Redefining the role of surgical management in the evolving therapeutic landscape of melanoma

Towards a more holistic approach

Daniëlle Verver

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Redefining the Role of Surgical Management in the Evolving Therapeutic Landscape of Melanoma

Towards a more holistic approach

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Richting een meer holistische benadering

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TABLE OF CONTENTS

GENERAL INTRODUCTION

DISEASE PRESENTATION

The incidence of melanoma has increased steeply over the past decade in many European countries, including the Netherlands[1, 2]. From 2006 to 2016, the number of patients with newly diagnosed melanoma almost doubled from approximately 3600 to over 6500 Dutch patients per year[3]. This increase is not only explained by overdiagnosis, as incidence and mortality rates increased among all Breslow thickness categories[4]. It is more likely explained by an interplay of overdiagnosis, increased awareness, rise in ultraviolet exposure (natural and artificial) and changed market forces in the Dutch healthcare system[5].

Approximately 10% of patients present with detectable metastases at time of diagnosis; locoregional in ~7% and distant in ~3%[6-10]. The large majority of patients (around 90%) do not have detectable metastases at presentation, but up to one fifth will develop recurrent disease later on[6, 8]. The most widely accepted approach to melanoma staging is the American Joint Committee on Cancer (AJCC) classification, which is based on the tumour-node-metastasis (TNM) criteria[11, 12]. Breslow thickness and ulceration, two fundamental prognostic factors, define the T-category. The N-category is defined by presence of clinically occult (i.e. positive sentinel nodes) or clinically detected positive lymph nodes, and the M-category is determined by anatomic site of distant metastasis and serum lactate dehydrogenase (LDH). Prognosis is extremely dependent on disease stage. Five-year overall survival ranges between 100 to 19% for patients diagnosed with stage I to stage IV melanoma, respectively[3]. The introduction of effective novel therapies has significantly improved survival, but advanced melanoma remains a serious and fatal disease.

REGIONAL NODAL MANAGEMENT

SENTINEL LYMPH NODE BIOPSY

The Halstedian hypothesis describes the sequential spread of microscopic metastases from the primary melanoma, via regional lymph nodes, to eventually distant sites, and provided the rationale for elective lymph node dissection (ELND)[13]. The procedure improved tumour staging, but controversial results were reported concerning the therapeutic effect[14-16]. A clear survival benefit was lacking as most patients (~80%) harboured negative lymph nodes. Hence, researchers sought a method to identify the patients with positive lymph nodes, considering they were the most likely to benefit. In the early nineties, the sentinel lymph node biopsy (SLNB) was introduced as a less-

invasive technique enabling selective detection and histopathological inspection of the primary draining regional lymph node[17]. The SLNB has been studied extensively since then, and at present, the sentinel node status is one of the most essential prognostic factors[18-20]. It has been steadily incorporated in the AJCC classification since 2001[12, 21, 22]. The tumour burden in the sentinel node, most commonly defined as the longest diameter of the longest metastatic lesion according to the Rotterdam criteria, further improves prognostication, but has not (yet) been integrated in the AJCC classification[22-24]. One randomized trial investigated the potential therapeutic value of SLNB, the Multicenter Selective Lymphadenectomy Trial 1 (MSLT-1), and found no survival benefit[19, 20].

COMPLETION LYMPH NODE DISSECTION

Until recently, patients with a positive sentinel node routinely underwent completion lymph node dissection (CLND). Based on outcomes of two randomized trials, this strategy has been largely abandoned. Both trials compared survival outcomes between an immediate CLND and regular nodal observation using ultrasound examination in patients with a positive sentinel node. The DECOG-SLT trial was first to report, and although underpowered, showed no survival benefit for CLND[25, 26]. These results were quickly followed by results from the larger MSLT-2 study. Similarly, CLND was not associated with improved survival[27]. A possible explanation for this lacking benefit can be found in the hypothesis of melanoma spreading in a parallel manner as opposed to a cascade of orderly progression. In other words, positive (sentinel) lymph nodes act as an indicator for distant disease rather than being an incubator[28].

THERAPEUTIC LYMPH NODE DISSECTION

A therapeutic lymph node dissection (TLND) is currently still standard of care for patients with macroscopic positive lymph nodes detected by serial imaging or physical examination. Besides providing relevant staging information, it can achieve regional control (e.g. prevent mass effect or skin breakdown) and may even be curative in approximately one in every four patients[29]. The extent of surgery in this stage is also subject of discussion, due to conflicting outcomes and associated morbidity. Generally, oncological surgeons are tending towards a more conservative approach. In patients with positive groin lymph nodes it is possible to perform an inguinal or ilioinguinal dissection. As the latter is more extensive, an increased risk of morbidity may be expected. Interestingly, this seems not to be the case[30]. Nevertheless, several studies indicate there is no survival difference between these two approaches, thereby implying that an ilioinguinal dissection can be safely omitted[31, 32]. The

currently ongoing randomized EAGLE-FM trial comparing outcomes between patients undergoing an inguinal or ilioinguinal dissection is expected to provide more conclusive evidence. Until then, it seems safe to omit the ilioinguinal dissection in selected patients [33] although this selection may be hampered as most predictive factors are unknown prior to inguinal dissection[34]. In contrast to the groin, data on extent of surgery for axillary nodes is limited, in particular regarding the necessity of resecting level III nodes. Reported level III node positivity rates range between 17 to 32%[35-37]. To date, randomized studies comparing outcomes between level I-II versus level I-III dissections in this setting are lacking[36].

SYSTEMIC MANAGEMENT

ADVANCED SETTING

The landscape of therapeutic options for patients with advanced melanoma has transformed drastically over the past decade with the introduction of novel systemic therapies. Prior to this era, systemic treatment included cytostatic or cytotoxic agents with limited therapeutic benefit[38]. Surgical resection generally served a palliative purpose and occasionally resulted in effective disease control, especially in patients with limited or oligometastatic disease[39-44]. Nonetheless, one-year overall survival was observed to be only ~40%[45, 46].

The novel systemic therapies can be classified into immune checkpoint inhibition and targeted therapy. Immune checkpoint inhibitors are monoclonal antibodies that enhance anti-tumor T-cell-mediated immune responses. Ipilimumab targets the cytotocix T-lymphocyte-associated protein 4 (CTLA-4) and was the first checkpoint inhibitor to be approved in 2011. In the following years two other checkpoint inhibitors were approved, pembrolizumab and nivolumab, both targeting the programmed death-1 (PD-1) receptor. Targeted therapy has a different mechanism of action by blocking cancer cell proliferation and includes the selective BRAF inhibitors (i.e. vemurafenib, dabrafenib) and MEK inhibitors (i.e. trametinib, cobimetinib).

One-year reported overall survival rates for patients treated with ipilimumab is 46-58%, for vemurafenib 64%, for dabrafenib 68%, for nivolumab 73%, for pembrolizumab 68-74%, for vemurafenib and cobimetinib 75%, for dabrafenib and trametinib 73-74% and for ipilimumab plus nivolumab 73%[47]. Of note, only patients with BRAF-mutated melanomas (~50% of the patients) are eligible to receive targeted therapy, in contrast to immune checkpoint inhibition which is available for all patients with advanced melanoma.

ADJUVANT THERAPY

Despite radical surgery, patients with stage IIB/C and III disease have high risk of recurrence. Five-year recurrence-free survival rates of 59% for stage II disease and only 17% for stage III disease have been reported, in contrast to 85% for stage I disease[8]. Considering these poor outcomes, there was an obvious need for adjuvant therapy. Interferon IFN-α-2b was the first approved adjuvant agent for patients with high-risk melanoma. Larger trials and a recent individual patient data meta-analysis including patients treated with adjuvant IFN showed only marginal improved recurrence-free survival and no significant effect on overall survival[48-52]. Only patients with an ulcerated melanoma seemed to exclusively benefit from adjuvant IFN therapy[49, 52, 53]. Considering this, ulcerated melanomas may represent a distinct biological entity. Due to recent developments, IFN is only considered an option in countries with limited resources and without access to novel agents, in particular for patients with ulcerated melanoma.

The successes of immune checkpoint inhibition and targeted therapy in the advanced setting have led to various clinical trials evaluating their potential in the adjuvant setting. Ipilimumab, at a dose of 10mg/kg, was first to show significantly prolonged recurrence-free, distant metastasis-free and overall survival in patients with completely resected stage III melanoma[54]. Unfortunately it was also associated with substantial toxicity. Preliminary results from a trial investigating a lower dose suggest similar recurrence-free survival along with a milder toxicity profile[55]. At present, ipilimumab has been relegated to the background due to more promising results with anti-PD1 agents nivolumab and pembrolizumab[56-58]. In patients with BRAF-mutated melanoma, dabrafenib plus trametinib has also significantly improved recurrence-free survival[59, 60]. Of note, a convincing benefit in recurrence-free survival has been found in these more recent studies, yet results on overall survival are still pending. Though it appears that recurrence-free survival is a valid surrogate end point for overall-survival[61].

AIM AND OUTLINE OF THIS THESIS

This thesis aims to provide insight in the highly evolving landscape of melanoma management with special focus on the role of surgery. In early stage melanoma, surgical oncologists plays a central role in staging through the SLNB and thereby selection of patients for adjuvant therapy. The first part of this thesis mainly focuses on several aspects of the sentinel node. In **chapter 2** new selection criteria for SLNB are evaluated in patients with pT1 melanoma. A potential more minimal invasive

alternative for SLNB is investigated in **chapter 3**. In **chapter 4**, a nomogram predicting recurrence and melanoma-specific mortality in patients with negative sentinel lymph nodes is presented. The extent of CLND in the groin after a positive sentinel node is investigated in **chapter 5**. In **chapter 6**, the prognostic value of CLND and whether it is possible to select patients for adjuvant therapy solely based on information from the primary melanoma and SLNB is discussed. A novel nomogram predicting recurrence, distant metastasis and overall mortality in patients with positive sentinel nodes is presented in **chapter 7**. **Chapter 8** focusses on the biology of ulceration, which is one of the strongest prognostic factors in melanoma and has been shown to be a predictive factor for adjuvant IFN therapy. The second part of this thesis focuses on treatment and outcomes in patients with advanced melanoma. **Chapters 9** and **10** focus on patients diagnosed with metastatic melanoma without a detectable primary site, which is relatively uncommon. **Chapter 9** illustrates the changes in treatment of patients with an unknown primary melanoma and their survival since the introduction of novel therapies. Whether they have better survival on these novel therapies compared with patients with melanoma of known primary is explored in **chapter 10**. With the introduction of novel systemic therapies, the role for surgical management in advanced melanoma is changing. In **chapter 11**, a possible new role is proposed. It describes a protocol for a randomized trial evaluating whether reduction of tumour burden achieved through local therapies such as surgical resection might result in improved responses to immunotherapy.

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EARLY STAGE

MELANOMA

Improved stratification of pT1 melanoma according to the 8th American Joint Committee on Cancer staging edition criteria:

A Dutch population -based study

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ABSTRACT

INTRODUCTION The 8th American Joint Committee on Cancer (AJCC) staging edition includes revisions regarding pT1 melanomas. We aimed to evaluate the expected impact of this edition on staging and survival in the Dutch pT1 melanoma population.

METHODS In total, 32,935 pT1 melanoma patients, whose data were retrieved from the Netherlands Cancer Registry between 2003 and 2015, were included in the study. Patients were stratified by the 6th AJCC edition (cohort 1: 2003-2009) and 7th edition (cohort 2: 2010-2015) and all reclassified according to the 8th edition. Stage migration, sentinel lymph node biopsy (SLNB) positivity rates and relative survival were analysed. Agreement between staging systems was calculated by Cohen's kappa coefficient.

RESULTS In cohort 2, restaging according to the 8th edition led to an increase of 7% in the total number of patients staged pT1b. The kappa score for agreement between the 6th and 8th edition was 0.15, and 0.25 for agreement between 7th and 8th edition. Restaging according to the 8th edition resulted in a higher SLNB positivity rate for pT1b patients than pT1a patients (8 versus 5%, $p = 0.08$). Relative survival curves were predominantly similar between the staging editions.

CONCLUSION Implementation of the 8th AJCC staging edition will presumably not have major impact on the total number of Dutch pT1b patients. Consequently, the number of patients eligible for SLNB would roughly remain similar. In terms of SLNB positivity, the selection of high-risk pT1 melanoma patients is likely to improve. In addition, the 8th edition criteria for pT1 melanoma seem more workable for pathologists.

INTRODUCTION

Melanoma is the major cause of skin cancer-associated mortality, and its incidence has been rising sharply in the past decades, worldwide and in Europe[1-5]. The majority of newly diagnosed melanomas (up to 70%) are thin melanomas which generally have good prognosis but paradoxically, they do cause approximately 29% of melanoma deaths in absolute terms[6-11]. Accurate classification of melanoma patients into different disease stages is essential, both for prognostic assessment and guidance for patient management decisions. The tumour-node-metastasis classification for melanoma according to the American Joint Committee on Cancer (AJCC) staging system is used most often and has been internationally accepted[12-15]. Recently, the 8th AJCC staging edition has been introduced, which will be implemented in the Netherlands in 2018. Revisions include criteria regarding the pT1a and pT1b classification. According to the revised edition, melanomas with a Breslow thickness of <0.8 mm without ulceration will be classified pT1a, and melanomas with a Breslow thickness of ≥0.8-1.0 mm or a Breslow thickness of <0.8 mm with ulceration will be classified pT1b[16]. The sentinel lymph node biopsy (SLNB) technique that was developed to enable further nodal staging is not routinely recommended in these thin melanomas[17,18]. It is recommended in only a subset of patients with thin melanoma who have a higher risk of SLNB positivity and worse prognosis[13,15,19,20]. Previous studies have shown that not only Breslow thickness is one of the most important prognostic factors, but also ulceration, Clark level and mitotic rate are also important prognostic indicators for both positive SLNB and survival[12,17,20].The Dutch melanoma guidelines (published in 2012) recommend SLNB for patients with stage IB melanoma or higher, which includes pT1b melanoma, to optimise staging and provide relevant prognostic information[21]. These recommendations were based on the 7th AJCC staging edition, where pT1b patients are those with a Breslow thickness of ≤1.0 mm with ulceration or mitoses ≥1/mm2. The aim of this study was to evaluate the expected impact of the implementation of the 8th edition on staging, SLNB positivity and survival in the Dutch melanoma population focussing on the pT1 stadium compared with the 6th and 7th AJCC editions.

METHODS

STUDY POPULATION

This study included all pT1 melanoma patients diagnosed between 2003 and 2015 whose data were retrieved from the Netherlands Cancer Registry (NCR), embedded within the Netherlands Comprehensive Cancer Organisation[1]. The NCR is annually linked to the Municipal Personal Records database to retrieve information regarding vital status, which has been updated till January 1st 2017. Data on gender, age at diagnosis, year of diagnosis, Breslow thickness, mitotic rate, pT classification, SLNB, lymph node dissection(LND), the number of removed and positive lymph nodes and vital status were retrieved. Between 2003 and 2009, data on SLNB were not accurately recorded due to applied registration methods. LND data sometimes overruled the SLNB data. Before 2010, these methods were altered, thus from 2010 onwards data on SLNB were separately recorded and therefore accurate.

STAGING

Patients diagnosed between 2003 and 2009 were classified according to the 6th AJCC staging edition (pT1a: Breslow thickness ≤1.0 mm and Clark level II or III without ulceration; pT1b: Breslow thickness ≤1.0 mm and Clark level IV or V or present ulceration) and were regarded as cohort 1. Patients diagnosed between 2010 and 2015 were classified according to the 7th AJCC staging edition (pT1a: Breslow thickness ≤1.0 mm without ulceration and mitosis <1/mm2; pT1b: Breslow thickness ≤1.0 mm with ulceration or mitoses ≥1/mm2) and were regarded as cohort 2. All patients were restaged according to the 8th AJCC staging edition (pT1a: Breslow thickness <0.8 mm without ulceration; pT1b: Breslow thickness ≥0.8 mm or Breslow thickness <0.8 mm with ulceration). Decimal values in the hundredth's place were rounded down in those ending in 1 to 4 (e.g. 0.74 mm was recorded as 0.7mm) and rounded up to those ending in 5 to 9 (e.g. 0.75mm was recorded as 0.8 mm). Ulceration status is not registered by the NCR. Since 2010, mitotic status was registered, which made it possible to derive the ulceration status from this variable in some patients. Patients with <0.8 mm Breslow thickness, for who ulceration status could not be derived, were restaged as pT1a. Patients with unknown Breslow thickness (n=650) were restaged as pT1 not otherwise specified(nos).

STATISTICAL ANALYSIS

Univariable analysis consisted of Mann-Whitney U test for continuous variables and chi-squared tests for categorical variables. The proportions were determined per

cohort and per year of diagnosis. The agreement between the AJCC staging editions was calculated by the Cohen's kappa coefficient. A commonly used scale for Kappa value interpretation is as follow: 0.01-0.20 slight agreement; 0.21-0.40 fair agreement; 0.41-0.60 moderate agreement; 0.61-0.80 substantial agreement and 0.81-0.99 almost perfect agreement[22]. Relative survival is an estimation of the disease-specific survival and was calculated correcting for age- and gender-specific background mortality[23]. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 21 (IBM Corp, Armonk, NY, USA) and SAS, version 9.4 (SAS Institute Inc, Cary, NC). A two-sided P value <0.05 was considered to be statistically significant.

RESULTS

PATIENT CHARACTERISTICS

A consecutive series of 32,935 patients were diagnosed and registered with pT1 melanoma from 2003 to 2015 in the Netherlands. The annual number of newly diagnosed pT1 melanoma more than doubled over these years; from 1499 in 2003 to 3567 in 2015. The proportion of male patients, patients aged ≥65 years, Breslow thickness of ≥0.8 mm, staged pT1b and performed SLNBs throughout the incidence years are depicted in **Figure 1**.

Cohort 1 (2003-2009) consisted of 13,660 patients (42%) with a median follow-up (FU) of 9.5 years (interquartile range [IQR] 7.8-11.5 years) and cohort 2 (2010-2015) consisted of 19,275 patients (59%) with a median FU of 3.6 years (IQR 2.3-5.2 years). In cohort 2, mitotic rate was known in 10,734 patients, of whom 29% had mitosis ≥1/mm^2. Median Breslow thickness in patients with mitosis <1/mm^2 was 0.50 (IQR 0.40-0.70 mm) versus 0.70 mm (IQR 0.58-0.90 mm) for patients with mitosis ≥1/mm^2 (p<0.001). Of the 7615 patients with mitosis <1/mm^2, there were 114 patients (2%) originally staged pT1b with a median Breslow thickness of 0.70 mm (IQR 0.55-0.85 mm). We presumed that these patients were staged as such due to the presence of ulceration. Patient and tumour demographics per cohort are summarised in **Table 1**.

STAGE MIGRATION

The shift of pT1 patients when staged by the 6th or 8th AJCC staging edition in cohort 1 and 7th or 8th AJCC staging edition in cohort 2 is illustrated in **Figure 2**. In cohort 1, staging by the 6th or 8th edition showed concordant staging in 68%, upstaging (pT1a to pT1b) in 22% and downstaging (pT1b to pT1a) in 4%. The kappa score for agreement between these two staging editions was 0.15 (standard error [SE] 0.007).

Table 1 Patient and tumour demographics of all 32,935 consecutive pT1 melanoma patients diagnosed in the Netherlands between 2003 and 2015

Patient and tumour demographics		No. (%) or median (IQR)		p value[a]
		Cohort 1 (2003 - 2009) (*n* = 13,660)	Cohort 2 (2010 - 2015) (*n* = 19,275)	
Gender	Male	5,296 (38.8)	8,532 (44.3)	<0.001
	Female	8,364 (61.2)	10,743 (55.7)	
Age	years	53 [41 - 64]	58 [46 - 68]	<0.001[b]
Breslow	mm	0.60 (0.40 - 0.80)	0.57 (0.40 - 0.75)	<0.001[b]
Breslow	<0.8 mm	9,522 (69.7)	14,007 (72.7)	<0.001
	≥0.8 mm	3,896 (28.5)	4,898 (25.4)	
	Unknown	242 (1.8)	370 (1.9)	
Mitosis ≥1mm^2	Yes	0	3,119 (16.2)	n/a
	No	0	7,615 (39.5)	
	Unknown	13,660 (100)	8,541 (44.3)	
T stadium	T1a	11,553 (84.6)	14,496 (75.2)	<0.001
	T1b	1,365 (10.0)	4,632 (24.0)	
	T1 nos	742 (5.4)	147 (0.8)	
SLNB	Yes	445 (3.3)	1,162 (6.0)	<0.001
	No	13,215 (96.7)	18,113 (94.0)	
SLNB result	Positive	34 (7.6)	81 (7.0)	0.028
	Negative	409 (91.9)	1,048 (90.2)	
	Not found	2 (0.4)	20 (1.7)	
	Unknown	0	13 (1.1)	
LND	Yes	64 (0.5)	63 (0.3)	0.041
	No	13,589 (99.5)	19,189 (99.7)	
LND result	Positive	40 (62.5)	34 (54.0)	0.597
	Negative	9 (14.1)	12 (19.0)	
	Unknown	15 (23.4)	17 (27.0)	

IQR, interquartile range; SLNB, sentinel lymph node biopsy; LND, lymph node dissection; n/a, not applicable.
[a]Chi-squared test.
[b]Mann-Whitney *U* test.

Figure 1 Proportion of male patients, aged ≥65 years, Breslow thickness ≥0.8 mm, staged pT1b and performed sentinel lymph node biopsies (SLNBs) throughout the incidence years

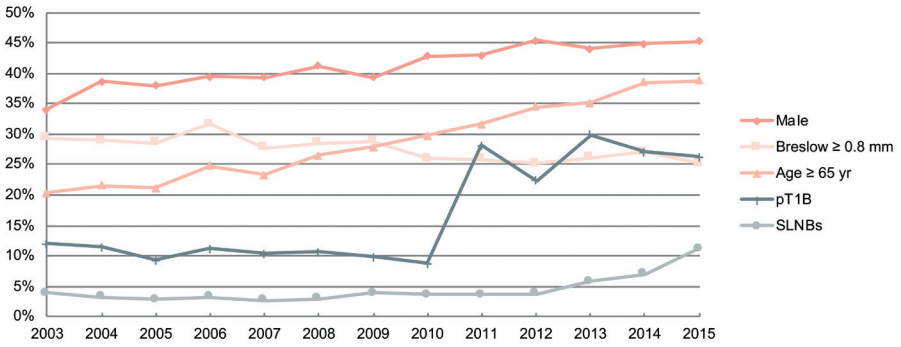
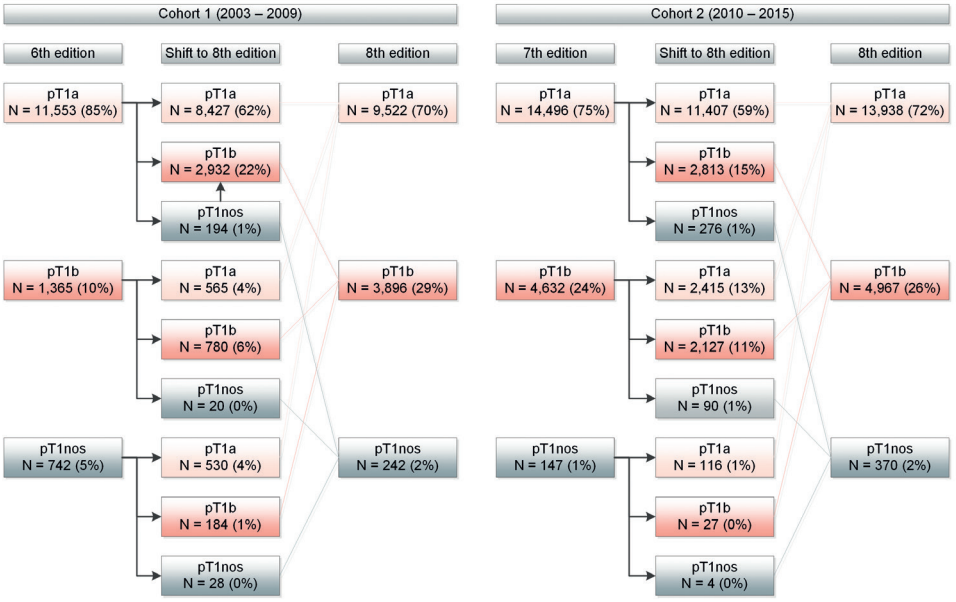


Figure 2 Shift of Dutch pT1 melanoma patients when staged according to 6th or 8th AJCC staging edition in cohort 1 and 7th or 8th AJCC staging edition in cohort 2



In cohort 1, staging by the 7th or 8th edition showed concordant staging in 72%, upstaging in 15% and downstaging in 13%. The kappa score for agreement between these two staging editions was 0.25 (SE 0.007). In cohort 2, restaging according to the 8th edition led to an increase of 7% in the total number of patients staged pT1b (from 4632 to 4967 patients).

SENTINEL LYMPH NODE BIOPSIES

The SLNB results of the 1162 patients (6%) in cohort 2, who underwent a SLNB staged according to the 7th and 8th edition, are depicted in **Table 2**. There were 4632 patients in cohort 2 originally staged pT1b according to the 7th edition, of whom 817 patients underwent SLNB (18%). The overall SLNB positivity rate was 7% (81/1162).

RELATIVE SURVIVAL

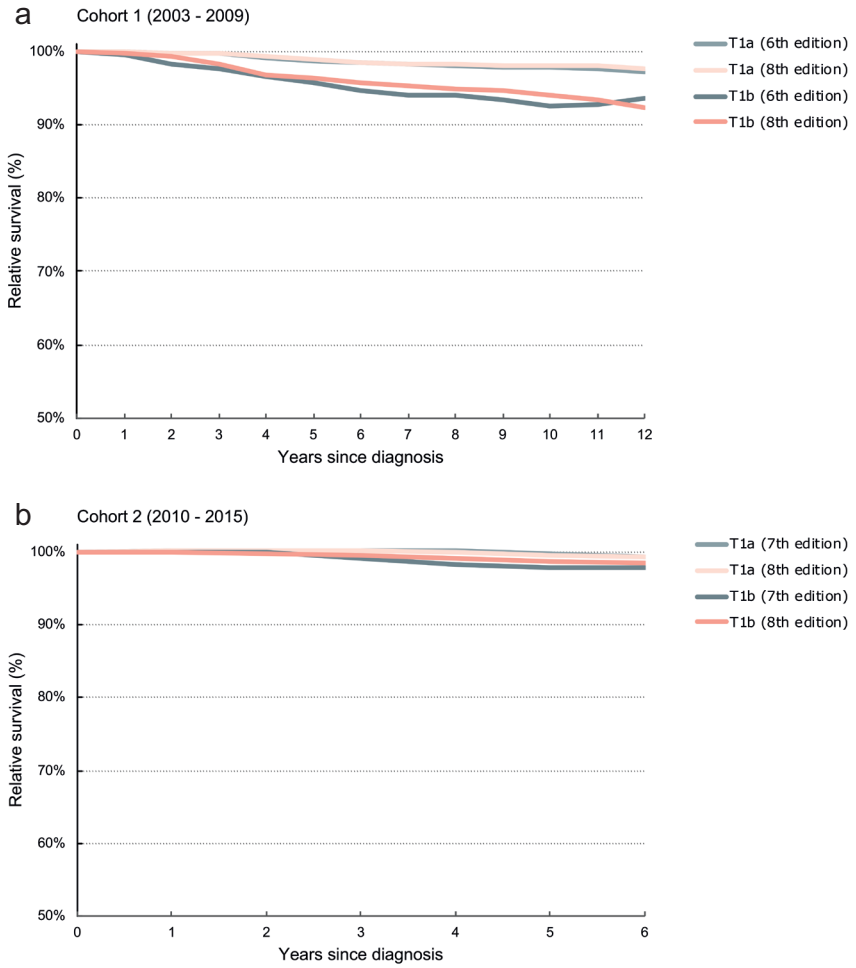
Figure 3 shows the relative survival curves for the patients of cohort 1 and 2 staged according to the 6th, 7th or 8th AJCC staging edition. In cohort 1, the 10-year relative survival rates for patients staged pT1a and pT1b according to the 6th edition were 98 and 92%, respectively, and 98 and 95%, respectively, when restaged according to the 8th edition. In cohort 2, the 5-year relative survival rates for patients staged pT1a and pT1b according to the 7th edition were 100 and 98%, respectively, and 100 and 99%, respectively, when restaged according to the 8th edition.

Table 2 SLNB results in cohort 2 (2010 - 2015) staged according to the 7th AJCC staging edition and staged according to the 8th AJCC staging edition (n = 1162)

SLNB result	7th AJCC staging edition, No. (%)				8th AJCC staging edition, No. (%)			
	pT1a	pT1b	pT1nos	*p* value[a]	pT1a	pT1b	pT1nos	*p* value[a]
Positive	26 (8)	54 (7)	1 (100)		20 (5)	60 (8)	1 (2)	
Negative	301 (88)	747 (91)	0	0.473[b]	352 (92)	661 (90)	35 (81)	0.076[b]
Not found	7 (2)	13 (2)	0		10 (3)	10 (1)	0	
Unknown	10 (3)	3 (0)	0		2 (1)	4 (1)	7 (16)	

AJCC, American Joint Committee on Cancer; SLNB, sentinel lymph node biopsy;
[a] Chi-squared test.
[b] *p* value when only the positive or negative results are compared between the pT1a and pT1b group (thus pT1nos, not found and unknown results are excluded in the analysis).

Figure 3 Relative survival in pT1a and pT1b patients in (A) cohort 1 staged according to the 6th and 8th AJCC staging edition and in (B) cohort 2 staged according to the 7th and 8th AJCC staging edition (pT1nos was excluded)



DISCUSSION

In this Dutch population-based study, it is shown that the incidence of cutaneous pT1 melanoma is still rising and has doubled over the last two decades. This observed trend is not only due to overdiagnosis because the incidence of all melanomas increased as well as the mortality rates[24]. The conjunction of overdiagnosis with ultraviolet exposure (natural and artificial), changed market forces in the Dutch healthcare system and increased awareness are all contributing factors[25]. Other striking trends observed in the Dutch population were the steep increase in the proportion of melanoma patients classified as pT1b and the increase in SLNBs performed from 2011 onwards. Both are linked to the introduction of the 7th AJCC staging edition and the adherence to local guidelines based on this edition.

The proportion of pT1b patients increased substantially from 10% for patients diagnosed between 2003 and 2009 staged according to the 6th AJCC staging edition to 24% for patients diagnosed between 2010 and 2015 staged according to the 7th edition. In 2009, the 6th AJCC staging edition was replaced by the 7th edition and involved alterations regarding the pT1 criteria. High level of invasion (Clark level) was replaced by mitotic rate (≥1 mitosis/mm^2) because Clark level was no longer an independent prognostic factor when mitotic rate was included in the analysis[12]. Since approximately 29% of the Dutch pT1 melanoma patients diagnosed between 2010 and 2015 have ≥1 mitosis/mm^2, the increase in the proportion of pT1b patients was to be expected. However, there is debate whether stratification for mitotic rate correlates to a clinically relevant decrease in survival rate and whether it increases the risk of SLNB positivity[26,27]. In the Netherlands, it was concluded that the 7th AJCC staging edition did not improve selection of high-risk pT1 patients[28]. In any case, it is striking that mitotic rate is no longer included as a criterion for pT1a or pT1b stratification in the 8th AJCC staging edition. However, it has little impact on the total number of patients that will be staged pT1b. In the current study, it was observed that restaging according to the new criteria resulted in an 7% increase in total number of patients staged pT1b. Consequently, we would expect a corresponding increase in the total number of patients eligible for SLNB if recommendations for performing an SLNB remain unchanged.

Although the proportion of performed SLNBs increased over the past years, still only 18% of the patients classified as pT1b ultimately underwent SLNB. This indicates that the Dutch guidelines, which recommend the use of SLNB in pT1b patients, are not strictly adhered to. Even more so, there is considerable regional variation in SLNB practice in the Netherlands, which is partly explained by patient and tumour characteristics

and the coherent comorbidity[29]. It has been reported that Dutch melanoma patients with a Breslow thickness >1.0 mm are less likely to undergo an SLNB in case of head and neck melanoma, older age and low socioeconomic status and are more likely to undergo SLNB when diagnosed in an university hospital[30]. Perhaps these reasons could partly explain the omission of SLNB in thin melanomas as well. In addition, the guidelines are not mandatory, and many clinicians debate the question whether or not to perform SLNB in thin melanomas at all. Commonly, a yield of 5% SLNB positivity has been recognised as a threshold for justification of performing SLNB[17,18]. In the current study, an overall positivity rate of 7% was observed in pT1 melanomas in cohort 2. Patients who were originally staged pT1a or pT1b according to the 7th edition had similar SLNB positivity rates (8 versus. 7% respectively, p=0.47), suggesting an inadequate selection of high-risk patients. Several studies have reported that Breslow thickness is associated with SLNB positivity and have suggested that cut-off values of 0.75 mm might better stratify high-risk pT1 patients[13,15,19,31]. Reported SLNB positivity rates for melanomas ≤0.75 mm range between 0 and 6.1% and for >0.75 mm range between 8.2 and 12.8% [19,31-33]. When patients were restaged pT1a or pT1b according to the 8th edition, which represents applying a cut-off value of 0.8 mm, the SLNB positivity rate was higher for patients restaged pT1b compared with pT1a (8 versus 5%, p=0.08), suggesting an improvement in selection of high-risk thin melanoma patients. However, the overall yield is still fairly low, and thus, careful consideration for performing SLNB in thin melanoma remains necessary. Perhaps including patient age in the decision-making could aid in achieving higher yields because it was recently demonstrated that age seems to be an important discriminant of nodal positivity as well; patients aged <40 years have a higher risk as compared with patients aged ≥65 years[34].

The present study did not reveal any clinically relevant changes in relative survival (proxy for disease-specific survival) between the 6th, 7th or 8th AJCC staging editions. This might be explained by the fact that (disease-specific) survival in patients with thin melanoma is already relatively high, and it is therefore difficult to demonstrate a clear survival difference between the pT1 substages.

Prognosis and management of melanoma patients are principally determined by pathological parameters of the primary tumour. It is therefore essential that these parameters are accurately analysed and documented by pathologists. Breslow thickness has universally been found to be the most reliable reproducible parameter with the highest concordance between pathologists[35].The concordance for ulceration has been reported to be excellent as well, and the concordance for Clark level is lower but still good[35]. In contrast, it has been reported that the interobserver

reliability of mitosis in thin melanoma is poor[36,37]. These findings would suggest that the new 8th edition criteria for pT1 melanoma seems to be more workable in everyday practice compared with the 6th and 7th AJCC staging edition criteria.

