKN2A* germline mutation-negative melanoma patients.[14] In contrast, a Swedish cohort of *CDKN2A*-mut patients with familial melanoma had worse survival than *CDKN2A* germline mutation-negative patients with familial or sporadic melanoma.[12] Another study from the same Swedish group demonstrated that *CDKN2A*-mut MPM patients had worse survival than *CDKN2A* germline mutation-negative MPM patients.[13] The type and location of the *CDKN2A* germline mutation might be of influence on the effect that this mutation has on survival. In the current study, patients had the p16-Leiden mutation, a *CDKN2A* germline mutation which mainly inactivates p16, while the function of p14ARF is only slightly impaired. In the study by Dalmasso et al. most patients harbored the G101W mutation, a *CDKN2A* missense mutation, while in the study by Helgadottir et al. the Swedish founder mutation, p.Arg112dup, was most often found.[12–14,20]

The aims and design of these studies and our study differ on several points. In one of the Swedish studies, only MPM patients were included, while in the other two studies and in this study also single primary melanoma (SPM) patients were included.[12–14] MPM patients have worse survival than SPM patients, complicating comparison of these studies.[21] The selection and size of the control group, i.e. sporadic melanoma patients, is also different. We used a nationwide control group of almost 60,000 patients, which made it possible to control for a large number of confounders. Previous studies did not control for primary site, ulceration, melanoma subtype, and SN status.[12,14] A drawback of our approach is the fact that *CDKN2A* mutation status was unknown for patients in the control group. Since all newly diagnosed clinically localized cutaneous melanoma patients in the Netherlands were included in this study, the 89 *CDKN2A*-mut patients will most likely also be present in the control group. However, since this concerns less than 0.2% of the control patients, we do not expect this to reduce the validity of the results.[22] The outcome measures also differ between the above studies. OS was assessed in all four studies, while in the current study RFS was studied instead of melanoma-specific survival.[12–14]

The patient and tumor characteristics of *CDKN2A*-mut and sporadic melanoma patients differed considerably in our study. In accordance with earlier studies, *CDKN2A*-mut patients were much younger when their first melanoma was diagnosed and were more prone to develop MPMs.[5,12,14,23,24] In the current study, melanomas of sporadic melanoma patients were thicker and more often nodular and ulcerated. Prior studies comparing histological features of *CDKN2A*-mut and *CDKN2A* germline mutation-negative melanomas have found conflicting results. In some studies, melanomas of *CDKN2A*-mut were less advanced at diagnosis, while in others no difference between the groups was found.[5,12,14,23–26]

To detect melanomas at earlier stages, Dutch *CDKN2A* mutation carriers are subjected to thorough surveillance. Biannual total skin examination with the use of dermoscopy and total body photography is recommended to *CDKN2A*-mut patients from the age of 12. Furthermore, patients are instructed to perform skin self-examination. From the age of 40, annual pancreatic screening by MRI and/or endoscopic ultrasound is performed in proven mutation carriers who are enrolled in several prospective studies.[27–29] Close surveillance of *CDKN2A*-mut patients is probably one of the reasons why melanomas of *CDKN2A*-mut patients were diagnosed at less advanced stages. As previously demonstrated, *CDKN2A*-mut patients are at increased risk of misdiagnosis of their benign melanocytic lesion as melanoma.[30] Melanoma overdiagnosis of *CDKN2A*-mut patients might falsely skew their prognosis.[30,31]

There are several limitations affecting this study. Due to the fact that cause of death for sporadic melanoma patients is not registered in the Dutch Cancer Registry, melanoma-specific survival could not be calculated. However, we were able to calculate RFS, which was not assessed in prior studies comparing survival of *CDKN2A*-mut and sporadic melanoma patients.[12–14] Ascertainment bias and longevity bias might also limit the results of this study. Ascertainment bias is difficult to prevent in mutation-based studies. Pedigrees with many affected relatives and MPM patients are more likely to be identified, registered and genetically tested. In addition, patients who survive longer are more likely to be offered genetic testing, thus causing an overestimation of survival (longevity bias).[32] The *CDKN2A*-mut patients are at increased risk of developing MPM and pancreatic cancer. These competing risks might have influenced the outcomes of interest. Other limitations were the retrospective design, the relatively small number of *CDKN2A*-mut patients, and some missing values.

CONCLUSION

The presence of germline *CDKN2A* mutation was not associated with melanoma survival in the present study. Melanomas of *CDKN2A*-mut patients were diagnosed at an earlier stage. This emphasizes the importance of early dermatological surveillance of *CDKN2A*-mut patients.

REFERENCES

1. Read J, Wadt KAW, Hayward NK. Melanoma genetics. *J Med Genet*. 2016;53(1):1-14.
2. Goldstein AM, Fraser MC, Struewing JP, et al. Increased risk of pancreatic cancer in melanoma-prone kindreds with p16 INK4 mutations. *N Engl J Med*. 1995;333(15):970-975.
3. Goldstein AM, Chan M, Harland M, et al. High-risk melanoma susceptibility genes and pancreatic cancer, neural system tumors, and uveal melanoma across GenoMEL. *Cancer Res*. 2006;66(20):9818-9828.
4. de Snoo FA, Bishop DT, Bergman W, et al. Increased risk of cancer other than melanoma in CDKN2A founder mutation (p16-Leiden)-positive melanoma damilies. *Clin Cancer Res*. 2008;14(21):7151-7157.
5. 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.
6. Helgadottir H, Höiom V, Jönsson G, et al. High risk of tobacco-related cancers in CDKN2A mutation-positive melanoma families. *J Med Genet*. 2014;51(8):545-552.
7. Vasen HF, Gruis NA, Frants RR, van Der Velden PA, Hille ET, Bergman W. Risk of developing pancreatic cancer in families with familial atypical multiple mole melanoma associated with a specific 19 deletion of p16 (p16-Leiden). *Int J Cancer*. 2000;87(6):809-811.
8. Mukherjee B, DeLancey JO, Raskin L, et al. Risk of non-melanoma cancers in first-degree relatives of CDKN2A mutation xarriers. *J Natl Cancer Inst*. 2012;104(12):953-956.
9. Borg A, Sandberg T, Nilsson K, et al. High frequency of multiple melanomas and breast and pancreas carcinomas in CDKN2A mutation-positive melanoma families. *J Natl Cancer Inst*. 2000;92(15):1260-1266.
10. Potjer TP, Kranenburg HE, Bergman W, et al. Prospective risk of cancer and the influence of tobacco use in carriers of the p16-Leiden germline variant. *Eur J Hum Genet*. 2015;23(5):711-714.
11. Baretta Z, Mocellin S, Goldin E, Olopade OI, Huo D. Effect of BRCA germline mutations on breast cancer prognosis: A systematic review and meta-analysis. *Medicine*. 2016;95(40):e4975.
12. 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.
13. 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.
14. 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.
15. Vasen HFA, Bergman W, van Haeringen A, Scheffer E, van Slooten EA. The familial dysplastic nevus syndrome. *Eur J Cancer Clin Oncol*. 1989;25(2):337-341.
16. Casparie M, Tiebosch ATMG, Burger G, et al. Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. *Cell*. 2007;29(1):19-24.
17. Johansson ÅM, Karlsson MO. Comparison of methods for handling missing covariate data. *AAPS J*. 2013;15(4):1232-1241.

18. Groenwold RHH, White IR, Donders ART, Carpenter JR, Altman DG, Moons KGM. Missing covariate data in clinical research: when and when not to use the missing-indicator method for analysis. *Can Med Assoc J*. 2012;184(11):1265-1269.
19. Marshall A, Altman DG, Royston P, Holder RL. Comparison of techniques for handling missing covariate data within prognostic modelling studies: a simulation study. *BMC Med Res Methodol*. 2010;10(1):7.
20. 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.
21. El Sharouni M-A, Witkamp AJ, Sigurdsson V, van Diest PJ. Comparison of Survival Between Patients With Single vs Multiple Primary Cutaneous Melanomas. *JAMA Dermatol*. 2019;155(9):1049.
22. Harland M, Cust AE, Badenas C, et al. Prevalence and predictors of germline CDKN2A mutations for melanoma cases from Australia, Spain and the United Kingdom. *Hered Cancer Clin Pract*. 2014;12(1):1-10.
23. Måsbäck A, Olsson H, Westerdahl J, et al. Clinical and histopathological features of malignant melanoma in germline CDKN2A mutation families. *Melanoma Res*. 2002;12(6):549-557.
24. 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.
25. 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.
26. Sargen MR, Kanetsky PA, Newton-Bishop J, et al. Histologic features of melanoma associated with CDKN2A genotype. *J Am Acad Dermatol*. 2015;72(3):496-507.e7.
27. Halk AB, Potjer TP, Kukutsch NA, Vasen HFA, Hes FJ, van Doorn R. Surveillance for familial melanoma: Recommendations from a national centre of expertise. *Br J Dermatol*. 2019;181(3):594-596.
28. van der Rhee JI, Boonk SE, Putter H, et al. Surveillance of second-degree relatives from melanoma families with a CDKN2A germline mutation. *Cancer Epidemiol Biomark Prev*. 2013;22(10):1771-1777.
29. van der Rhee JI, de Snoo FA, Vasen HFA, et al. Effectiveness and causes for failure of surveillance of CDKN2A-mutated melanoma families. *J Am Acad Dermatol*. 2011;65(2):289-296.
30. Van Der Rhee JI, Mooi WJ, Kukutsch NA, De Snoo FA, Bergman W. Iatrogenic melanoma. Comment on: Melanoma epidemic: a midsummer night's dream? *Br J Dermatol*. 2010;162(2):457-458.
31. Welch HG, Mazer BL, Adamson AS. The rapid rise in cutaneous melanoma diagnoses. *N Engl J Med*. 2021;384(1):72-79.
32. Hujoel MLA, Parmigiani G, Braun D. Statistical approaches for meta-analysis of genetic mutation prevalence. *Genet Epidemiol*. 2021;45(2):154-170.