Some limitations of the present study must be considered. Since the data used are derived from the nationwide NCR, only data on diagnosis and initial treatment are available. Data on SLNB in cohort 1 were not accurate; therefore, we could not analyse the impact of the staging editions on SLNB results in this cohort. Several important factors that are critical for evaluation are not routinely registered by the NCR, which is another disadvantage because it was therefore impossible to account for these factors. We presumed that ~2% of the Dutch population with thin melanoma had ulceration, which is similar to previously reported rates that range from 1.5 to 4.8%, with even lower rates in patients with Breslow thickness ≤0.8 mm[6,38]. However, because ulceration status has not been registered, it is possible that the number of patients restaged pT1b is slightly underestimated. Even more so, according to the new criteria patients with a Breslow thickness of 1.01-1.04 mm should now be staged pT1b as well whereas they were staged pT2a or pT2b according to the 7th or 6th edition. We only included pT1 patients, thus those with a Breslow thickness up to 1.00 mm. Consequently, our results may not completely reflect the impact of the 8th edition. There was slight agreement between the 6th and 8th staging editions in cohort 1 and fair agreement between the 7th and 8th staging editions in cohort 2. Consequently, which staging edition is applied has major impact on a large proportion of patients because they would have been staged pT1a according to one but pT1b to another and the other way round.

Despite these limitations, our study has important strengths as well, including its large size and generalisability. A population-based database can serve as a powerful tool to explore the prognostic details of thin melanoma, and therefore, our study provides valuable insight. To enhance the quality of healthcare for pT1 melanoma patients in the Netherlands, it would be useful to set up a registry similar to the Dutch Melanoma Treatment Registry, which represents a nation-wide collaboration of all stakeholders involved in melanoma care regarding Dutch patients with unresectable stage IIIC or IV melanoma[39].

In conclusion, it can be presumed that the implementation of the 8th AJCC staging edition will not have major impact on the total number of Dutch pT1b patients. Consequently, the number of patients eligible for SLNB would roughly remain similar. In terms of SLNB positivity, the selection of high-risk pT1 melanoma patients is likely to improve. In addition, the 8th edition criteria for pT1 melanoma seem more workable for pathologists in clinical practice.

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Gamma probe and ultrasound-guided fine needle aspiration cytology of the sentinel node (GULF) trial

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ABSTRACT

PURPOSE Sentinel lymph node biopsy (SLNB) was introduced as a minimally invasive technique for nodal staging. Since associated morbidity is not negligible, it is highly relevant to pursue a more minimally invasive alternative. The purpose of this study was to prospectively evaluate the sensitivity of fine needle aspiration cytology (FNAC) with combined gamma probe and ultrasound (US) guidance in comparison with the gold standard histology of the sentinel node (SN) after SLNB for detecting metastasis.

METHODS The study was designed as a prospective, multicentre, open-label, single-arm trial enrolling patients with newly diagnosed cutaneous melanoma or breast cancer between May 2015 and August 2017. Sample radioactivity was measured using a Mini 900 scintillation monitor. After FNAC, all patients underwent SLNB. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were estimated.

RESULTS Accrual was terminated early following an unplanned interim analysis indicating that a FNAC sensitivity of at least 80% could not be achieved. In total 58 patients of the originally planned 116 patients underwent FNAC with gamma probe and US guidance. There were no true-positive FNAC results, 14 false-negative results and one false-positive result, and thus the sensitivity, specificity, PPV and NPV of FNAC were 0, 98, 0 and 75%, respectively. At least 75% of the FNAC samples had a radioactivity signal higher than the background signal.

CONCLUSION FNAC with gamma probe and US guidance is not able to correctly detect metastases in the SN and is therefore not able to replace SLNB. Gamma probe-guided US is a highly accurate method for correctly identifying the SN, which offers possibilities for future research.

INTRODUCTION

The sentinel lymph node biopsy (SLNB) procedure was introduced in the early 1990s as a less-invasive technique than elective lymph node dissection, enabling selective detection and histopathological inspection of the primary draining lymph node in the regional lymph node basin related to the primary tumour site, e.g. melanoma or breast cancer[1, 2]. The status of the (sentinel) lymph nodes is one of the most important prognostic indicators for recurrence and survival[3–5]. In addition, (sentinel) lymph node status guides locoregional treatment decisions and will probably soon guide the choice of systemic treatment.

Although less invasive than elective lymph node dissection, the morbidity associated with SLNB is not negligible. This is of particular concern since a positive sentinel node (SN) is found in only 20–30% of patients[5–7]. Morbidity occurs in approximately 11% of patients, with the most common early postoperative complications being seroma (about 5%) and infection (about 3%)[8]. Lymphoedema has been reported to occur in at least 6% of patients[9, 10]. It is important to note that SLNB does not improve survival but only provides accurate and important staging information[5, 11]. In this light, it seems highly relevant to pursue a more minimally invasive alternative to SLNB. Fine needle aspiration cytology (FNAC) with ultrasound (US) guidance may provide a good minimally invasive alternative. Several studies have focused on US examination with or without FNAC in melanoma patients, but sensitivity rates vary greatly and most studies lacked a method to accurately identify the SN prior to US examination and FNAC[12]. This problem could be overcome by using a hand-held gamma probe as an aid to US identification of the SN after lymphoscintigraphy. This has been shown to be feasible in several studies in breast cancer patients, in which the SN was correctly identified in 75–100% of patients[13–16].

The purpose of this study was to prospectively evaluate the sensitivity of FNAC with combined gamma probe and US guidance compared with the gold standard, histology of the SN after SLNB, for detecting SN metastasis.

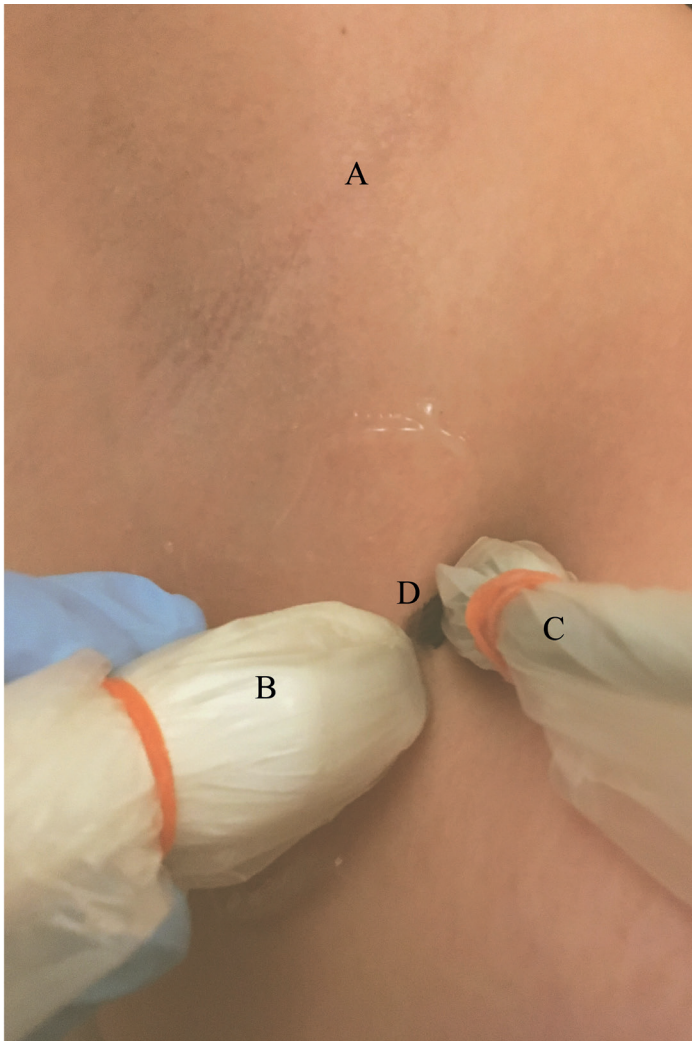
MATERIALS AND METHODS

STUDY DESIGN

Details of the study design and protocol have been published previously[12]. Briefly, the trial was designed as a prospective, multicentre, open-label, single-arm trial and was performed in two Dutch hospitals. The Ethical Review Board approved the

study protocol. This trial was registered with The Netherlands Trial Registry (NTR; ID NRT5193, 1 May 2015). The study was prepared in accordance with the Standards for Reporting of Diagnostic Accuracy Studies[17].

Figure 1 Sentinel node identification using gamma probe guided US



Presentation of the identification of the presumed sentinel node in the axilla (A) using the ultrasound probe (B), gammaprobe (C) and the skin mark (D).

PROCEDURES

After peritumoral or intradermal injection of 99mTc-nanocolloid, lymphoscintigraphy was performed according to the institution's standard protocol during the 24 h before surgery to define the location of the SN. A nuclear medicine specialist reported information regarding the identified SN basin(s) and primary tier SN(s). The presumed SN(s) were distinguished from the second-tier nodes by visualization of the first node or a direct drainage pathway. Following successful lymphoscintigraphy, a dedicated radiologist identified the hot spot over the skin using a hand-held gamma probe (16mm Europrobe 3) and the area was examined using US (Aloka ProSound alpha10) with a 1–15 MHz linear transducer to attempt to visualize the assumed SN (a visible lymph node at the centre of the hotspot as identified with the gamma probe; **Figure 1**). Fine needle aspiration of all visualized assumed SN(s) was performed using a 21-gauge needle, regardless of suspicion of metastasis on US examination, with usually one or two cortical samples per SN (depending on the visual yield of each sample). A Mini 900 scintillation monitor with a sodium iodide crystal was used, when available, to measure radioactivity of the samples. All FNAC samples were subsequently transported to and analysed in the pathology laboratory of the Erasmus MC Cancer Institute.

Cytological smears were prepared according to a standard protocol. Cytomorphology was assessed on haematoxylin and eosin (H&E) stained smears. The remainder of the aspirate was expressed into a CytoLyt solution from which a Cellient cell block was prepared, provided that an adequate amount of material was obtained. Cytomorphology was assessed again. In addition immunohistochemical staining was performed using S-100 and Melan-A for melanoma samples and Ker8-18 for breast cancer samples. US examination findings are reported according to the Berlin morphological criteria[18]. The SN identified on US examination was regarded as malignant when the lymph node appeared "balloon shaped", and suspicious if peripheral perfusion, loss of central echoes, asymmetrical broadening of the parenchyma, or an echo-poor island within an otherwise normal lymph node was present[18, 19]. After FNAC, all patients proceeded directly to the operating room for SLNB. The SNs were handled and assessed in each centre according to the European Organization for Research and Treatment of Cancer (EORTC) SN pathology protocol[20].

OUTCOMES

The primary objective of the trial was to assess the sensitivity of FNAC with combined gamma probe and US guidance in detecting SN metastasis in patients with melanoma or breast cancer. Prespecified secondary end-points evaluated in the pilot phase of the study included the SN identification rate and the histological results of core needle biopsy (CNB) in comparison with FNAC and SLNB.

STATISTICAL ANALYSIS

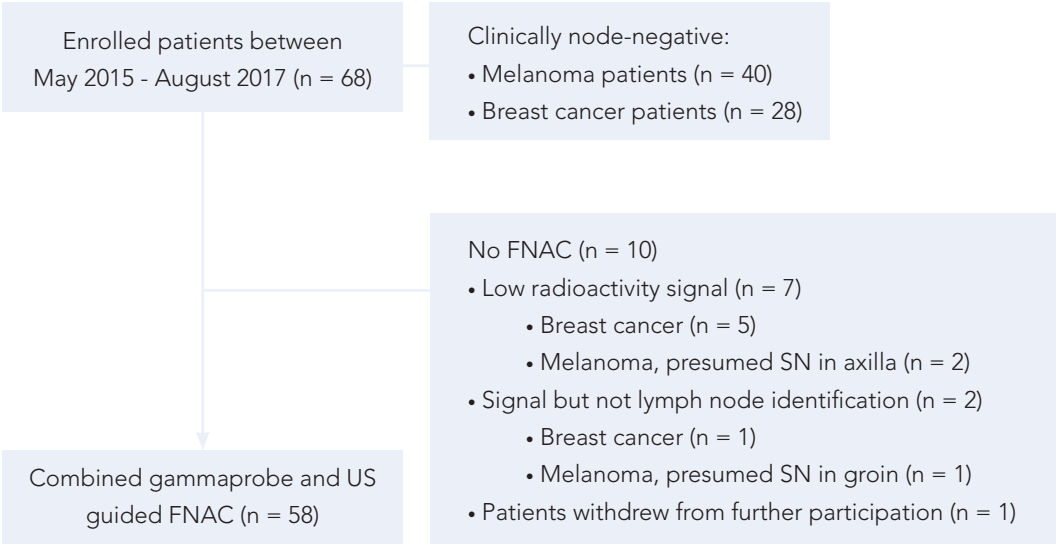
Power and sample size calculations are described in detail elsewhere[12]. Considering a 30% prevalence, the required sample size was 116 patients to detect metastatic SN(s) with a sensitivity of 90% and a 95% confidence interval (CI) of 80– 100%, with a two-sided significance level α of 0.05 and a power 1 − β of 0.80. The estimated recruitment goal was 120 patients. Negative FNAC was defined as absence of metastatic tumour cells (i.e. negative cytology) or unrepresentative cytology due to collection of an insufficient number of cells. Based on comparison with final histology, four types of FNAC results were defined: false-negative, true-negative, false-positive and true-positive. The same definitions were applied to the CNB results and the US results according to the Berlin criteria. A malignant or suspicious SN on US examination according to the Berlin criteria was recorded as positive. Continuous data are presented as medians with interquartile ranges (i.q.r.) and categorical data as frequencies with their respective percentages. The number of true-positives, false-positives, false-negatives and true-negatives were calculated. Positive predictive value, negative predictive value, specificity and sensitivity were estimated. When clinical data were missing, the clinical characteristic was categorized as unknown. Two-sided P values <0.05 were considered statistically significant. SPSS version 22.0 (IBM, Armonk, NY) was used for all statistical analyses.

RESULTS

A total of 68 clinically node-negative patients with melanoma or breast cancer were enrolled, of whom 10 (15%) did not undergo FNAC and therefore were excluded from further analysis (**Figure 2**). Baseline characteristics of the 58 patients who did undergo FNAC with combined gamma probe and US guidance are shown in **Table 1**. The median number of identified SNs on lymphoscintigraphy was 1 (i.q.r. 1–2) and the median number of SN basins on lymphoscintigraphy was 1 (i.q.r. 1–1). The median number of identified SNs on FNAC with gamma probe and US guidance was 1 (i.q.r. 1–2) and the median number of retrieved SNs during SLNB was 2 (i.q.r. 1– 3; P < 0.001). Originally 116 patients were planned to be included. Slow accrual prompted extension of the predefined study period, but before this was done, an unplanned (non-protocol-specified) interim efficacy analysis was carried out. Evaluation of the results indicated that even in the best-case scenario a FNAC sensitivity of at least 80% could not be achieved, and accrual was therefore terminated. Early termination was not related to any safety concerns related to FNAC with gamma probe and US guidance. The interim analysis was performed in July 2017 with available data on the first 53 patients

who had undergone FNAC (an additional five patients had been included but data on these patients were not yet complete). The technique was considered successful if a sensitivity of 90% with an upper and lower 95% CI of 80–100% was achieved. Calculations based on the data available showed that in the best-case scenario, the highest achievable sensitivity would be 63%

Figure 2 Flow of participants



FNAC, fine needle aspiration cytology; SN, sentinel node; US, ultrasound

Table 1 Baseline patient and tumour characteristics (n = 58)

Characteristic	Value
Gender	
Male	18 (31)
Female	40 (69)
Age (years)	56 (44 – 64)
Body mass index (kg/m^2)	25.1 (22.4 – 27.1)
Breast cancer	21 (36)
Size (mm)	15.0 (6.5 – 28.3)
Side	
Right	14 (67)
Left	7 (33)
Quadrant	
Left upper	8 (38)
Left lower	3 (14)
Medial lower	3 (14)
Medial upper	4 (19)
Histology	
Ductal	15 (71)
Other	6 (29)
Oestrogen receptor status	
Negative	3 (14)
Positive	16 (76)
Unknown	2 (10)
Progesterone receptor status	
Negative	5 (24)
Positive	14 (67)
Unknown	2 (10)
Her2neu status	
Negative	15 (71)
Positive	4 (19)
Unknown	2 (10)

Table 1 Continued

Characteristic	Value
Melanoma	37 (63.8)
Breslow thickness (mm)	1.85 (1.13 – 4.00)
Location	
Arm	8 (21.6)
Leg	14 (37.8)
Trunk	15 (40.5)
Histology	
Superficial spreading	18 (48.6)
Nodular	10 (27.0)
Acral lentiginous	2 (5.4)
Lentigo Maligna	2 (5.4)
Other	2 (5.4)
Unknown	3 (8.1)
Ulceration	
Present	10 (27.0)
Absent	25 (67.6)
Unknown	2 (5.4)
Regression	
Complete	1 (2.7)
Partial	2 (5.4)
Absent	32 (86.5)
Unknown	2 (5.4)
Micro satellites	
Present	1 (2.7)
Absent	33 (89.2)
Unknown	3 (8.1)

Table 2 Measures of diagnostic accuracy of US examination, FNAC and core needle biopsy

Results, n (%)	FNAC (n = 58)	US[a] (n = 58)	CNB (n = 10)
True positive	0	2 (3)	0
Tumour			
Breast cancer	n/a	1 (50)	n/a
Melanoma	n/a	1 (50)	n/a
SN location			
Axilla	n/a	1 (50)	n/a
Groin	n/a	1 (50)	n/a
False positive	1 (2)	6 (10)	0
Tumour			
Breast cancer	0	2 (33)	n/a
Melanoma	1 (100)	4 (66)	n/a
SN location			
Axilla	1 (100)	6 (100)	n/a
Groin	0	0	n/a
True negative	43 (74)	38 (66)	9 (90)
Tumour			
Breast cancer	17 (40)	15 (40)	9 (100)
Melanoma	26 (61)	23 (61)	0
SN location			
Axilla	34 (79)[b]	29 (76)	9 (100)
Groin	9 (21)	9 (24)	0
False negative	14 (24)	12 (21)	1 (10)
Tumour			
Breast cancer	4 (29)	3 (25)	1 (100)
Melanoma	10 (71)	9 (75)	0
SN location			
Axilla	6 (43)	5 (42)	1 (100)
Groin	8 (57)	7 (58)	0
Measures, % (95% CI)			
Sensitivity	0%	14% (3% - 38%)	0%
Specificity	98% (90% - 100%)	86% (74% - 94%)	100%
Positive predictive value	0%	25% (5% – 59%)	0%
Negative predictive value	75% (63% - 85%)	76% (63% - 86%)	90% (63% - 99%)

CI, confidence interval; CNB, core needle biopsy; FNAC, fine needle aspiration cytology; n/a, not applicable; US, ultrasound

[a] According to Berlin criteria, a malignant/suspicious sentinel node on US examination was recorded positive

[b] Two patients had SNs identified in the groin and axilla

DISCUSSION

The pilot phase of this prospective trial showed that gamma probe-guided US can accurately identify the SN in up to 90% of patients. This is in line with previous reported correct identification rates of 75–100%[13–16]. Furthermore, at least 75% of the FNAC samples had a radioactivity signal more than twice the background signal, which supports the high accuracy of SN identification. However, FNAC lacked sensitivity as it was not able to correctly detect metastases in the SN.

A high body mass index, a high background signal, the presence of a cluster of multiple nodes and a presumed SN location close to the injection site might hamper SN visualization and identification. In the present study, accurate radiographic SN visualization and identification was impossible in 10 of 68 patients (15%). This was predominantly caused by a low transcutaneous radioactivity signal, presumably as a result of poor tracer uptake or tracer migration. SLNB was successful in all ten patients and in at least two patients the signal was also recorded as low during the surgical procedure.

All 51 patients (88%) with representative cytological smears stained with H&E showed normal cytomorphology. Additional cytomorphology and immunohistochemistry analysis on Cellient blocks was possible in 39 patients (67%). One of these patients showed abnormal morphology with positive immunohistochemistry in the Cellient block, and was recorded as having a positive FNAC. However, on histology the SLNB turned out to be negative, even after additional slides had been reviewed. Thus, this FNAC was recorded as false-positive. In 15 patients (39%) the H&E-stained smears and Cellient blocks showed normal morphology but a nonspecific immunohistochemistry. The FNAC in these patients was regarded and recorded as negative. Remarkably, histology after SLNB showed metastasis in 6 of these 15 patients (40%).

The Mini 900 scintillation monitor with a sodium iodide crystal was used on 44 samples, and showed a signal more than twice the background signal in 75% of the samples. This supports the high accuracy of SN identification using FNAC with gamma probe and US guidance, as was previously demonstrated in the trial phase with a correct identification rate of 90%[12]. A signal equal to or lower than the background count does not necessarily mean that the sample is not radioactive, but could be a result of a higher background count that occurs occasionally or a low sample volume. So with respect to the false-positive FNAC, the degree of certainty that the lymph node from which FNAC was done was similar to the excised lymph node is at least 75% tending towards 90%. Fusion of US guidance with free-hand SPECT connected to a gamma detection device might become an alternative method that could further increase the correct identification rate of the SN[21].

The FNAC technique would be considered successful when a sensitivity of 90% was achieved. This was based on the assumption that submicrometastases in melanoma (i.e. <0.1 mm at any site or 0.4 mm subcapsular) or isolated tumour cells in breast cancer (i.e. <0.2 mm) are less likely to be detected using FNAC and occur in approximately 10% of patients. Though our hypothesis for detecting positive SNs using FNAC with combined gamma probe and US guidance seemed promising, unfortunately the technique failed, as micrometastatic and also macrometastatic lesions were not detected. This failure can partially be explained by the relatively small sizes of the metastatic lesions in the SN in nine of 14 patients (<1.0 mm for melanoma and <2.0 mm for breast cancer) and in five of these patients the lesions were even submicrometastases. The histological pattern of a subcapsular metastasis is often small and spread in line with the capsule, thus presenting the physician performing the FNAC with a great challenge since it is easy to puncture through the lesion into the parenchyma[22]. On the other hand, a detection limit (the smallest diameter of SN melanoma metastasis that can be detected) for a positive FNAC of 0.3 mm has been reported[23]. This suggests that the detection of micrometastases, and certainly macrometastases, should be possible.

In the previous promising studies on US-guided FNAC almost all procedures were performed by the same senior sonologist who usually obtained four cortical samples per SN[18, 19, 22, 23]. In our study the procedure was performed by several dedicated radiologists with usually one or two cortical samples per SN. Thus, operator dependency and fewer cortical samples per SN (which increases the chance of missing smaller areas of interest within the SN) might also be possible explanations for our results. In this light, the use of large-lumen needles (10-gauge or 12-gauge) might increase the effectiveness of the procedure in detecting metastasis[24]. However, we performed CNB using a 14-gauge needle in ten patients in the pilot phase, and of these patients one turned out to have macrometastasis in the SN which was not detected by CNB. This suggests that piecemeal sampling, regardless of the needle lumen, compromises pathological evaluation. Technical difficulties could also have contributed (e.g. small node, difficult location and/or recognition of the presumed SN, blood in the SN after the first sample). This illustrates the difficulty in the broad implementation of the technique. It is noteworthy that in most previous studies investigating the sensitivity of US-guided FNAC, FNAC was performed only if there was suspicion of metastasis on US examination[12]. This increases the likelihood of a positive FNAC considerably and explains some of the higher reported sensitivity rates,and might explain our sensitivity rate since we performed FNAC regardless of suspicion of metastasis on US examination. Nonetheless, low sensitivity and moderate negative predictive values remain an issue[25].

Our study clearly had some limitations mainly due to the premature termination of the trial. This naturally resulted in a smaller number of included patients than initially planned. Nevertheless, it represents prospectively collected data and the interim analysis showed that even in the best case scenario it would have been impossible to achieve the desired sensitivity of at least 80%. Thus, continuation of the trial would not have led to substantially different conclusions.

Although the main outcome of this trial was negative and was not in accordance with our hypothesis, valuable information was obtained. FNAC with gamma probe and US guidance is not able to correctly detect metastases in the SN and the technique used is therefore not able to replace the SLNB procedure. On the other hand, gamma probe-guided US was found to be highly accurate in correctly identifying the SN. This offers possibilities for evaluating other minimally invasive techniques that incorporate gamma probe-guided US for SN identification.

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SUPPLEMENTARY MATERIAL

RESULTS OF FNAC WITH GAMMA PROBE AND US GUIDANCE

The cytological smears were representative in 51 of 58 patients (88%) and additional cytomorphology and immunohistochemistry analysis on Cellient blocks was possible in 39 of 58 patients (67%). In patients with insufficient cytology FNAC was recorded as negative. One of these patients turned out to have a positive SN on histology, and the FNAC result was recorded as false-negative. The radioactivity signal of the material, measured using the Mini 900 scintillation monitor with a sodium iodide crystal, was reported to be more than twice the background signal in 33 of the 44 tested samples (75%).

The results of the FNAC, US examination (according to the Berlin criteria) and CNB and measures of diagnostic accuracy are shown in Table 2. A positive SN on histology after SLNB was found in 14 patients (24.1%), 10 with melanoma and 4 with breast cancer. Submicrometastases were present in 5 of 14 patients (35.7%), 3 with melanoma (<0.1 mm at any site or 0.4 mm subcapsular) and 2 with breast cancer (≤0.2 mm isolated tumour cells). Micrometastases were present in four patients (28.6%), 3 with melanoma (>0.1–1.0 mm) and 1 with breast cancer (>0.2–2.0 mm). Macrometastases were present in the remaining five patients (35.7%), 4 with melanoma (>1.0 mm) and 1 with breast cancer (>2.0 mm). In the pilot phase of the study an additional CNB of the SN was done in ten breast cancer patients. The biopsy was not representative in four patients due to collection of an insufficient amount of tissue. Histology after SLNB revealed a macrometastasis in one patient.

3

Development and validation of a nomogram to predict recurrence and melanoma-specific mortality in patients with negative sentinel lymph nodes

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ABSTRACT

BACKGROUND Patients with melanoma and negative sentinel nodes (SNs) have varying outcomes, dependent on several prognostic factors. Considering all these factors in a prediction model might aid in identifying patients who could benefit from a personalized treatment strategy. The objective was to construct and validate a nomogram for recurrence and melanoma-specific mortality (MSM) in patients with melanoma and negative SNs.

METHODS A total of 3220 patients with negative SNs were identified from a cohort of 4124 patients from four EORTC Melanoma Group centres who underwent sentinel lymph node biopsy. Prognostic factors for recurrence and MSM were studied with Cox regression analysis. Significant factors were incorporated in the models. Performance was assessed by discrimination (c-index) and calibration in cross-validation across the four centres. A nomogram was developed for graphical presentation.

RESULTS There were 3180 eligible patients. The final prediction model for recurrence and the calibrated model for MSM included three independent prognostic factors: ulceration, anatomical location and Breslow thickness. The c-index was 0.74 for recurrence and 0.76 for the calibrated MSM model. Cross-validation across the four centres showed reasonable model performance. A nomogram was developed based on these models. One-third of the patients had a 5-year recurrence probability of 8.2% or less, and one-third had a recurrence probability of 23.0% or more.

CONCLUSION A nomogram for predicting recurrence and MSM in patients with melanoma and negative SNs was constructed and validated. It could provide personalized estimates useful for tailoring surveillance strategies (reduce or increase intensity), and selection of patients for adjuvant therapy or clinical trials.

INTRODUCTION

Sentinel lymph node biopsy (SLNB), introduced in 1991 as a staging procedure for cutaneous melanoma, evaluates the presence of lymph node involvement[1]. The impact of SLNB has been studied extensively and is one of the most important prognostic indicators for recurrence and survival in patients with melanoma[2,3]. Consequently, sentinel node (SN) status has significant implications for treatment strategy. Patients with a positive SN usually had completion lymph node dissection (CLND), but the landmark DeCOG-SLT trial[4] and Multicenter Selective Lymphadenectomy Trial (MSLT) II trial[5] concluded there was no significant survival benefit for CLND compared with nodal observation. In future, most patients with positive SNs will be offered routine adjuvant therapy, conceivably without preceding CLND[4–9]. Patients with negative SNs have not been included in recent adjuvant therapy trials and are usually offered regular surveillance examinations instead.

Reported recurrence rates for patients with negative SNs vary between 6 and 29%[10,11]. When accounting for histological subtype and ulceration, the recurrence rate may increase up to 43%, which, strikingly, approximates the recurrence rate in patients with positive SNs[11]. Perhaps these high-risk patients with negative SNs might benefit from adjuvant therapy as well.

The eighth edition of the AJCC staging manual categorizes melanoma with a negative SN into stages IA–IIC based on ulceration and Breslow thickness (T category) [12]. Several other independent factors have been identified that contribute to risk of recurrence and/or melanoma-specific mortality (MSM)[13]. Considering these additional clinicopathological factors in a prediction model might provide more accurate patient-specific estimates that could be used for treatment strategy decision-making. The objective of the present study was to identify independent prognostic factors in a large European melanoma population with negative SNs to develop and validate a prediction model for recurrence and MSM, presented in the form of a nomogram.

METHODS

COHORT CHARACTERISTICS

A retrospective cohort collected and described previously was used for this study[14]. The cohort contained 4124 patients who underwent a SLNB between 1997 and 2013 in one of four European Organization for Research and Treatment of Cancer (EORTC)

Melanoma Group centres. The study was approved and performed in accordance with local ethics committee guidelines and national legislation. For purposes of the present study, a total of 3220 patients with a negative SN were identified from this cohort. Data on sex, age, diagnosis date, date of SLNB, primary tumour characteristics (Breslow thickness, ulceration), and details on recurrence and follow-up were collected.

PROCEDURES AND FOLLOW-UP

Histopathological examination of an excision biopsy of the primary melanoma led to the diagnosis in all patients. The excision biopsy was performed with total thickness excision and a narrow circumferential margin[15]. Eligibility for SLNB in all centres was assessed according to international guideline criteria: Breslow thickness greater than 1.0 mm or presence of risk factors, including ulceration, Clark level IV or V according to the sixth edition of the AJCC staging manual up to 2009[16], and regression or mitosis greater than 1/mm^2 according to the seventh edition of the AJCC staging manual from 2009[17]. In general, a wide local excision was performed simultaneously with the SLNB, as described elsewhere[1,14]. Histopathological analysis of the SN was conducted according to the EORTC Melanoma Group pathology protocol[18]. Follow-up strategies in EORTC centres varied, but usually consisted of clinical examination two to four times per year for 5–10 years[15,19].

OUTCOMES

Outcomes of interest were recurrence and MSM, calculated from date of SLNB to date of first recurrence or death. When there was multisite first recurrence, the site with the worst prognosis was scored as the first site. Subsequently, recurrence was defined as new locoregional recurrence only: in-transit metastasis or satellites, regional nodal recurrence in similar SN basin (with or without concurrent locoregional disease), or distant nodal or systemic recurrence (with or without concurrent regional nodal and/or locoregional disease). As the type of recurrence does not have clinical consequences at first (all patients with recurrence will undergo several diagnostic tests anyway) and to retain as much statistical power and as few methodological issues as possible, all recurrence was the outcome used for the prediction model. Median follow-up from date of SLNB to date of last follow-up was calculated, applying the reversed Kaplan–Meier method; deaths were censored. Disease-free survival (DFS) was calculated from date of SLNB to date of first recurrence; lost to follow-up or death was censored. Melanoma-specific survival (MSS) was calculated from date of SLNB to date of MSM; lost to follow-up or death from other causes was censored.

STATISTICAL ANALYSIS

The checklist proposed by the AJCC was used for guidance in building a high-quality prediction model[20]. Associations between possible prognostic factors and recurrence were studied with Cox regression analysis. The following nine variables were identified as possible prognostic factors based on clinical experience, literature review and availability of sufficient data: sex, age, ulceration, location, histology, Breslow thickness, level of invasion (Clark level), total number of SNs removed and multiple SN fields. To make efficient use of the available data an advanced multiple imputation of missing values strategy (5 imputations) was applied[21]. The possible non-linearity of the continuous variables (age, Breslow thickness and total number of SNs removed) was modelled by logarithmic transformation. Independent prognostic factors were selected with multivariable backward selection. Linear predictor values (the sum of truncated predictor values times their predictor effects) were scaled and rounded to a risk score with integer values between 0 and 100. Because recurrence and MSM are strongly related, the final recurrence prediction model based on data from all four EORTC centres was used as a basis for predicting MSM, where the baseline hazard and the slope of the recurrence prediction model were calibrated to MSM[22]. The advantage of this approach is that it is possible to obtain a unique risk score for each patient that translates into probabilities of both outcomes of interest: recurrence and MSM. This is in contrast to developing two independent prediction models that result in two independent risk scores with corresponding probabilities. To test the validity of this approach, the performance of an independently developed MSM prediction model was compared with that of the calibrated MSM prediction model. The absolute risk prediction of each of the two outcomes was plotted against the risk score. To reduce the overestimation of events occurring in patients with extremely high scores, the score was truncated at an integer of 15, which corresponded to the 95th percentile of score distribution in the cohort. Model performance was assessed by examining discrimination and calibration. Discrimination was measured using the concordance index (c-index); the closer the c-index is to 1, the better the discrimination, and a value of 0.5 indicates that the model is no better than chance[23]. Calibration was assessed visually by plotting the predicted probability against the actual observed frequency in quintiles of predicted recurrence and melanoma-specific mortality. A 45° line indicates perfect calibration (when the predictive value of the model perfectly matches the patient's actual risk). Any deviation above or below the 45° line indicates underprediction or overprediction respectively. To evaluate the generalizability of the model across different centres, an internal–external cross-validation was performed in which the model was fitted using data from three centres and validated in the centre

that was left out[24]. A nomogram was developed for graphical presentation of the models. All statistical tests were two-sided with a statistical significance level set at P <0.05. Statistic analyses were performed with IBM SPSS® 22.0 (IBM, Armonk, New York, USA) and R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

From the 3220 patients identified with melanoma and negative SNs, 3180 were eligible for inclusion in the present study. Patients were excluded due to duplicates (9), urogenital melanoma (8), in situ melanoma (7), SLNB for recurrent disease (2), missing data (4), or discrepancy between date of recurrence and date of diagnosis and/or SLNB (10). Baseline patient and tumour characteristics for all patients and per EORTC centre are shown in **Table 1**.

Median duration of follow-up for all survivors was 61 (i.q.r. 29–99) months. Recurrence occurred in 496 patients (15.6%). The DFS rate at 5 and 10 years was 86.7% (s.e. 0.7) and 72.8% (s.e. 1.3) respectively. Some 277 patients (8.7%) died from melanoma. The MSS rate at 5 and 10 years was 91.5% (s.e. 0.6) and 84.8% (s.e. 1.0) respectively. Details of outcome and follow-up for all patients and per EORTC centre are depicted in **Table 2**.