CHAPTER 6

Sentinel node biopsy in cutaneous melanoma patients with germline *CDKN2A* mutations

Norbertus A. Ipenburg
Jos A. van der Hage
Julia A. Newton-Bishop
Mark Harland
Nicole A. Kukutsch
Hildur Helgadottir
Maarten H. Vermeer
Omgo E. Nieweg
Remco van Doorn

Melanoma Research. 2020 Dec;30(6):630-631

INTRODUCTION

Sentinel node biopsy (SNB) has become a routine staging procedure with prognostic and therapeutic impact in patients with cutaneous melanoma. Sentinel node status is the strongest prognostic factor for survival in clinically localized melanoma patients.[1] Approximately 10% of melanoma patients have a family history of this disease. Germline mutations in the *CDKN2A* gene, encoding the p16 and p14 tumor suppressor proteins, are the most common cause of familial melanoma.[2] These patients with familial atypical multiple mole melanoma (FAMMM) syndrome have a life-time melanoma risk of approximately 70%.[2,3] Melanoma-specific survival of patients with germline *CDKN2A* mutations has been reported to be worse than of patients with sporadic melanoma.[3] Since the biology of melanoma in *CDKN2A* mutation carriers appears to be more aggressive, we hypothesized that the frequency and predictive value of sentinel node-positivity might be different in this patient group. This study reports the characteristics and outcomes of patients with hereditary melanoma carrying germline *CDKN2A* mutations who underwent SNB.

METHODS

In this multicenter, retrospective case series, all CDKN2A mutation carriers with clinically-localized cutaneous melanoma who underwent SNB at 4 tertiary referral centers (Leiden University Medical Center, Leiden, the Netherlands; Melanoma Institute Australia, Sydney, Australia; Leeds Institute of Medical Research, Leeds, UK; and Karolinska Institutet, Stockholm, Sweden) between January 2000 and April 2015 were included. Demographics, tumor characteristics and follow-up data were collected.

RESULTS

SNB was performed in 23 melanoma patients carrying germline *CDKN2A* mutations. Fifteen patients were female and eight male; the median age was 47 years (range 20–70 years). Seven patients had previously been diagnosed with primary melanoma. Melanomas were located on the trunk in nine patients, lower limb in eight patients, upper limb in four patients, and two patients had their melanoma located in the head and neck region. The median Breslow-thickness was 1.5mm (range 0.8– 3.3mm), four melanomas were ulcerated, and 15 had a tumor mitotic rate ≥1/mm^2. Lymphoscintigraphy showed drainage to a median of two sentinel nodes and five melanomas drained to multiple nodal regions. Sentinel node was

positive in five patients. Breslow-thickness of the sentinel node-positive melanomas ranged from 1.1 to 2.9 mm, one was ulcerated and all had tumor mitotic rate ≥1/mm^2. Completion lymph node dissection (CLND) was performed in three sentinel node-positive patients, while two patients declined the procedure. Only one patient who underwent CLND had metastasis in a non- sentinel node lymph node.

During a median follow-up time of 100 months, three patients experienced a locoregional recurrence and three patients developed systemic metastatic disease. At the end of this period, 17 patients were still alive, two patients had died of melanoma and three patients of other causes. Of the five sentinel node-positive patients, one had a local recurrence and another developed systemic metastases and died of melanoma. This female patient had been diagnosed with stage IIIA (pT2aN2a) melanoma on the trunk and survived for 3years. Two of the 17 sentinel node-negative patients (12%) developed a local recurrence and two had systemic metastases. One female patient with a stage IB (pT2aN0) melanoma on the left lower leg died 6 years later from her disease. Three patients, all sentinel node-negative, died of other causes.

DISCUSSION

This is the first study to present results from SNB in patients with hereditary melanoma due to germline *CDKN2A* mutations (FAMMM syndrome), whose melanomas have been reported to behave more aggressively.[3] The emergence of effective adjuvant systemic treatment in SN-positive patients and the recent report of superior immunotherapy responses in *CDKN2A* mutations carriers make SNB an even more important staging tool.[4,5] Although *CDKN2A* mutation carriers were reported to have worse survival than sporadic melanoma patients, we did not observe a higher sentinel node-positivity rate in our case series. The sentinel node-positivity rate of 22% in our study is not inconsistent with what has been reported for patients with sporadic intermediate-thickness melanomas.[1,3] As only two melanoma-related deaths occurred in this cohort we cannot draw reliable conclusions regarding the prognostic value of SNB. Genetic testing for a germline *CDKN2A* mutation is recommended in patients suspected of hereditary melanoma. In our clinics, we see these patients more regularly than patients without the mutation and advise screening for pancreatic cancer from the age of 40 years.

In our experience, there may be reluctance to perform SNB in this particular patient group. Since over 40% of *CDKN2A* mutation carriers have multiple primary melanomas, many of them would have been excluded from clinical trials investigating SNB.[1,3] Patients with multiple

primary melanomas probably often have multiple sentinel nodes and drainage to more than one basin. Although the procedure might be more extensive, having multiple melanomas is not a contraindication for SNB. Due to surveillance, melanomas are on average diagnosed at an earlier stage in *CDKN2A* mutation carriers than in sporadic melanoma patients, limiting the experience with SNB in this patient group.[3]

There are several limitations affecting this study of which the small size of the cohort is the most important one. Other limitations are the retrospective design and potential selection bias.

CONCLUSION

The sentinel node-positivity rate for patients with *CDKN2A* mutation and for patients with sporadic melanoma appears to be similar. There should be no reluctance to perform SNB in these patients.

REFERENCES

1. 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.
2. Read J, Wadt KAW, Hayward NK. Melanoma genetics. *J Med Genet*. 2016;53(1):1-14.
3. 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).
4. 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.
5. 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.

CHAPTER 7

External validation of a prognostic model to predict survival of sentinel node-negative melanoma patients

Norbertus A. Ipenburg
Omgo E. Nieweg
Tasnia Ahmed
Remco van Doorn
Richard A. Scolyer
Georgina V. Long
John F. Thompshon
Serigne Lo

British Journal of Surgery. 2019 Sep;106(10):1319-1326

ABSTRACT

Background. Identifying patients with sentinel node-negative melanoma at high risk of recurrence or death is important. The European Organisation for Research and Treatment of Cancer (EORTC) recently developed a prognostic model including Breslow thickness, ulceration and site of the primary tumor. The aims of the present study were to validate this prognostic model externally and to assess whether it could be improved by adding other prognostic factors.

Methods. Patients with sentinel node-negative cutaneous melanoma were included in this retrospective single-institution study. The beta values of the EORTC prognostic model were used to predict recurrence-free survival and melanoma-specific survival. The predictive performance was assessed by discrimination (c-index) and calibration. Seeking to improve the performance of the model, additional variables were added to a Cox proportional hazards model.

Results. Some 4235 patients with sentinel node-negative cutaneous melanoma were included. The median follow-up time was 50 (IQR 18.5–81.5) months. Recurrences and deaths from melanoma numbered 793 (18.7%) and 456 (10.8%) respectively. Validation of the EORTC model showed good calibration for both outcomes, and a c-index of 0.69. The c-index was only marginally improved to 0.71 when other significant prognostic factors (sex, age, tumor type, mitotic rate) were added.

Conclusion. This study validated the EORTC prognostic model for recurrence-free and melanoma-specific survival of patients with negative sentinel nodes. The addition of other prognostic factors only improved the model marginally. The validated EORTC model could be used for personalizing follow-up and selecting high-risk patients for trials of adjuvant systemic therapy.