Table 3 gives the results of the multivariable Cox model for recurrence including all nine candidate variables. After backwards selection and manual exclusion of Clark level (due to limited additional effect and current clinical practice), the final model for recurrence included three independent prognostic factors: ulceration, anatomical site and Breslow thickness (**Table 4**). The non-linearity of Breslow thickness was highly significant (P <0.001) and well represented by logarithmic transformation. The c-index for the final recurrence model was 0.74 (95% c.i. 0.71 to 0.76). In cross-validation, the c-index for a model based on three centres and applied to the centre that was left out ranged from 0.70 to 0.77. The additional prognostic value of mitotic rate (at least 1 mitosis/mm^2 present in 112 of 151 observations), tested in a model with the linear predictor as an offset, was not significant (P =0.678). The recurrence model was reasonably calibrated across the four centres in cross-validation (**Supplementary Figure 1**).

The association between the linear predictors of recurrence and MSM was even stronger (calibration slope 1.10, 95% c.i. 0.96 to 1.24). The c-index for the calibrated MSM model was 0.76 (0.73 to 0.79). In cross-validation, the c-index for a calibrated model based on three centres applied to the centre that was left out ranged from 0.73 to 0.80. The calibrated model was reasonably calibrated across the four centres

Table 1 Baseline patient and tumour characteristics by centre

	All (n = 3180)	EORTC centres			
		Centre 1 (n = 398)	Centre 2 (n = 1082)	Centre 3 (n = 953)	Centre 4 (n = 747)
Age (years)*	55 (44–67)	51 (40–62)	63 (49–71)	51 (42–62)	55 (44–65)†
Sex					
F	1668 (52.5)	211 (53.0)	478 (44.2)	589 (61.8)	390 (52.2)
M	1510 (47.5)	187 (47.0)	604 (55.8)	364 (38.2)	355 (47.5)
Missing	2 (0.1)	0 (0)	0 (0)	0 (0)	2 (0.3)
Anatomical site					
Arm	556 (17.5)	74 (18.6)	187 (17.3)	180 (18.9)	115 (15.4)
Leg	996 (31.3)	146 (36.7)	255 (23.6)	369 (38.7)	226 (30.3)
Trunk	1360 (42.8)	162 (407)	517 (47.8)	390 (40.9)	291 (39.0)
Head and neck	259 (8.1)	16 (4.0)	123 (11.4)	13 (1.4)	107 (14.3)
Missing	9 (0.3)	0 (0)	0 (0)	1 (0.1)	8 (1.1)
Histological type					
SSM	1739 (54.7)	204 (51.3)	762 (70.4)	307 (32.2)	466 (62.4)
NM	885 (27.8)	134 (33.7)	204 (18.9)	353 (37.0)	194 (26.0)
ALM	93 (2.9)	10 (2.5)	39 (3.6)	23 (2.4)	21 (2.8)
LMM	139 (4.4)	5 (1.3)	42 (3.9)	75 (7.9)	17 (2.3)
Other	46 (1.4)	9 (2.3)	1 (0.1)	4 (0.4)	32 (4.3)
Missing	278 (8.7)	36 (9.0)	34 (3.1)	191 (20.0)	17 (2.3)
Breslow thickness (mm)*	(*n* = 3125) 1.70 (1.10–3.00)	(*n* = 392) 1.90 (1.40–2.80)	(*n* = 1069) 1.30 (0.88–2.40)	(*n* = 926) 2.00 (1.00–4.00)	(*n* = 738) 1.70 (1.20–2.70)
Clark level					
I–II	271 (8.5)	13 (3.3)	60 (5.5)	180 (18.9)	18 (2.4)
III	1230 (38.7)	147 (36.9)	400 (37.0)	479 (50.3)	204 (27.3)
IV	1354 (42.6)	188 (47.2)	569 (52.6)	219 (23.0)	378 (50.6)
V	140 (4.4)	18 (4.5)	31 (2.9)	41 (4.3)	50 (6.7)
Missing	185 (5.8)	32 (8.0)	22 (2.0)	34 (3.6)	97 (13.0)
Ulceration					
No	2264 (71.2)	242 (60.8)	874 (80.8)	604 (63.4)	544 (72.8)
Yes	788 (24.8)	92 (23.1)	182 (16.8)	339 (35.6)	175 (23.4)
Missing	128 (4.0)	64 (16.1)	26 (2.4)	10 (1.0)	28 (3.7)
Mitosis					
No	39 (1.2)	11 (2.8)	0 (0)	0 (0)	28 (3.7)
Yes	112 (3.5)	59 (14.8)	0 (0)	0 (0)	53 (7.1)
Missing	3029 (95.3)	328 (82.4)	1082 (100)	953 (100)	666 (89.2)
Total no. of SNs*	(*n* = 3039) 1 (1–2)	(*n* = 397) 2 (1–2)	(*n* = 1072) 1 (1–2)	(*n* = 823) 1 (1–1)	(*n* = 747) 2 (2–3)
Multiple SN fields					
No	2768 (87.0)	337 (84.7)	918 (84.8)	953 (100)	560 (75.0)
Yes	412 (13.0)	61 (15.3)	164 (15.2)	0 (0)	187 (25.0)

Values in parentheses are percentages unless indicated otherwise; *values are median (i.q.r.). †Based on 741 patients. EORTC, European Organization for Research and Treatment of Cancer; SSM, superficial spreading melanoma; NM, nodular melanoma; ALM, acral lentiginous melanoma; LMM, lentigo maligna melanoma; SN, sentinel node

Table 2 Outcomes and follow-up by centre

	All (n = 3180)	EORTC centres			
		Centre 1 (n = 398)	Centre 2 (n = 1082)	Centre 3 (n = 953)	Centre 4 (n = 747)
Recurrence					
Yes	496 (15.6)	91 (22.9)	94 (8.7)	191 (20.0)	120 (16.1)
No	2684 (84.4)	307 (77.1)	988 (91.3)	762 (80.0)	627 (83.9)
Recurrence type†					
Locoregional	142 (28.6)	34 (37.0)	25 (27)	38 (19.9)	45 (37.5)
Regional nodal	122 (24.6)	14 (15.0)	32 (34)	48 (25.1)	28 (23.3)
Distant	194 (39.1)	43 (47.0)	37 (39)	67 (35.1)	47 (39.2)
Unknown	38 (7.7)	0 (0)	0 (0)	38 (19.9)	0 (0)
Additional surgery‡					
Yes	13 (0.4)	13 (3.3)	0 (0)	0 (0)	0 (0)
n.r.	3167 (99.6)	385 (96.7)	1082 (100)	953 (100)	747 (100)
Radiotherapy					
Yes	24 (0.8)	24 (6.0)	0 (0)	0 (0)	0 (0)
n.r.	3156 (99.2)	374 (94.0)	1082 (100)	953 (100)	747 (100)
Chemotherapy					
Yes	12 (0.4)	12 (3.0)	0 (0)	0 (0)	0 (0)
n.r.	3168 (99.6)	386 (97.0)	1082 (100)	953 (100)	747 (100)
Novel therapy§					
Yes	22 (0.7)	16 (4.0)	0 (0)	0 (0)	6 (0.8)
n.r.	3158 (99.3)	382 (96.0)	1082 (100)	953 (100)	741 (99.2)
Duration of follow-up for survivors (months)*	61 (29–99)	94 (59–131)	33 (12–68)	87 (50–127)	57 (35–84)
Status					
No evidence of disease	2736 (86.0)	318 (79.9)	984 (90.9)	786 (82.5)	648 (86.7)
Alive with disease	90 (2.8)	12 (3.0)	23 (2.1)	20 (2.1)	35 (4.7)
Died from disease	277 (8.7)	56 (14.1)	41 (3.8)	139 (14..6)	41 (5.5)
Died from other cause	75 (2.4)	12 (3.0)	34 (3.1)	8 (0.8)	21 (2.8)
n.r.	2 (0.1)	0 (0)	0 (0)	0 (0)	2 (0.3)

Values in parentheses are percentages unless indicated otherwise; *values are median (i.q.r.). †Defined as follows: locoregional recurrence only (for example in-transit metastasis or satellites), regional nodal recurrence similar to sentinel node basin (with or without concurrent locoregional disease) and distant recurrence (with or without concurrent locoregional and/or regional nodal disease). ‡Includes resection of metastases or lymph node dissection. §Includes vaccines, targeted therapy and immunotherapy. EORTC, European Organization for Research and Treatment of Cancer; n.r., not reported.

in cross-validation (**Supplementary Figure 2**). The performance of this calibrated MSM prediction model, based on the baseline hazard and the slope of the recurrence model, was similar to that of the independently developed MSM prediction model (c-index 0.77, 0.74 to 0.80) (**Table 5**).

Table 3 Multivariable Cox analysis of recurrence

	Hazard ratio	P
Age (i.q.r. 67 versus 44 years)	1.06 (0.82, 1.36)	0.920
Sex (male versus female)	1.20 (0.99, 1.45)	0.065
Breslow thickness (i.q.r. 3.0 versus 1.1 mm)	2.47 (1.94, 3.13)	< 0.001
Ulceration (yes versus no)	1.84 (1.50, 2.26)	< 0.001
Clark level		0.005
I–II	1.00 (reference)	
III	1.59 (0.97, 2.61)	
IV	1.68 (1.02, 2.75)	
V	2.70 (1.51, 4.80)	
Anatomical location		0.001
Arm	1.00 (reference)	
Leg	1.38 (1.03, 1.87)	
Trunk	1.54 (1.15, 2.07)	
Head and neck	2.12 (1.45, 3.11)	
Histology		0.336
SSM	1.00 (reference)	
NM	1.18 (0.93, 1.49)	
ALM	1.53 (0.94, 2.51)	
LMM	1.25 (0.77, 2.03)	
Other	0.89 (0.45, 1.79)	
No. of SNs (i.q.r. 2 versus 1)	1.06 (0.87, 1.29)	0.800
Multiple SN fields (yes versus no)	1.15 (0.82, 1.62)	0.411

Values in parentheses are 95% confidence intervals. SSM, superficial spreading melanoma; NM, nodular melanoma; ALM, acral lentiginous melanoma; LMM, lentigo maligna melanoma; SN, sentinel node.

A three-item risk score was developed, assigning points to each prognostic factor based on the magnitude of association with recurrence. A nomogram to calculate the score and the risk of recurrence and MSM is presented in **Figure 1**. The scores were divided into three classes based on the score distribution (each consisting of approximately one-third of the cohort): low risk, score 0–6; intermediate risk, score 7–9; high risk, score 10 or more (**Figure 1**). For recurrence, these risk classes correspond to the following probabilities: low risk, 1.6–8.2%; intermediate risk, 11.0–18.0%; high risk, 23.0% or above. For MSM, these risk classes correspond to the following probabilities: low risk, 0.5–3.2%; intermediate risk, 4.4–8.0%; high risk, 11.0% or more.

Table 4 Final model for recurrence

	Hazard ratio	P
Breslow thickness (i.q.r. 3.0 versus 1.1 mm)	2.22 (1.97, 2.51)	< 0.001
Ulceration (yes versus no)	1.85 (1.52, 2.25)	< 0.001
Anatomical site		
Arm	1.00 (reference)	
Leg	1.35 (1.01, 1.81)	0.044
Trunk	1.55 (1.17, 2.05)	0.002
Head and neck	2.39 (1.66, 3.44)	< 0.001

Values in parentheses are 95% confidence intervals.

Table 5 Final model for melanoma-specific mortality

	Hazard ratio	P
Breslow thickness (i.q.r. 3.0 versus 1.1 mm)	2.37 (2.03, 2.78)	< 0.001
Ulceration (yes versus no)	2.11 (1.62, 2.75)	< 0.001
Anatomical site		
Arm	1.00 (reference)	
Leg	0.97 (0.66, 1.44)	0.881
Trunk	1.70 (1.18, 2.44)	0.004
Head and neck	1.80 (1.07, 3.03)	0.028

Values in parentheses are 95% confidence intervals.

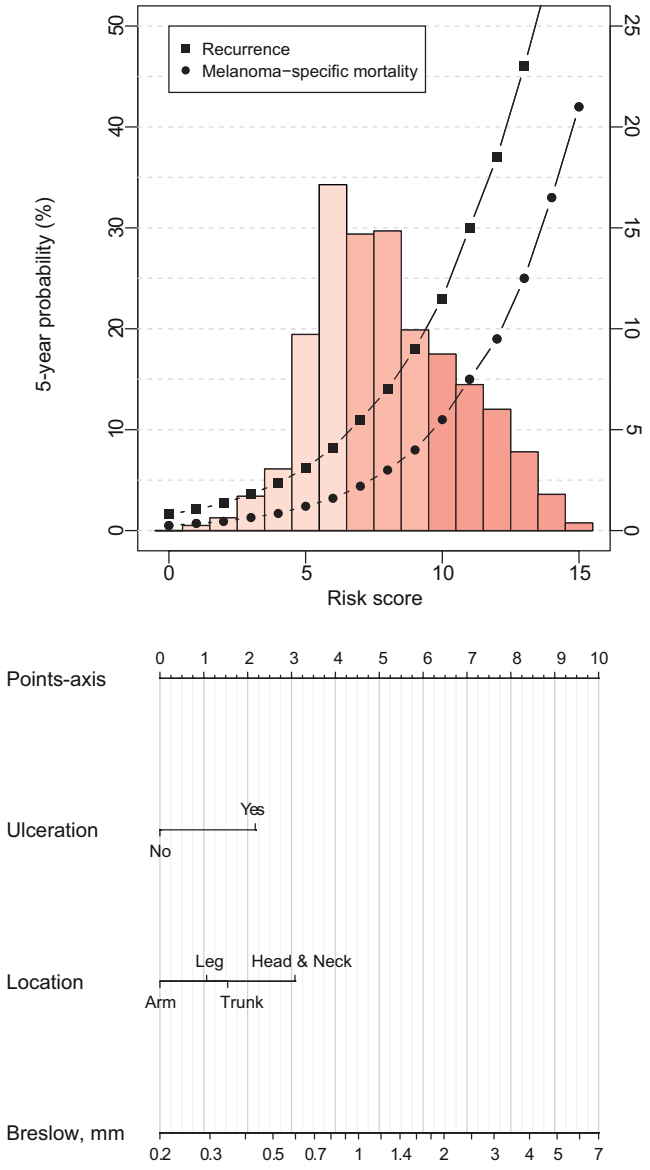

Figure 1 Nomogram and corresponding probabilities

The curves refer to predicted recurrence or melanoma-specific mortality (MSM) at 5 years. The histogram refers to the risk score distribution in the cohort; each bar represents the proportion of patients in the cohort that was assigned that specific score. The histogram was divided in tertiles: light pink bars, first tertile (low risk); medium pink bars, second tertile (intermediate risk); dark pink bars, third tertile (high risk). The nomogram incorporates three factors: ulceration, anatomical location and Breslow thickness. To calculate an individual's probability of 5-year recurrence and MSM, values for the prognostic factors must be determined first (for example: ulceration; leg; Breslow thickness 2.5 mm). Second, for each value the corresponding points can be obtained by drawing a line from each value towards the points axis (in example: 2, 1 and 7 points respectively). Third, the points must be added up to obtain the total risk score (in example: risk score of 10). Finally, the 5-year recurrence and MSM probability can be read by moving vertically from the x-axis (total risk score) to the predicted risk curves and corresponding probabilities on the left y-axis (for example: 23.0% for recurrence and 11.0% for MSM). The percentage of patients in the entire population (3180) that also had a total risk score of 10 can be determined from the histogram, as well as the corresponding percentage of patients on the right y-axis (for example: 17.5%).

DISCUSSION

Patients with melanoma are staged according to the AJCC staging system, based on TNM criteria[12]. Within these stage groupings there is still marked prognostic heterogeneity, and several clinical prognostic tools have been developed to improve predictive accuracy[25]. None of these tools focuses specifically on outcomes in patients with negative SNs, and most predict only survival[25,26].

The present study developed and validated a nomogram to predict recurrence and MSM in patients with melanoma and negative SNs. Focusing on specifically these patients is important for several reasons. They comprise a large group with highly varying prognosis, who are generally offered regular surveillance examinations with the intent to detect early (locoregional) recurrence. Follow-up strategies vary, but usually focus on regular clinical examination for 5–10 years[15,19]. Some guidelines support a one-off follow-up visit with instructions for subsequent self-examination after treatment for stage IA melanoma[27]. The recurrence rate for stage IA disease is reported to be 5%[28]. Besides personalized outcome prediction, the nomogram could be used to group patients. In the present study population, approximately one-third of patients with a negative SN had an 8.2% or less predicted probability of recurrence (risk score 6 or less). Surveillance strategies could be reduced in these patients, particularly as most recurrences are self-detected, and less frequent follow-up seems to have no effect on recurrence and self-detection rates, and no adverse effects[29,30]. However, a 5-year risk of relapse of 48% has been reported for stage IIIA melanoma[31]. In the present study population, approximately one-fifth had a 30% or greater predicted recurrence probability (risk score 11 or more). Surveillance strategies could be intensified in these patients, or they could be considered for adjuvant therapy (trials). The present nomogram could aid in designing clinical trials by defining inclusion criteria, or help gain better equivalence between study arms. In the current era of effective novel therapies in both the adjuvant and therapeutic setting it is highly relevant to focus on negative SN melanoma, as it is likely that most patients with negative SNs will not be offered adjuvant therapy before first recurrence. Mortality predictions in these patients might be partly affected in the present study, as those who developed recurrent disease after 2011 were eligible to receive effective therapy.

This study has important strengths, including its large size, widely available and easily ascertainable characteristics, multicentre composition, and outcomes that are of interest to both clinicians and patients. In multivariable analysis, ulceration, anatomical site and Breslow thickness proved to be significant independent prognostic factors,

in concordance with previous reports[10,11,13,16,32]. Clark level is no longer part of the seventh AJCC staging edition for melanoma because it was shown not to be an independent prognostic factor when corrected for mitotic rate[17]. As its effect was marginal in the multivariable model for recurrence, Clark level was excluded manually. All patients were treated at multidisciplinary high-volume European melanoma centres that applied similar international guideline criteria. This minimizes variability in the interpretation of results and, as generally a policy of centralized referral of patients with melanoma eligible for SLNB is recommended, the cohort is likely to be representative of the European melanoma population with a negative SN. Another strength of the nomogram is the model performance. Discrimination and calibration were good for both the recurrence model and the calibrated model for MSM. The performance of the calibrated model for MSM was comparable to the independently developed model for MSM, indicating the validity of the applied approach. Furthermore, the models were successful in cross-validation and showed good agreement between prediction and actual observation. Validation of the nomogram is essential to avoid overfitting and to determine generalizability[33]. In the present study, the prediction models were validated using the recommended internal–external validation procedure. One centre at a time was left out to cross-validate a model developed in the other centres; as this split was not random, it qualifies as external validation[24]. Previous prediction models did not focus on SN-negative melanoma[25,26]. The AJCC online prognostic calculator focused on localized melanoma but included both clinical and pathological stage I–II disease (thus also patients who did not undergo SLNB) and predicted only melanoma-specific survival[34]. In addition, all tools but one predict survival (disease-specific or overall)[25,35]. The online Sunbelt predictor (MelanomaCalculator.com) included patients staged by SLNB (both positive and negative) and calculates overall survival, as well as DFS and locoregional recurrence-free survival; however, only the methodology for predicting overall survival was published[36].

This study also has several limitations. The first is the retrospective design, which has inherent biases. In addition, other prognostic factors such as regression or lymphatic invasion could not be incorporated in the present models due to insufficient data[37,38]. They could be incorporated in next-generation nomograms. Another variable shown to have an independent prognostic effect is mitotic rate[17,32]. The prognostic effect of mitotic rate was tested by introducing it as an offset term, but it was not significant. This study did not perform competing-risk analysis, which has been done before[26]. Consequently the predictions are an overestimate of the actual risk, but, owing to relatively few competing events, this overestimation is expected to be limited. Presently, there is no online version of the nomogram, but it is hoped to have this available soon.

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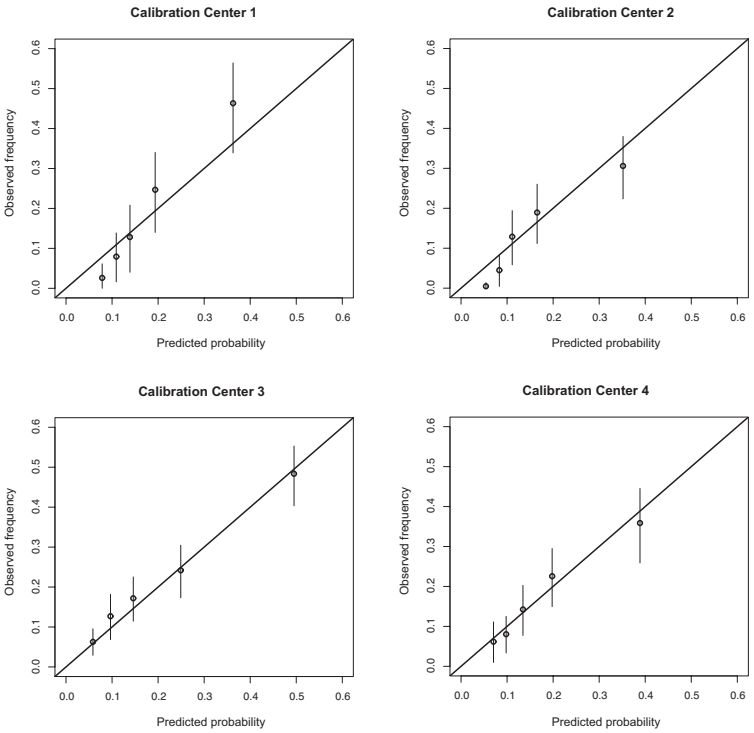
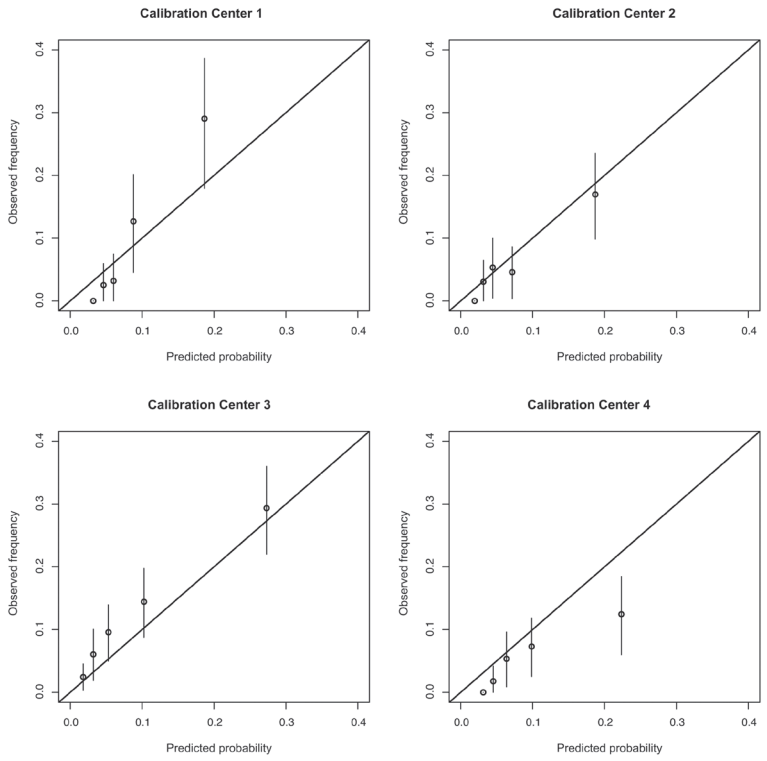
SUPPLEMENTARY MATERIALS

Supplementary Figure 1 Calibration plots for the recurrence model



The predicted probability is plotted on the x-axis, the actual probability on the y-axis. A plot along the 45° line would indicate perfect calibration in which the predicted probabilities were identical to the actual outcomes.

Supplementary Figure 2 Calibration plots for the calibrated melanoma-specific mortality model



The predicted probability is plotted on the x-axis, the actual probability on the y-axis. A plot along the 45° line would indicate perfect calibration in which the predicted probabilities were identical to the actual outcomes.

ANNEX 1

Comment on: External validation of a prognostic model to predict survival of patients with sentinel node-negative melanoma

Daniëlle Verver, Alex Rekkas, Dirk J. Grünhagen, Cornelis (Kees) Verhoef

Br J Surg. 2020 Apr;107(5):615-616.

Editor,

We have read with great interest the article by Ipenburg et al.[1] published recently in the British Journal of Surgery. This study externally validated the developed prognostic European Organisation for Research and Treatment of Cancer (EORTC) model predicting risk of recurrence and melanoma-specific mortality in patients with sentinel-node negative melanoma[2]. As the models appeared well calibrated and showed good performance in 4235 patients from the Melanoma Institute Australia, the authors confirmed the prognostic accuracy.

We would like to point out some relevant findings. First, the authors demonstrated that the EORTC models were reproducible in an independent non-European population, thereby confirming the generalizability. Performance in terms of the concordance index was lower in external validation (0.69 for both the recurrence and melanoma-specific mortality model compared with 0.74 and 0.76 in the European population) but may be explained by differences in clinicopathological variables (e.g. more head and neck melanomas) and possibly patterns of clinical care. Second, distribution of patients across the risk groups was reasonably balanced (20% low risk, 46% intermediate risk and 34% high risk) and the Kaplan–Meier plots showed distinct survival curves, indicating the clinical usefulness. Third, the possible additional value of several factors that could not be sufficiently tested in the EORTC models was examined, including mitotic rate and regression. The latter factor did not show independent prognostic value, and the extended model including mitotic rate among others showed only marginally improved performance.

In conclusion, the EORTC nomogram is a validated easy-applicable tool predicting recurrence and melanoma-specific mortality in patients with sentinel-node negative melanoma. Accurately identifying high-risk patients could aid in selecting candidates for adjuvant therapy. To facilitate its use, an online calculator has been developed and can be accessed at www.evidencio.com/models/show/1890

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4

Optimal extent of completion lymphadenectomy for patients with melanoma and a positive sentinel node in the groin

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ABSTRACT

BACKGROUND The optimal extent of groin completion lymph node dissection (CLND) (inguinal or ilioinguinal dissection) in patients with melanoma is controversial. The aim of this study was to evaluate whether the extent of groin CLND after a positive sentinel node biopsy (SNB) is associated with improved outcome.

METHODS Data from all sentinel node-positive patients who underwent groin CLND at four tertiary melanoma referral centres were retrieved retrospectively. Baseline patient and tumour characteristics were collected for descriptive statistics, survival analyses and Cox proportional hazards regression analyses.

RESULTS In total, 255 patients were included, of whom 137 (53.7%) underwent inguinal dissection and 118 (46.3%) ilioinguinal dissection. The overall CLND positivity rate was 18.8%; the inguinal positivity rate was 15.5% and the pelvic positivity rate was 9.3%. The pattern of recurrence, and 5-year melanoma-specific survival, disease-free survival and distant-metastasis free survival rates were similar for both dissection types, even for patients with a positive CLND result. Cox regression analysis showed that type of CLND was not associated with disease-free or melanoma-specific survival.

CONCLUSION There was no significant difference in recurrence pattern and survival rates between patients undergoing inguinal or ilioinguinal dissection after a positive SNB, even after stratification for a positive CLND result. An inguinal dissection is a safe first approach as CLND in patients with a positive SNB.

INTRODUCTION

Although evidence for a therapeutic benefit is still lacking pending the final results of Multicentre Selective Lymphadenectomy Trial (MSLT) II, many current melanoma guidelines advise consideration of completion lymphadenectomy (CLND) in case of a positive sentinel node biopsy (SNB)[1–4]. This is in line with the opinion of 91.8% of 193 melanoma surgeons worldwide, but in practice only half of patients with a positive sentinel node (SN) actually undergo CLND[5,6]. In the groin area, CLND can be classified as an inguinal dissection with removal of all femoral and inguinal lymph nodes, or an ilioinguinal dissection with additional removal of all iliac (up to the bifurcation of the common iliac artery) and obturator lymph nodes.

The optimal surgical extent of CLND in the groin is controversial. Some authors advocate ilioinguinal dissection to optimize regional control and possibly increase survival[7–9]. Others disagree and advocate an inguinal dissection, especially in patients with low suspicion of pelvic nodal metastasis, because ilioinguinal dissection is believed to be associated with increased morbidity and does not seem to affect outcome[10–16].

Few studies have compared the therapeutic benefit of inguinal and ilioinguinal dissection solely in patients with melanoma and a positive SNB. The majority of studies comparing these two types of dissection have been limited to those with palpable disease[7,11,15], or did not differentiate between patients with a positive SNB or palpable disease[8,17]. It has been demonstrated, however, that patients with a positive SNB differ from those with palpable disease in tumour biology, rate of pelvic nodal involvement, recurrence pattern and survival rate[8,13,14,17–19].

The aim of the present study was to evaluate whether the extent of groin CLND in patients with a positive SNB was associated with better outcome. For this purpose, data from four tertiary large melanoma centres in the Netherlands were retrieved. Recurrence patterns, disease-free survival (DFS), distant metastasis-free survival (DMFS) and melanoma-specific survival (MSS) were compared after inguinal and ilioinguinal dissection.

METHODS

Patients with a positive SNB and subsequent CLND in the groin were identified from retrospective SNB melanoma databases in four tertiary melanoma centres in the Netherlands, two of which routinely performed inguinal dissection and two ilioinguinal

dissection. Patient characteristics (age, sex), tumour characteristics (histology, Breslow thickness), SN characteristics (tumour burden), CLND outcomes and follow-up data were extracted from the databases of the participating centres.

SENTINEL NODE BIOPSY

SNB was performed for primary melanomas at least 1.00mm thick, or shallower than 1.00mm but with ulceration or other adverse tumour characteristics (Clark level IV–V or at least 1 mitosis/mm^2 depending on the AJCC staging edition at the time of diagnosis). The triple technique was used, as described previously[20–22].

COMPLETION LYMPHADENECTOMY

In general, the Dutch Melanoma Guidelines were adhered to by all participating centres for preoperative and postoperative management; preoperative or postoperative imaging was not indicated[2].

The local policy of centres 1 and 4 was inguinal dissection with removal of inguinal nodes only as standard treatment, whereas in centres 2 and 3 the routine practice was ilioinguinal dissection with additional removal of all iliac and obturator nodes (**Figure 1**). Sometimes surgeons deviated from this routine practice, based on factors such as age, co-morbidities, drainage patterns during lymphoscintigraphy and number of positive SNs. Unfortunately, these reasons are heterogeneous and not amenable to retrospective analysis. Ilioinguinal dissection was performed either via a single inguinal elliptical incision extending cranially, or via two separate transverse incisions, as described previously[15,23].

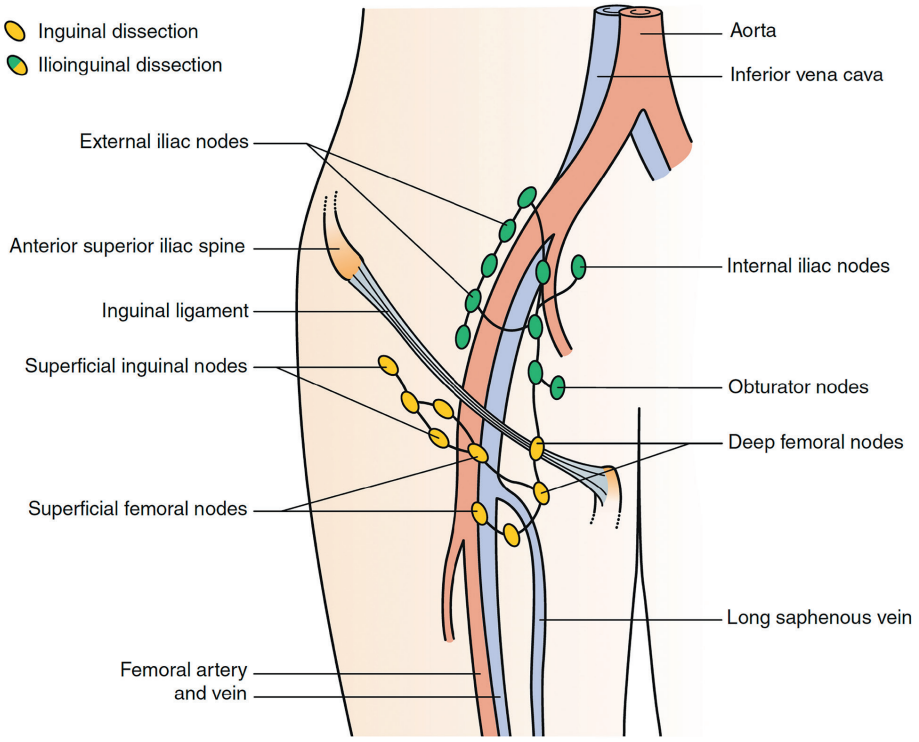
PATHOLOGY

SNs were processed according to the European Organisation for Research and Treatment of Cancer (EORTC) SN pathology protocol[24]. CLND specimens were processed in a standard fashion; all lymph nodes were bisected or trisected, and stained with haematoxylin and eosin. Pathology reports were considered adequate when the total number of removed and involved lymph nodes was mentioned. For ilioinguinal specimens, the number of both inguinal and pelvic nodes removed, and number of positive nodes were also recorded, if available.

STATISTICAL ANALYSIS

Differences between the two treatment groups were calculated using χ^2 tests, Fisher's exact tests or non-parametric Mann–Whitney U tests, as appropriate. Where data were missing or unknown, an 'unknown' subcategory was created and included in the analysis.

Figure 1 Nodes removed in inguinal versus ilioinguinal completion lymph node dissection in the groin



MSS was calculated from the date of CLND until last follow-up or death from melanoma; deaths from other causes were censored. DFS was calculated from the date of CLND to the date of first recurrence or the date of last-follow-up or death. DMFS was calculated from the date of CLND to the date of first distant metastasis or date of last follow-up or death. The Kaplan–Meier method was used to estimate survival, and differences between groups were assessed by means of the log rank test. Multivariable Cox proportional hazards regression analyses were performed to identify prognostic co-variables. Two-sided P <0.05 was considered statistically significant. SPSS® version 22.0 was used for all statistical analyses (IBM, Armonk, New York, USA).

RESULTS

A total of 283 patients treated between 1994 and 2014 were identified from the SNB databases. Twenty-eight patients were excluded for the following reasons: palpable disease or distant metastases before surgery (9); missing data on CLND date and resected specimen (7); additional positive SNB outside the groin (9); no available follow-up (2); and altered choice of surgery owing to pregnancy (1). The remaining 255 patients were analysed. Median follow-up for all patients was 51 (i.q.r. 26–99) months. Baseline patient and tumour characteristics are shown in Table 1. An inguinal dissection was performed in 137 patients (53.7%) and an ilioinguinal dissection in 118 (46.3%). The ilioinguinal group included more men (P =0.040) and had a significantly higher SN tumour burden (P =0.003).

Forty-eight patients (18.8%) had additional lymph node metastases in the CLND specimen (positive CLND), 15 in the inguinal dissection group and 33 in the ilioinguinal dissection group. The overall inguinal positivity rate (with or without additional pelvic positivity) was 15.7% (40 of 255), and the overall pelvic positivity rate (with or without additional inguinal positivity) was 9.3% (11 of 118). The median number of inguinal lymph nodes removed was similar for both dissection types (P =0.417), but the median number of positive inguinal lymph nodes was significantly greater for patients undergoing ilioinguinal dissection (P =0.014) (Table 2). In patients with a positive CLND, the median numbers of both removed and positive inguinal lymph nodes were similar for both dissection types (P =0.062 and P =0.842 respectively). Twenty patients participated in an adjuvant immunotherapy trial, ten in an EORTC interferon-α trial[25] and ten in a dendritic cell therapy trial[26]. Another ten patients received adjuvant radiotherapy.

RECURRENCE

The overall recurrence rate was 47.4% (65 of 137) after inguinal dissection and 49.2% (58 of 118) after ilioinguinal dissection (P =0.786). For both dissection types, most patients presented with locoregional recurrence only (such as in-transit metastasis) or distant recurrence (distant subcutaneous, distant lymph nodes or distant visceral) at first presentation of relapse. First relapse in the regional lymph node basin (similar to the CLND basin) occurred less often, in 12% (8 of 65) after inguinal dissection and 7% (4 of 58) after ilioinguinal dissection (P =0.394). During follow-up, another nine patients in the inguinal dissection group and five in the ilioinguinal dissection group presented with a second relapse located in the regional lymph node basin. Thus, the overall regional lymph node recurrence rate was 12.4% (17 of 137) after inguinal dissection

Table 1 Patient and tumour characteristics all patients and stratified per positive CLND result

	All patients			Positive CLND result		
	Inguinal dissection (n=137)	Ilioinguinal dissection (n=118)	P†	Inguinal dissection (n=15)	Ilioinguinal dissection (n=33)	P†
Baseline data						
Treatment centre						
1	67 (48.9)	5 (4.2)	<0.001	8 (53)	2 (6)	<0.001§
2	34 (24.8)	44 (37.3)		4 (27)	8 (24)	
3	17 (12.4)	63 (53.4)		1 (7)	22 (67)	
4	19 (13.9)	6 (5.1)		2 (13)	1 (3)	
Age (years)*	52 (39 – 62)	50 (38 – 63)	0.915‡	52 (40 – 56)	57 (44 – 65)	0.201‡
Sex (F : M)	78 : 59	52 : 66	0.040	11 : 4	8 : 25	0.001
Primary site						
Leg	105 (76.6)	96 (81.4)	0.358	12 (80)	31 (94)	0.307
Trunk	32 (23.4)	22 (18.6)		3 (20)	2 (6)	
Histological type						
SSM	69 (50.4)	62 (52.5)	0.098	10 (67)	16 (48)	0.828
NM	36 (26.3)	35 (29.7)		4 (27)	10 (30)	
ALM	10 (7.3)	10 (8.5)		1 (7)	4 (12)	
other	2 (1.5)	5 (4.2)		0 (0)	2 (6)	
unknown	20 (14.6)	6 (5.1)		0 (0)	1 (3)	
Breslow thickness (mm)*	2.90 (1.74 – 4.50)	2.80 (1.80 – 4.70)	0.720‡	2.70 (2.00 – 5.50)	3.50 (2.40 – 5.20)	0.367‡
pT category (mm)						
pT1 (<1.00)	1 (0.7)	4 (3.4)	0.656§	0 (0)	1 (3)	0.465§
pT2 (1.01-2.00)	38 (27.7)	31 (26.3)		4 (27)	3 (9)	
pT3 (2.01-4.00)	60 (43.8)	48 (40.7)		6 (40)	16 (48)	
pT4 (>4.00)	37 (27.0)	34 (28.8)		5 (33)	13 (39)	
Unknown	1 (0.7)	1 (0.8)		0 (0)	0 (0)	
Ulceration						
No	60 (43.8)	64 (54.2)	0.003	6 (40)	15 (45)	0.158§
Yes	57 (41.6)	51 (43.2)		6 (40)	17 (52)	
Unknown	20 (14.6)	3 (2.5)		3 (20)	1 (3)	
SN analysis						
No. of SNs*	2 (1 – 2)	2 (1 – 3)	0.226‡	1 (1 – 2)	2 (1 – 3)	0.057‡
No. of nonSNs*	0 (0 – 0)	0 (0 – 0)	0.144‡	0 (0 – 0)	0 (0 – 0)	0.176‡
No. of positive SNs*	1 (1 – 1)	1 (1 – 2)	0.225‡	1 (1 – 1)	1 (1 – 2)	0.025‡
No. of positive non-SNs*	0 (0)	0 (0)	1.000	0 (0)	0 (0)	1.000
SN tumour burden (mm)						
<0.1	16 (11.7)	4 (3.4)	0.003	0 (0)	0 (0)	0.164
0.1-1.0	52 (38.0)	45 (38.1)		4 (27)	12 (36)	
>1.0	30 (21.9)	46 (39.0)		5 (33)	16 (48)	
Unknown	39 (28.5)	23 (19.5)		6 (40)	5 (15)	

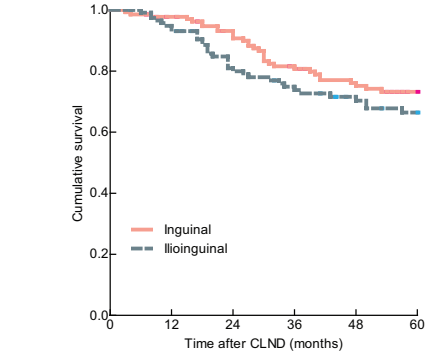
Values in parentheses are percentages unless indicated otherwise; *values are median (i.q.r.). CLND, completion lymph node dissection; SSM, superficial spreading melanoma; NM, nodular melanoma; ALM, acral lentiginous melanoma; SN, sentinel node.
†χ^2 test, except ‡Mann–Whitney U test and §Fisher's exact test.

Table 2 Outcomes all patients and stratified per positive CLND result

	All patients			Positive CLND result		
	Inguinal dissection (n=137)	Ilioinguinal dissection (n=118)	P‡	Inguinal dissection (n=15)	Ilioinguinal dissection (n=33)	P‡
CLND result						
No. of LNs*	8 (5 – 11)	14 (10 – 20)	<0.001§	7 (4 – 11)	15 (10 – 23)	<0.001§
No. of positive LNs*	0 (0 – 0)	0 (0 – 1)	<0.001§	1 (1 – 2)	2 (1 –4)	0.125§
No. of LNs including SNBtot*	10 (7 – 13)	16 (12 – 22)	<0.001§	9 (5 – 12)	18 (13 – 25)	<0.001§
No. of positive LNs including SNBtot*	1 (1 – 2)	1 (1 – 3)	0.007§	2 (2 – 3)	3 (3 – 6)	0.009§
No. of inguinal LNs*	8 (5 – 10) (*n*=135)	8 (5 – 11) (*n*=96)	0.417§	7 (4 – 11)	9 (7 – 16) (*n*=29)	0.062§
No. of positive inguinal LNs*	0 (0 – 0) (*n*=135)	0 (0 – 0) (*n*=114)	0.014§	1 (1 – 2)	1 (1 – 3) (*n*=29)	0.842§
No. of pelvic LNs*	-	5 (3 – 9) (*n*=96)	-	-	5 (2 – 9) (*n*=29)	-
No. of positive pelvic LNs*	-	0 (0 – 0) (*n*=114)	-	-	0 (0 – 1) (*n*=29)	-
Positive LNs						
Inguinal only	15 (100)	18 (55)	0.018	15 (100)	18 (55)	0.018
Pelvic only	0 (0)	4 (12)		0 (0)	4 (12)	
Inguinal and pelvic	0 (0)	7 (21)		0 (0)	7 (21)	
Unknown	0 (0)	4 (12)		0 (0)	4 (12)	
Follow-up						
Adjuvant immunotherapy‡						
No	7 (5.1)	3 (2.5)	0.024	5 (33)	1 (3)	0.004
Yes	16 (11.7)	4 (3.4)		1 (7)	1 (3)	
Unknown	114 (83.2)	111 (94.1)		9 (60)	31 (94)	
Adjuvant radiotherapy						
No	36 (26.3)	98 (83.1)	<0.001	4 (27)	23 (70)	0.001
Yes	4 (2.9)	6 (5.1)		1 (7)	6 (18)	
Unknown	97 (70.8)	14 (11.9)		10 (67)	4 (12)	
Recurrence						
No	72 (52.6)	60 (50.8)	0.786	2 (13)	9 (27)	0.287
Yes	65 (47.4)	58 (49.2)		13 (87)	24 (73)	
Site of first recurrence						
Locoregional	31 (48)	34 (59)	0.394	2 (15)	9 (38)	0.125
Regional LN	8 (12)	4 (7)		0 (0)	3 (13)	
Distant	26 (40)	20 (34)		11 (85)	12 (50)	
Any regional LN recurrence						
No	120 (87.6)	110 (93.2)	0.132	13 (87)	28 (85)	1.000
Yes	17 (12.4)	8 (6.8)		2 (13)	5 (15)	
Site of regional recurrence						
Inguinal only	5 (29)	1 (13)	0.181	1 (50)	0 (0)	0.095
Inguinal and pelvic	6 (35)	1 (13)		1 (50)	0 (0)	
Pelvic only	5 (29)	4 (50)		0 (0)	3 (60)	
Popliteal	1 (6)	0 (0)		0 (0)	0 (0)	
Unknown	0 (0)	2 (25)		0 (0)	2 (40)	

Values in parentheses are percentages unless indicated otherwise; *values are median (i.q.r.). †Interferon-α or dendritic cell therapy. CLND, completion lymph node dissection; LN, lymph node; SNBtot, number of sentinel nodes plus non-sentinel nodes during sentinel node biopsy. ‡χ^2 test, except §Mann–Whitney U test and ¶Fisher's exact test.

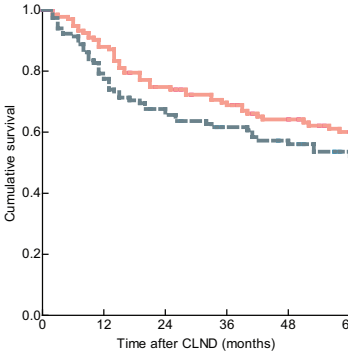
and 6.8% (8 of 118) after ilioinguinal dissection (P =0.132). The specified locations of regional lymph node recurrences are shown in **Table 2**. The overall recurrence rate for patients with a positive CLND result was 87% (13 of 15) after inguinal dissection and 73% (24 of 33) after ilioinguinal dissection (P =0.287). The overall regional lymph node recurrence rate was 13% (2 of 15) and 15% (5 of 33) respectively (P =1.000) (**Table 2**).



No. at risk

Inguinal	137	129	116	95	80	68
Ilioinguinal	118	110	82	68	58	44

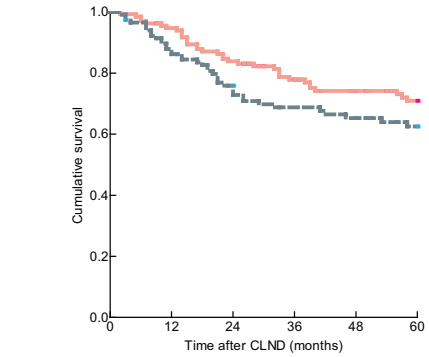
a Five-year melanoma specific survival



No. at risk

Inguinal	137	114	92	80	67	5
Ilioinguinal	118	91	69	57	49	3

b Five-year disease free survival



No. at risk

Inguinal	137	124	103	88	75	63
Ilioinguinal	118	101	75	61	53	42

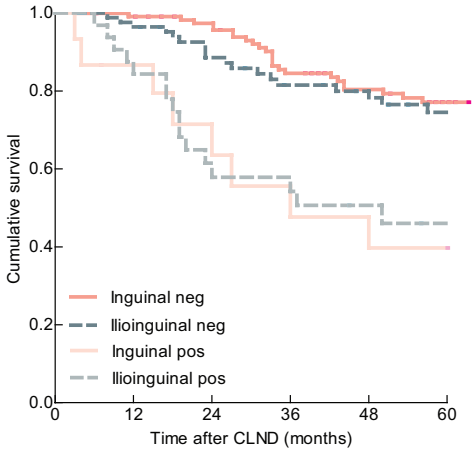
c Five-year distant metastasis free survival

Figure 2 Five-year a melanoma-specific survival, b disease-free survival and c distant metastasis-free survival after inguinal and ilioinguinal completion lymph node dissection (CLND)

a *P* =0.184, b *P* =0.169, c *P* =0.143 (log rank test)

SURVIVAL

Five-year estimated MSS, DFS and DMFS rates were 73.2, 59.2 and 70.4% respectively after inguinal dissection, and 66.4, 53.1 and 62.5% after ilioinguinal dissection (P =0.184, P =0.169 and P =0.143 respectively) (**Figure 2**). For patients with a positive CLND, the 5-year estimated MSS, DFS and DMFS rates were 40, 26 and 26% respectively after inguinal dissection, compared with 46, 30 and 36% after ilioinguinal dissection (P =0.767, P =0.978 and P =0.651 respectively). Results for MSS are illustrated in **Figure 3**. Univariable Cox proportional hazards regression analyses for DFS and MSS included all baseline and treatment characteristics. In multivariable analysis for DFS, advanced age, unknown histology, higher SN tumour burden and a positive CLND result were adverse prognostic factors (**Table 3**). In multivariable analysis for MSS, only advanced age and positive CLND were adverse prognostic factors (**Table 4**). In univariable analysis of prognostic factors in the subgroup of 48 patients with a positive CLND, type of dissection was not a significant prognostic factor for DFS (hazard ratio (HR) (ilioinguinal versus inguinal dissection) 0.88, 95% c.i. 0.44 to 1.76; P =0.713) or for MSS (HR 0.82, 0.38 to 1.79; P =0.622).



No. at risk

Inguinal neg	122	117	107	88	74	63
Ilioinguinal neg	85	82	65	52	47	36
Inguinal pos	15	12	9	7	6	5
Ilioinguinal pos	33	28	17	16	11	8

Figure 3 Five-year melanoma-specific survival for patients with a positive or negative result of inguinal or ilioinguinal completion lymph node dissection (CLND)

P =0.767, inguinal positive versus ilioinguinal positive (log rank test)

Table 3 Cox proportional hazards regression model for disease-free survival

		Univariable analysis		Multivariable analysis	
Variable	n	Hazard ratio	P	Hazard ratio	P
Age	255	1.02 (1.01, 1.04)	<0.001	1.02 (1.01, 1.03)	0.002
Breslow thickness	253	1.10 (1.04, 1.15)	0.001	1.03 (0.96 , 1.11)	0.377
Ulceration					
No	124	1.00 (reference)		1.00 (reference)	
Yes	108	1.70 (1.17, 2.48)	0.005	1.36 (0.90, 2.04)	0.143
Unknown	23	1.15 (0.60, 2.21)	0.671	0.61 (0.29, 1.28)	0.192
Histology					
SSM	131	1.00 (reference)		1.00 (reference)	
NM	71	1.66 (1.09, 2.52)	0.017	1.39 (0.89, 2.18)	0.148
ALM	20	2.04 (1.09, 3.83)	0.027	1.80 (0.91, 3.53)	0.090
Other	7	1.25 (0.39, 4.02)	0.704	0.65 (0.19, 2.22)	0.495
Unknown	26	1.91 (1.09, 3.36)	0.024	1.94 (1.01, 3.75)	0.048
SN tumour burden (mm)					
<0.1	20	1.00 (reference)		1.00 (reference)	
0.1-1.0	97	6.12 (1.48, 25.30)	0.012	4.42 (1.05, 18.58)	0.042
>1.0	76	10.33 (2.49, 42.86)	0.001	6.78 (1.60, 28.78)	0.009
Unknown	62	7.87 (1.90, 32.65)	0.004	6.12 (1.46, 25.73)	0.013
CLND type					
Inguinal	137	1.00 (reference)		1.00 (reference)	
Ilioinguinal	118	1.14 (0.80, 1.63)	0.464	0.80 (0.54, 1.19)	0.271
CLND result					
Negative	207	1.00 (reference)		1.00 (reference)	
Positive	48	2.83 (1.92, 4.17)	<0.001	2.82 (1.84, 4.33)	<0.001

Values in parentheses are 95 per cent confidence intervals. SSM, superficial spreading melanoma; NM, nodular melanoma; ALM, acral lentiginous melanoma; SN, sentinel node; CLND, completion lymph node dissection.
The multivariable analysis was adjusted for age (continuous), Breslow thickness (continuous), ulceration, Rotterdam criteria, CLND type and CLND result. Not shown (not significant in univariable analysis): treatment centre, sex, location, total number of SNs, number of positive SNs and SN ratio. The categories adjuvant immunotherapy and radiotherapy were not included in the multivariable analysis; both were significant in univariable analysis, but this was no longer the case when the analysis was corrected for CLND result.

Table 4 Cox proportional hazards regression model for melanoma-specific survival

		Univariable analysis		Multivariable analysis	
Variable	n	Hazard ratio	P	Hazard ratio	P
Age	255	1.02 (1.00, 1.03)	0.015	1.02 (1.00, 1.03)	0.023
Breslow thickness	253	1.09 (1.02, 1.16)	0.012	1.03 (0.95, 1.12)	0.538
Ulceration					
No	124	1.00 (reference)		1.00 (reference)	
Yes	108	1.64 (1.05, 2.56)	0.031	1.38 (0.84, 2.26)	0.206
Unknown	23	1.18 (0.55, 2.54)	0.637	0.90 (0.41, 2.00)	0.795
SN tumour burden (mm)					
<0.1	20	1.00 (reference)		1.00 (reference)	
0.1-1.0	97	1.99 (0.60, 6.67)	0.260	1.37 (0.40, 4.64)	0.618
>1.0	76	4.93 (1.51, 16.2)	0.008	2.82 (0.83, 9.59)	0.097
Unknown	62	3.22 (0.98, 10.7)	0.055	2.51 (0.75, 8.48)	0.137
CLND type					
Inguinal	137	1.00 (reference)		1.00 (reference)	
Ilioinguinal	118	1.24 (0.81, 1.90)	0.319	0.91 (0.57, 1.46)	0.704
CLND result					
Negative	207	1.00 (reference)		1.00 (reference)	
Positive	48	3.12 (1.99, 4.90)	<0.001	2.97 (1.82, 4.83)	<0.001

Values in parentheses are 95 per cent confidence intervals. SN, sentinel node; CLND, completion lymph node dissection. The multivariable analysis was adjusted for age (continuous), Breslow thickness (continuous), ulceration, Rotterdam criteria, CLND type and CLND result. Not shown (not significant in univariable analysis): treatment centre, sex, location, histology, total number of SNs, number of positive SNs, SN ratio and adjuvant immunotherapy (interferon-α or dendritic cell therapy). The category adjuvant radiotherapy was not included in the multivariable analysis; it was significant in univariable analysis but this was no longer the case when the analysis was corrected for CLND result.

SURVIVAL

Five-year estimated MSS, DFS and DMFS rates were 73.2, 59.2 and 70.4% respectively after inguinal dissection, and 66.4, 53.1 and 62.5% after ilioinguinal dissection (P =0.184, P =0.169 and P =0.143 respectively) (**Figure 2**). For patients with a positive CLND, the 5-year estimated MSS, DFS and DMFS rates were 40, 26 and 26% respectively after inguinal dissection, compared with 46, 30 and 36% after ilioinguinal dissection (P =0.767, P =0.978 and P =0.651 respectively). Results for MSS are illustrated in **Figure 3**. Univariable Cox proportional hazards regression analyses for DFS and MSS included all baseline and treatment characteristics. In multivariable analysis for DFS, advanced age, unknown histology, higher SN tumour burden and a positive CLND result were adverse prognostic factors (**Table** 3). In multivariable analysis for MSS, only advanced age and positive CLND were adverse prognostic factors (**Table 4**). In univariable analysis of prognostic factors in the subgroup of 48 patients with a positive CLND, type of dissection was not a significant prognostic factor for DFS (hazard ratio (HR) (ilioinguinal versus inguinal dissection) 0.88, 95% c.i. 0.44 to 1.76; P =0.713) or for MSS (HR 0.82, 0.38 to 1.79; P =0.622).

DISCUSSION

The extent of groin CLND (inguinal or ilioinguinal dissection) did not affect recurrence patterns and survival rates in patients with melanoma and a positive SNB. Even when stratified for a positive CLND result, outcomes were not significantly different.

The overall CLND positivity rate was 18.8%; the inguinal positivity rate was 15.7% (including patients with additional positive pelvic nodes) and the pelvic positivity rate 9.3% (including patients with additional positive inguinal nodes). Similar rates have been reported previously[27–29]. The inguinal positivity rate after ilioinguinal dissection was significantly higher than that after inguinal dissection, presumably as a result of unfavourable preoperative characteristics (such as higher SN tumour burden) as the median number of removed inguinal nodes was similar for both dissection types. Both in the overall cohort and in the subgroup of patients with a positive CLND result there were no significant differences in recurrence patterns between dissection types, including regional lymph node recurrence. These results indicate that the extent of surgery was not associated with recurrence, even though the pelvic nodes remained in situ after inguinal dissection, with the theoretical possibility of microscopic disease being present already. It also seems that ilioinguinal dissection was not associated with superior regional disease control. A previous smaller study of 94 patients reported a

regional lymph node recurrence rate of 12% after inguinal dissection compared with 17% after ilioinguinal dissection (P =0.66)[19].

Estimated 5-year MSS, DFS and DMFS rates did not differ significantly between patients undergoing inguinal or ilioinguinal dissection, both in the overall cohort and in the CLND-positive subgroup. Moreover, Cox regression showed that dissection type was not associated with DFS and MSS. These results indicate that a more radical dissection in the groin area in patients with a positive SNB is not associated with superior survival rates. Previous small studies reported an overall survival rate of 72% after inguinal dissection compared with 69% after ilioinguinal dissection (P =0.38), and 76 versus 80% respectively (P =0.80)[29]. In another small study, there was no significant difference in estimated 5-year overall survival (P =0.604)[30]. Previously reported DFS rates were 54% after inguinal dissection and 61% after ilioinguinal dissection (P =0.69)[29]. Another study reported no significant differences in pelvic node recurrence-free survival (P =0.80) and DFS (P =0.44) between the two dissection types[19].

The overall pelvic positivity rate was 9.3% in this study. In contrast, pelvic positivity rates of approximately 30% have been reported in patients with palpable disease[15,31]. However, even in these patients the extent of surgery does not seem to affect outcome[15]. Many patients, both those with a positive SNB and those with palpable disease, who undergo ilioinguinal dissection are therefore exposed to a potentially higher risk of morbidity but may not benefit from any therapeutic effect.

One limitation that must be considered when interpreting the present results is the retrospective study design, which is subject to numerous biases. Another is selection bias. The decision to deviate from routine practice differed by centre. Patients undergoing ilioinguinal dissection in centres where this was not standard practice presumably had an unfavourable preoperative prognosis. The potential therapeutic benefit of ilioinguinal dissection could therefore be partly counterbalanced by unfavourable prognostic factors. However, even in patients with a positive CLND result, recurrence patterns and estimated 5-year MSS, DFS and DMFS did not differ between the two dissection types. This indicates that the extent of CLND does not influence recurrence and survival positively or negatively. Other selection and treatment-based factors may also have played a considerable role, such as variation in local population, proportion of patients who underwent SNB, SN positivity rate per centre, the extent to which radical surgery was performed, the pathology protocol used, and the extent to which pathologists searched for nodes. Unfortunately, details of complications were not available for all patients in the present series, so this aspect could not be evaluated. The timing of CLND after diagnosis of melanoma was not assessed in this study, but it has been demonstrated recently that this does not seem to influence tumour load, DFS or MSS[32].

To date, the therapeutic value of CLND in patients with a positive SNB has not yet been proven in prospective randomized trials[33,34]. The DeCOG-SLT multicentre trial randomized patients with a positive SNB to undergo axillary or inguinal CLND, or observation. The trial showed no difference in DMFS, overall survival or DFS, not even a trend towards better survival for the CLND group. However, it was underpowered, and was criticized for having a majority of patients with a relatively low SN tumour load[34]. A more definitive answer to this controversial and long-standing question will be provided by MSLT-II, which has included a larger number of patients with long follow-up[4]. The EAGLE FM trial[35] is focusing on the question of whether to perform inguinal or ilioinguinal dissection in patients with groin metastases; patients with a positive SNB or palpable nodal metastases in the groin will be randomized to inguinal or ilioinguinal dissection. However, if MSLT-II does not show a survival benefit for CLND, it will be less important to know whether to perform an inguinal or ilioinguinal dissection.

Despite these forthcoming developments, there remains a role for CLND in the near future. Currently all adjuvant therapy trials require complete pathological nodal staging of patients with stage III disease (by lymph node dissection) before inclusion. Eggermont and colleagues reported that 10mg/kg ipilimumab resulted in a significant increase of 11% in recurrence-free and overall survival compared with placebo[36]. The mortality risk was 28% lower with ipilimumab than with placebo, and the risk of DMFS was 24% higher. Although more research is necessary before ipilimumab can be implemented safely as standard adjuvant therapy, these results seem promising. Ongoing trials with other agents may also report a survival benefit in the next few years, all based on adjuvant therapy after lymph node dissection. Thus, CLND will remain a standard procedure for a while, either as a criterion for entry into trials or, for example, as a prerequisite for Food and Drug Administration/European Medicines Agency-approved adjuvant therapy.

The present study found no significant difference in recurrence pattern and survival rates between patients undergoing either inguinal or ilioinguinal dissection for a positive SNB, even in the subgroup with a positive CLND result. The risk of pelvic nodal involvement was low (9.3%). Therefore, inguinal dissection seems a safe first approach to CLND in patients with a positive SNB.

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Risk stratification of sentinel node-positive melanoma patients defines surgical management and adjuvant therapy treatment considerations

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ABSTRACT

BACKGROUND In light of the evolving landscape of adjuvant therapy in melanoma and the recently confirmed absent survival benefit of completion lymph node dissection (CLND), it becomes important to explore possible consequences of omitting CLND, and whether it is possible to adequately stratify positive sentinel node (SN) patients solely based on information retrieved from the melanoma up to the sentinel lymph node biopsy (SLNB).

METHODS A retrospective cohort from nine European Organization for Research and Treatment of Cancer Melanoma Group centres was used. Patients were staged based on SLNB and CLND result according to the American Joint Committee on Cancer (AJCC) criteria and stratified by ulceration and SN tumour burden. These were incorporated in Cox regression models. Predictive ability was assessed using Harrell's concordance index (c-index) and the Akaike information criterion (AIC).

RESULTS In total, 1015 patients were eligible. CLND led to upstaging in N-category in 19% and in AJCC stage in 5-6%. The model incorporating only ulceration and SN tumour burden performed equally well as the model incorporating substages after CLND. The model incorporating substages based on SLNB had the lowest predictive ability. Stratifying by ulceration and SN tumour burden resulted in four positive SN groups from which low-, intermediate and high-risk prognostic classes could be derived.

CONCLUSIONS Adequate stratification of positive SN patients was possible based on ulceration and SN tumour burden category. The identification of low-, intermediate- and high-risk patients could guide adjuvant therapy in clinical practice. Omitting CLND seems to have little consequences.

INTRODUCTION

The landscape of systemic therapy for melanoma is evolving rapidly, both in the metastatic setting as in the potentially curative adjuvant setting. The introduction of immune checkpoint inhibitors and BRAF/MEK inhibitors has significantly improved the perspective of patients with stage IV melanoma[1,2]. This success has led to various clinical trials evaluating the potential of these therapies in the adjuvant setting in high-risk stage III melanoma. Positive results in this setting have already been published[3-5]. Until recently, complete nodal staging through lymph node dissection was standard-of care in stage III melanoma, either as a therapeutic lymph node dissection for clinically apparent disease, or as a completion lymph node dissection (CLND) preceded by a positive sentinel lymph node biopsy (SLNB; occult disease). As such, complete nodal staging was, and still is, a prerequisite for inclusion in clinical trials in the adjuvant setting. However, the landmark Multicentre Selective Lymphadenectomy Trial (MSLT) II trial lately concluded that CLND was not associated with a significant survival benefit compared with nodal observation[6]. These results are supported by the underpowered DeCOG-SLT trial with similar conclusions[7]. Consequently, clinical practice will change dramatically since CLND is expected to no longer be standard procedure after a positive sentinel node (SN). In the current landscape of (systemic) adjuvant therapy, a vacuum remains for patients classified stage III solely based on the SLNB result since results from published trials cannot be extrapolated, nor do these patients qualify for trial participation due to the present inclusion criteria. In that light, CLND after a positive SLNB may remain valuable since it could potentially lead to upstaging and subsequently yield additional prognostic information.

Considering the recent developments, it becomes important to adequately identify those positive SN patients that are at high-risk of recurrence and/or melanoma- specific death, preferably by only using information retrieved from the primary melanoma up to the SLNB. The number of positive lymph nodes, Breslow thickness and the presence of ulceration in the primary tumour have long been identified as poor prognostic characteristics[8-11]. Sentinel node tumour burden, according for example to the Rotterdam and/or Dewar criteria, has also been identified as an important prognostic factor for survival and also as a predictive factor for additional positive (non-sentinel) lymph nodes[12-14]. The current 8th and previous 7th American Joint Committee on Cancer (AJCC) staging editions, however, do not account for SN tumour burden[10,11]. On the contrary, the maximum diameter of the largest tumour lesion in the SN (≤1.0 mm versus >1.0 mm) has already been implemented as an additional criterion for participation in adjuvant clinical trials[3,5]. The aim of the present study was to

explore the potential upstaging of CLND, its prognostic value and whether patients with a positive SN could be adequately stratified into distinct risk classes solely based on information retrieved from the melanoma up to the SLNB procedure, including ulceration status and SN tumour burden category.

METHODS

For purposes of the present study, a retrospective cohort of SN-positive patients previously collected and described, was used[15-17]. This cohort contained 1080 SN-positive melanoma patients that underwent a SLNB between 1993 and 2008 in one of nine European Organization for Research and Treatment of Cancer (EORTC) Melanoma Group centres. The study was performed in accordance with local ethics committee guidelines and national legislation. Patient characteristics (age, sex), tumour characteristics (location, histology, Breslow thickness, Clark level, ulceration), SN characteristics (number of retrieved SNs, number of positive SNs, tumour burden) and follow-up data were extracted from the databases of the participating centres. Patients that did not undergo CLND were excluded.

PROCEDURES

Excisional biopsy of the primary melanoma was performed with total thickness excision and a narrow circumferential margin. All centres assessed SLNB eligibility according to international guideline criteria; Breslow thickness of >1.0 mm or the presence of risk factors ulceration or Clark level IV or V (according to the 6th AJCC staging edition at the time of diagnosis)[8]. In general, a wide local excision (WLE) was performed simultaneously with SLNB. The SLNB technique used has been described elsewhere[15,18]. Histopathological analysis of the SN was generally conducted according to the EORTC Melanoma Group Pathology Protocol and was centrally revised for purposes of the study in 2014[19]. CLND consisted of an axillary, inguinal, ilioinguinal or cervical lymphadenectomy, depending on the localisation of the positive SN.

CLASSIFICATION

Patients were staged according to the 8th AJCC staging edition criteria, which takes into account T category (Breslow thickness and ulceration) and N-category (number of positive nodes), into AJCC substages (IIIA-T1a, T1b or T2a-N1a, IIIA-T1a, T1b or T2a -N2a, IIIB -T2b or T3a-N1a, IIIB-T2b or T3a-N2a, IIIC-T3b, T4a or T4b-N1a, IIIC -T3b,

T4a orT4b-N2a, IIIC-T1a, T1b, T2b, T3a, T3b or T4a-N3a or IIID-T4b-N3a) and AJCC stages (IIIA, IIIB, IIIC or IIID)[11]. In addition, patients were staged according to the 7th AJCC staging edition criteria, which takes into account ulceration and N-category, into AJCC substages (IIIA-N1a, IIIA-N2a, IIIB-N1a, IIIB-N2a or IIIC-N3) and AJCC stages (IIIA, IIIB or IIIC)[10]. Patients were staged solely based on the total number of positive nodes retrieved solely after SLNB and also based on the total number of positive nodes retrieved after CLND.

Furthermore, all patients were stratified by ulceration and SN tumour burden category (low: ≤1.0 mm; high: >1.0 mm) into four groups: 1) absent ulceration and low SN tumour burden; 2) absent ulceration and high SN tumour burden; 3) present ulceration and low SN tumour burden; and 4) present ulceration and high SN tumour burden.

STATISTICAL ANALYSIS

Data were analysed using descriptive statistical methods. The median follow-up (FU) length of the survivors was calculated from the date of SLNB until last FU using the reversed Kaplan-Meier method; deaths were censored. Melanoma-specific survival (MSS) was calculated from the date of SLNB until last FU or death from melanoma; deaths from other causes were censored. The variables AJCC substages according to both the 8th and 7th edition, ulceration and SN tumour burden category were incorporated in a multivariable Cox regression analysis. Harrell's concordance index (c-index) and the Akaike information criterion (AIC) values were analysed to compare the predictive ability of the different Cox regression models[20,21]. A higher c-index and lower AIC value indicate a better model for predicting outcome. The AIC incorporates a trade-off between the goodness-of-fit and complexity of the model. The Kaplan-Meier method was used to estimate survival, and differences between groups were assessed by means of the log rank test. To make efficient use of the available data an advanced multiple imputation of missing values strategy (5 imputations) was applied before multivariable analysis[22]. All other analyses were based on complete cases. When data were missing or unknown, an unknown subcategory was presented in the basic characteristics table. A P value of 0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 22.0 (IBM, Armonk, NEW York, USA) and R (version 2.15, R Foundation for Statistical Computing, Vienna, Austria, 2011).

RESULTS

There were 1080 melanoma patients with a positive SN in one of nine EORTC Melanoma Group centres between 1993 and 2008, of whom 1015 patients were eligible for the present study. Patients who had not undergone CLND (n=63) or duplicate cases (n=2) were excluded. Recurrence occurred in 498 patients (49%) and melanoma-specific death in 420 patients (41%). Median FU time for all survivors was 107 months (95% confidence interval (CI) 102-113 months). The patient and tumour characteristics for all patients are depicted in **Table 1**.