INTRODUCTION

Sentinel node biopsy (SNB) has become a standard staging procedure in patients with clinically-localized primary cutaneous melanoma. The status of the sentinel node (SN) is the strongest independent prognostic factor in clinical stage I and II melanoma.[1] SN-negative melanoma has a better survival rate than SN-positive melanoma.[1,2] However, a negative SN does not guarantee disease-free survival, with reported recurrence rates in this group varying between 6% and 29%.[3–12] Initial trial results showed that adjuvant postoperative systemic therapies are effective for stage III melanoma, and trials with adjuvant programmed cell death protein 1 inhibitors in high-risk SN-negative stage II melanoma have recently been initiated (NCT03553836 and NCT03405155).[13–16] As these drugs can have serious side-effects, identifying patients who are at high risk of recurrence is important. Multiple smaller studies have identified risk factors for recurrence in SN-negative melanoma.[3,5–9,17,18] However, combining risk factors is essential when estimating the recurrence risk of an individual patient.

A recently published prognostic model and nomogram for recurrence and melanoma-specific mortality addressed this issue.[11] This prognostic model was built using 3180 patients from four European Organisation for Research and Treatment of Cancer (EORTC) Melanoma Group centers, and included as parameters: Breslow thickness, ulceration and primary tumor site. Clinical prognostic models must be validated externally to ensure that the prediction is accurate and applicable to other populations [19] This EORTC model has not yet been externally validated. Therefore, it is not known how applicable it is to other populations. The primary aim of the present study was to validate the EORTC model in a large external cohort of patients with SN-negative melanoma. The secondary aim was to assess whether adding other known prognostic fac- tors would improve the accuracy of the model.

METHODS

Patients

This study used prospectively collected data from the database of Melanoma Institute Australia (MIA). Data were extracted from the MIA Research Database, with written informed patient consent and institutional review board approval (Sydney South West Area Health Service institutional ethics review committee Protocol Number X15-0081).

Lymphoscintigraphy and SNB

A SN was defined as a lymph node on the direct lymphatic drainage pathway from the primary tumor.[20] SNB was offered to patients without clinical evidence of metastatic disease whose melanoma was ≥ 1 mm thick, or thinner if adverse histopathological features were present, such as ulceration, Clark level IV or V, or tumor mitotic rate of 1 per mm^2 or higher. Technical details of lymphoscintigraphy and SNB at MIA have been described previously.[21,22] In short, preoperative dynamic and static lymphoscintigraphy were performed using ^{99m}Tc labeled antimony sulphide colloid. Since 2008, single photon emission computed tomography with integrated computerized tomography (SPECT/CT) has been routinely added. The biopsy was performed using Patent Blue Dye and, since May 1995, a gamma ray detection probe has also been employed. Pathologists examined multiple sections and used S100, HMB-45 and, since 2010, MelanA immunohistochemistry.[23]

Data collection

Data on patient demographics (sex, age), primary tumor characteristics (location, Breslow thickness, Clark level, tumor type, ulceration, tumor mitotic rate, regression, lymphovascular invasion, vascular invasion), SN characteristics (number of SNs, drainage sites), recurrence (date, site and type of recurrence), type of treatment after recurrence and follow-up (date of last follow-up, status at last follow-up) were recorded.

Statistical analysis

Patient characteristics were summarized using median (interquartile range (IQR)) for continuous variables and proportions for categorical variables. Baseline characteristics of the MIA cohort were compared with those of the EORTC cohort that was used to build the prognostic model. Comparison of continuous variables was performed using the Mann-Whitney *U* test and values of categorical variables were compared using the Pearson's χ^2 test. Melanoma-specific survival (MSS) was calculated as the interval from initial diagnosis to melanoma-related death. Patients who died from a non-melanoma cause and those still alive at last follow-up were censored. Recurrence-free survival (RFS) was calculated from the date of diagnosis to the date of recurrence or death from any cause. Censoring occurred at the end of follow-up.

The final EORTC model for RFS and MSS included Breslow thickness (logarithmically transformed), ulceration and primary tumor site.[11] To assess model discrimination, Harrell's

concordance index (c-index) was calculated.[24] For each patient in the cohort, a risk score was calculated using the EORTC nomogram. Based on these risk scores, patients were classified as having a low risk (score 0 – 6), an intermediate risk (score 7 – 9) or a high risk (score 10 or more) of recurrence or melanoma-specific death.[11] Kaplan-Meier curves were produced for each risk group. Internal validation was performed on the MIA cohort using the bootstrap method. Model calibration was assessed by plotting the predicted survival and recurrence against the observed frequency.

New co-variables were added to investigate whether the predictive performance of the EORTC model could be improved. The American Joint Committee on Cancer (AJCC) acceptance criteria for individualized prognostic models were taken into account when building the model.[25] The following potential prognostic factors were selected based on clinical experience and literature review: gender, age, ulceration, Breslow thickness, primary tumor site, melanoma subtype, Clark level, tumor mitotic rate, regression, number of SN fields, and total number of SNs.[3,5,6,11,26,27] To address the possibility of a non-linear association with outcomes, the continuous variables age and Breslow thickness were modelled by logarithmic transformation.[11] A full model was built with all variables that had a $P \leq 0.20$ in univariable analysis. Variables were removed from the full model by backward stepwise elimination using the Akaike information criterion to achieve the smallest value.[28] Model performance was assessed with calibration plots and c-indices. The proportional hazards assumption was checked for all variables using Schoenfeld residual plots and corresponding test statistics. P-values were two-sided and considered statistically significant if <0.05. Statistical analyses were performed with SAS 9.4 (SAS Institute, Cary, North Carolina, USA) and R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Between January 1992 and December 2015, 5443 patients with a clinically localized primary cutaneous melanoma underwent SNB at MIA. Of these, 4431 (81.4%) were SN-negative and 1012 (18.6%) were SN-positive. Patients were excluded if they had melanoma in situ (7), (micro)satellites (135), in-transit metastases (10) or if preoperative ultrasound examination had revealed nodal metastasis (6). Thirty-eight patients who participated in the Multicenter Selective Lymphadenectomy Trial II and had a negative SN on histological assessment, but a positive reverse transcriptase-PCR finding in their SNs, were also excluded. Ultimately, 4235 patients were included in this study.

Cohort characteristics

Baseline characteristics of the 4235 SN-negative patients from MIA and 3180 in the EORTC cohort are shown in Table 1. Compared with the EORTC cohort, patients in the MIA cohort were significantly more often male (58% vs 52%, P < 0.001), and had more head and neck melanomas (17% vs 8%, P < 0.001). Superficial spreading melanoma was more common in EORTC patients, whereas patients at MIA presented more frequently with desmoplastic melanomas (P < 0.001). The MIA cohort more often had SNs in multiple node fields (19% vs. 13%, P < 0.001) and had more SNs identified and removed (median 2 vs. 1).

Table 1. Clinicopathologic characteristics of the model development (EORTC) and validation cohort (MIA).

Characteristic	**EORTC**	**MIA**	**P-value#**
Total number of patients	3180	4235	
Gender			< 0.001
Male	1668 (52.5)	2463 (58.2)	
Female	1510 (47.5)	1772 (41.8)	
Missing	2 (0.1)	0 (0)	
Age at diagnosis (years)*	55 (44 – 67)	58 (47.5 – 68.5)	
Primary tumor site			< 0.001
Head and neck	259 (8.1)	716 (16.9)	
Upper limb	556 (17.5)	844 (19.9)	
Lower limb	996 (31.3)	1060 (25.0)	
Trunk	1360 (42.8)	1615 (38.1)	
Breslow thickness*	1.7 (1.1 – 3.0)	1.8 (1.0 – 2.6)	
Tumor mitotic rate/mm²*	NA	3.0 (0.5 – 5.5)	
0	39 (1.2)	417 (9.8)	< 0.001
≥1	112 (3.5)	3631 (85.7)	
Missing	3029 (95.3)	187 (4.4)	
Ulceration			0.944
Absent	2264 (71.2)	2890 (68.2)	
Present	788 (24.8)	1002 (23.7)	
Missing	128 (4.0)	343 (8.1)	
Melanoma subtype			< 0.001
Superficial spreading melanoma	1739 (54.7)	1731 (40.9)	
Nodular melanoma	885 (27.8)	1295 (30.6)	
Acral lentiginous melanoma	93 (2.9)	62 (1.5)	
Lentigo maligna melanoma	139 (4.4)	85 (2.0)	
Other	46 (1.4)	442 (10.4)	
Missing	278 (8.7)	620 (14.6)	

Clark level			< 0.001
I-II	271 (8.5)	58 (1.4)	
III	1230 (38.7)	1147 (27.1)	
IV	1354 (42.6)	2615 (61.7)	
V	140 (4.4)	326 (7.7)	
Missing	185 (5.8)	89 (2.1)	
Regression			
Absent	NA	1228 (29.0)	
Early/Intermediate	NA	2011 (47.5)	
Late	NA	348 (8.2)	
Missing	NA	648 (15.3)	
Vascular invasion			
Absent	NA	3371 (79.6)	
Present	NA	81 (1.9)	
Missing	NA	783 (18.5)	
Lymphovascular invasion			
Absent	NA	2876 (67.9)	
Present	NA	77 (1.8)	
Missing	NA	1282 (30.3)	
Total no. of SNs*	1 (1 – 2)	2 (1 – 3)	
Drainage site of identified SNs			
Axilla	NA	2215 (52.3)	
Groin	NA	1174 (27.7)	
Neck	NA	794 (18.7)	
Other	NA	52 (1.2)	
No. of drainage sites			
1	NA	3436 (81.1)	
2	NA	717 (16.9)	
3	NA	73 (1.7)	
4	NA	9 (0.2)	
No. of SN fields			< 0.001
1	2768 (87.0)	3436 (81.1)	
>1	412 (13.0)	799 (18.9)	

Values in parentheses are percentages unless indicated otherwise; *values are median (IQR); #Pearson's χ^2 test; NA not available.

Survival

The median duration of follow-up was 50 (IQR 18.5 – 81.5) months. Melanoma recurred in 793 patients (19%), with a median time to recurrence of 26 (IQR 8.5 – 43.5) months. A first recurrence occurred 5 years or more after the melanoma diagnosis in 144 of these patients

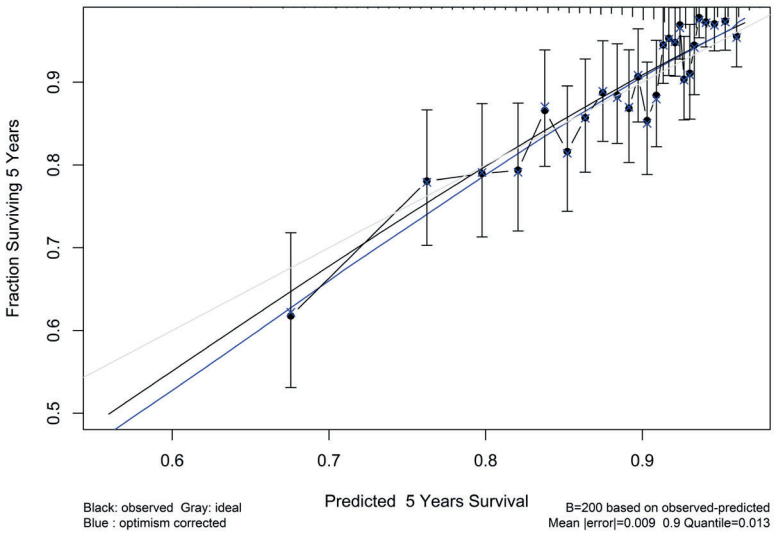
(18%) and 28 patients (4%) had their first recurrence after 10 years or more. Regional node recurrence was seen in 192 of the patients (24%) and 335 (42%) had a distant site as the first site of recurrence. The incidence of false-negative SNB, defined as a regional nodal recurrence in a patient whose SNs had been found to be tumor-free, was 16%. MSS rates at 5 and 10 years were 89% (95% confidence interval (CI): 87% – 90%) and 80% (95% CI: 78% – 82%). RFS rates at 5 and 10 years were 80% (95% CI: 78% – 81%) and 71% (95% CI: 69% – 73%).

External validation and improvement of the EORTC model

The predictive ability of the EORTC model was assessed by calculating the c-index. The c-indices of the externally validated EORTC model were 0.69 (95% CI: 0.67 – 0.71) and 0.69 (95% CI: 0.66 – 0.72) for RFS and MSS respectively. The prognostic models appeared well calibrated as observed 5-year survival rates were close to the predicted 5-year rates (Figure 1). Figure 2 displays the Kaplan-Meier curves for the three risk classes. Eight potential prognostic factors for RFS and MSS were added to the EORTC models: gender, age, melanoma subtype, Clark level, mitotic rate, regression, total number of SNs removed and number of SN fields, were added to the EORTC models.

After backward selection, regression, Clark level, total number of SNs removed and multiple SN fields did not add enough to the prediction of the outcomes to justify their inclusion in the final model. Table 2 shows the final model that included gender, age, melanoma subtype, tumor mitotic rate, Breslow thickness, ulceration, and primary tumor site. The c-index was 0.71 (95% CI: 0.69 – 0.73) for the RFS model and also 0.71 (95% CI: 0.68 – 0.74) for the MSS model.

Figure 1. Calibration plots of the Cox proportional hazards model for the prediction of five-year recurrence-free survival and melanoma-specific survival.
1A Melanoma-specific survival
1B Recurrence-free



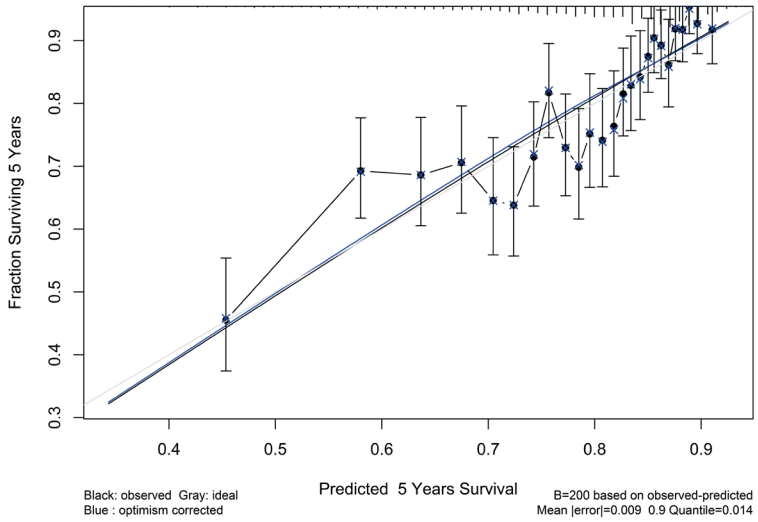
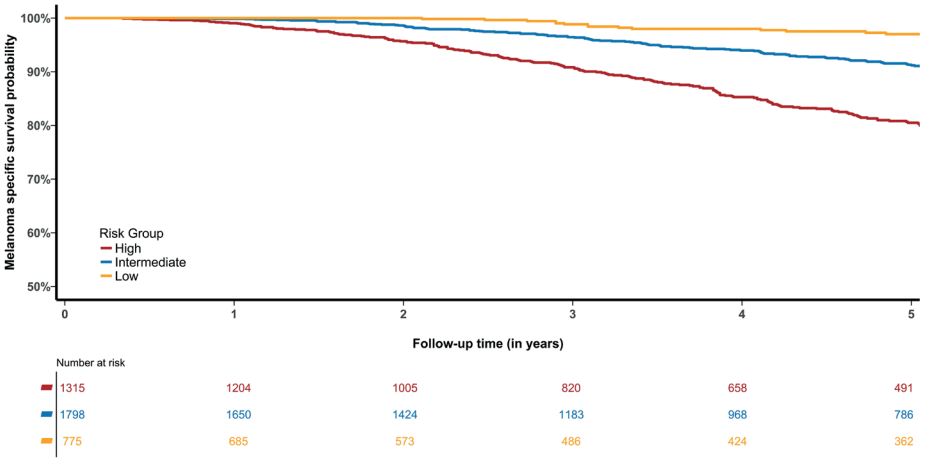


Figure 2. Kaplan-Meier plots for the low-, intermediate- and high-risk group.
2A Melanoma-specific survival
2B Recurrence-free survival



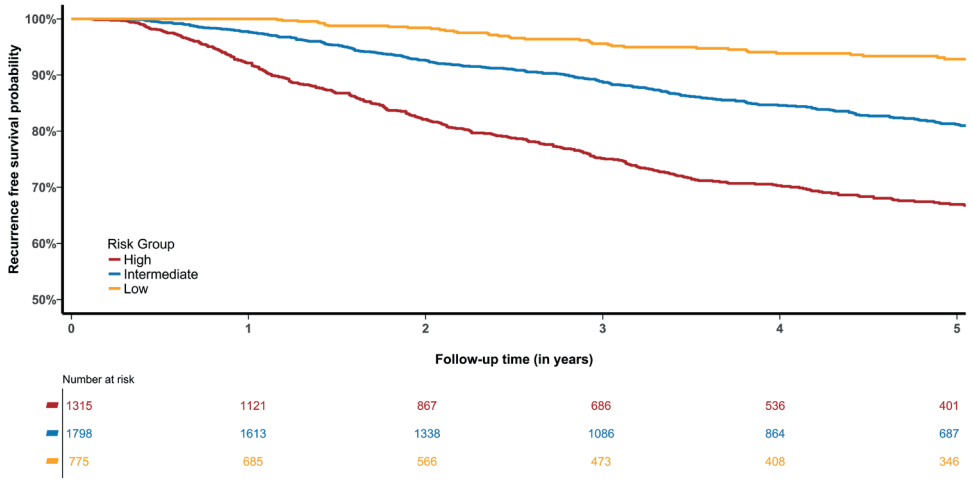


Table 2. Updated model for prediction of recurrence-free survival and melanoma-specific survival.
2A Melanoma-specific survival