Details on upstaging after CLND are depicted in **Table 2**. Upstaging in N-category occurred in 19% of the patients (168/891); from N1a to N2a in 116 patients, from N1a to N3a in 26 patients and from N2a to N3a in 26 patients. Upstaging in AJCC stage 8th edition occurred in 5% of the patients (41/891); from IIIA to IIIC in three patients, from IIIB to IIIC in 14 patients and from IIIC to IIID in 24 patients. Upstaging in AJCC stage 7th edition after CLND occurred in 6% of the patients (52/892); from IIIA to IIIC in 18 patients and from IIIB to IIIC in 34 patients (**Supplementary Table 1**).

MULTIVARIABLE COX MODELS FOR 5-YEAR MELANOMA-SPECIFIC SURVIVAL

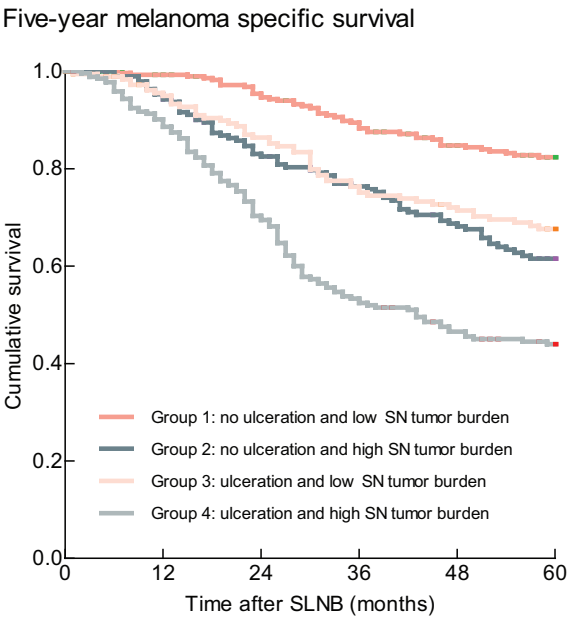
The multivariable models for 5-year MSS based on the substages according to the 8th AJCC edition are shown in **Table 3**. Model 1 incorporated the substages after CLND and represents the current situation. Model 2 incorporated the substages according to solely the SLNB result, which had the lowest predictive ability (highest AIC value and lowest c-index). In model 3, SN tumour burden category was added to the substages based on the SLNB result, which resulted in a more desirable model and was at least comparable to model 1. Model 4 only incorporated ulceration and SN tumour burden category and was also comparable to model 1. Similar results were observed when the multivariable models for 5-year MSS were based on the substages according to the 7th AJCC edition (**Supplementary Table 2**).

POSITIVE SENTINEL NODE GROUPS AND DERIVED RISK CLASSES

Stratifying patients according to ulceration and SN tumour burden category resulted in four positive SN groups: group 1 (ulceration absent, low SN tumour burden), group 2 (ulceration absent, high SN tumour burden), group 3 (ulceration present, low SN tumour burden) and group 4 (ulceration present, high SN tumour burden). **Table 4** depicts the distribution and 5-year MSS estimates per group. The corresponding Kaplan-Meier curves are shown in **Figure 1**.

The 5-year MSS estimates were significantly different between group 1 and 2 (82.4% versus 61.5%, P < 0.001), group 1 and 3 (82.4% versus 67.6%, P < 0.001), group 1 and 4 (82.4% versus 44.0%, P < 0.001), group 2 and 4 (61.5% versus 44.0%, P < 0.001) and group 3 and 4 (67.6% versus 44.0%, P < 0.001). Estimates between group 2 and 3 were not statistically different (61.5% versus 67.6%, P = 0.295). From these estimates, three prognostic risk classes were derived: low risk (group 1); intermediate risk (groups 2 and 3); and high risk (group 4).

Figure 1 Five-year melanoma-specific survival per positive sentinel node group



No. at risk						
Group 1	302	286	265	235	213	198
Group 2	199	182	176	131	115	97
Group 3	185	168	147	127	117	100
Group 4	272	233	166	116	92	81

Table 1 Patient and tumour characteristics

Characteristic	Patients (n = 1015)
	No. (%) or Median [IQR]
Age (years), n = 1014	51 [40 - 61]
Gender (F: M)	475:540
Primary site	
Extremity	566 (55.8)
Trunk	417 (41.1)
Head & neck	32 (3.2)
Histological type	
SSM	412 (40.6)
NM	364 (35.9)
LLM	14 (1.4)
ALM	33 (3.3)
Other	5 (0.5)
Unknown	187 (18.4)
Breslow thickness (mm), n = 1013	3.00 [1.94 - 5.00]
T category[a]	
pT1a	8 (0.8)
pT1b	41 (4.0)
pT2a	167 (16.4)
pT2b	59 (5.8)
pT3a	203 (20.0)
pT3b	179 (17.6)
pT4a	87 (8.6)
pT4b	213 (21.0)
Unknown	59 (5.8)
Ulceration	
Absent	501 (49.4)
Present	457 (45.0)
Unknown	57 (5.6)
Clark level	
ii	31 (3.1)
iii	257 (25.3)
iv	573 (56.5)
v	110 (10.8)
Unknown	44 (4.3)
No. of SNs removed, n = 934	2 [1 - 3]
No. of positive SNs, n = 936	1 [1 - 1]
No. of LNs removed, n = 918	14 [10 - 20]
No. of positive LNs, n = 933	1 [1 - 2]

Table 1 Continued

Abbreviations: ALM, acral lentiginous melanoma; IQR, interquartile range; LMM, lentigo maligna melanoma; NM, nodular melanoma; SN, sentinel node; SSM, superficial spreading melanoma.

[a] T category based on 8th AJCC edition criteria.

Table 2 Upstaging in AJCC (sub)stage according to the 8th AJCC edition by CLND compared to SLNB only

		AJCC substage based on SLNB only								
		IIIA - T1a, T1b, T2a - N1a	IIIA - T1a, T1b, T2a - N2a	IIIB - T2b, T3a - N1a	IIIB - T2b, T3a - N2a	IIIC - T3b, T4a, T4b - N1a	IIIC - T3b, T4a, T4b - N2a	IIIC - T1a, T1b, T2a, T2b, T3a, T3b, T4a - N3a	IIID - T4b - N3a	Total
AJCC substage based on CLND	IIIA - T1a, T1b, T2a - N1a	141	0	0	0	0	0	0	0	141
	IIIA - T1a, T1b, T2a - N2a	21	36	0	0	0	0	0	0	57
	IIIB - T2b, T3a - N1a	0	0	147	0	0	0	0	0	147
	IIIB - T2b, T3a - N2a	0	0	30	48	0	0	0	0	78
	IIIC - T3b, T4a, T4b - N1a	0	0	0	0	257	0	0	0	257
	IIIC - T3b, T4a, T4b - N2a	0	0	0	0	65	87	0	0	152
	IIIC - T1a, T1b, T2a, T2b, T3a, T3b, T4a - N3a	0	3	7	7	4	8	2	0	31
	IIID - T4b - N3a	0	0	0	0	15	8	0	5	28
	Total	162	39	184	55	341	103	2	5	891

Abbreviations: AJCC, American Joint Committee on Cancer; CLND, completion lymph node dissection; SLNB, sentinel lymph node biopsy.

Table 3 Multivariable models for 5-year melanoma specific survival based on substages according to the 8th AJCC edition

	Model 1		Model 2		Model 3		Model 4	
Variables	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
AJCC substages CLND								
IIIA - T1a, T1b or T2a - N1a	Reference							
IIIA - T1a, T1b or T2a - N2a	2.32 (1.16 - 4.65)	0.018						
IIIB - T2b or T3a - N1a	1.98 (1.02 - 3.84)	0.042						
IIIB - T2b or T3a - N2a	3.37 (1.78 - 6.39)	<0.001						
IIIC - T3b, T4a or T4b - N1a	3.63 (2.11 - 6.24)	<0.001						
IIIC - T3b, T4a or T4b - N2a	5.61 (3.18 - 9.89)	<0.001						
IIIC - T1a, T1b, T2a, T2b, T3a, T3b or T4a - N3a	7.23 (3.69 - 14.20)	<0.001						
IIID - T4b - N3a	14.39 (7.56 - 27.42)	<0.001						
AJCC substages SLNB								
IIIA - T1a, T1b or T2a - N1a			Reference		Reference			
IIIA - T1a, T1b or T2a - N2a			2.23 (1.06 - 4.66)	0.034	1.95 (0.94 - 4.05)	0.075		
IIIB - T2b or T3a - N1a			2.17 (1.23 - 3.84)	0.008	1.97 (1.11 - 3.49)	0.021		
IIIB - T2b or T3a - N2a			2.67 (1.39 - 5.15)	0.003	2.06 (1.06 - 4.01)	0.034		
IIIC - T3b, T4a or T4b - N1a			4.07 (2.57 - 6.34)	<0.001	3.30 (2.07 – 5.25)	<0.001		
IIIC - T3b, T4a or T4b - N2a			4.78 (2.83 - 8.09)	<0.001	3.44 (2.01 - 5.87)	<0.001		
IIIC - T1a, T1b, T2a, T2b, T3a, T3b or T4a - N3a			4.69 (0.63 - 35.09)	0.132	3.46 (0.49 - 24.26)	0.212		
IIID - T4b - N3a			7.95 (2.44 - 25.88)	0.001	4.71 (1.43 - 15.49)	0.011		
SN tumour burden								
£ 1.0 mm					Reference		Reference	
> 1.0 mm					2.13 (1.68 - 2.71)	<0.001	2.35 (1.86 - 2.97)	<0.001
Ulceration								
Absent							Reference	
Present							1.94 (1.54 - 2.45)	<0.001
Model performance								
AIC	4172.16		4201.50		4161.69		4159.20	
C-index	0.66 (0.63 - 0.70)		0.63 (0.60 - 0.66)		0.67 (0.64 - 0.70)		0.66 (0.63 - 0.69)	

Abbreviations: AIC, Akaike Information Criterion; AJCC, American Joint Committee on Cancer; c-index, concordance index; CI, confidence interval; CLND, completion lymph node dissection; HR, hazard rate; SLNB, sentinel lymph node biopsy; SN, sentinel node.

Table 4 Positive SN groups and derived risk classes: distribution and 5-year MSS estimates (*n* = 958)

			5-year MSS			
Group	Ulceration	SN tumour burden	No. (%)	Rate %	No. at risk	Risk class
1	Absent	≤ 1.0 mm	302 (31.5)	82.4	198	Low risk
2	Absent	> 1.0 mm	199 (20.8)	61.5	97	Intermediate risk
3	Present	≤ 1.0 mm	185 (19.3)	67.6	100	Intermediate risk
4	Present	> 1.0 mm	272 (28.4)	44.0	81	High risk

Abbreviations: MSS, melanoma-specific survival; SN, sentinel node.

DISCUSSION

This large EORTC multicentre cohort study evaluated the potential upstaging after CLND, its prognostic value and whether it was possible to adequately stratify melanoma patients with a positive SN into distinct risk classes solely based on information retrieved from the primary melanoma up to the SLNB procedure. Stratification by ulceration and SN tumour burden category resulted in the adequate identification of low-, intermediate- and high-risk positive SN patients.

The remarkable breakthroughs in treatment for patients with advanced and metastatic melanoma offered possibilities for therapy in the potentially curative adjuvant setting, focussing on 'high-risk' patients. Eggermont et al.[3] reported that ipilimumab (an immune checkpoint inhibitor) at a dose of 10 mg/kg in completely resected stage III melanoma patients showed significantly prolonged recurrence-free, overall and distant metastasis-free survival as compared with a placebo. However, it is conceivable that ipilimumab will be replaced by nivolumab since Weber et al.[4] recently reported that nivolumab showed improved recurrence-free survival and lower toxicity rates as compared with ipilimumab in resected stage IIIB, IIIC or IV melanoma. Also recently, Long et al.[5] reported that adjuvant use of combination therapy with dabrafenib and trametinib (BRAF/MEK inhibitors) resulted in a significantly lower risk of recurrence in stage III melanoma patients with BRAF V600E or V600K mutations as compared with a placebo. At present, multiple potentially revolutionary clinical trials are still on-going, and results are expected in the next several years (Table 5). All these trials require(d) complete nodal staging on inclusion, except for the MIND-DC trial which recently amended the inclusion criteria in order for positive SN patients to be eligible for inclusion without having to undergo CLND. This is in line with the expectation

Table 5 Overview of recent high-profile phase 3 adjuvant clinical trials for stage III melanoma

Trial name	Start year	Results expected	Study details	Inclusion details[a]	ClinicalTrials.gov identifier
MIND-DC	2016	2021	Active immunization with natural dendritic cells versus placebo	IIIB, IIIC	NCT02993315
SWOG S1404	2015	2020	Interferon alpha-2b, ipilimumab or pembrolizumab	IIIA-N2a – IIIC	NCT02506153
EORTC-1325/ KEYNOTE-054	2015	2025	Pembrolizumab versus placebo	IIIA-N1a > 1.0 mm – IIIC	NCT02362594
CHECKMATE 238[3]	2015	Published 2017	Nivolumab versus ipilimumab	IIIB – IV	NCT02388906
COMBI-AD[4]	2013	Published 2017	Dabrafenib plus trametinib versus placebo	IIIA-N1a > 1.0 mm – IIIC	NCT01682083
BRIM8	2012	Presented 2017	Vemurafenib versus placebo	IIC, IIIA-N1a > 1.0 mm – IIIC	NCT01667419
ECOG 1609	2011	2018	Ipilimumab versus interferon alfa-2b	IIIB, IIIC, IV	NCT01274338
EORTC 18071[2]	2008	Published 2016	Ipilimumab versus placebo	IIIA-N1a > 1.0 mm – IIIC	NCT00636168
ECOG-S0008[29]	2000	Published 2014	High-dose interferon alpha 2b versus cisplatin, vinblastine, DTIC plus IL-2 and interferon	IIIA-N2a – IIIC	NCT00006237
EORTC 18991[30]	2000	Published 2008	Pegylated interferon-alpha 2b versus observation	IIIA – IIIC	NCT00006249

[a] Staging according to the 7th AJCC staging edition, all trials required completely resected disease before inclusion.

that the number of CLND will decrease since it was shown to not have a significant survival benefit and exposes many patients to a potentially unnecessary morbid procedure[6,7]. On the other hand, it can be argued that omitting CLND will lead to a loss of prognostic information. Therefore, it becomes important to evaluate possible consequences of omitting CLND and assess whether it is possible to appropriately stratify positive SN patients solely based on information retrieved from the primary melanoma up to the SLNB.

Our results showed that CLND led to upstaging in the N-category in 19% of the patients, but in AJCC stage in only 5-6%. So, few patients would actually have a meaningful change in staging. Surprisingly, the inclusion criteria for the (on-going) adjuvant therapy trials vary, whereas all trials state that they will focus on 'high-risk' stage III melanoma patients (**Table 5**). Some include from stage IIIA-N1a with >1.00 mm SN tumour burden, others from stage IIIA-N2a or from stage IIIB (according to the 7th AJCC edition). This initiates discussion regarding which patients should receive adjuvant therapy in future daily clinical practice. The therapeutic consequences of omitting CLND depend on these criteria. When all patients with stage IIIA-N1a with >1.00 mm SN tumour burden are eligible to receive adjuvant therapy, omitting CLND has no consequences since it is not required to obtain this information. If having stage IIIC (7th AJCC edition) disease becomes the threshold for receiving adjuvant therapy, omitting CLND would have consequences for ~6% of the patients, namely those that would have been upstaged from IIIA/IIIB to IIIC.

Currently, the 8th AJCC edition has been implemented which adds even more difficulty to this discussion. As a result, Grob et al. [23] propose to adhere to the 7th AJCC edition criteria for stage III melanoma to prevent issues regarding the interpretation of study results. Nonetheless, both editions do not reflect the expected new clinical standard of omitting CLND. As we demonstrated, predictive ability of a model incorporating the AJCC substages based on solely the SLNB result (which reflects this new clinical situation), is relatively poor compared with a model that incorporates the CLND result. In that light, performing CLND would lead to better prognostication. However, we also demonstrated that a model incorporating ulceration and SN tumour burden category showed at least similar performance compared with a model based on the CLND result. Thus, solely stratifying for ulceration and SN tumour burden category provides accurate prognostic information. Therefore, we argue to not routinely perform CLND in case of a positive SN. Others share this opinion and have suggested other alternatives such as routinely harvesting 4-5 nodes during a SLNB[24] or using a prognostic model that incorporates the disseminated cancer cell density in the SN[25].

Stratifying patients according to ulceration and SN tumour burden category resulted in four groups. Based on the corresponding 5-year MSS estimates of each group, it was possible to derive three distinct prognostic risk classes: low-, intermediate- and high-risk positive SN patients. These prognostic risk classes might aid in selecting patients for adjuvant therapy (trials). However, stratifying for survival is not necessarily equivalent to stratifying for therapeutic benefit. This is reflected by the outcomes of the EORTC 18071 trial[3]. Individuals with clinically palpable (macrometastatic) disease are associated with worse survival when compared with those with micrometastatic

disease (positive SLNB)[26]. By contrast, it was shown that ipilimumab actually had more beneficial effect in those patients with micrometastatic disease[3]. Similarly, a post hoc meta-analysis of two large adjuvant interferon (IFN)/PEG-IFN trials showed that patients with favourable stage (IIB and III-N1) significantly benefited from treatment, whereas patients staged III-N2 disease did not[27]. In addition, it has been reported that only patients with an ulcerated primary appear to benefit from IFN [27-29]. Unfortunately, these results cannot be easily extrapolated. So, we are unable to conclude on whether our positive SN groups and derived risk classes provide an accurate stratification for therapeutic benefit as well.

One of the limitations of the present study is its retrospective design. Selection bias may also be present since part of the analyses was based on complete case analysis. However, missing data were fairly limited and a multiple imputation strategy was performed for the multivariable Cox models. Preferably, it needs to be evaluated whether the presented risk classes are able to stratify accurately for therapeutic benefit as well.

In conclusion, omitting CLND seems to have little consequences for risk stratification since only few patients had a relevant shift in staging that would possibly lead to a meaningful change in management. Furthermore, it was demonstrated that adequate stratification of positive SN patients was possible based on ulceration and SN tumour burden category only, with at least similar predictive ability compared with the model incorporating the AJCC stages based on the CLND result. The stratification into low-risk, intermediate-risk and high-risk patient classes could guide adjuvant therapy in clinical practice.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1 Upstaging in AJCC (sub)stage according to the 7th AJCC edition by CLND compared to SLNB only

		AJCC (sub)stage based on SLNB only					
7th edition		IIIA - N1a	IIIA - N2a	IIIB - N1a	IIIB - N2a	IIIC - N3	Total
AJCC (sub) stage based on CLND	IIIA - N1a	301	0	0	0	0	301
	IIIA - N2a	58	87	0	0	0	145
	IIIB - N1a	0	0	245	0	0	245
	IIIB - N2a	0	0	58	84	0	142
	IIIC - N3a	7	11	19	15	7	59
	Total	366	98	322	99	7	892

Abbreviations: AJCC, American Joint Committee on Cancer; CLND, completion lymph node dissection; SLNB, sentinel lymph node biopsy

Supplementary Table 2 Multivariable models for 5-year melanoma-specific survival based on substages according to the 7th AJCC edition

	Model 1		Model 2		Model 3		Model 4	
Variables	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
AJCC substages CLND								
IIIA - N1a	Reference							
IIIA - N2a	1.61 (1.07 - 2.40)	0.021						
IIIB - N1a	2.00 (1.43 - 2.78)	<0.001						
IIIB - N2a	3.55 (2.54 - 4.96)	<0.001						
IIIC - N3a	5.58 (3.71 - 8.38)	<0.001						
AJCC substages SLNB								
IIIA - N1a			Reference		Reference			
IIIA - N2a			1.39 (0.90 - 2.14)	0.137	1.15 (0.76 - 1.76)	0.508		
IIIB - N1a			2.22 (1.70 - 2.90)	<0.001	1.94 (1.48 - 2.54)	<0.001		
IIIB - N2a			2.72 (1.87 - 3.96)	<0.001	2.14 (1.46 - 3.12)	<0.001		
IIIC - N3a			3.64 (1.32 - 10.03)	0.012	2.47 (0.92 - 6.63)	0.072		
SN tumour burden								
£ 1.0 mm					Reference		Reference	
> 1.0 mm					2.30 (1.81 - 2.92)	<0.001	2.35 (1.86 - 2.97)	<0.001
Ulceration								
Absent							Reference	
Present							1.94 (1.54 - 2.45)	<0.001
Model performance								
AIC	4173.27		4208.23		4162.18		4159.20	
C-index	0.65 (0.62 - 0.68)		0.61 (0.58 - 0.64)		0.66 (0.64 - 0.69)		0.66 (0.63 - 0.69)	

Abbreviations: AIC, Akaike Information Criterion; AJCC, American Joint Committee on Cancer; c-index, concordance index; CI, confidence interval; CLND, completion lymph node dissection; HR, hazard rate; SLNB, sentinel lymph node biopsy; SN, sentinel node

The EORTC-DeCOG nomogram adequately predicts outcomes of patients with sentinel node-positive melanoma without the need for completion lymph node dissection

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ABSTRACT

PURPOSE Based on recent advances in the management of sentinel node (SN) positive melanoma patients, we aimed to develop prediction models for recurrence, distant metastasis (DM) and overall mortality (OM).

METHODS The derivation cohort consisted of 1080 SN-positive melanoma patients from nine EORTC-centres. Prognostic factors for recurrence, DM and OM were studied with Cox regression analysis. Significant factors were incorporated in the models. Performance was assessed by discrimination (c-index) and calibration in cross-validation across centres. The models were externally validated using a prospective cohort consisting of 705 German SN-positive patients; 473 DeCOG-SLT trial participants and 232 screened patients. A nomogram was developed for graphical presentation.

RESULTS The final model for recurrence and the calibrated models for DM and OM included ulceration, age, SN tumour burden and Breslow thickness. The models showed reasonable calibration. The c-index for the recurrence, DM and OM model was 0.68, 0.70 and 0.70 respectively, and 0.70, 0.72 and 0.74 respectively in external validation. The EORTC-DeCOG model identified a robust low risk group, with all identified low risk patients (approximately 4% of the entire population) having a 5-year recurrence probability of <25% and an overall 5-year recurrence rate of 13%. A model including information on completion lymph node dissection (CLND) showed only marginal improvement in model performance.

CONCLUSIONS The EORTC-DeCOG nomogram provides an adequate prognostic-tool for patients with SN-positive melanoma, without the need for CLND. It showed consistent results across validation. The nomogram could be used for patient counselling and might aid in adjuvant therapy decision-making.

INTRODUCTION

The American Joint Committee on Cancer (AJCC) staging system is the most widely accepted approach to melanoma staging[1, 2]. Patients are classified into distinct stages based on the Tumour Node Metastasis (TNM) criteria where nodal status is based on number of positive lymph nodes after completion lymph node dissection (CLND) in case of a positive sentinel node (SN) or after a therapeutic lymph node dissection in case of clinically apparent nodal disease. Recently there have been many advances in the care of patients with SN-positive melanoma that also affect staging, namely CLND is no longer routine practice as the Multicenter Selective Lymphadenectomy Trial-II (MSLT-II) and the German Dermatologic Cooperative Oncology Group study (DeCOG-SLT) demonstrated no survival benefit for CLND[3-6], and as immune checkpoint inhibition and targeted therapy have been introduced in the adjuvant setting with highly encouraging results[7-10]. Consequently, the AJCC staging system is likely to be less appropriate for SN-positive melanoma patients not undergoing CLND due to decreased discriminatory ability[11] as the number of positive nodes after SLNB is not an independent prognostic factor[3, 4] (in contrast to involved non-SNs retrieved after CLND[3]). As a result, omitting CLND could result in poorer risk stratification and impaired selection for adjuvant therapy. On the other hand, SN tumour burden has been shown to be an independent predictor of involved non-SNs[12-14] and therefore SN tumour burden may serve as a surrogate.

The objective of the present study was to identify independent prognostic factors in a large European SN positive melanoma population, using solely information from the primary melanoma and the SLNB, in order to develop a prediction model for recurrence, distant metastasis (DM) and overall mortality (OM), presented in the form of a nomogram. The resulting model could aid in adjuvant therapy decision making. The prediction models were externally validated using a large prospective German cohort.

PATIENTS AND METHODS

COHORT CHARACTERISTICS

DERIVATION COHORT

The retrospective derivation cohort consisted of 1080 SN-positive melanoma patients who underwent SLNB between 1993 and 2008 in one of nine EORTC Melanoma Group centres that has been previously collected and described[11, 15-17]. The current study

only excluded duplicate cases (n = 2), leading to a total of 1078 eligible SN-positive patients. The two duplicate cases concerned an error in that database. The applied procedures have been described previously[11].

VALIDATION COHORT

The prospective German validation cohort involved two sets of patients. The first set consisted of 473 patients who were included in the DeCOG-SLT multicentre randomised phase 3 trial comparing survival between SN-positive melanoma patients who did or did not undergo CLND[4]. The second set consisted of an additional 219 patients from a single centre (University Hospital Tuebingen) who were initially screened for inclusion in the DeCOG-SLT trial but were not included due to meeting the trial's exclusion criteria (e.g. head & neck melanoma, age > 75 years), were unwilling to participate, or no known reason. They also did or did not undergo CLND and were followed and prospectively registered according to similar protocols. All patients had a tumour thickness of at least 1 mm and underwent surgery between 2006 and 2014. The study design, applied procedures and follow-up protocols have been described in detail elsewhere[4]. There was no overlap between the derivation cohort and validation cohort.

OUTCOMES

Outcomes of interest were first recurrence, first DM and OM. Time to recurrence was calculated from date of SLNB to date of first recurrence or date of death by any cause. Time to first DM was calculated from date of SLNB to date of first DM or date of death by any cause. Time to OM was calculated from date of SLNB to date of death by any cause.

STATISTICAL ANALYSIS

The checklist proposed by the AJCC was used for guidance in building a high-quality prediction model[18]. Associations between possible prognostic factors and recurrence were studied with Cox regression analysis. The following eight variables were identified as possible prognostic factors based on clinical experience, literature review and availability of sufficient data: sex, age, ulceration, location, histology, Breslow thickness, total number of SNs removed, and total number of positive SNs. To make efficient use of available data an advanced multiple imputation of missing values strategy (5 imputations) was applied[19]. This was done separately for each derivation centre to avoid using information of missings in cross-validation. The possible non-linearity of continuous variables was modelled by logarithmic transformation.

Independent prognostic factors were selected with multivariable backward selection. Linear predictor values (the sum of truncated predictor values times their predictor effects) were scaled and rounded to a risk score with integer values between 0 and 100. Because recurrence, DM and OM are strongly related, the final recurrence prediction model based on data from all nine EORTC centres was used as a basis for predicting DM and OM, where the baseline hazard and the slope of the recurrence prediction model were calibrated to DM and OM[20]. This approach is beneficial as it provides an unique risk score for each individual that translates into probabilities of all outcomes of interest, instead of developing three independent prediction models. In order to test the validity of our approach, we did develop these independent models and compared them with the calibrated models. The absolute risk prediction of each outcome was plotted against the risk score. To reduce overestimation of events occurring in patients with extremely high scores, scores were truncated at an integer of 22, corresponding to the 99th percentile of score distribution. Model performance was assessed by examining discrimination and calibration. Discrimination was measured using the concordance index (c-index); the closer to 1, the better the discrimination, and a value of 0.5 indicates that the model is no better than chance[21]. Calibration was assessed visually by plotting the predicted probability against the actual observed frequency in quintiles of predicted outcomes. A 45° line indicates perfect calibration (when the predictive value of the model perfectly matches the patient's actual risk). Any deviation above or below the 45° line indicates underprediction or overprediction respectively. A nomogram was developed for graphical presentation of the models. To evaluate generalizability of the models across different centres, an internal-external cross-validation was performed in which the model was fitted using data from eight centres and validated in the centre that was left out[22]. In addition, we performed external validation using the prospective German cohort. We first needed to develop a model for recurrence where we replaced the continuous variable SN tumour burden with the categorical substitute used in the prospective German cohort (single cells, <0.5 mm, 0.5 – 1.0 mm, >1.0 – 2.0 mm, >2.0 – 5.0 mm and >5.0 mm). For the derivation cohort, single cells were defined as <0.1 mm according to the Rotterdam criteria[23]. Single cells in the validation cohort were not specifically defined, but as the Rotterdam criteria were used for measuring SN tumour burden, definitions are likely to correlate. The performance of this altered model was compared to the final recurrence model used for the nomogram. Subsequently, the altered model was externally validated with the 692 patients from the prospective German cohort. To test how much the information on additional positive nodes retrieved after CLND would add to the discrimination of the prediction model, we also developed a prediction model in which the variable,

additional positive nodes after CLND, was added. This model was based on 1015 patients that underwent CLND in the derivation cohort.
Furthermore, we calculated the model performance for recurrence, DM and OM of the AJCC 7th edition classification, AJCC 8th edition classification and the simple classification that was published previously (i.e. absent/present ulceration and low/high SN tumour burden)[11]. Lastly, the observed outcomes per group for all classifications were estimated using the Kaplan Meier analysis. All statistical tests were two-sided, with a $P < 0.05$ considered statistically significant. All statistical analyses were performed using SPSS version 22.0 (IBM, Armonk, NEW York, USA) and R (version 2.15, R Foundation for Statistical Computing, Vienna, Austria, 2011).

RESULTS

The retrospective derivation cohort consisted of 1078 and the prospective validation cohort of 692 SN-positive patients. Patients in the validation cohort had less extensive disease in terms of Breslow thickness, number of positive SNs and tumour burden in the SN compared to those in the derivation cohort (**Table 1**).
In the derivation cohort, recurrence at five-years occurred in 496 patients (46.0%), DM in 437 patients (40.5%) and OM in 364 patients (33.8%). Median follow-up time for all survivors was 106 months (interquartile range (IQR) 61 – 130 months). In the prospective validation cohort, recurrence at five-years occurred in 267 patients (38.6%), DM in 223 patients (32.2%) and OM in 174 patients (25.1%). Median follow-up time for all survivors was 66 months (IQR 48 – 94 months).

MODELS FOR RECURRENCE, DISTANT METASTASIS AND OVERALL MORTALITY

The final multivariable Cox model for recurrence after backwards selection included four independent prognostic factors: ulceration, age, Breslow thickness, and SN tumour burden (**Table 2**). Logarithmic transformation of the continuous variables adequately represented their effects. The c-index for the final recurrence model was 0.68 (95% confidence interval (CI) 0.65 – 0.70). In cross-validation, the recurrence model was reasonably calibrated across nine centres in general, only in smaller centres there was substantial underestimation of the risk (**Supplementary Figure 1**).
The association between linear predictors of recurrence and DM was of the same size (calibration slope 1.01, 95% CI 0.87 – 1.16). The c-index for the calibrated model for DM was 0.70 (95% CI 0.67 – 0.72), and was reasonably calibrated across nine centres in cross-validation (**Supplementary Figure 2**).

Table 1 Baseline characteristics of derivation and validation cohort

Characteristic	Derivation cohort (n = 1078)	Validation cohort (n = 692)	P value[b]
Age, years[a]	*n = 1077*		<0.001[c]
	51 (40 - 62)	57 (46 - 68)	
Gender			<0.001
Female	509 (47.2)	267 (38.6)	
Male	569 (52.8)	425 (61.4)	
Breslow, mm[a]	*n = 1076*		<0.001[c]
	3.0 (1.9 - 4.8)	2.4 (1.6 - 4.0)	
Ulceration	*n = 1015*	*n = 596*	0.570
Absent	536 (52.8)	306 (51.3)	
Present	479 (47.2)	290 (48.7)	
Location			<0.001
Extremity	614 (57.0)	335 (47.0)	
Trunk	426 (39.5)	355 (51.3)	
Head & Neck	38 (3.5)	12 (1.7)	
Positive SNs	*n = 984*	*n = 690*	<0.001
1 node	775 (78.8)	623 (90.3)	
2 nodes	164 (16.7)	60 (8.7)	
>2 nodes	45 (4.6)	7 (1.0)	
SN tumour burden, mm	0.9 (0.4 – 2.5)	-	-
SN tumour burden, extended		*n =626*	<0.001
Single cells[d]	113 (10.5)	187 (29.9)	
<0.5 mm	221 (20.5)	57 (9.1)	
0.5 - 1.0 mm	235 (21.8)	208 (33.2)	
>1.0 - 2.0 mm	200 (18.6)	114 (18.2)	
>2.0 - 5.0 mm	195 (18.1)	36 (5.8)	
>5.0 mm	114 (10.6)	24 (3.8)	
SN tumour burden, simple		*n =626*	<0.001
≤ 1.0 mm	569 (52.8)	452 (72.2)	
> 1.0 mm	509 (47.2)	174 (27.8)	
CLND			<0.001
No	63 (5.8)	384 (55.5)	
Yes	1015 (94.2)	308 (44.5)	

Table 1 Continued

Characteristic	Derivation cohort (n = 1078)	Validation cohort (n = 692)	P value[b]
Positive non-SNs[e]	*n =1007*	*n =302*	0.088
None	804 (79.8)	229 (75.8)	
1 node	127 (12.6)	53 (17.5)	
>1 node	76 (7.5)	20 (6.6)	

CLND, completion lymph node dissection; IQR, interquartile range; SN, sentinel node. Values in parentheses are percentages unless indicated otherwise; [a]values are median (IQR). [b]Chi-square test, except [c]Mann-Whitney U test. [d]For the derivation cohort, single cells were defined as metastasis <0.1mm. [e]Information retrieved after CLND.

The performance of this calibrated model, based on the baseline hazard and the slope of the recurrence model, was similar to that of the independently developed prediction model for DM (c-index 0.70, 95% CI 0.68 – 0.73). The association between linear predictors of recurrence and OM was of the same size (calibration slope 1.04, 95% CI 0.88 – 1.20). The c-index for the calibrated model for OM was 0.70 (95% CI 0.67 – 0.73), and was reasonably calibrated across nine centres in cross-validation (**Supplementary Figure 3**). The performance of this calibrated model was similar to that of the independently developed prediction model for OM (c-index 0.70, 95% CI 0.68 – 0.73). A four-item risk score was developed, assigning points to each prognostic factor based on the magnitude of association with recurrence. A nomogram to calculate the score and the risk of recurrence, DM and OM is presented in **Figure 1**. The scores were divided into four risk groups based on the 5-year probability of recurrence: <25% (low risk; score 6-9; 4.1% of the population); 25 – 50% (intermediate risk; score 10-15; 52.9% of the population); 50 – 75% (high risk; score 16-19; 33.2% of the population); and >75% (very high risk; score 20-23; 10.0% of the population). The observed outcomes for recurrence, DM and OM per risk group are shown in **Table 3**.