	Univariable		Multivariable		Multivariable	
Variable	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR**	**P-value**
Gender						
Female	1	**< 0.001**	1	**< 0.001**	1	**< 0.001**
Male	1.71 (1.40 - 2.08)		1.50 (1.19 - 1.90)		1.50 (1.18 - 1.89)	
Age at diagnosis (years)	2.75 (1.94 - 3.89)	**< 0.001**	1.76 (1.18 - 2.61)	**0.005**	1.79 (1.21 - 2.66)	**0.004**
Breslow thickness (mm)	2.04 (1.79 - 2.33)	**< 0.001**	1.46 (1.18 - 1.81)	**< 0.001**	1.56 (1.29 - 1.89)	**< 0.001**
Tumor mitotic rate	1.04 (1.03 - 1.05)	**< 0.001**	1.02 (1.00 - 1.03)	**0.017**	1.02 (1.00 - 1.03)	**0.039**
Ulceration						
Absent	1	**< 0.001**	1	**< 0.001**	1	**< 0.001**
Present	2.30 (1.90 - 2.79)		1.60 (1.27 - 2.01)		1.57 (1.25 - 1.96)	
Primary tumor site						
Head and Neck	1	**< 0.001**	1	**0.026**	1	**0.037**
Lower Limb	0.74 (0.56 - 0.98)		0.88 (0.64 - 1.22)		0.91 (0.66 - 1.26)	
Trunk	0.88 (0.68 - 1.13)		0.92 (0.69 - 1.23)		0.92 (0.69 - 1.21)	
Upper Limb	0.49 (0.35 - 0.68)		0.57 (0.39 - 0.84)		0.59 (0.41 - 0.86)	
Clark level						
II/III	1	**< 0.001**	1	0.260		
IV	1.41 (1.12 - 1.77)		1.21 (0.93 - 1.57)			
V	2.54 (1.84 - 3.52)		1.40 (0.88 - 2.21)			
Melanoma subtype						
Superficial spreading	1	**< 0.001**	1	**0.008**	1	**0.013**
Nodular	1.75 (1.41 - 2.17)		1.14 (0.89 - 1.47)		1.15 (0.90 - 1.48)	
Acral lentiginous	3.60 (2.21 - 5.88)		2.89 (1.62 - 5.15)		2.80 (1.58 - 4.96)	
Desmoplastic	1.20 (0.85 - 1.70)		0.86 (0.58 - 1.29)		0.92 (0.63 - 1.35)	
Lentigo maligna	0.96 (0.42 - 2.16)		0.86 (0.37 - 1.98)		0.90 (0.39 - 2.06)	
Other	1.91 (0.47 - 7.72)		1.57 (0.38 - 6.49)		1.56 (0.38 - 6.39)	

	Univariable		Multivariable		Multivariable	
Variable	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR**	**P-value**
Regression						
Absent	1	0.762				
Early/Intermediate	0.96 (0.78 - 1.19)					
Late	0.87 (0.59 - 1.28)					
Total no. of SNs	1.02 (0.94 - 1.10)	0.676				
No. of SN fields						
1	1	**0.054**	1	0.648		
>1	1.25 (1.00 - 1.56)		0.94 (0.71 - 1.24)			

2B Recurrence-free survival

	Univariable		Multivariable		Multivariable	
Variable	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR**	**P-value**
Gender						
Female	1	**< 0.001**	1	**0.014**	1	**0.002**
Male	1.42 (1.24 - 1.64)		1.25 (1.05 - 1.49)		1.31 (1.11 - 1.55)	
Age at diagnosis (years)	2.92 (2.26 - 3.77)	**< 0.001**	1.67 (1.23 - 2.26)	**0.001**	1.66 (1.24 - 2.21)	**< 0.001**
Breslow thickness (mm)	2.06 (1.87 - 2.27)	**< 0.001**	1.52 (1.28 - 1.80)	**<0.001**	1.50 (1.28 - 1.76)	**< 0.001**
Tumor mitotic rate	1.05 (1.04 - 1.05)	**< 0.001**	1.03 (1.02 - 1.04)	**<0.001**	1.02 (1.01 - 1.03)	**< 0.001**
Ulceration						
Absent	1	**< 0.001**	1	**<0.001**	1	**< 0.001**
Present	2.07 (1.79 - 2.39)		1.46 (1.22 - 1.74)		1.43 (1.20 - 1.69)	
Primary tumor site						
Head and Neck	1	**<0.0001**	1	**0.001**	1	**< 0.001**
Lower Limb	0.77 (0.63 - 0.93)		0.98 (0.77 - 1.25)		0.98 (0.78 - 1.24)	
Trunk	0.64 (0.53 - 0.77)		0.73 (0.58 - 0.91)		0.71 (0.58 - 0.88)	
Upper Limb	0.50 (0.40 - 0.63)		0.67 (0.51 - 0.88)		0.63 (0.48 - 0.82)	

	Univariable		Multivariable		Multivariable	
Variable	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR**	**P-value**
Clark level						
II/III	1	**<0.001**	1	0.138		
IV	1.50 (1.26 - 1.78)		1.21 (0.99 - 1.47)			
V	2.86 (2.25 - 3.64)		1.33 (0.94 - 1.88)			
Melanoma subtype						
Superficial spreading	1	**<0.001**	1	**0.006**	1	**0.003**
Nodular	1.73 (1.48 - 2.04)		1.14 (0.94 - 1.38)		1.13 (0.94 - 1.36)	
Acral lentiginous	3.28 (2.21 - 4.88)		2.03 (1.27 - 3.26)		2.05 (1.29 - 3.24)	
Desmoplastic	1.21 (0.94 - 1.56)		0.82 (0.60 - 1.11)		0.86 (0.64 - 1.15)	
Lentigo maligna	1.63 (1.02 - 2.60)		1.25 (0.73 - 2.14)		1.33 (0.81 - 2.17)	
Other	3.13 (1.40 - 7.04)		2.15 (0.86 - 5.35)		2.46 (1.07 - 5.65)	
Regression						
Absent	1	**0.046**	1	0.763		
Early/Intermediate	0.87 (0.75 - 1.02)		0.94 (0.79 - 1.11)			
Late	0.72 (0.54 - 0.96)		0.98 (0.71 - 1.35)			
Total no. of SNs	1.00 (0.95 - 1.06)	0.894				
No. of SN fields						
1	1	0.236				
>1	1.11 (0.94 - 1.31)					

DISCUSSION

This single institution study successfully validated the EORTC model for prediction of RFS and MSS in patients with SN-negative melanoma. External validation is an essential step in assessing the generalizability of a prognostic model.[19,25] As expected, the model performance was not as good as in the derivation data.[19] The c-indices for the recurrence and melanoma-specific mortality models were both 0.69 in our population, compared to 0.74 and 0.76 in the EORTC cohort.[11] A c-index of 0.69 means that the model correctly predicted recurrence or melanoma-specific death in 69% of the patients.[29]

The present cohort of patients with SN-negative melanoma differed from the EORTC cohort with respect to several important clinicopathological characteristics. More of the present patients were men, more had head and neck primary melanomas, and the melanomas drained more frequently to multiple node fields and to more SNs. Tumors in the EORTC cohort had a lower Clark level in general and superficial spreading melanomas were more numerous. Despite these differences in patient characteristics, the EORTC model proved to be a strong predictive tool in the present population.

Simplicity is a strength of the EORTC model, as it is based on three common tumor characteristics. Although ease of use in clinical practice is important, this should not come at the cost of leaving out strong but more complex prognostic factors. The present study therefore investigated whether the model performance could be improved by adding co-variables, and confirmed the independent prognostic value of sex, age, primary tumor site, Breslow thickness, ulceration, melanoma subtype and tumor mitotic rate. The tumor mitotic rate is one of the most important risk factors for recurrence and melanoma-specific mortality[26,30] It was an essential part of the AJCC/UICC melanoma staging classification for almost 10 years.[2,30] Smaller studies, some with up to 95% missing values, failed to show an association of tumor mitotic rate with survival in SN-negative melanoma.[5,8,11] In multivariable analysis, the present study con- firmed the independent prognostic effect of this parameter. Another tumor characteristic of interest is regression. Regression has been found to be an independent prognostic factor for patients with melanoma in general.[27] In line with previous studies, the independent prognostic value was not proven for SN-negative melanoma in our analysis.[5,6] Adding gender, age, melanoma subtype and tumor mitotic rate to the EORTC model improved the predictive ability of the models by only 2% (with overlapping confidence intervals). The authors consider that this improvement is insufficient to justify changing the simple EORTC model.