Table 2 Final model for 5-year recurrence

	Hazard ratio	Lower 95	Upper 95
Age	1.28	1.12	1.45
Breslow	1.41	1.23	1.61
SN tumour burden	1.59	1.39	1.81
Ulceration			
Absent	Reference		
Present	1.41	1.16	1.73

SN, sentinel node

EXTERNAL VALIDATION

For external validation purposes, an altered recurrence model was developed using the categorized SN tumour burden variable used in the prospective German cohort (**Supplementary Table 1**). This altered model showed similar performance compared to the final recurrence model (c-index 0.68, 95% CI 0.65 – 0.70). In external validation, the c-index for the altered recurrence model was 0.70 (95% CI 0.67 – 0.74), for DM 0.72 (95% CI 0.68 – 0.75) and for OM 0.74 (95% CI 0.71 – 0.78). The calibration plots indicate good calibration, though there may be slight underestimation for higher risk patients in the recurrence and OM models (**Supplementary Figure 4**).

ADDITIONAL PROGNOSTIC VALUE OF CLND

An extended model for recurrence was created by adding the variable, number of additional positive nodes after CLND, to the final recurrence model. This extended model for recurrence had a c-index of 0.69 (95% CI 0.67 – 0.72). The calibrated extended models for DM and OM showed c-indices of 0.72 (95% CI 0.69 – 0.74) and 0.72 (95% CI 0.69 – 0.75), respectively.

SIMPLE CLASSIFICATION

A simplified version of the model stratifies patients into four groups based on ulceration and SN tumour burden: 1) absent ulceration and ≤ 1.0 mm; 2) absent ulceration and > 1.0 mm; 3) present ulceration and ≤ 1.0 mm; 4) present ulceration and > 1.0 mm[11]. The c-indices for this classification in predicting recurrence, DM and OM were 0.63 (95% CI 0.61 – 0.65), 0.64 (95% CI 0.62 – 0.67) and 0.64 (95% CI 0.61 – 0.67), respectively. The observed outcomes for recurrence, DM and OM per risk group are shown in **Table 3**.

AMERICAN JOINT COMMITTEE ON CANCER (AJCC) CLASSIFICATIONS

Patients were classified based on the 7th AJCC classification into IIIA ≤ 1.0 mm, IIIA >1.0 mm, IIIB and IIIC and based on the 8th edition into IIIA ≤ 1.0 mm, IIIA >1.0 mm, IIIB, IIIC and IIID. The c-indices for predicting recurrence, DM and OM for the 7th AJCC edition were 0.61 (95% CI 0.59 – 0.63), 0.62 (95% CI 0.60 – 0.65) and 0.62 (95% CI 0.59 – 0.65), respectively, and for the 8th AJCC edition 0.62 (95% CI 0.59 – 0.64), 0.63 (95% CI 0.60 – 0.65) and 0.63 (95% CI 0.61 – 0.66), respectively. The observed outcomes for recurrence, DM and OM for both AJCC classifications are shown in **Table 3**. A cross-table comparing the patients staged according to the AJCC classifications and the risk groups based on the EORTC-DeCOG model is illustrated in **Table 4**. An overview of c-indices for all the different models is presented in **Table 5**.

Table 3 Observed outcomes per classification in the derivation cohort (95% CI)

Risk groups	Recurrence	Distant metastasis	Overall mortality
EORTC-DeCOG model			
Low risk (<25% recurrence)	0.13 (0.06-0.20)	0.10 (0.04-0.16)	0.07 (0.02-0.13)
Intermediate risk (25-50% recurrence)	0.38 (0.33-0.43)	0.31 (0.26-0.36)	0.25 (0.21-0.30)
High risk (50-75% recurrence)	0.61 (0.56-0.66)	0.55 (0.49-0.60)	0.49 (0.43-0.54)
Very high risk (>75% recurrence)	0.82 (0.73-0.88)	0.78 (0.69-0.84)	0.70 (0.61-0.77)
Simple classification			
Group 1	0.32 (0.26-0.36)	0.26 (0.21-0.30)	0.21 (0.16-0.25)
Group 2	0.52 (0.44-0.58)	0.48 (0.40-0.54)	041 (0.33-0.47)
Group 3	0.49 (0.41-0.55)	0.42 (0.34-0.48)	0.35 (0.28-0.42)
Group 4	0.73 (0.67-0.77)	0.69 (0.63-0.74)	0.60 (0.53-0.66)
AJCC 7th edition			
IIIA ≤ 1.0 mm	0.32 (0.26-0.37)	0.25 (0.20-0.30)	0.20 (0.15-0.25)
IIIA >1.0mm	0.50 (0.42-0.57)	0.46 (0.38-0.53)	0.40 (0.32-0.46)
IIIB	0.63 (0.58-0.67)	0.57 (0.52-0.62)	0.49 (0.44-0.53)
IIIC	0.60 (0.02-0.84)	0.62 (0.02-0.85)	0.63 (0.02-0.86)
AJCC 8th edition			
IIIA ≤ 1.0 mm	0.27 (0.20-0.34)	0.21 (0.15-0.28)	0.15 (0.09-0.21)
IIIA >1.0mm	0.37 (0.23-0.49)	0.34 (0.20-0.46)	0.27 (0.14-0.38)
IIIB	0.43 (0.36-0.48)	0.35 (0.29-0.41)	0.30 (0.24-0.36)
IIIC	0.64 (0.59-0.68)	0.48 (0.53-0.63)	0.50 (0.45-0.55)
IIID	0.66 (0.00-0.90)	0.68 (0.00-0.91)	0.70 (0.00-0.92)

AJCC, American Joint Committee on Cancer; CI, confidence interval;

Table 4 Cross-table comparing EORTC-DeCOG risk groups with the 7th and 8th AJCC classification, based on 937 complete cases

	AJCC 7th classification				
EORTC-DeCOG classification	IIIA ≤1.0mm	IIIA >1.0mm	IIIB	IIIC	Total
Low risk (score 6-9)	82	2	5	0	89
Intermediate risk (score 10-15)	207	83	93	2	385
High risk (score 16-19)	15	89	230	1	335
Very high risk (score 20-23)	0	11	113	4	128
Total	304	185	441	7	

	AJCC 8th classification					
EORTC-DeCOG classification	IIIA ≤1.0mm	IIIA >1.0mm	IIIB	IIIC	IIID	Total
Low risk (score 6-9)	62	3	22	2	0	89
Intermediate risk (score 10-15)	96	43	157	89	0	385
High risk (score 16-19)	0	10	71	253	1	335
Very high risk (score 20-23)	0	0	2	122	4	128
Total	158	56	252	466	5	937

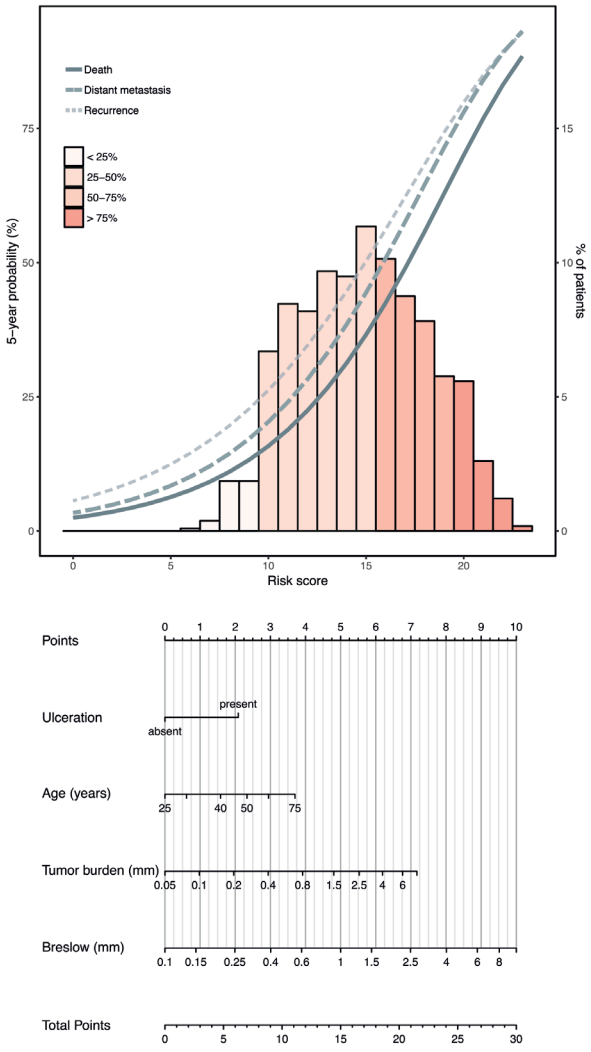
AJCC, American Joint Committee on Cancer

Table 5 C-indices with 95% confidence intervals for the different prediction models

	Recurrence	Distant metastasis[a]	Overall mortality[a]
EORTC-DeCOG prediction model	0.68 (0.65 – 0.70)	0.70 (0.67 – 0.72)	0.70 (0.67 – 0.73)
EORTC-DeCOG altered model			
Derivation cohort	0.68 (0.65 – 0.70)	0.70 (0.67 – 0.72)	0.70 (0.67 – 0.73)
External validation	0.70 (0.67 – 0.74)	0.72 (0.68 – 0.75)	0.74 (0.71 – 0.78)
EORTC-DeCOG extended model	0.69 (0.67 – 0.72)	0.72 (0.69 – 0.74)	0.72 (0.69 – 0.75)
EORTC-DeCOG simple classification	0.63 (0.61 – 0.65)	0.64 (0.62 – 0.67)	0.64 (0.61 – 0.67)
AJCC 7th edition[b]	0.61 (0.59 – 0.63)	0.62 (0.60 – 0.65)	0.62 (0.59 – 0.65)
AJCC 8th edition[c]	0.62 (0.59 – 0.64)	0.63 (0.60 – 0.65)	0.63 (0.61 – 0.66)

[a] Calibrated models; [b] For IIIA ≤1.0 mm, IIIA >1.0mm, IIIB, IIIC; [c] For IIIA ≤1.0 mm, IIIA >1.0mm, IIIB, IIIC, IIID.
AJCC, American Joint Committee on Cancer; SN, sentinel node

Figure 1 Nomogram and risk distribution



The curves refer to predicted recurrence, distant metastasis or overall mortality at 5 years. The histogram refers to the risk score distribution in the cohort; each bar represents the proportion of patients in the cohort that was assigned that specific score. The histogram was divided in four risk groups based on the risk of recurrence: low risk: <25%, intermediate risk: 25-50%, high risk: 50-75% and very high risk: >75%. The nomogram incorporates four factors: ulceration, age, SN tumour burden and Breslow thickness. To calculate an individual's probability of 5-year recurrence, distant metastasis and overall mortality, values for the prognostic factors must be determined first (for example: absent ulceration; 35 years; SN tumour burden 0.8 mm, Breslow thickness 1.0 mm). Second, for each value the corresponding points can be obtained by drawing a line from each value towards the points axis (in example: 0, 1, 4 and 5 points respectively). Third, the points must be added up to obtain the total risk score (in example: risk score of 10). Finally, the 5-year recurrence, distant metastasis and overall mortality probability can be read by moving vertically from the x-axis (total risk score) to the predicted risk curves and corresponding probabilities on the left y-axis (in example: 26% for recurrence, 20% for distant metastasis and 16% for overall mortality). The percentage of patients in the entire population (1078) that also had a total risk score of 10 can be determined from the histogram, as well as the corresponding percentage of patients on the right y-axis (in example: 7%).

DISCUSSION

The present study developed and validated a nomogram to predict five-year recurrence, DM and OM in patients with SN-positive melanoma, by solely using information from the primary melanoma and the SLNB. The resulting patient-specific probabilities could be used to tailor adjuvant therapeutic strategies for patients with SN-positive melanoma, without the prerequisite to undergo CLND and thereby avoiding potential significant morbidity. The greatest contemporary value of our prognostic nomogram is the possibility of identifying patients at sufficiently low risk for recurrence, DM and OM in whom adjuvant therapy could be omitted.

Although the FDA and EMA pragmatically approved adjuvant therapy for all stage III patients, it is still under debate which patients should not be considered candidates. Patients with stage IIIA ≤ 1.0 mm (AJCC 7th edition) were considered low risk in most adjuvant therapy trials and were therefore not included (one even excluded all IIIA patients)[7-9, 24, 25]. The current study indicates that when the AJCC 8th edition criteria are used for defining IIIA ≤ 1.0 mm instead of the 7th edition, it results in improved selection of low risk patients in terms of predicted prognosis (e.g. 5-year recurrence probability of 27% versus 32%, respectively). A recent study also showed that including SN tumour burden to the 8th AJCC staging system has crucial prognostic relevance[26]. Of note, our EORTC-DeCOG model is able to identify an even more robust low risk group, as all identified low risk patients (which approximately concerned 4% of the entire population after imputation) had a 5-year recurrence probability of <25% and an overall 5-year observed recurrence rate of 13%. However, identifying more robust low risk groups comes at the cost of fewer patients being assigned low risk (see **Table 4**). Nonetheless, a major advantage of our EORTC-DeCOG model is that it provides a more continuous type of predicted probabilities. As a result it is possible to derive risk groups based on outcome probabilities and/or risk scores (e.g. low risk; scores 6-9; recurrence probability of <25%) which is in contrast to the AJCC classifications where exact patient/tumour characteristics define the risk groups (e.g. IIIA ≤ 1.0 mm: T1a/b-T2a + N1a-N2a with ≤ 1.0 mm SN tumour burden). In the current study we choose to derive risk groups based on the recurrence probability, as this seems the most relevant outcome in the context of selecting patients for adjuvant therapy. But, other cut-off values and/or outcomes are possible. In conclusion, the EORTC-DeCOG model not only outperforms the AJCC classifications in terms of overall model discrimination (see **Table 5**), but also seems to be able to identify a more robust low risk group in whom it may be justified to forego adjuvant therapy.

The previously published simplified model, based on ulceration and SN tumour burden, harboured the least performance, though still reasonable, and showed similar predicted prognosis for the low risk group as the 7th AJCC edition. Whether to implement a more complex model versus a less robust model is a balance between performance and simplicity. In our opinion, the simple model could serve as an easy user-friendly prognostic tool for daily clinical practice and to generally inform patients, but for more adequate risk estimates and decisions upon (adjuvant) treatment, we advocate to use the comprehensive EORTC-DeCOG model. Noteworthy, besides the common prognostic factors (i.e. ulceration, Breslow thickness and SN tumour burden), the current study also identified increasing age as an independent prognostic factor for recurrence, DM and OM. This finding is supported by other studies reporting on the significance of the patient's age[27].

Stratifying for ulceration and SN tumour burden only, was previously demonstrated to yield similar discriminatory ability for melanoma-specific mortality as stratifying for AJCC substages which included information on nodal status after CLND[11]. The additional value of non-SN status retrieved after CLND was also tested in the current study, by developing an extended model. This model showed only marginal improvement in performance (e.g. c-index for the recurrence model increased from 0.68 to 0.69), thereby indicating that omitting CLND has very limited consequences for prognostication if SN tumour burden is taken into account.

This study has several limitations. First is the retrospective design of the derivation cohort, which has inherent biases. However, the models proved to be successful in external validation. Performance was comparable between the derivation and prospective validation cohort, even though the latter cohort included patients with relatively better prognosis (e.g. less extensive disease) and largely represents a clinical trial population. Adjuvant interferon-α therapy was intended in approximately 60% of the patients included in the DeCOG-SLT trial, which is another possible limitation[4]. It could have potentially influenced outcomes, especially in patients with ulcerated melanomas as ulceration seems to be a predictive factor for IFN sensitivity[28, 29]. Furthermore, it is unknown how many patients in the validation cohort received effective novel therapy after recurrence. Since patients were included from 2006 through 2014, it is likely some patients did. As patients in the derivation cohort were included from 1993 through 2008, novel therapies probably had limited effect. To date, no novel biomarker has been validated that suffices to predict long-term clinical benefits and subsequently could be incorporated in the models, despite efforts in this direction (e.g. PD-L1)[30]. In addition, other prognostic factors such as mitotic rate or microsatellites could not be incorporated in the present models due to insufficient data. Another limitation is the

inadequate representation of SN-positive patients with a head and neck melanoma in both cohorts. For the validation cohort this is largely explained as it was an exclusion criterion in the DeCOG-SLT trial, and for the derivation cohort this might be partially explained by the historical concerns of poor safety, accuracy and prognostication. Similar numbers (~5%) have been reported in other European cohorts[31, 32] , while particularly American cohorts have reported higher numbers (>10%)[3, 33]. With the introduction of adjuvant therapies, the number of performed SLNBs in head and neck melanomas is likely to increase.

Considering the advances in the management of SN-positive melanoma patients, it becomes highly relevant to have a prediction model that provides precise patient-specific probabilities based on solely factors from the primary melanoma and the SLNB. The EORTC-DeCOG nomogram is the first that meets these demands, and as a result it could be used for patient counselling and assist in trial design. In addition, it might aid in adjuvant therapy decision-making. To facilitate its use, an online calculator has been developed and can be accessed at www.evidencio.com/models/show/2010.

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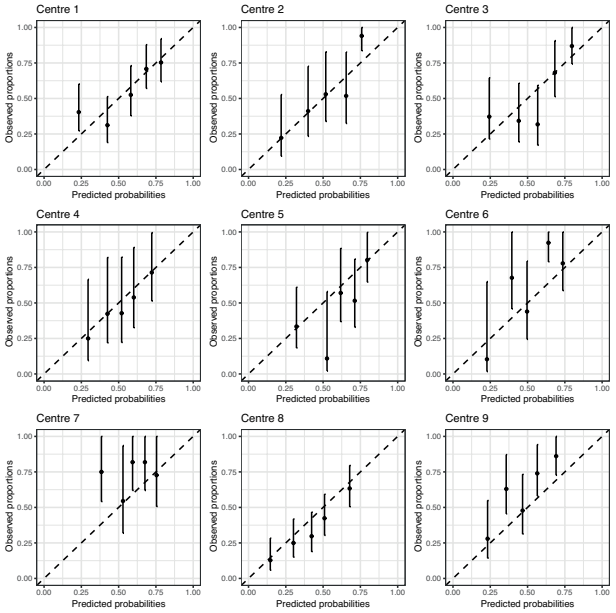
SUPPLEMENTARY MATERIAL

Supplementary Tabel 1 Altered model for 5-year recurrence

	Hazard ratio	Lower 95	Upper 95
Age	1.28	1.13	1.46
Breslow	1.42	1.24	1.63
SN tumour burden category			
Single cells	0.47	0.30	0.74
<0.5 mm	0.84	0.62	1.13
0.5 – 1.0 mm	Reference		
>1.0 – 2.0 mm	1.19	0.90	1.57
>2.0 – 5.0 mm	1.57	1.20	2.06
>5.0 mm	1.64	1.21	2.23
Ulceration			
Absent	Reference		
Present	1.42	1.16	1.73

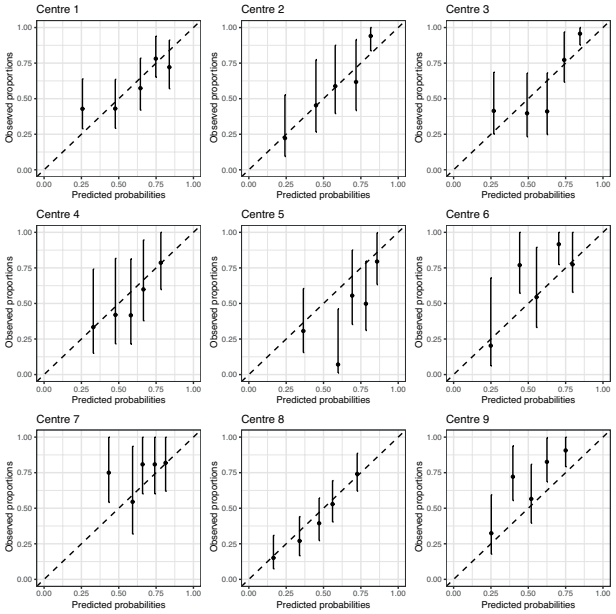
SN, sentinel node

Supplementary Figure 1 Cross validation for the final recurrence model



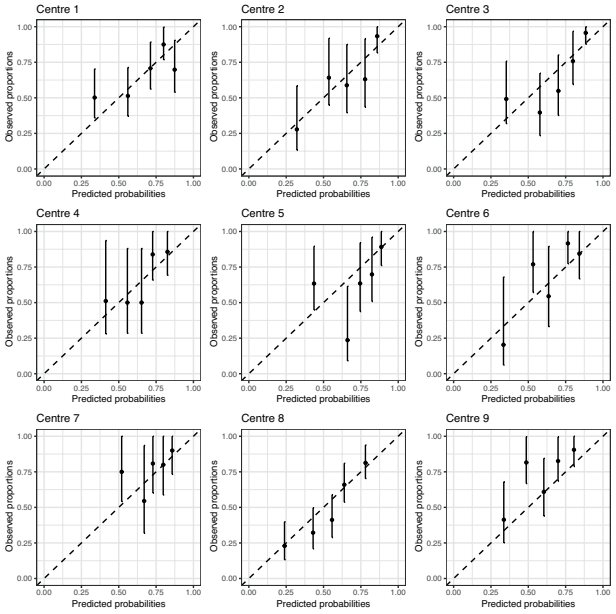
Calibration plots for the final recurrence model. The predicted probability is plotted on the x-axis, the actual probability on the y-axis. A plot along the 45° line would indicate perfect calibration in which the predicted probabilities were identical to the actual outcomes

Supplementary Figure 2 Cross validation for the final distant metastasis model



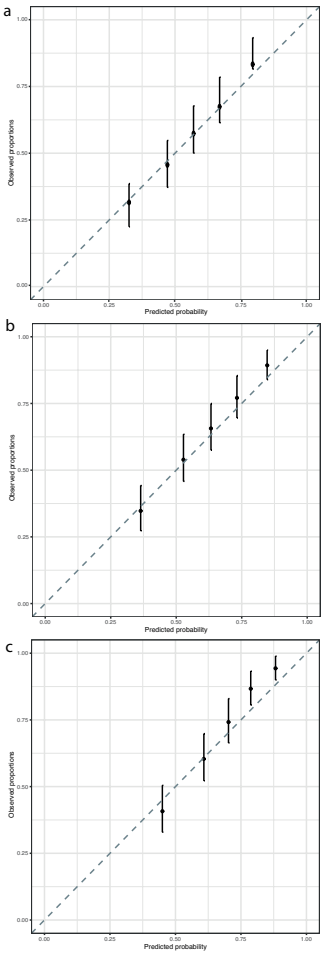
Calibration plots for the final distant metastasis model. The predicted probability is plotted on the x-axis, the actual probability on the y-axis. A plot along the 45° line would indicate perfect calibration in which the predicted probabilities were identical to the actual outcomes

Supplementary Figure 3 Cross validation for the final overall mortality model

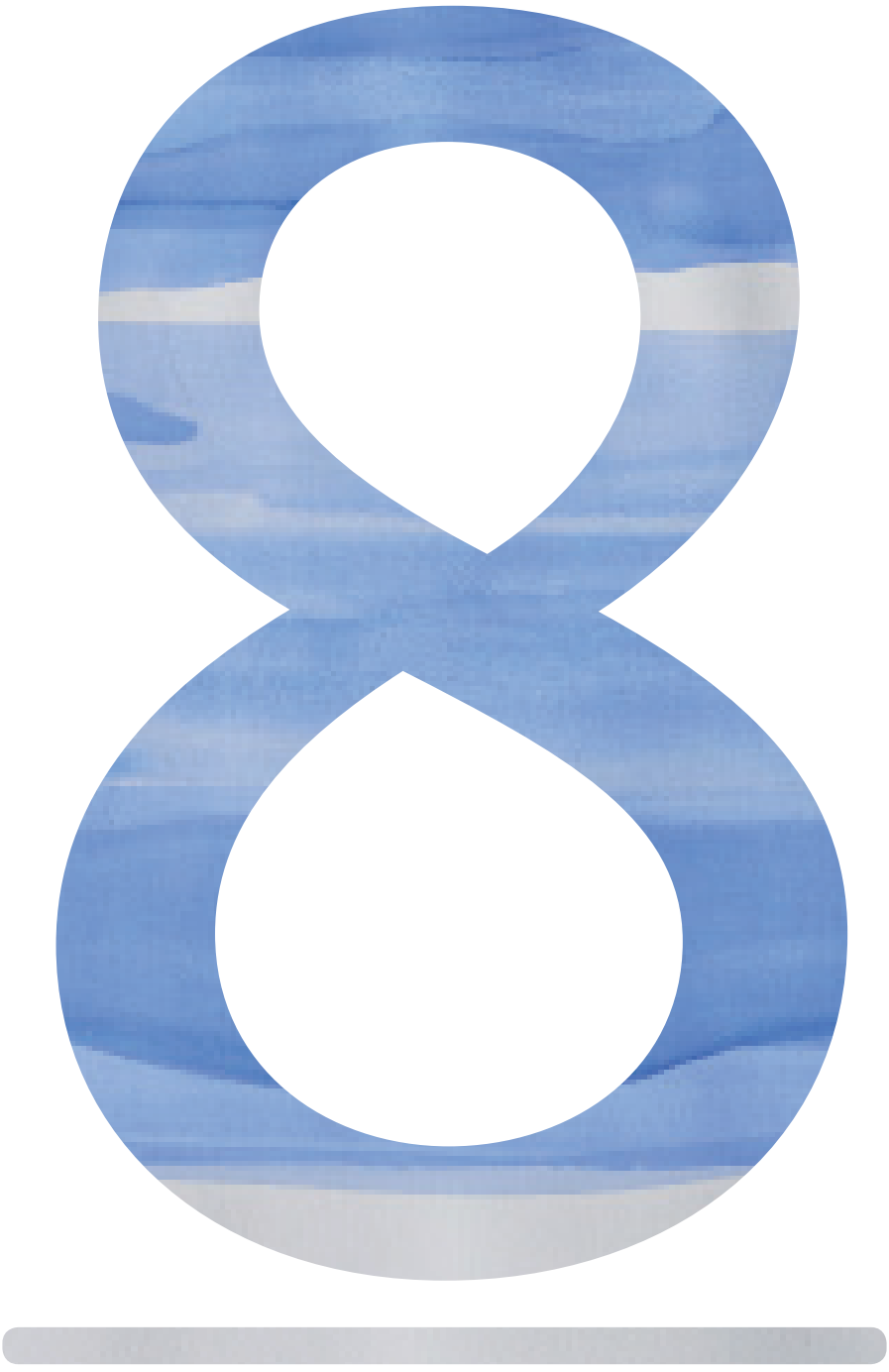


Calibration plots for the final overall mortality model. The predicted probability is plotted on the x-axis, the actual probability on the y-axis. A plot along the 45° line would indicate perfect calibration in which the predicted probabilities were identical to the actual outcomes

Supplementary Figure 4 Calibration plots external validation



Calibration plots in external validation (A, recurrence; B, distant metastasis; C, overall mortality). The predicted probability is plotted on the x-axis, the actual probability oå the y-axis. A plot along the 45° line would indicate perfect calibration in which the predicted probabilities were identical to the actual outcomes.



Upregulation of intratumoral HLA class I and peritumoral Mx1 in ulcerated melanomas

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ABSTRACT

Before the era of immune checkpoint blockade, a meta-analysis encompassing fifteen trials reported that adjuvant IFN-α significantly reduces the risk of relapse and improves survival of ulcerated melanoma (UM) with no benefit for higher doses compared to lower doses. IFN-α-2b affects many cell intrinsic features of tumor cells and modulates the host innate and cognate immune responses. To better understand the biological traits associated with ulceration that could explain the efficacy of prophylactic type 1 IFN, we performed immunohistochemical analysis of various molecules (major histocompatibility complex class (MHC) I and class II, MX Dynamin Like GTPase 1 (Mx1), inducible Nitric-Oxide Synthase (iNOS) or CD47) in two retrospective cohorts of melanoma patients, one diagnosed with a primary cutaneous melanoma (1995–2013, N = 172, among whom 49% were ulcerated melanoma (UM)) and a second one diagnosed with metastatic melanoma amenable to lymph node resection (EORTC 18952 and 18991 trials, N = 98, among whom 44% were UM). We found that primary and metastatic UM exhibit higher basal expression of MHC class I molecules, independently of Breslow thickness, histology and lymphocytic infiltration compared with NUM and that primary UM harbored higher constitutive levels of the antiviral protein Mx1 at the border of tumor beds than NUM. These findings suggest that UM expand in a tumor microenvironment where chronic exposure to type 1 IFN could favor a response to exogenous IFNs.

INTRODUCTION

Ulceration of primary melanoma has first been recognized as a poor prognostic factor in 1953[1] and has been steadily incorporated in the American Joint Committee on Cancer (AJCC) staging systems since 2001[2–4]. It was originally defined as the absence of intact epidermis overlying a major portion of the primary melanoma, but this definition was refined in 2003 based on the observation that ulceration seems to be more than just the loss of epidermal lining[5]. Besides a full-thickness epidermal defect, including absence of stratum corneum and basement membrane, there must also be evidence of a host response (i.e. fibrin deposition, neutrophils) and thinning, effacement or reactive hyperplasia of the surrounding epidermis[5]. This definition allows to better distinguish artefactual causes such as biopsy trauma, scratching or technical issues during the preparation of the slides, from actual tumor ulceration. In addition, it firstly implied that an ulcerated melanoma might represent more than just a phenotype, or in other words a biologic entity.

Over the years, researchers have made several attempts to unravel the biological significance of ulcerated melanomas. Several differences have been observed in terms of histopathological, genetic and immunological findings. A common histopathological observation is that ulcerated melanomas have an increased tumor vascularity[6-9] and are associated with loss of cell-to-cell adhesion[10]. Also, ulcerated melanomas have been shown to have a different gene profile than non-ulcerated melanomas[8,11]. Furthermore, the immune microenvironment seems distinct between non-ulcerated and ulcerated melanomas, since ulcerated melanomas have been correlated with the presence of lymphatic invasion[8], increased density of neutrophils, mainly in the superficial part of the tumor[10,12], stronger infiltration of CD11c+ dendritic cells[13], greater intratumoral macrophage count[8,13] and an increased number of PD-L1 positive tumors[13], which all may contribute to promoting invasion and tumor cell dispersal. They have also been associated with a lower mature dendritic cell density in sentinel lymph nodes[14].

Ulceration as a specific biological entity, represents a very strong prognostic factor associated with dissemination or relapse in stage I, II and III melanoma, and therefore is integrated in multivariable analyses during all systemic therapies currently in randomized trials. Hence, ulcerated primary melanomas seem to exclusively benefit from adjuvant interferon (IFN). Indeed, adjuvant IFN-α significantly reduces the risk of relapse and improves survival with no benefit for higher doses compared to lower doses. This finding was first addressed in the final report of the European Organization for Research and Treatment of Cancer (EORTC) 18991 trial, where 1256 patients

with stage III melanoma were randomized between adjuvant pegylated-IFN and observation only[15]. Similar findings were published regarding high dose IFN therapy in the Sunbelt Trial[16]. Later, it was confirmed in long term outcomes in the EORTC 18991 and EORTC 18952 trials, where 1388 patients with stage IIB-III melanoma were randomized between adjuvant IFN-α and observation only[17–20]. The most definite evidence that ulceration may be predictive of response to IFN comes from a recent meta-analysis of all available data from 15 randomized trials of adjuvant IFN versus observation[21].

Type 1 IFNs exhibit cell autonomous and host related fundamental functions for tumor immunosurveillance, including cell proliferation, angiogenesis, antigen presentation, and B and T cell differentiation[22]. To further unravel some mechanistic features associated with ulceration that could pave the way for a better and elective therapeutic efficacy of IFN in melanomas, we aimed to explore whether there are significant differences between non-ulcerated melanoma (NUM) and ulcerated melanoma (UM) in the immunohistochemical expression profile for markers related to tumor antigen presentation [Major Histocompatibility Complex Class I and II (HLA class I and class II)], indicative of intrinsic type I IFN signaling [MX Dynamin Like GTPase 1 (Mx1)], associated with cytotoxic activity of macrophages [inducible Nitric-Oxide Synthase (iNOS)], or involved in the anti-phagocytotic pathway (CD47).

METHODS

SAMPLE COHORTS

Primary melanomas from institute gustave-roussy

The study group was derived from 1373 consecutive resections of primary cutaneous melanomas between October 1995 and October 2013 from the Institute Gustave-Roussy. Samples were excluded based on chart review (e.g. wrong coding, history of resection with previous biopsy, mucosal melanoma, in situ melanoma, Breslow thickness <1.0 mm) and based on pathology review (e.g., biopsies, non-confirmed ulceration status, traumatic ulceration, and Breslow thickness >10.0 mm). A total of 172 samples were eligible for staining of which 87 samples represented NUM and 85 UM. Data on patient characteristics (e.g., age, gender) and tumor characteristics (e.g. ulceration, Breslow thickness, histology) were retrieved from electronic patient files. Follow-up information was not available. Codes were used to maintain patient confidentiality throughout the study. Human archival tissue specimens were used. Informed consent was obtained prior to storage of the human tissue specimens.

Lymph nodes from EORTC 18952 & 18991 trials

The EORTC 18952 trial evaluated the role of intermediate doses of regular IFN-α2b in 1388 stage IIB-III patients compared with observation only[18,19]. The EORTC 18991 trial evaluated pegylated-IFN (PEG-IFN) in 1256 stage III patients compared with observation only[15,20]. From both trials, 98 positive lymph nodes were selected, of which 55 samples belonged to patients with NUM and 43 to UM. Data on patient characteristics (e.g., age, gender) and tumor characteristics (e.g. ulceration, Breslow thickness, histology, microscopic or macroscopic involvement) were retrieved. Follow-up information was not retrieved.

IMMUNOHISTOCHEMICAL STAINING

Formalin-fixed paraffin-embedded tumor samples were stained with hematoxylin-eosin (H&E) and assed immunohistochemically on a Ventana BenchMark Ultra Platform, using antibodies against HLA class I (clone: EMR8-5, supplier: MBL, catalog: D226-3, source: mouse, concentration: 1:2000), antibodies against HLA class II (clone: CR3/43, supplier: Dako, catalog: M0775, source: mouse, concentration: 1:200), antibodies against Mx1 (clone: polyclonal, supplier: Sigma, catalog: HPA030917, source: rabbit, concentration: 1:100), antibodies against iNOS (clone: L14-B, supplier: Creative Diagnostics, catalog: DMAB5712RH, source: rabbit, concentration: 1:420) and antibodies against CD47 (clone: polyclonal, supplier: Sigma, catalog: HPA044659, source: rabbit, concentration: 1:50). An UltraView detection kit was used with diaminobenzidine (DAB) as a chromogen for HLA class I and alkaline phosphatase (AP) red as a chromogen for HLA class II, Mx1, iNOS and CD47. Human colon carcinoma tissue sections were used as positive control for HLA class I, HLA class II and iNOS. Human pancreas tissue sections were used as positive control for Mx1 and CD47. Negative controls were obtained by omitting the primary antibody.

IMMUNO-HISTOCHEMICAL SCORING

Evaluation of immunohistochemistry was performed by two pathologists (KC and GT), who were blinded to the subject's ulceration status. Disagreement was resolved by discussion. For each slide objective fields at 5x, 10x, 20x or 40x magnification were reviewed using a Leica DM 2000 microscope. Immunohistochemical staining of the primary melanomas was evaluated in two ways: 1) evaluation of intratumoral staining using semi-quantitative scoring for the intensity of positive staining (0, negative; 1, weak; 2, moderate; 3, strong) and for the percentage of positive staining (0–100%). A cumulative "H-score" was obtained by multiplying the intensity score (0–3) by the percentage of positive staining (0–100%; with any intensity of staining). The final score

ranges from 0 to 300. This approach gives more weight to higher-intensity staining in a given tumor sample; and 2) evaluation of staining in the peritumoral epidermis adjacent to and/or overlying the primary melanoma, by assigning the following score: absent, weak, moderate or strong. In addition, lymphocyte infiltrate was evaluated on the H&E slides, and the following score was assigned: diffuse, (pluri)focal or absent. Diffuse was defined as presence of lymphocytes throughout the vertical growth phase or across the entire base of the tumor. (Pluri)focal was defined as lymphocytes focally infiltrating the tumor. Absent was defined as absent lymphocytes or when they were present but not infiltrating the tumor. Immunohistochemical staining of the metastatic lymph nodes was evaluated using only the semi-quantitative H-score.

STATISTICAL ANALYSES

Continuous data according to the ulceration status were presented as median with interquartile range (IQR) and statistically compared using the Mann-Whitney U test. Categorical data according to the ulceration status were presented by number and percentage and statistically compared using the chi-square test. Associations between intra- and peritumoral staining in primary melanomas and ulceration status, were tested in multivariable logistic regression models, adjusting for Breslow thickness, histology and TILs. Associations between intratumoral staining in lymph nodes and ulceration status, were also tested in multivariable logistic regression models, adjusting for Breslow thickness and type of lymph node involvement. Associations with ulceration status were reported as odds ratios (OR) and their 95% confidence intervals (CI), and the corresponding P-values. For all statistical tests the threshold for significance was set a $P < .05$. All statistical analyses were performed in SPSS, version 24.

RESULTS

TIL INFILTRATION DOES NOT CONTRAST ULCERATED FROM NON-ULCERATED PRIMARY MELANOMA

We analyzed two retrospective cohorts of melanoma bearing patients, one diagnosed with a primary cutaneous melanoma (1995–2013, N = 172) and a second one diagnosed with metastatic melanoma amenable to lymph node resection (EORTC 1895218[19] and 1899115[20] trials, N = 98).

The primary melanoma cohort from Gustave Roussy was composed of 172 patients, of which 87 patients had NUM and 85 UM (**Table 1**). Median Breslow thickness was significantly higher in UM compared with NUM, namely 4.00 mm (IQR 2.55–6.00) and 1.70 mm (IQR 1.30–3.20), respectively ($P < .001$). In UM and NUM, a diffuse TIL infiltrate was observed in similar frequencies, namely 25.0% (21/84) and 15.7% (13/83), respectively ($P = .33$). When adjusted for Breslow thickness and histology, there was still no association between ulceration status and TIL infiltrate (**Table 2**).

The metastatic lymph node (LN) cohort was composed of 95 patients who participated in the EORTC 18952 & 18991 trials, of which 53 patients had NUM and 42 UM (**Table 1**). Median Breslow thickness was significantly higher in UM compared with NUM, namely 3.05 mm (IQR 2.05–6.01) and 1.95 mm (IQR 1.02–3.08), respectively ($P < .001$). For both UM and NUM, most patients had macroscopic nodal disease, namely 73.8% (31/42) and 81.1% (43/53), respectively ($P = .39$).

In two independent cohorts of early stage melanoma, ulceration characterized 44–49% cases, was associated with higher Breslow thickness but not distinguishable from the non-ulcerated lesions according to T cell infiltration.

MHC CLASS I MOLECULES ARE OVEREXPRESSED IN ULCERATED MELANOMA

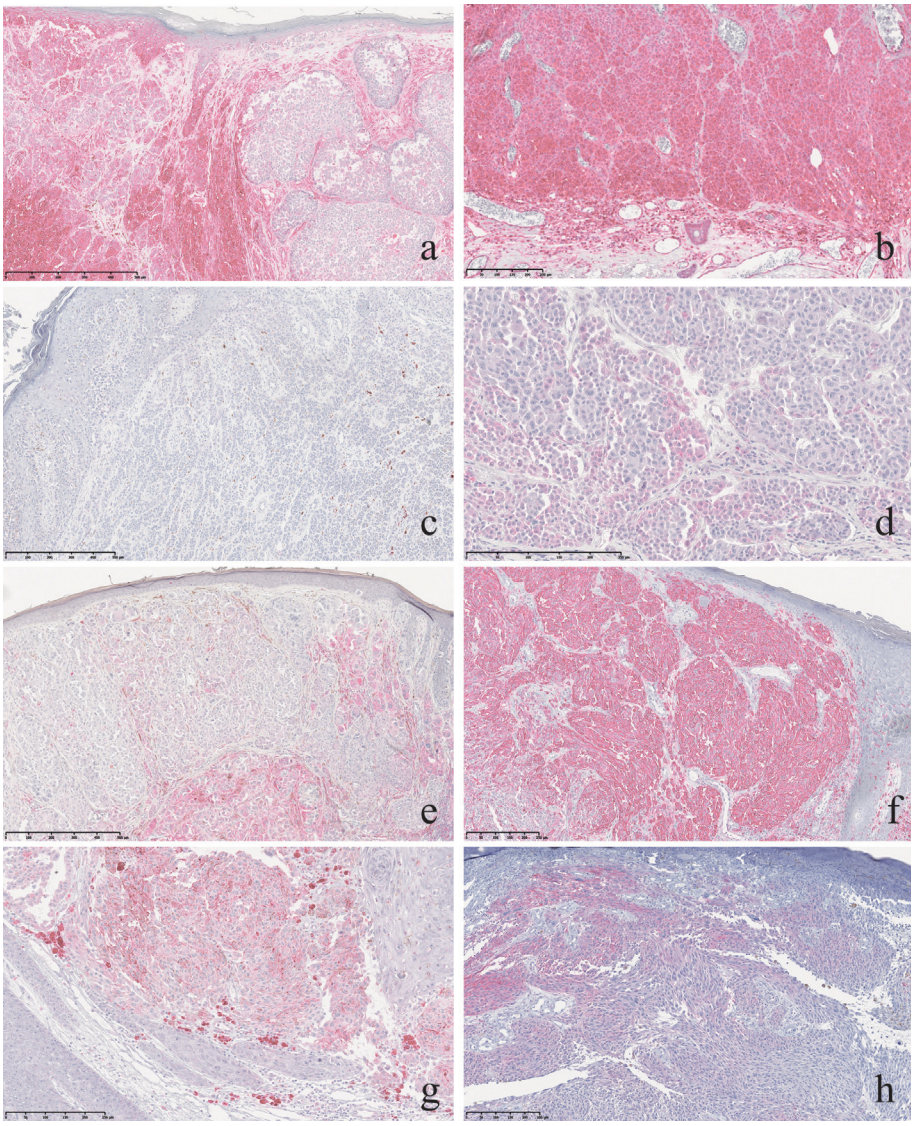
MHC class I expression is gradually lost with tumor progression[23], as a result of positive selection following CTL pressure[24–27]. Type 1 IFNs are known to restore or upregulate transcription and translation of MHC molecules on epithelial and antigen presenting cells[28,29]. Hence, we explored intra- and peri-tumoral expression of MHC class I molecules in NUM and UM in our cohorts (examples in **Figure 1**). In the primary melanoma cohort, the median H-score for intratumoral HLA class I expression, which takes into account staining intensity as well as percentages of positive cells, tended to be significantly higher in UM than in NUM, namely 208 (IQR 120–260) versus 180 (IQR 83–230), respectively, ($P = .05$) (**Table 3**, **Figure 2**). In multivariable logistic regression analysis, the H-score for intratumoral HLA class I expression was independently associated with UM ($P = .004$) (**Table 3**).

Table 1 Baseline characteristics

	Primary melanomas Institute Gustave Roussy			Lymph nodes EORTC 18952 & 18991 trials		
	NUM (N = 87)	UM (N = 85)	P-value[b]	NUM (N = 53)	UM (N = 42)	P-value[b]
Age[a]	-	-	-	48 (42 - 57)	51 (39 - 61)	0.460[c]
Gender			0.882			0.711
Male	42 (48.3)	42 (49.4)		27 (50.9)	23 (54.8)	
Female	45 (51.7)	43 (50.6)		26 (49.1)	19 (45.2)	
Histology	*n = 82*	*n = 83*	0.006			-
SSM	59 (72.0)	40 (48.2)		-	-	
NM	6 (7.3)	15 (18.1)		-	-	
Other	17 (20.7)	28 (33.7)		-	-	
Breslow thickness, mm[a]	1.70 (1.30 - 3.20)	4.00 (2.55 - 6.00)	<0.001[c]	*n = 52* 1.95 (1.02 - 3.08)	*n = 41* 3.05 (2.06 - 6.01)	<0.001[c]
TILs	*n = 84*	*n = 83*	0.325			-
Absent	33 (39.3)	37 (44.6)		-	-	
(Pluri)focal	30 (35.7)	33 (39.8)		-	-	
Diffuse	21 (25.0)	13 (15.7)		-	-	
LN involvement			-			0.393
Microscopic	-	-		10 (18.9)	11 (26.2)	
Macroscopic	-	-		43 (81.1)	31 (73.8)	
Reliable staining			-			-
HLA class I	80 (92.0)	84 (98.8)		51 (96.2)	40 (95.2)	
HLA class II	84 (96.6)	83 (97.6)		-	-	
Mx-1	80 (92.0)	83 (97.6)		49 (92.5)	41 (97.6)	
iNOS	83 (95.4)	83 (97.6)		-	-	
CD47	84 (96.6)	82 (96.5)		-	-	

LN, lymph node; NM, nodular melanoma; SSM, superficial spreading melanoma; TILs, tumor infiltrating lymphocytes. [a]median with interquartile range. [b]Chi-square test, unless indicated otherwise: Mann-Whitney U test.

Figure 1 Examples of different immunohistochemical expressions in non-ulcerated primary melanomas (NUM) and ulcerated primary melanomas (UM)



a) HLA class I expression in NUM, b) HLA class I expression in UM, c) Mx-1 expression in NUM, d) Mx-1 expression in UM, e) HLA class II expression in NUM, f) HLA class II expression in UM, g) iNOS expression in UM, h) CD47 expression in UM.

Table 2 Logistic regression analysis for evaluation of the association between TIL pattern in primary melanomas and ulceration

	Multivariable logistic analysis	
	OR (95% CI)[a]	P value
Breslow thickness, mm	1.99 (1.55 - 2.55)	<0.001
Histology		
SSM	1.00	
NM	1.67 (0.48 - 5.87)	0.421
Other	1.62 (0.68 - 3.84)	0.273
TILs		
Absent	1.00	
Focal/plurifocal	1.48 (0.65 - 3.39)	0.353
Diffuse	0.70 (0.24 - 2.00)	0.503

OR, odds ratio; TILs, tumor infiltrating lymphocytes.
[a] Based on complete case analysis.

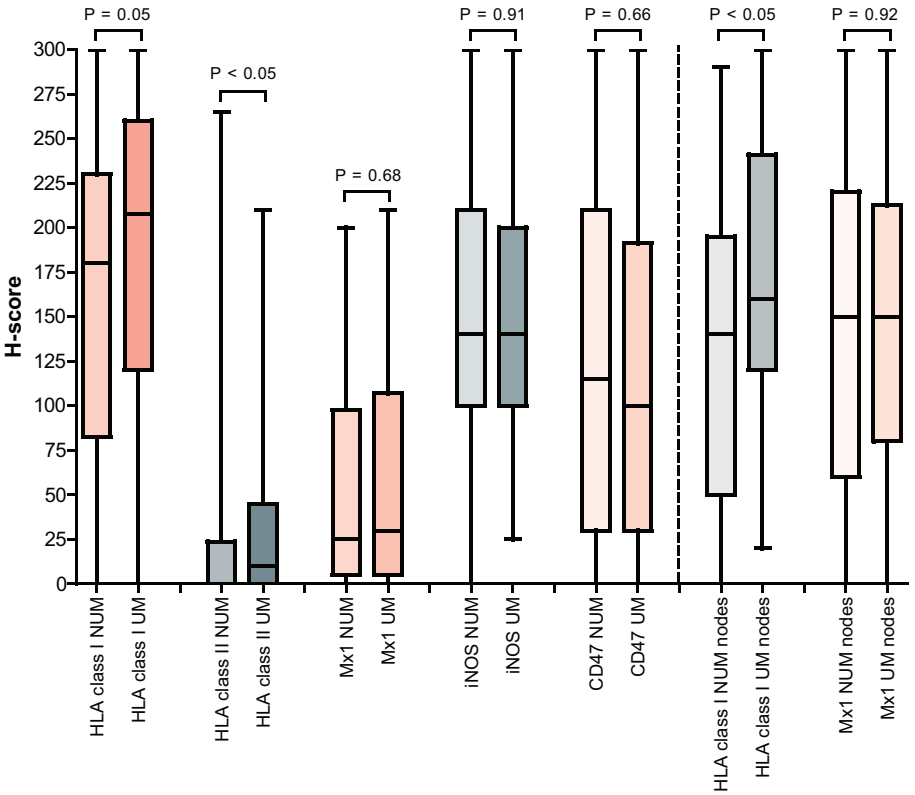
This trend was confirmed in the LN cohort. The median H-score for intratumoral HLA class I expression was significantly higher in UM than in NUM, namely 160 (IQR 120–237) and 140 (IQR 50–200), respectively (P < .05) (**Table 4**, **Figure 2**). In multivariable analysis, the H-score for intratumoral HLA class I expression tended to be independently associated with UM (P = .08) (**Table 4**). Of note, there was no difference in the peritumoral expression levels of MHC class I molecules in the primary melanoma cohort, with 63.8% (51/80) and 61.0% (50/82) strong/moderate peritumoral HLA class I expression in NUM and UM respectively (P = .72) (**Table 3**, **Figure 3**). Also, it was not independently associated with UM in multivariable logistic regression analysis (P = .79) (**Table 3**).

Recent articles revealed that MHC class II associated molecules (such as its invariant chain CD74) and more generally the IFNγ-regulated antigen processing machinery conferred a favorable prognosis and was a predictor of response to nivolumab in metastatic melanoma[30,31]. Here, the median H-score for intratumoral expression of HLA class II was significantly higher in UM compared with NUM, namely 10 (IQR 0–45) and 0 (IQR 0–24) respectively, (P < .05). However, in multivariable logistic regression analysis, the H-score for intratumoral HLA class II expression was not independently associated with UM (P = .84) (**Table 3**). The number of strong/moderate peritumoral HLA class II expression was not significantly different between NUM and UM (7.1%

(6/84) and 9.6% (8/83), respectively (P = .56)) and in addition, it was not independently associated with UM in multivariable logistic regression analysis (P = .35) (**Figure 3**, **Table 3**).

In conclusion, primary and metastatic ulcerated melanoma exhibit higher basal expression of MHC class I molecules, independently from Breslow thickness, histology and TILs.

Figure 2 H-scores for intratumoral expression of HLA class I, HLA class II, Mx1, iNOS and CD47 in the primary melanoma cohort and of HLA class I and Mx1 in the lymph node cohort



The coloured bars represent the median H-score (middle line) with interquartile range (two outer lines of the coloured bar). The horizontal line endings represent the range of the H-score. NUM, non-ulcerated melanomas; UM, ulcerated melanomas.

CONSTITUTIVE EXPRESSION OF MX1 IN THE PERITUMORAL AREAS OF ULCERATED MELANOMA

Given the observed overexpression of MHC class I molecules on UM, we hypothesized that constitutive IFN signaling might take place in this immunopathology. Dynamin-like GTPase myxovirus resistance protein 1 (Mx1) is an intracellular anti-viral protein following the activation of type I and type III interferon signaling. Mx1 inhibits viral replication by blocking the transcription of viral RNA, and a deficiency in this protein enhances susceptibility to viral infection[32]. Higher Mx1/MxA expression in breast or glioblastoma than in normal tissue has been reported, suggesting that IFN signaling is constitutively active in these tumors[33,34]. Therefore, we explored Mx1 expression in our two cohorts (examples in **Figure 1**).

In the primary melanoma cohort, the median H-score for intratumoral Mx1 expression was relatively low and not significantly different between NUM and UM, namely 25 (IQR 5–98) and 30 (IQR 5–108), respectively (P = .68) (**Table 3**, **Figure 2**). The H-score for intratumoral Mx1 expression was also not independently associated with UM in multivariable logistic regression analysis (P = .55) (**Table 3**). Similar findings were observed in the LN cohort. Median H-scores for intratumoral Mx1 expression were relatively higher than in the primary melanomas, but not significantly different between NUM and UM (130 (IQR 60–220) and 150 (80–205), respectively, P = .92) (**Table 4**, **Figure 2**). In addition, the H-score for intratumoral Mx1 expression was not independently associated with UM in multivariable logistic regression analysis (P = .78) (**Table 3**).

Interestingly, in contrast to intralesional stainings, peritumoral expression in primary melanomas was different between UM and NUM. Moderate or strong peritumoral Mx1 expression was dominant in UM compared with NUM (50.6% (42/83) and 31.3% (25/80), respectively, P = .01) (**Table 3**, **Figure 3**). It was also independently associated with UM in multivariable logistic regression analysis (P = .02) (**Table 3**).

In conclusion, as expected from the upregulation of MHC class I molecules, we observed a constitutive high level of antiviral protein Mx1 at the epidermal border of tumor beds in primary melanoma.

Table 3 Association between detection of HLA class I, HLA class II, Mx1, iNOS and CD47 in the primary melanoma cohort and ulceration

	Univariable analysis			Multivariable logistic regression	
	NUM	UM	P value[a]	OR adj (95% CI)[c]	P value
HLA class I	N = 80	N = 84			
Intratumoral H score[d]	180 (83 - 230)	208 (120 - 260)	0.054[b]	1.01 (1.00 - 1.01)	0.004
Peritumoral epidermis		*N = 82*	0.716		
Absent/weak	29 (36.3)	32 (39.0)		1.00	
Strong/moderate	51 (63.8)	50 (61.0)		0.89 (0.38 - 2.08)	0.791
Breslow thickness, mm[d]	1.66 (1.30 - 2.62)	4.00 (2.64 - 6.00)	<0.001[b]	2.11 (1.61 - 2.76)	<0.001
Histology	*N = 75*	*N = 82*	0.025		
SSM	52 (69.3)	40 (48.8)		1.00	
NM	6 (8.0)	15 (18.3)		1.25 (0.32 - 4.88)	0.750
Other	17 (22.7)	27 (32.9)		1.16 (0.46 - 2.93)	0.753
TILs	*N = 79*	*N = 82*	0.315		
Absent	31 (39.2)	37 (45.1)		1.00	
(Pluri)Focal	29 (36.7)	33 (40.2)		1.06 (0.43 - 2.61)	0.901
Diffuse	19 (24.1)	12 (14.6)		0.28 (0.08 - 0.99)	0.048
HLA class II	N = 84	N = 83			
Intratumoral H score[d]	0 (0 - 24)	10 (0 - 45)	0.046[b]	1.00 (0.99 - 1.01)	0.838
Peritumoral epidermis			0.561		
Absent/weak	78 (92.9)	75 (90.4)		1.00	
Strong/moderate	6 (7.1)	8 (9.6)		1.87 (0.51 - 6.92)	0.348
Breslow thickness, mm[d]	1.70 (1.30 - 3.08)	4.00 (2.50 - 6.00)	<0.001[b]	2.08 (1.60 - 2.71)	<0.001
Histology	*n = 79*	*n = 81*	0.010		
SSM	56 (70.9)	39 (48.1)		1.00	
NM	6 (7.6)	15 (18.5)		1.54 (0.42 - 5.60)	0.513
Other	17 (21.5)	27 (33.3)		1.39 (0.57 - 3.38)	0.471
TILs	*n = 83*	*n = 81*			
Absent	32 (38.6)	36 (44.4)		1.00	
(Pluri)Focal	30 (36.1)	32 (39.5)		1.45 (0.61 - 3.46)	0.403
Diffuse	21 (25.3)	13 (16.0)		0.74 (0.24 - 2.28)	0.598
Mx1	N = 80	N = 83			
Intratumoral H score[d]	25 (5 - 98)	30 (5 - 108)	0.675[b]	1.00 (1.00 - 1.01)	0.546
Peritumoral epidermis			0.012		
Absent/weak	55 (68.8)	41 (49.4)		1.00	
Strong/moderate	25 (31.3)	42 (50.6)		2.87 (1.21 - 6.80)	0.016
Breslow thickness, mm[d]	1.66 (1.30 - 3.08)	4.00 (2.75 - 6.00)	<0.001[b]	2.11 (1.61 - 2.76)	<0.001
Histology	*n = 75*	*n = 81*	0.020		
SSM	53 (70.7)	40 (49.4)		1.00	
NM	6 (8.0)	15 (18.5)		1.54 (0.41 - 5.87)	0.525
Other	16 (21.3)	26 (32.1)		1.59 (0.62 - 4.11)	0.337
TILs	*n = 79*	*n = 82*	0.403		
Absent	31 (39.2)	37 (45.1)		1.00	
(Pluri)Focal	30 (38.0)	33 (40.2)		1.14 (0.47 - 2.76)	0.779
Diffuse	18 (22.8)	12 (14.6)		0.46 (0.14 - 1.56)	0.212

Table 3 Continued

	Univariable analysis			Multivariable logistic regression	
	NUM	UM	P value[a]	OR adj (95% CI)[c]	P value
iNOS	N = 83	N = 83			
Intratumoral H score[d]	140 (100 - 210)	140 (100 - 200)	0.907[b]	1.00 (0.99 - 1.01)	0.800
Peritumoral epidermis	*n = 81*	*n = 80*	0.189		
Absent/weak	64 (79.0)	56 (70.0)		1.00	
Strong/moderate	17 (21.0)	24 (30.0)		1.76 (0.62 - 5.01)	0.289
Breslow thickness, mm[d]	1.70 (1.30 - 3.20)	4.00 (2.60 - 6.00)	<0.001[b]	2.23 (1.68 - 2.96)	<0.001
Histology	*n = 78*	*n = 81*	0.014		
SSM	55 (70.5)	39 (48.1)		1.00	
NM	6 (7.7)	14 (17.3)		1.50 (0.38 - 5.86)	0.564
Other	17 (21.8)	28 (34.6)		1.34 (0.52 - 3.42)	0.541
TILs	*n = 80*	*n = 81*	0.461		
Absent	32 (40.0)	37 (45.7)		1.00	
(Pluri)Focal	29 (36.3)	31 (38.3)		1.51 (0.63 - 3.64)	0.358
Diffuse	19 (23.8)	13 (16.0)		0.47 (0.14 - 1.55)	0.213
CD47	N = 84	N = 82			
Intratumoral H score[d]	115 (30 - 210)	100 (30 - 191)	0.661[b]	1.00 (0.99 - 1.00)	0.149
Peritumoral epidermis	*n = 83*		0.627		
Absent/weak	23 (27.7)	20 (24.4)		1.00	
Strong/moderate	60 (72.3)	62 (75.6)		1.56 (0.64 - 3.78)	0.326
Breslow thickness, mm[d]	1.70 (1.30 - 3.20)	4.00 (2.58 - 6.00)	<0.001[b]	2.03 (1.57 - 2.63)	<0.001
Histology	*n = 79*	*n = 81*	0.015		
SSM	56 (70.9)	40 (49.4)		1.00	
NM	6 (7.6)	15 (18.5)		1.96 (0.53 - 7.22)	0.312
Other	17 (21.5)	26 (32.1)		1.85 (0.76 - 4.54)	0.179
TILs			0.341		
Absent	33 (39.3)	37 (45.1)		1.00	
(Pluri)Focal	30 (35.7)	32 (39.0)		1.52 (0.65 - 3.57)	0.336
Diffuse	21 (25.0)	13 (15.9)		0.80 (0.27 - 2.39)	0.688

adj, adjusted; NM, nodular melanoma; NUM, non-ulcerated melanoma; OR, odds ratio; SSM, superficial spreading melanoma; TILs, tumor infiltrating lymphocytes; UM, ulcerated melanoma [a] Chi-square analysis, unless indicated otherwise: [b]Mann-Whitney U test. [c] Based on complete case analysis. [d] Median with interquartile range.

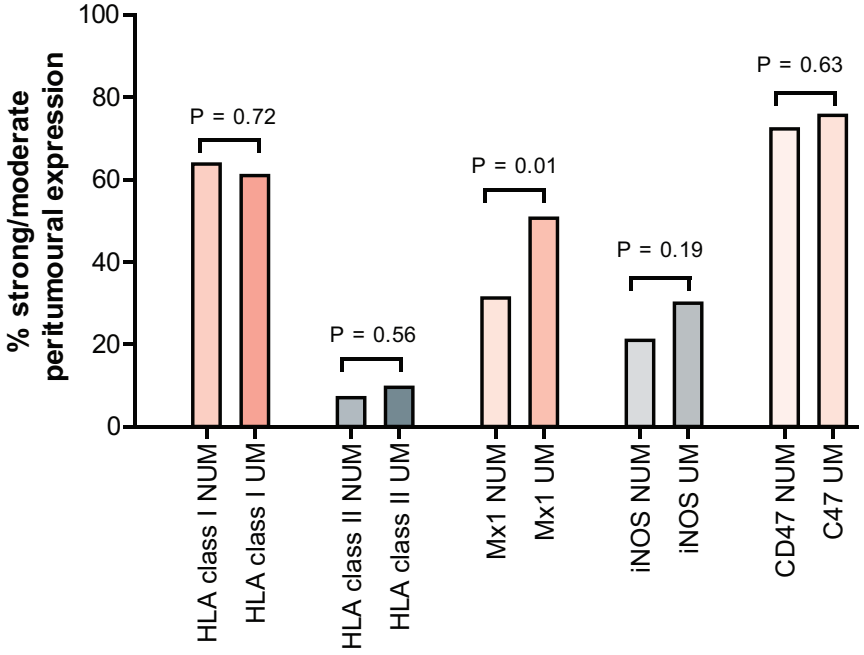
NO RELEVANCE OF OTHER KEY MOLECULES INVOLVED IN SCAVENGING AND PHAGOCYTOSIS

We next analyzed the expression of nitric oxide synthase 2 (NOS2) in NUM versus UM. The production of nitric oxide (NO) in cells results from the conversion of L-arginine to L-citrulline by the enzyme NOS. NO regulates neurotransmission, immune-responses and antimicrobial responses. In addition, its role during the various stages of oncogenesis has been well exemplified. The anti-tumor effects of NO produced by the immune defence were reported in various human tumors, while the pro-tumorigenic and immunosuppressive effects of NO were demonstrated in progressive tumors and metastases[35,36]. NOS2 is primarily regulated at the expression level by inflammatory cytokines (TNF-α, IL-1β, IL-6 and IFNγ), lipopolysaccharide, hypoxia, oxidative stress and HSP70[35]. NOS2 can be dependent on type I IFN signaling, especially STAT1, STAT2, Irf3 and NF-κB[37] leading to various outcomes during infection[38–40]. In melanoma, NOS2 activation in γδT cells promoted dissemination in mouse melanoma models[41] and was associated with poor survival in melanoma patients[30].

Therefore, we examined whether NOS2/iNOS would be associated with ulceration and chronic inflammation in our cohorts. The median H-score for intratumoral iNOS expression was not significantly different between NUM and UM (140 (IQR 100–210) and 140 (IQR 100–200), P = .91) (**Table 3**, **Figure 2**). Also, peritumoral expression was not significantly different between NUM and UM (strong/moderate expression in 21.0% (17/81) and 30.0% (24/83), respectively, P = .19) (**Table 3, Figure 3**). In multivariable logistic regression analysis, both the H-score for intratumoral expression of iNOS as the peritumoral expression were not independently associated with ulceration (P = .80 and P = .29, respectively) (**Table 3**).

CD47 is a "don't eat me signal" associated molecule with an immunoglobulin-like domain that is expressed on the tumor cell surface and inhibits macrophage phagocytosis via binding the signal regulatory protein α (SIRPα) on phagocytes[42]. In gastric and ovarian cancer as well as in uveal melanoma, CD47 expression is an independent negative prognostic factor[43] often upregulated following exposure to inflammatory stimuli[44]. Anti-CD47 Ab could blunt melanoma dissemination[45]. We next explored the potential discriminative value of this don't eat me signal in UM versus NUM in our two cohorts. The median H-score for intratumoral CD47 expression was not significantly different between NUM and UM (115 (IQR 30–210) and 100 (IQR 30–191), P = .66) (**Table 3**, **Figure 2**). Also, peritumoral expression was not significantly different between NUM and UM (strong/moderate expression in 72.3% (60/84) and 75.6% (62/82), respectively, P = .63) (**Table 3**, **Figure 3**). In multivariable logistic regression analysis, both the H-score for intratumoral expression of CD47 as the peritumoral expression were not independently associated with ulceration (P = .15 and P = .33, respectively) (**Table 3**).

Figure 3 Percentage of strong/moderate peritumoral expression of HLA class I, HLA class 2, Mx1, iNOS and CD47 in primary melanomas



The coloured bars represent the percentage of strong/moderate peritumoral expression per protein. NUM, non-ulcerated melanomas; UM, ulcerated melanomas.

Table 4 Association between detection of HLA class I and Mx1 in the lymph node cohort and ulceration

	Univariable analysis			Multivariable logistic regression	
	NUM	UM	P value[a]	OR adj (95% CI)[c]	P value
HLA Class I	N = 51	N = 40			
Intratumoral H score[d]	140 (50 - 200)	160 (120 - 237)	0.045[b]	1.01 (1.00 - 1.01)	0.079
	n = 50	*n = 39*			
Breslow thickness, mm[d]	2.00 (1.06 - 3.10)	3.60 (2.20 - 6.02)	<0.001[b]	1.30 (1.07 - 1.58)	0.007
Nodal involvement			0.538		
Microscopic	10 (19.6)	10 (25.0)		1.00	
Macroscopic	41 (80.4)	30 (75.0)		1.00 (0.32 - 3.14)	0.998
Mx1	N = 49	N = 41			
Intratumoral H score[d]	130 (60 - 220)	150 (80 - 205)	0.919[b]	1.00 (0.99 - 1.00)	0.777
	n = 48	*n = 40*			
Breslow thickness, mm[d]	2.00 (1.03 - 3.08)	2.93 (2.05 - 6.02)	0.001[b]	1.26 (1.05 - 1.51)	0.012
Nodal involvement			0.336		
Microscopic	9 (18.4)	11 (26.8)		1.00	
Macroscopic	40 (81.6)	30 (73.2)		0.87 (0.29 - 2.64)	0.812

adj, adjusted; NUM, non-ulcerated melanoma; OR, odds ratio; UM, ulcerated melanoma.
[a] Chi-square analysis, unless indicated otherwise: [b]Mann-Whitney U test. [c] Based on complete case analysis.
[d] Median with interquartile range.

DISCUSSION

Over the past decade, researchers have made several attempts to unravel the biological significance of ulcerated melanomas. There are several explanations for the adverse prognostic value of ulceration in melanoma: ulceration is a surrogate of an intrinsic biological attribute of the tumor or the host that favors its dissemination, or ulceration directly favors the dissemination of the tumor, for example, by modifying the local environment. Proliferation of the tumor in the vicinity of the epidermis may erode it by contact and thus favor tumor expansion. Among the intrinsic properties of melanoma that might favor ulceration, proliferation and dissemination, the most convincing evidence is for the loss of E-cadherin expression[10], the dual role of the matrix protein osteoponti[46–50], and/or the lack of the matricellular CCN3 which inhibits melanocyte proliferation and stimulates adhesion to collagen type IV[51–53]. In addition, ulceration may have a direct influence on the local microenvironment that subsequently may favor dissemination. The presence of increased density of activated tumor-associated neutrophils in the superficial part of the lesion[10] and

gene signatures associated with the wound healing pathway and pro-inflammatory cytokines (such as IL1b, IL6 and IL8)[8] in ulcerated melanomas, compared with non-ulcerated ones reinforce this hypothesis. However, our study did not support the fact that ulceration may result from defects in clearance of dying cells or phagocytosis, since the main don't eat me signal CD47 was not differentially expressed between the two melanoma entities. However, our results fuel the assumption that ulceration may result from chronic exposure to IFNs. Indeed, we observed increased MHC class I and Mx1 expression in ulcerated lesions compared with non-ulcerated melanoma. But this Mx1-associated inflammation found in ulcerated lesions may unlikely be associated with type II IFN since MHC class II molecules and inducible NOS were not relevant in our analyses.

Our study revealed unexpected findings. First, the observation that higher tumor HLA class I expression represented a hallmark of ulceration could appear surprising. Indeed, IFNγ-mediated cell surface expression of MHC molecules predicts superior antigen presentation, subsequent activation of CD8+ cytotoxic T lymphocytes, and better prognosis, ensuring prediction to respond to immune checkpoint blockade in numerous studies[31,54,55]. As a matter of fact, the presence of tumor infiltrating lymphocytes has been reported as an independent favorable prognostic factor for survival in ulcerated melanomas, though the quantity of TILs was similar in both non-ulcerated and ulcerated melanomas[56]. It is conceivable that higher MHC class I expression may represent a surrogate marker for IFNγ-related hallmarks of immunoresistance, such as PD-L1 or IDO and other ligands for inhibitory receptors. Supporting this view, higher proportion of tumor cell PD-L1 expression, associated with increased intratumoral CD163+ macrophage infiltrates suggesting a dominance of M2 geered-tumor microenvironment were reported in ulcerated melanomas[13].