Only one other prognostic model for predicting melanoma recurrence in SN-negative patients has been published.[3] In that study, combining Breslow thickness, ulceration and microsatellites yielded a c-index of 0.75. Microsatellites are caused by lymphovascular dissemination and their presence is well known to be associated with worse survival.[31,32] Patients with non-nodal regional metastases (microsatellites, satellites, or in-transit metastases) are already regarded as high-risk patients and should not have been included. According to the AJCC 8th edition melanoma staging system, these patients are classified as having at least stage IIIB melanoma and they are eligible for adjuvant systemic therapy.[2]

The recurrence rate of 19% in the present cohort is comparable to previously reported rates ranging from 6 to 29%.[3–11] Importantly, all previous studies with a median follow-up of at least 5 years reported a recurrence rate of over 14%.[4,6,7,11,12] The present study has shown that first recurrences are frequently (18%) found after more than 5 years of follow-up. Identifying these patients is important, as follow-up is considered unnecessary after 5 years in some countries.[33] This prediction model could help in designing individualized follow-up regimens. As 42% of all patients with a recurrence had their first relapse at a distant site, these patients with aggressive tumor biology might be those who could benefit most from adjuvant systemic therapy. The externally validated EORTC model could help to identify patients with the highest risk of recurrence or melanoma-related death.

The present study has several limitations. Lymphatic invasion is a known prognostic factor in melanoma, but could unfortunately not be assessed reliably in this study because there were too many missing values (30%).[34,35] The retrospective design and short follow-up of some patients are other limitations.

CONCLUSION

This external validation confirmed the value of the EORTC prognostic model for RFS and MSS of SN-negative melanoma. Addition of other known prognostic factors only marginally improved the model. The validated EORTC model can be used for patient counselling, personalizing follow-up and selection of high-risk patients for clinical trials of adjuvant systemic therapies.

REFERENCES

1. 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.
2. 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.
3. 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.
4. Ward CE, MacIsaac JL, Heughan CE, Weatherhead L. Metastatic Melanoma in Sentinel Node-Negative Patients: The Ottawa Experience. *J Cutan Med Surg*. 2018;22(1):14-21.
5. Faut M, Wevers KP, van Ginkel RJ, et al. Nodular histologic subtype and ulceration are tumor factors associated with high risk of recurrence in sentinel node-negative melanoma patients. *Ann Surg Oncol*. 2017;24(1):142-149.
6. Egger ME, Bhutiani N, Farmer RW, et al. Prognostic factors in melanoma patients with tumor-negative sentinel lymph nodes. *Surgery*. 2016;159(5):1412-1421.
7. Jones EL, Jones TS, Pearlman NW, et al. Long-term follow-up and survival of patients following a recurrence of melanoma after a negative sentinel lymph node biopsy result. *JAMA Surg*. 2013;148(5):456-461.
8. Yee VSK, Thompson JF, McKinnon JG, et al. Outcome in 846 cutaneous melanoma patients from a single center after a negative sentinel node biopsy. *Ann Surg Oncol*. 2005;12(6):429-439.
9. Gershenwald JE, Colome MI, Lee JE, et al. Patterns of recurrence following a negative sentinel lymph node biopsy in 243 patients with stage I or II melanoma. *J Clin Oncol*. 1998;16(6):2253-2260.
10. Chao C, Wong SL, Ross MI, et al. Patterns of early recurrence after sentinel lymph node biopsy for melanoma. *Am J Surg*. 2002;184(6):520-524; discussion 525.
11. 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.
12. O'Connell EP, O'Leary DP, Fogarty K, Khan ZJ, Redmond HP. Predictors and patterns of melanoma recurrence following a negative sentinel lymph node biopsy. *Melanoma Res*. 2016;26(1):66-70.
13. 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.
14. 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.
15. Long G V, 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.
16. 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.
17. Zogakis TG, Essner R, Wang H, Foshag LJ, Morton DL. Natural history of melanoma in 773 patients with tumor-negative sentinel lymph nodes. *Ann Surg Oncol*. 2007;14(5):1604-1611.
18. Gambichler T, Scholl L, Bechara FG, Stockfleth E, Stücker M. Worse outcome for patients with recurrent melanoma after negative sentinel lymph biopsy as compared to sentinel-positive patients. *Eur J Surg Oncol*. 2016;42(9):1420-1426.

19. Altman DG, Royston P. What do we mean by validating a prognostic model? *Stat Med*. 2000;19(4):453-473.

20. Nieweg OE, Tanis PJ, Kroon BB. The definition of a sentinel node. *Ann Surg Oncol*. 2001;8(6):538-541.

21. Verwer N, Scolyer RA, Uren RF, et al. Treatment and prognostic significance of positive interval sentinel nodes in patients with primary cutaneous melanoma. *Ann Surg Oncol*. 2011;18(12):3292-3299.

22. Uren RF, Howman-Giles R, Chung D, Thompson JF. Guidelines for lymphoscintigraphy and F18 FDG PET scans in melanoma. *J Surg Oncol*. 2011;104(4):405-419.

23. Scolyer RA, Murali R, McCarthy SW, Thompson JF. Pathologic examination of sentinel lymph nodes from melanoma patients. *Semin Diagn Pathol*. 2008;25(2):100-111.

24. Harrell FE, Califf RM, Pryor DB, Lee KL, Rosati RA. Evaluating the yield of medical tests. *JAMA*. 1982;247(18):2543-2546.

25. Kattan MW, Hess KR, Amin MB, et al. American Joint Committee on Cancer acceptance criteria for inclusion of risk models for individualized prognosis in the practice of precision medicine. *CA Cancer J Clin*. 2016;66(5):370-374.

26. 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.

27. Gualano MR, Osella-Abate S, Scaioli G, et al. Prognostic role of histological regression in primary cutaneous melanoma: a systematic review and meta-analysis. *Br J Dermatol*. 2018;178(2):357-362.

28. Ambler G, Brady AR, Royston P. Simplifying a prognostic model: a simulation study based on clinical data. *Stat Med*. 2002;21(24):3803-3822.

29. Uno H, Cai T, Pencina MJ, D'Agostino RB, Wei LJ. On the C-statistics for evaluating overall adequacy of risk prediction procedures with censored survival data. *Stat Med*. 2011;30(10):1105-1117.

30. Balch CM, Gershenwald JE, Soong SJ, et al. Final version of 2009 AJCC melanoma staging and classification. *J Clin Oncol*. 2009;27(36):6199-6206.

31. Rao UNM, Ibrahim J, Flaherty LE, Richards J, Kirkwood JM. Implications of microscopic satellites of the primary and extracapsular lymph node spread in patients with high-risk melanoma: pathologic corollary of Eastern Cooperative Oncology Group Trial E1690. *J Clin Oncol*. 2002;20(8):2053-2057.

32. León P, Daly JM, Synnestvedt M, Schultz DJ, Elder DE, Clark WH. The prognostic implications of microscopic satellites in patients with clinical stage I melanoma. *Arch Surg*. 1991;126(12):1461-1468.

33. Trotter SC, Sroa N, Winkelmann RR, Olencki T, Bechtel M. A global review of melanoma follow-up guidelines. *J Clin Aesthetic Dermatol*. 2013;6(9):18-26.

34. Moy AP, Mochel MC, Muzikansky A, Duncan LM, Kraft S. Lymphatic invasion predicts sentinel lymph node metastasis and adverse outcome in primary cutaneous melanoma. *J Cutan Pathol*. 2017;44(9):734-739.

35. Xu X, Chen L, Guerry D, et al. Lymphatic invasion is independently prognostic of metastasis in primary cutaneous melanoma. *Clin Cancer Res*. 2012;18(1):229-237.

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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

CHAPTER 9

Nederlandse samenvatting

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Dankwoord

Nederlandse samenvatting

INTRODUCTIE

Het melanoom van de huid is een vorm van kanker die ontstaat in een moedervlek of in de cellen die het normale pigment produceren. Na het plaveiselcelcarcinoom en basaalcelcarcinoom is melanoom de meest voorkomende vorm van huidkanker. Het aantal mensen met melanoom neemt dramatisch toe. In 2018 kregen bijna 7000 mensen in ons land een melanoom en 800 mensen overleden aan de ziekte. Er zijn meerdere factoren die het risico op de ontwikkeling van een melanoom verhogen. De belangrijkste daarvan is ultraviolet licht.

Deze vorm van huidkanker presenteert zich meestal als een donkere huidafwijking die nieuw of veranderd is. Nadat de verdachte plek chirurgisch is verwijderd, stelt de patholoog de diagnose en beoordeelt verschillende kenmerken van de afwijking. De dikte van het gezwel, zweervorming en het aantal delende cellen zijn de belangrijkste kenmerken en essentieel voor het bepalen van het stadium van de ziekte. Dit stadium vormt de leidraad voor de keuze van de behandeling en geeft informatie over de prognose. De prognostische factoren bij verschillende groepen patiënten zijn een centraal onderwerp in dit proefschrift.

SENTINEL-NODEBIOPSIE

Na het stellen van de diagnose wordt het litteken van het melanoom ruimer weggesneden. Deze tweede operatie verkleint de kans op terugkeer van het melanoom. Afhankelijk van het stadium van de ziekte wordt een sentinel-nodebiopsie (SNB) uitgevoerd. De sentinel node (SN) is een lymfeklier die rechtstreeks in verbinding staat met het melanoom. De aan- of afwezigheid van tumorcellen in de SN is van belang voor de prognose van de patiënt. Patiënten bij wie tumorcellen worden gevonden in de SN hebben namelijk een slechtere prognose. Helaas is SNB niet geschikt voor alle patiënten.

Hoofstuk twee beschrijft 160 patiënten op hoge leeftijd of met onderliggend lijden (observatiegroep) bij wie geen SNB werd verricht. De lymfeklieren werden bij deze patiënten in kaart gebracht middels lymfescintigrafie en bij ieder controlebezoek werden er echo's gemaakt van hun lymfeklieren. Deze 160 patiënten werden vergeleken met 2945 patiënten die wel een SNB ondergingen (SNB-groep). Patiënten in de observatiegroep hadden dikkere melanomen, die vaker in het hoofd-hals gebied zaten dan patiënten in de SNB-groep. Tevens hadden de melanomen van de patiënten in de observatiegroep gemiddeld meer SNs. Terugkeer van het

melanoom in een lymfeklier werd bij 1 op de 3 patiënten in de observatiegroep gevonden door middel van echo-onderzoek. Patiënten in de observatiegroep overleden niet vaker aan het melanoom dan patiënten in de SNB-groep. Deze studie toont aan dat lymfescintigrafie gevolgd door echo-onderzoek bij ieder controlebezoek een goed alternatief is voor SNB bij patiënten op leeftijd of met onderliggend lijden.

In **hoofdstuk drie** worden de 2945 patiënten uit de SNB-groep vergeleken met 203 patiënten waarbij de SNB werd gecanceld (gecancelde-groep). Bij deze patiënten was de SNB wel gepland, maar na het maken van het lymfescintigram, werd besloten om de SNB te annuleren. Ieder controlebezoek werden er echo's gemaakt van de lymfeklieren. De belangrijkste reden voor het annuleren van de SNB was de aanwezigheid van meerdere SNs of als de SNs op meerdere locaties zaten. De operatie is dan namelijk complexer. Patiënten uit de gecancelde-groep waren ouder, hadden dunnere melanomen en hadden vaker een melanoom in het hoofd-halsgebied. Eerdere studies tonen aan dat melanomen in de hoofd-halsregio een minder voorspelbare lymfeafvloed hebben. Ruim een derde van de patiënten met een hoofd-halsmelanoom heeft een SN in de oorspeekselklier. Het verwijderen van deze SN is riskanter vanwege het risico op schade aan de aangezichtszenuw. Patiënten in de beide groepen hadden een vergelijkbaar risico om te overlijden aan het melanoom. Dit onderzoekt toont aan dat follow-up van SNs middels echo-onderzoek een acceptabele behandelstrategie is voor melanoompatiënten waarbij SNB te complex is.

MELANOOM BIJ KINDEREN EN ADOLESCENTEN

Kinderen en adolescenten krijgen zelden een melanoom. Melanomen bij deze jonge patiënten zijn lastiger te herkennen dan bij volwassenen met een melanoom. Bij volwassenen is het aantal delende cellen in het melanoom een belangrijke graadmeter voor de kans om te overlijden aan de ziekte. Eerdere studies toonden aan dat deze delende cellen minder frequent worden gezien in melanomen van kinderen en adolescenten dan in die van volwassenen. **Hoofdstuk vier** beschrijft een onderzoek naar de waarde van deze delende cellen voor kinderen (jonger dan 12 jaar) en adolescenten (12 tot 20 jaar) met een melanoom. Tevens werden melanomen van kinderen en adolescenten vergeleken. Van de 156 patiënten in deze studie waren slechts 13 patiënten (8%) jonger dan 12 jaar. Melanomen bij kinderen waren dikker dan bij adolescenten en werden vaker in het hoofd-hals gebied gevonden. Een verschil in het aantal delende cellen of overleving werd niet gezien tussen de groepen. Het aantal delende cellen was hoger bij dikkere melanomen. In totaal keerde het melanoom bij 28 patiënten (18%) terug en overleden er 16 patiënten (10%) aan hun melanoom. Bij 14

overleden patiënten (88%) waren er delende cellen aanwezig in het melanoom. Kinderen en adolescenten met deze delende cellen in het melanoom hadden een grotere kans om te overlijden aan hun melanoom. Deze studie laat zien dat de aanwezigheid van delende cellen in het melanoom een belangrijke voorspeller is voor kinderen en adolescenten met een melanoom. Deze eigenschap van het gezwel kan bijdragen aan een preciezere inschatting van de overlevingskansen en het maken van een behandelplan voor deze patiëntencategorie.

ERFELIJK MELANOOM

Bij een deel van de melanoompatiënten speelt erfelijke aanleg een rol. In Nederland is de meest voorkomende oorzaak een verandering (mutatie) in het gen *CDKN2A*. Deze patiënten hebben niet alleen 70% kans om in hun leven een melanoom te krijgen, maar hebben ook een verhoogd risico op alvleesklierkanker, hoofd-halskanker en longkanker. De melanomen van patiënten met een *CDKN2A*-mutatie lijken zich agressiever te gedragen, maar het is onduidelijk of deze patiënten ook vaker aan hun melanoom overlijden dan patiënten zonder een *CDKN2A*-mutatie. **Hoofdstuk vijf** beschrijft een studie naar de invloed van een *CDKN2A*-mutatie op de overlevingskans van melanoompatiënten. Deze studie vergelijkt 89 melanoompatiënten met een *CDKN2A*-mutatie en 56.929 melanoompatiënten zonder deze mutatie. Patiënten met een *CDKN2A*-mutatie waren gemiddeld 15 jonger (42 vs. 57 jaar) dan patiënten zonder de mutatie. Ook waren hun melanomen dunner en was zweervorming minder vaak aanwezig. De overleving van de twee patiëntengroepen was vergelijkbaar. Aanwezigheid van een *CDKN2A*-mutatie was dus geen voorspeller voor overleving in onze studie.

In **hoofdstuk zes** wordt een studie beschreven naar kenmerken en uitkomsten van 23 melanoompatiënten met een *CDKN2A*-mutatie die een SNB ondergingen. Bij 5 patiënten (22%) waren er tumorcellen aanwezig in de SN. Na de patiënten gemiddeld 8 jaar gevolgd te hebben, bleek het melanoom bij zes patiënten teruggekeerd te zijn. Twee patiënten waren overleden aan hun melanoom, 1 met en 1 zonder tumorcellen in de SN, en drie patiënten waren overleden aan een andere oorzaak. Het deel patiënten met tumorcellen in de SN is vergelijkbaar met cijfers die in eerdere studies gevonden werden bij melanoompatiënten zonder erfelijke aanleg. Door het lage aantal patiënten kon er geen uitspraak worden gedaan over de voorspellende waarde van SNB in deze unieke populatie. De studie toont dat er geen reden is voor terughoudendheid bij het verrichten van SNB bij patiënten met een *CDKN2A*-mutatie.

VOORSPELMODEL

Patiënten zonder melanoomcellen in de SN (SN-negatieve patiënten) hebben een betere prognose dan patiënten bij wie melanoomcellen gevonden worden in de SN (SN-positieve patiënten). Ondanks de betere prognose keert het melanoom bij 6 tot 29% van de SN-negatieve patiënten terug. Het is belangrijk om te achterhalen welk risico een individuele SN-negatieve patiënt loopt op terugkeer van het melanoom. Individuele risicofactoren geven richting, maar een voorspelmodel is essentieel voor het bepalen van de prognose van de individuele patiënt. De "European Organisation for Research and Treatment of Cancer" (EORTC) heeft een voorspelmodel ontwikkeld. Bij dit model wordt de prognose van een SN-negatieve patiënt ingeschat aan de hand van tumordikte, zweervorming en locatie van het melanoom. De studie beschreven in **hoofdstuk zeven** had als doel om te onderzoeken of het EORTC-model toepasbaar was op 4235 SN-negatieve patiënten uit Australië. Tevens werd onderzocht of het model verbeterd kon worden door het toevoegen van andere kenmerken. Na de patiënten gemiddeld 50 maanden gevolgd te hebben, was bij 793 patiënten (19%) het melanoom teruggekeerd en waren 456 patiënten (11%) overleden aan hun melanoom. Bij 144 patiënten (18%) keerde het melanoom na meer dan vijf jaar terug. Het EORTC-model kon bij 69% van de patiënten correct voorspellen of het melanoom terugkeert of dat de patiënt aan het melanoom overlijdt. Om te bepalen of het model verbeterd kon worden, werden er acht kenmerken toegevoegd aan het model. Het voorspelmodel verbeterde hierdoor met slechts 2%. De resultaten van deze studie tonen aan dat het voorspelmodel van de EORTC ook toepasbaar is op andere populaties. Het EORTC-model kan gebruikt worden voor het personaliseren van het behandelplan van patiënten met een SN-negatief melanoom.

LIST OF ABBREVIATIONS

ACD	ACD shelterin complex subunit and telomerase recruitment factor
AJCC	American Joint Committee on Cancer
BAP1	BRCA1-associated protein-1
C-index	Concordance index
CDK4	Cyclin-dependent kinase inhibitor 4
CDKN2A	Cyclin-dependent kinase inhibitor 2A
CI	Confidence interval
CLND	Completion lymph node dissection
DeCOG-SLT	German Dermatologic Cooperative Oncology Group Selective Lymphadenectomy Trial
DRFS	Distant recurrence-free survival
ECOG	Eastern Cooperative Oncology Group
EORTC	European Organisation for Research and Treatment of Cancer
FAMMM	Familial atypical multiple mole melanoma
HR	Hazard ratio
IQR	Interquartile range
JAK2	Janus Kinase 2
MIA	Melanoma Institute Australia
MITF	Microphthalmia-associated transcription factor
MSLT	Multicenter selective lymphadenectomy trial
MSS	Melanoma-specific survival
MPM	Multiple primary melanoma
NA	Not applicable
NFDHT	Netherlands Foundation for Detection of Hereditary Tumors
OS	Overall survival
PCR	Polymerase chain reaction
POT1	Protection of telomeres 1
RFS	Recurrence-free survival
RLNFS	Regional lymph node-free survival
SD	Standard deviation
SEER	Surveillance, Epidemiology, and End Results
SN	Sentinel node
SNB	Sentinel node biopsy
SPECT/CT	Single photon emission computed tomography with integrated computerized tomography
SPM	Single primary melanoma
TERF1P	Telomeric repeat-binding factor 2-interacting protein

TERT	Telomerase reverse transcriptase
UICC	Union Internationale Contre le Cancer
US	Ultrasound
WLE	Wide local excision

List of publications

Dik EA, Willems SM, **Ipenburg NA**, Adriaansens SO, Rosenberg AJ, van Es RJ. Resection of early oral squamous cell carcinoma with positive or close margins: relevance of adjuvant treatment in relation to local recurrence: margins of 3 mm as safe as 5 mm. Oral Oncol. 2014 Jun;50(6):611-5

Dorresteijn PM, **Ipenburg NA**, Murphy KJ, Smit M, van Vulpen JK, Wegner I, Stegeman I, Grolman W. Rapid Systematic Review of Normal Audiometry Results as a Predictor for Benign Paroxysmal Positional Vertigo. Otolaryngol Head Neck Surg. 2014 Jun;150(6):919-24

Dik EA, **Ipenburg NA**, Adriaansens SO, Kessler PA, van Es RJ, Willems SM. Poor Correlation of Histologic Parameters Between Biopsy and Resection Specimen in Early Stage Oral Squamous Cell Carcinoma. Am J Clin Pathol. 2015 Oct;144(4):659-66

Ipenburg NA, Koole K, Liem KS, van Kempen PM, Koole R, van Diest PJ, van Es RJ, Willems SM. Fibroblast Growth Factor Receptor Family Members as Prognostic Biomarkers in Head and Neck Squamous Cell Carcinoma: A Systematic Review. Target Oncol. 2016 Feb;11(1):17-27

Dik EA, Willems SM, **Ipenburg NA**, Rosenberg AJ, Van Cann EM, van Es RJ. Watchful waiting of the neck in early stage oral cancer is unfavourable for patients with occult nodal disease. Int J Oral Maxillofac Surg. 2016 Aug;45(8):945-50

Ipenburg NA, Gruis NA, Bergman W, van Kester MS. The absence of multiple atypical nevi in germline *CDKN2A* mutations: Comment on "Hereditary melanoma: Update on syndromes and management: Genetics of familial atypical multiple mole melanoma syndrome". J Am Acad Dermatol. 2016 Oct;75(4):e157

Ipenburg NA, Peters E, van Doorn R. MBAITs en het BAP1-tumor-predispositiesyndroom NTvDV. 2016 Dec;26(11):653-56

Ipenburg NA, Kukutsch NA. Dermatoscopie xanthogranuloom. NTvDV. 2017 Mei;27(5):231

Ipenburg NA, Nieweg OE, Uren RF, Thompson JF. Outcome of Melanoma Patients Who Did Not Proceed to Sentinel Node Biopsy After Preoperative Lymphoscintigraphy. Ann Surg Oncol. 2017 Jan;24(1):117-126

Ipenburg NA, Mooi WJ, van Doorn R. A brown-red papule. Ned Tijdschr Geneeskd. 2017;161(0):D1687

Dik EA, **Ipenburg NA**, Kessler PA, van Es RJJ, Willems SM. The value of histological grading of biopsy and resection specimens in early stage oral squamous cell carcinomas. J Craniomaxillofac Surg. 2018 Jun;46(6):1001-1006

Ipenburg NA, Rustemeyer T. Parafenyleendiamine. NTvDV. 2018 Jun;28(6):35-36

Ipenburg NA, Nieweg OE, Ahmed T, van Doorn R, Scolyer RA, Long GV, Thompson JF, Lo S. External validation of a prognostic model to predict survival of patients with sentinel node-negative melanoma. Br J Surg. 2019 Sep;106(10):1319-1326

Ipenburg NA, Thompson JF, Uren RF, Chung D, Nieweg OE. Focused Ultrasound Surveillance of Lymph Nodes Following Lymphoscintigraphy Without Sentinel Node Biopsy: A Useful and Safe Strategy in Elderly or Frail Melanoma Patients. Ann Surg Oncol. 2019 Sep;26(9):2855-2863

Ipenburg NA, Lo SN, Vilain RE, Holtkamp LHJ, Wilmott JS, Nieweg OE, Thompson JE, Scolyer RA. The Prognostic value of tumor mitotic rate in children and adolescents with cutaneous melanoma: a retrospective cohort study. J Am Acad Dermatol. 2020 Apr;82(4):910-919

Ipenburg NA, Nieweg OE, Lo S. Author response to: Comment on: External validation of a prognostic model to predict survival of patients with sentinel node-negative melanoma. Br J Surg. 2020 Apr;107(5):616

Ipenburg NA, van der Hage JA, Newton-Bishop JA, et al. Sentinel node biopsy in cutaneous melanoma patients with germline *CDKN2A* mutations. Melanoma Res. 2020 June;30(6):630-631

Ipenburg NA, Fransen M, Rustemeyer T. Gallaten. NTvDV. 2021

Curriculum vitae

Norbert Ipenburg is geboren op 20 november 1988 in Kampen. Na het behalen van zijn atheneumdiploma aan het Lambert Franckens College in Elburg, begon hij in 2007 met de studie geneeskunde aan de Universiteit Utrecht. Gedurende zijn studie heeft hij wetenschappelijk onderzoek verricht op de afdeling mondziekten, kaak- en aangezichtschirurgie naar het plaveiselcelcarcinoom van de mondholte. In 2014 vertrok hij naar Sydney voor zijn wetenschapsstage bij Melanoma Institute Australia. Dit vormde het begin van zijn promotietraject onder begeleiding van prof. dr. M.H. Vermeer, prof. dr. O.E. Nieweg en dr. R. Van Doorn. Na het behalen van zijn artsexamen in november 2014 werkte hij als ANIOS op de afdelingen dermatologie van het Amphia Ziekenhuis en het Maastricht Universitair Medisch Centrum. In september 2015 startte hij met zijn opleiding tot dermatoloog in het Leids Universitair Medisch Centrum (opleider dr. A.P.M. Lavrijsen). Sinds september 2020 is hij werkzaam als dermatoloog in de Amsterdam UMC en de Roosevelt kliniek.

Dankwoord

Aert Samsom, AIOS, Alex Varey, Amanda Nijhuis, Antoinette Samsom, David Chung, Diederik Ipenburg, Domenique Müller, **Elise Philips,** Elsken van der Wall, Emilie Ipenburg, Emma Samsom-Aartsen, Erik Dik, Esther van Zuuren, Fatoş Çetinözman, Firdaus Ibrahim, Georgina Long, Hanke Ipenburg, Hazel Burke, Heleen van Randeraad, Herberth Samsom, Hildur Helgadottir, Isabel Ipenburg, James Wilmott, Jan Nico Bouwes Bavinck, Jasper van der Rhee, Jelle Goeman, John Thompson, Joke Philips-Wever, Julia Newton-Bishop, Justin Swartz, Kaye Oakley, Kees Philips, Kim Dowling, Koen Quint, Ly Nguyen, Maarten Vermeer, Magdalena van Heck, Marijke van Dijk, Mary-Ann el Sharouni, Mark Harland, Mars Samsom, Meelad Habib, Melvin Samsom, Neven en nichten, Nicole Kukutsch, Omgo Nieweg, Paul van Diest, Peter Steijlen, Petra Peeters, Remco van Doorn, Ricardo Vilain, Richard Scolyer, Rien Ipenburg, Robert van Es, Roel Genders, Roger Uren, Ronald Bierings, Seng Liem, Serigne Lo, Shirah Ipenburg, Simone Ipenburg, Sjan Lavrijsen, Stefan Willems, Stijn Kouwenhoven, StOET, Susan Elder, Sylvie Franken, Tasnia Ahmed, Thomas Rustemeyer, Valerie Jakrot, Victoria Steel, Wieke Holtkamp