Secondly, the notion that pre-existing type 1 IFN fingerprint could be associated with ulceration and dismal prognosis were not expected either. Indeed, type I IFNs, secreted by malignant cells or tumor-infiltrating dendritic cells, mainly function by stimulating host anticancer immune responses[22]. Moreover, intratumoral expression levels of type I IFNs or their downstream effectors such as IFN-stimulated genes (ISGs) correlate with favourable disease outcome in melanoma[54], in contrast to what was reported for breast cancer and glioblastoma[33,34]. The antiviral factor Mx dynamin-like GTPase 1 (Mx1) is one of the various type I ISGs. The current study did not show a difference in the intratumoral expression of Mx1 between non-ulcerated and ulcerated primary melanomas. This is in accordance with a previous study that also showed no difference in intratumoral expression of MxA (alternative name for Mx1) between ulcerated and non-ulcerated primary melanomas[13]. Interestingly, the current study did show

upregulation of peritumoral Mx1 expression in ulcerated melanomas, as indicated by a significantly higher percentage of strong/moderate expression in the adjacent epidermis. Many articles demonstrate the deleterious effects of chronic exposure to type 1 IFN or TLR3 signaling. Hence, IFN or STAT1–mediated epigenetic imprinting in tumor cells leads to a marked upregulation of major ligands for inhibitory receptors (such as Galectin 9 for TIM3). Crippling the program interfered with multiple inhibitory pathways and expanded distinct T cell populations expressing exhaustion markers[57]. Moreover, an IFN signature associated with DNA damage response paved the way to resistance to therapies inducing immunogenic cell death pathways[58]. Finally, TLR3 signaling stimulates stemcellness in many malignant processes or pathophysiological circumstances[59].

Notewithstanding, ulceration is the overriding determinant of activity of adjuvant IFN therapy. Kirkwood's group evaluated STAT1 and STAT3 jointly as mediators of type 1 IFN effects in the setting of a prospective neoadjuvant trial of high dose IFNa2b (HDI), in which tissue samples were obtained before and after 20 doses of HDI therapy. Double immunohistochemistry for pSTAT1 and pSTAT3 was performed on paired fixed or frozen biopsies in about 20 patients. HDI tilted the balance between pSTAT1 and pSTAT3, upregulating the former and down regulating the latter. Higher pSTAT1/pSTAT3 ratios in tumor cells or lymphocytes pretreatment were associated with longer overall survival. Moreover, TAP2 was augmented by HDI (but not TAP1 and MHC class I/II)[60]. pSTAT1 results from IFNAR1/IFNAR2 receptor signaling cascade, culminating in type 1 IFN or ISG transcription. Our data showing that MHC class I molecules and surrounding Mx1 expression characterized ulcerated lesions imply that the IFNAR signaling cascade may be intact and trained in tumor cells of ulcerated lesions, accounting for the ability of IFN-α-2b to promptly phosphorylate STAT1 downstream of this cascade.

We acknowledge the limitations of our study. Due to the retrospective nature of the analysis, the lack of clinical follow up of patients operated from their primary lesions, and the technology that failed to be multiplexed to encompass the type 1/type 2 IFN pathways and the whole MHC antigen processing machinery, it remains difficult to conclude on the clinical relevance of endogenous IFN signaling in ulcerated melanoma. Importantly, during the time of this study, adjuvant therapies for stage III melanoma have been revolutionized by the introduction of immune checkpoint inhibitors, such as ipilimumab followed by pembrolizumab[61,62], which are both distinctly governed by melanoma expression of MHC class I or class II molecules[31], suggesting that future studies using in depth tissue profiling through multiplexed immunohistochemical consecutive or concomitant stainings should revisit these notions accurately[63].

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ADVANCED

MELANOMA

Treatment of melanoma of unknown primary in the era of immunotherapy and targeted therapy:

A Dutch population-based study

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ABSTRACT

Melanoma of unknown primary (MUP) may have a different biology to melanoma of known primary, but clinical trials of novel therapies (e.g., immune checkpoint or BRAF/MEK inhibitors) have not reported the outcomes in this population. We therefore evaluated the overall survival (OS) among patients with MUP in the era of novel therapy. Data for stage III or IV MUP were extracted from a nationwide database for the period 2003–2016, with classification based on the eighth edition of the American Joint Committee on Cancer criteria. The population was divided into pre- (2003–2010) and post- (2011–2016) novel therapy eras. Also, OS in the post-novel era was compared between patients with stage IV MUP by whether they received novel therapy. In total, 2028 of 65,110 patients (3.1%) were diagnosed with MUP. Metastatic sites were known in 1919 of 2028 patients, and most had stage IV disease (53.8%). For patients with stage III MUP, the 5-year OS rates were 48.5% and 50.2% in the pre- and post-novel eras, respectively ($p = 0.948$). For those with stage IV MUP, the median OS durations were unchanged in the pre-novel era and post-novel era when novel therapy was not used (both 4 months); however, OS improved to 11 months when novel therapy was used in the post-novel era ($p < 0.001$). In conclusion, more than half of the patients with MUP are diagnosed with stage IV and the introduction of novel therapy appears to have significantly improved the OS of these patients.

INTRODUCTION

There is a continuing upward trend in melanoma incidence in many European countries, including the Netherlands[1]. Approximately 3% of patients who newly present with melanoma are diagnosed with melanoma of unknown primary (MUP)[2,3]. According to the eighth edition of the American Joint Committee on Cancer (AJCC) staging criteria, patients presenting with melanoma metastases in the (sub)cutis, soft tissue, and/or lymph nodes, without a detectable primary tumour, are diagnosed with stage III disease; by contrast, patients presenting with distant metastases, including visceral metastases, are diagnosed with stage IV disease[4].

Previous research has demonstrated improved survival in patients with stage III and IV MUP compared to stage-matched patients with melanoma of known primary (MKP) [3,5–8]. Therefore, it is conceivable that there is a difference in biology between MUP and MKP. It has also been hypothesised that primary melanomas remain undetected due to immune-mediated spontaneous regression[9,10]. These research findings may have implications for patients with MUP in the current era of targeted therapy and immunotherapy.

Until 2011, treatment regimens for patients with advanced melanoma (unresectable stage IIIC or stage IV) usually consisted of chemotherapy (e.g., dacarbazine) and were of limited therapeutic benefit[11]. Over the past decade, however, the introduction of novel therapies has dramatically improved survival[12]. Since 2011, seven systemic novel therapies have been approved by the Food and Drug Administration and subsequently by the European Medicines Agency and the Dutch Medicines Evaluation Board for the treatment of advanced melanoma, and these are broadly grouped into immune checkpoint inhibitors (immunotherapy) and BRAF and/or MEK inhibitors (targeted therapy). The CTLA-4 blocking antibody ipilimumab was the first immune checkpoint inhibitor to be approved in 2011, followed by the BRAF inhibitors vemurafenib (2012) and dabrafenib (2013) and the MEK inhibitor trametinib (2014). The PD-1 blocking antibodies nivolumab and pembrolizumab were then approved in 2015, along with the combined BRAF/MEK inhibitors dabrafenib plus trametinib and vemurafenib plus cobimetinib. The combination of ipilimumab and nivolumab was approved in 2016. Of note, immunotherapy is available for all patients with advanced melanoma, irrespective of mutation status, whereas only patients with BRAF-mutated melanomas are eligible for targeted therapy. Even though BRAF/MEK inhibitors are considered targeted therapies, yet these agents also appear to induce immune responses in melanoma[13].

While clinical trials of novel therapy have included patients with MUP, the outcomes in these patients have not been reported specifically[14]. Given the potential difference in biology compared to MKP, knowing the outcomes during novel therapy for patients with MUP could aid clinical decision-making. In the current study, we aimed to investigate the incidence, presentation, and treatment of MUP in the Netherlands and to assess overall survival (OS) associated with MUP in the era of novel therapy.

METHODS

STUDY DESIGN AND POPULATION

This was an exploratory, population-based observational study of all patients diagnosed with MUP between 2003 and 2016 for whom data were recorded in the Netherlands Cancer Registry(NCR)[15].The NCR is embedded in the Netherlands Comprehensive Cancer Organisation and is linked annually to the Municipal Personal Records database to retrieve information on vital status. Cancer registration in the Netherlands is based on notification of all new malignancies by the nationwide automated pathological archive. The national registry of hospital discharge diagnoses is an additional source of patient identification, accounting for up to 8% of new cases. After notification, trained and certified registrars from the NCR retrieve the patient's medical records and check all diagnoses. Besides details on patient and primary tumour characteristics, the NCR records details on the morphology, topography, and location of metastases for all newly diagnosed malignancies according to the classifications in the International Classification of Diseases-Oncology[16].The quality of the collected data is of a high standard thanks to computerised consistency checks and the reliance on trained and certified registrars who only obtain registration if they achieve a correctness score of ≥95%. Although details of first-line treatment are recorded, details of secondary treatment, disease recurrence, and/or progression are not recorded.

Prior to this study, vital status had last been updated on 1st January 2018. Patients diagnosed with MUP were identified by morphological codes 872–879 (nevi and melanoma) in combination with topographical code C80.9 (unknown primary site)[16]. Data on year of diagnosis, age, gender, number and location of involved lymph nodes, location and number of metastases, first-line treatment, and vital status were retrieved. First-line treatment was categorised as follows: no therapy; local therapy, including surgical excision, radiation therapy, radiofrequency ablation, and isolated limb perfusion; chemotherapy, with/without concurrent local therapy; and novel therapy, including immunotherapy and/or targeted therapy, with/without concurrent local therapy and/

or chemotherapy. Immunotherapy consisted of immune checkpoint inhibition with CTLA-4 blockade (ipilimumab), PD-1 blockade (either nivolumab or pembrolizumab), or combined CTLA-4 and PD-1 blockade (ipilimumab and nivolumab). Targeted therapy consisted of BRAF-inhibition (vemurafenib or dabrafenib), MEK-inhibition (trametinib) or combined BRAF and MEK inhibition (dabrafenib and trametinib or vemurafenib and cobimetinib).

STAGING

Classification was based on the eight edition of the AJCC criteria: stage IIIB was diagnosed if there was one involved lymph node or cutaneous/subcutaneous metastasis; stage IIIC, if there was more than one involved lymph node or involved lymph node(s) plus cutaneous/subcutaneous metastasis; stage IV–M1a, if there was distant metastasis to skin, soft tissue (including muscle), and/or non-regional lymph node(s); stage IV–M1b, if there was metastasis distant to the lungs, with or without concurrent M1a sites; stage IV–M1c, if there was metastasis distant to visceral sites, excluding the central nervous system (CNS), with or without concurrent M1a or M1b sites; and stage IV–M1d, if there was metastasis distant to the CNS with or without concurrent M1a, M1b, orM1c sites[4]. If the number of involved lymph nodes was unknown, classification was based on the number of involved lymph node basins, with the presence of one nodal basin regarded as stage IIIB and the presence of more than one nodal basin regarded as stage IIIC. Involvement of intra-thoracic or intra-abdominal nodes was considered non-regional nodal metastasis. In principle, parotid gland or submandibular gland involvement, and cervical, axillary, inguinal, or pelvic nodal involvement were each taken to indicate regional nodal metastasis, while breast involvement was considered to indicate regional soft tissue metastasis, unless anatomic distribution made regional coherence implausible (e.g., cervical nodal metastasis with a subcutaneous lesion on the arm). If the site of metastasis was unclear (C76: other and ill-defined sites; or C80: unknown site), disease was classified based on other available information (e.g., involvement of lymph nodes, lungs, and 'an overlapping lesion of ill-defined sites' were regarded as stage IV–M1b). If no other information was available, we labelled patients as 'not otherwise specified (NOS)' for the demographic analysis and excluded them from further analysis.

Patients with stage IV MUP were categorised according to the number of involved metastatic sites (≤2 versus >2). The following locations were regarded as distinct sites: cutis; subcutis/ soft tissue; lymph nodes; pulmonary tract; heart/mediastinum; liver; gallbladder; pancreas; adrenal gland; spleen; upper gastrointestinal tract, including the oesophagus, stomach, and duodenum; lower gastrointestinal tract, including the small

intestine, colon, sigmoid, rectum, and anus; retroperitoneum; peritoneum; urogenital; bone; head and neck, including tongue, tonsils, and (para)thyroid glands; and the CNS.

STATISTICAL ANALYSIS

To evaluate the impact of novel therapy on OS, the population was divided into two eras: 2003–2010 (pre-novel therapy era) and 2011–2016 (post-novel therapy era). For the latter era, patients with stage IV MUP were further categorised into those who received novel therapy as a first-line treatment (novel therapy group) and those who did not (no novel therapy group). In the novel therapy group, we also compared OS between patients who received first-line therapy in 2011–2012, 2013–2014, and 2015–2016 and between those who received first-line immunotherapy and those who received first-line targeted therapy. Patients who received both agents as first-line treatment were excluded from this analysis.

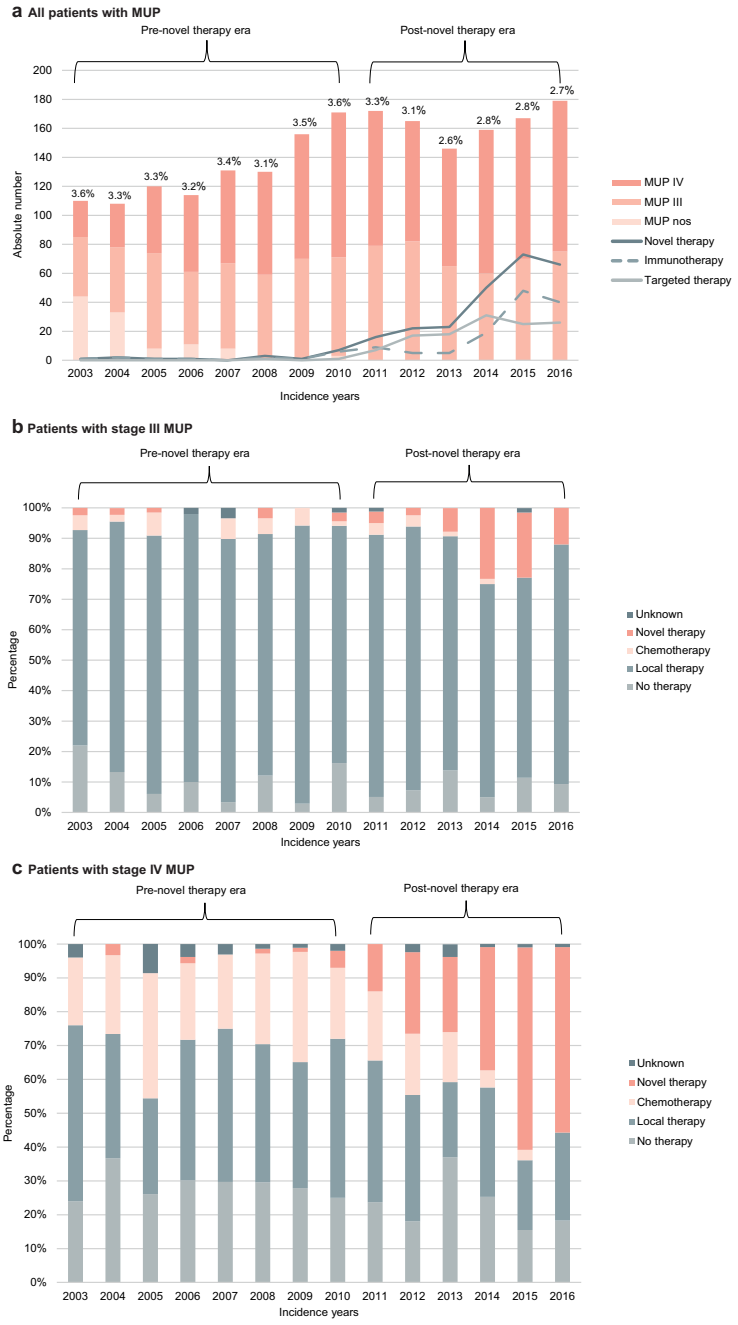
The proportion of patients with MUP relative to all newly diagnosed melanomas was determined. Univariable analysis consisted of Mann–Whitney *U* or Kruskal–Wallis tests for continuous variables and chi-square or Fisher exact tests for categorical variables, as appropriate. Where data were missing or unknown, an 'unknown' subcategory was created for analysis. OS was calculated from the date of diagnosis to the date of last follow-up or death. The Kaplan–Meier method was used to estimate survival, and differences between groups were assessed by the log-rank test. The median follow-up duration among survivors was calculated from the date of diagnosis to the date of last follow-up using the reversed Kaplan–Meier method (deaths were censored). Multivariable cox regression analysis was performed to identify whether type of novel therapy was an independent prognostic factor for OS. Statistical analyses were performed using IBM SPSS for Windows, Version 24 (IBM Corp., Armonk, NY). Two-sided p values of <0.05 were considered statistically significant.

RESULTS

PATIENT CHARACTERISTICS

During the study period, 2028 of 65,110 patients (3.1%) were diagnosed with MUP in the Netherlands (**Figure 1a**). Information on the metastatic site was available for 1919 of these patients (94.6%), with most presenting with visceral metastasis (n = 999; 51.2%), followed by nodal involvement alone (n = 594; 31.0%), (sub) cutaneous involvement alone (n = 243; 12.7%), (sub)cutaneous and nodal involvement (n = 50; 2.6%), and distant (sub) cutaneous and/or nodal involvement (n = 33; 1.7%).

Figure 1 Overview of patients diagnosed with MUP in the Netherlands and corresponding changes in first-line treatment use between 2003 and 2016



Nodal metastasis was predominantly located in the axilla, affecting 253 of 644 patients (39.3%). The distributions of nodal and (sub) cutaneous metastases are illustrated in **Supplementary Figure 1**.

COMPARISON BY DISEASE STAGE

According to the eight edition of the AJCC criteria, 887 patients had stage III disease (46.2%) and 1,032 patients had stage IV disease (53.8%) (**Tables 1 and 2**). The proportion of patients with MUP who presented with stage IV disease increased from 37.9% to 58.1% between 2003 and 2016. In patients with stage III and IV disease, the median follow-up durations were 85 months (interquartile range [IQR] 51–126 months) and 47 months (IQR 30–85 months), respectively.

In the post-novel therapy era, 41 of 431 patients with stage III MUP (9.5%) and 202 of 557 patients with stage IV MUP (36.3%) received novel therapy first-line. Patients with stage III MUP were typically treated with local therapies throughout the study (715 of 887 patients, 80.6%). Among patients with stage IV MUP, chemotherapy was used as a first-line treatment in 123 of 475 patients (25.9%) in the pre-novel therapy era and in 54 of 557 patients (9.7%) in the post-novel therapy era; its use was not recorded at all for 2016. The details of first-line treatment, by stage, are depicted for each year in **Figures 1b** and **1c**.

SURVIVAL

For patients with stage III MUP, the 5-year OS rates in the pre- and post-novel therapy eras were 48.5% (standard error, 2.3%) and 50.2% (standard error, 2.8%), respectively ($p = 0.948$) (**Figure 2a**). For patients with stage IV MUP, the median OS durations were 4 months (IQR 2–11) in the pre-novel therapy era and 4 months (IQR 2–16) in the post-novel era when not using novel therapy; however, this improved to 11 months (IQR 6–31) in the post-novel era when using novel therapy ($p < 0.001$) (**Figure 2b**). When this latter era was subdivided by year into 2011–2012, 2013–2014, and 2015–2016, the median OS durations were 8 months (IQR 6–14), 8 months (IQR 5–18), and 16 months (IQR 6–31), respectively ($p < 0.001$) (**Figure 2c**). In all subgroups, the median OS for patients with stage IV MUP was superior for those receiving novel therapy compared to those not receiving novel therapy (**Table 3**).

Table 1 Patient, tumour, and treatment characteristics by era among patients with stage III MUP

	Pre-novel therapy era	Post-novel therapy era	
	2003–2010	2011–2016	
Characteristics	(n = 456)	(n = 431)	P value[1]
Age[2]	62 (48–72)	64 (53–74)	0.009[3]
Gender			0.686
Male	252 (55.3)	244 (56.6)	
Female	204 (44.7)	187 (43.4)	
Substage III			0.897
IIIB	389 (85.3)	369 (85.6)	
IIIC	67 (14.7)	62 (14.4)	
Presentation stage III			0.324
(Sub)cutaneous only	115 (25.2)	128 (29.7)	
Nodal only	315 (69.1)	279 (64.7)	
(Sub)cutaneous + nodal	26 (5.7)	24 (5.6)	
Treatment strategy			<0.001
No therapy	46 (10.1)	37 (8.6)	
Local therapy	379 (83.1)	336 (78.0)	
Chemotherapy	20 (4.4)	8 (1.9)	
Novel therapy	7 (1.5)	48 (11.1)	
Unknown			
Novel therapy type			0.142
Immunotherapy	6 (85.7)	22 (45.8)	
Targeted therapy	1 (14.3)	25 (52.1)	
Both	0	1 (2.1)	

Values in parentheses are percentages, unless otherwise indicated.
Abbreviations: MUP, metastasis of unknown primary. [1]Chi-square test; [2]Values are median (interquartile range); [3]Mann–Whitney *U* test.

Table 2 Patient, tumour, and treatment characteristics by era and therapy type among patients with stage IV MUP

	Pre-novel therapy era 2003–2010	Post-novel therapy era 2011–2016		
Characteristics	(n = 475)	No novel (n = 355)	Novel (n = 202)	P value[1]
Age[2]	60 (49–71)	65 (55–73)	62 (51–69)	0.009[3]
Gender				0.561
Male	287 (60.4)	224 (63.1)	130 (64.4)	
Female	188 (39.6)	131 (36.9)	72 (35.6)	
Substage IV				0.043
IV–M1a	20 (4.2)	9 (2.5)	4 (2.0)	
IV–M1b	84 (17.7)	55 (15.5)	20 (9.9)	
IV–M1c	215 (45.3)	150 (42.3)	99 (49.0)	
IV–M1d	156 (32.8)	141 (39.7)	79 (39.1)	
Metastatic sites stage IV				<0.001
≤2 sites	352 (74.1)	268 (75.5)	96 (47.5)	
>2 sites	123 (25.9)	87 (24.5)	106 (52.5)	
Treatment strategy				n/a
No therapy	134 (28.2)	126 (35.5)	0	
Local therapy	196 (41.3)	167 (47.0)	0	
Chemotherapy	123 (25.9)	54 (15.2)	0	
Novel therapy	9 (1.9)	0	202 (100)	
Unknown	13 (2.7)	8 (2.3)	0	
Novel therapy type				0.066[4]
Immunotherapy	8 (88.9)	0	94 (46.5)	
Targeted therapy	1 (11.1)	0	99 (49.0)	
Both	0	0	9 (4.5)	

Values in parentheses are percentages unless indicated otherwise.
Abbreviations: MUP, metastasis of unknown primary. [1]Chi-square test; [2]Values are median (interquartile range); [3]Kruskal–Wallis test; [4]Fisher's exact test.

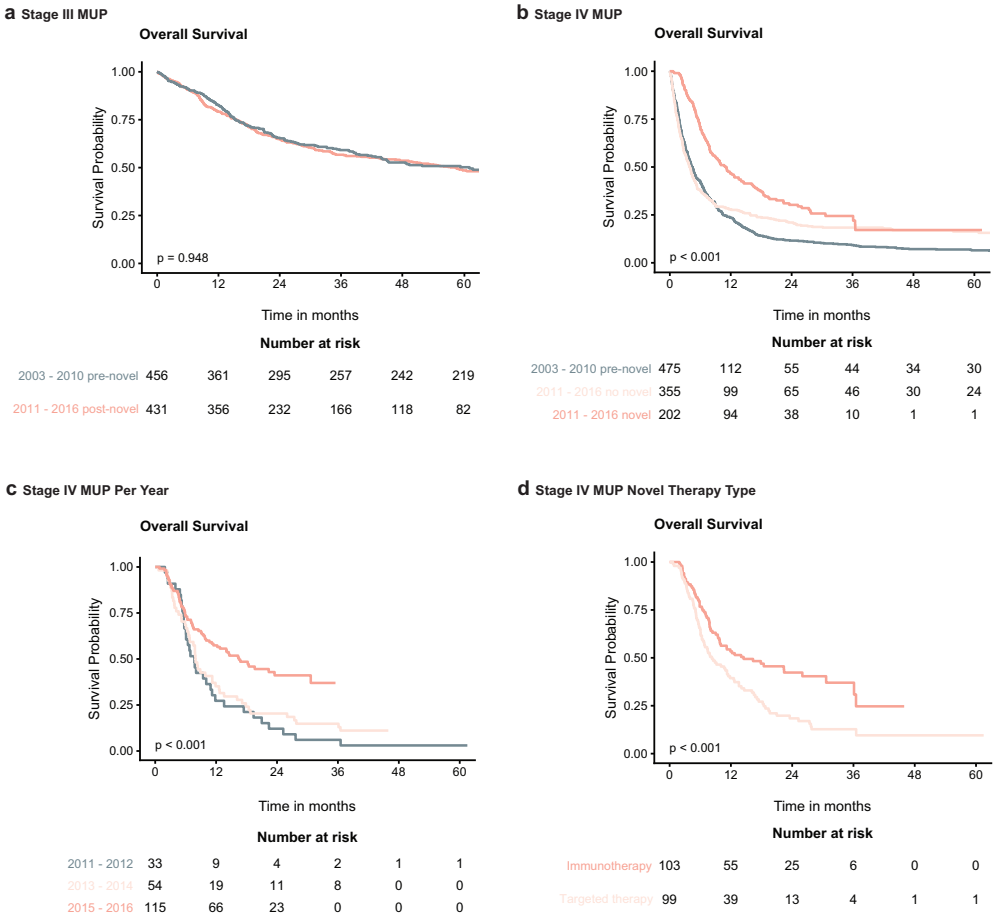
Table 3 Median OS compared between patients with stage IV MUP by whether they received novel therapy

	Novel therapy era, 2011–2016		
Subgroups	No novel therapy	Novel therapy	P value[1]
Age			
≤65 years	4 (2–42)	10 (6–37)	0.022
>65 years	4 (2–9)	12 (6–31)	<0.001
Metastatic sites			
≤2 sites	5 (2–23)	12 (6–nr)	0.001
>2 sites	3 (1–6)	10 (5–31)	<0.001
CNS metastasis			
No	4 (2–20)	14 (6–37)	<0.001
Yes	4 (2–14)	8 (5–20)	0.021

All data are shown in months. Values in parentheses are the interquartile ranges. Abbreviations: CNS, central nervous system; nr, not reached; MUP, metastasis of unknown primary; OS, overall survival. [1]Log-rank test.

For nine patients, both immunotherapy and targeted therapy were recorded as first-line therapy in the post-novel era, so these patients were excluded from further analysis. When patients with stage IV MUP received first-line treatment with immunotherapy or targeted therapy, the median OS rates were 18 months (IQR 7 to 'not reached') and 8 months (IQR 5–18), respectively ($p < 0.001$) (**Figure 2d**). Multivariable analysis of the factors affecting OS indicated that first-line immunotherapy remained a more favourable prognostic factor compared to first-line targeted therapy when adjusted for year (2011–2012, 2013–2014, and 2015–2016), gender, age, number of metastatic sites (≤ 2 or > 2), and the presence of CNS metastasis (no or yes) (**Table 4**).

Figure 2 Overall survival rates by stage, treatment era, and treatment type



Overall survival rates are shown for the following patient groups: (a) patients with stage III MUP in the pre- and post-novel therapy eras; (b) patients with stage IV MUP in the pre- and post-novel therapy eras, including those with and without novel therapy in the post-novel era; (c) patients with stage IV MUP who received novel therapy in 2011–2012, 2013–2014, and 2015–2016 in the post-novel therapy era; and (d) patients with stage IV MUP who received immunotherapy or targeted therapy first-line in the post-novel era. Abbreviations: MUP, melanoma unknown primary.

Table 4 Multivariable analysis for OS in patients with stage IV MUP receiving first-line novel therapy (n = 193)

Variables	Hazard ratio (95% CI)	P value
Incidence years		
2011–2012	Reference	
2013–2014	0.85 (0.53–1.36)	0.491
2015–2016	0.44 (0.27–0.72)	0.001
Age	1.01 (0.99–1.02)	0.269
Gender		
Male	Reference	
Female	0.92 (0.64–1.31)	0.632
Metastatic sites		
≤2 sites	Reference	
>2 sites	1.31 (0.92–1.86)	0.135
CNS metastasis		
No	Reference	
Yes	1.63 (1.14–2.33)	0.008
First-line novel therapy		
Targeted therapy	Reference	
Immunotherapy	0.64 (0.44–0.94)	0.021

Abbreviations: CNS, central nervous system; MUP, melanoma unknown primary; OS, overall survival.

DISCUSSION

The absolute number of Dutch patients diagnosed with MUP increased between 2003 and 2016, but the relative percentage compared to all newly diagnosed melanomas remained stable at approximately 3%. More than half of the patients presented with visceral metastases during this period and were diagnosed with stage IV MUP. However, 2006 was associated with a change in disease incidence, with stage III MUP being most common before 2006 and the relative incidence of stage IV MUP increasing to be higher in the subsequent years. This stage migration might be explained by the increased use of whole-body positron emission tomography, which in turn, might have increased the early detection of visceral metastases. In contrast to this result, previous studies have shown that patients with MUP tend to present with nodal involvement alone and less often with visceral metastasis[17,18]. This inconsistency may be explained by issues with the earlier studies, which used small sample sizes (65–88 patients) and data from single specialist centres for melanoma care. In contrast, we used a representative large Dutch nationwide database.

Currently, monotherapy with PD-1 blockade (pembrolizumab or nivolumab) or PD-1 blockade combined with CTLA-4 blockade (nivolumab and ipilimumab) are the preferred options for immunotherapy. The combination of BRAF and MEK inhibitors (dabrafenib and trametinib or vemurafenib and cobimetinib) are the preferred options for targeted therapy. In clinical trials, these immunotherapy and targeted therapy approaches have reported median OS durations that exceed 30 months and 20 months, respectively[19]. Although there are no specific treatment recommendations for patients with MUP, physicians tend to apply similar strategies for patients with stage-matched MKP[20]. This approach is supported by the results of a large study into the molecular characterisation of patients diagnosed with MUP, in which it was shown that the clinical behaviours and molecular patterns of BRAF/NRAS alterations were similar between patients with MUP and stage-matched MKP[21].

The percentage of Dutch patients diagnosed with stage IV MUP who were primarily treated with novel therapy increased considerably during the study period. Consistent with the respective approval dates, targeted therapies (approved 2012–2015) were prescribed at higher rates early in the novel therapy era, whereas immunotherapies were prescribed at higher rates later in that era (approved 2011 and 2015–2016). Parallel to this, the percentage of patients receiving chemotherapy decreased over time and was no longer prescribed by 2016. Together with the observed stage migration, these changes may have contributed to the significantly improved OS from 4 to 11 months among patients with stage IV MUP. Median OS durations even increased to 16 months

for patients receiving first-line novel therapy in 2015–2016. Indeed, improvement was observed in all subgroups, including those presumed to have worse outcomes (e.g., >2 metastatic sites or CNS metastasis). Patients who received first-line immunotherapy also showed a superior OS compared to those who received first-line targeted therapy, even after adjustment for year of presentation, age and gender, number of metastatic sites, and presence of CNS metastasis.

Given the hypothesis that MUP is a distinct biologic entity and the current exploratory results, one might suggest that immunotherapy may be considered the preferred first-line treatment for patients with stage IV MUP. However, the possibility of selection bias combined with the retrospective design denotes that such a conclusion should be interpreted with caution. Unfortunately, it was not possible to adjust for other potential confounding factors, such as the serum lactate dehydrogenase level, performance status, or BRAF mutation status because these factors were not recorded in the NCR. The optimal first-line treatment strategy for patients with BRAF-mutated melanoma has yet to be determined, as no randomised clinical trial has compared immunotherapy with targeted therapy for these patients[22].

We observed no benefit in OS for patients with stage III MUP after the introduction of novel therapies. This was expected because, in principle, only patients with unresectable stage IIIC were eligible to receive novel therapy. Very few patients with stage III MUP were registered in the NCR as having received novel therapy, with some of these patients even classified with stage IIIB disease. This could be explained by the likely underestimation of stage IIIC disease in our study, resulting from a lack of information about the number of involved lymph nodes in a considerable number of patients. Currently, the effective novel therapies are introduced in the adjuvant setting as routine care[23–27]. It is therefore likely that the survival of patients with stage III disease will significantly improve in the near future.

In this large population-based study, we reported the OS for patients with MUP since the introduction of immunotherapy and targeted therapy. A recent small, single-center study has also evaluated survival in patients with MUP who received novel therapy and showed that 23 patients had a median OS of 9 months after initiating immunotherapy[14]. In an accompanying systematic review, the authors only identified three other papers reporting on patients with MUP who received immunotherapy (a phase II study, a retrospective cohort study, and one case report) and seven papers concerning targeted therapy (six case reports and one small prospective observational study reporting on one patient). The phase II single-arm study reported a median OS duration of 9.9 months for patients with MUP who received ipilimumab[28]. Other studies have reported on the survival in patients with stage III and IV MUP, but they have not specifically addressed treatment with novel agents[2,5,29].

There are several limitations that must be considered when interpreting our data. Notably, the retrospective design is associated with inherent biases and must not be discounted. One example is selection bias. To partly overcome this issue, we divided the stage IV MUP population into three groups: pre-novel therapy era, 2011–2016 no novel therapy and 2011–2016 novel therapy. This division takes into account that patients in the pre-novel therapy era did not receive first-line novel therapy due to unavailability whereas patients in the 2011–2016 no novel therapy group did not receive first-line novel therapy due to unknown other reasons. In addition, patients in the 2011–2016 no novel therapy group might have received novel therapy as a second-line treatment, which might also have influenced overall survival. Another limitation is the possible misclassification of MUP, because there was no record of how many patients met the exclusion criteria proposed in 1963 by Das Gupta[30]. These include prior orbital exenteration or enucleation; evidence of a scar in the area of a positive lymph node; or prior skin excision, electrodessication, cauterization, or other surgical manipulation of a mole, freckle, birthmark, paronychia, or skin blemish. However, although we do not know the misclassification rate, we assume that it will have been low because of the high quality of recorded data and the observation that previous authors have reported a comparable incidence (approximately 3%)[2,3]. A final limitation is that the NCR does not register disease recurrence and/or progression and only records the primary treatment, meaning that we are unaware of later changes in who received novel therapy.

In conclusion, this nation-wide study showed that the incidence of MUP was approximately 3% and that this has remained largely unchanged over time. Throughout the study period, more than half of the patients with MUP presented with distant metastases and were diagnosed with stage IV MUP. We observed marked improvements in OS associated with the use of targeted therapy and immunotherapy in patients with stage IV MUP. These findings are highly relevant to clinical practice given the greater availability of novel therapy, and we anticipate continued improvements in OS should these trends persist, even among patients traditionally expected to have worse outcomes. To better understand the aetiology of MUP, additional (confirmatory) studies are needed that report on this particular type of melanoma in the era of novel therapies, and preferably that compare outcomes between patients with MUP and stage-matched MKP during novel therapy.

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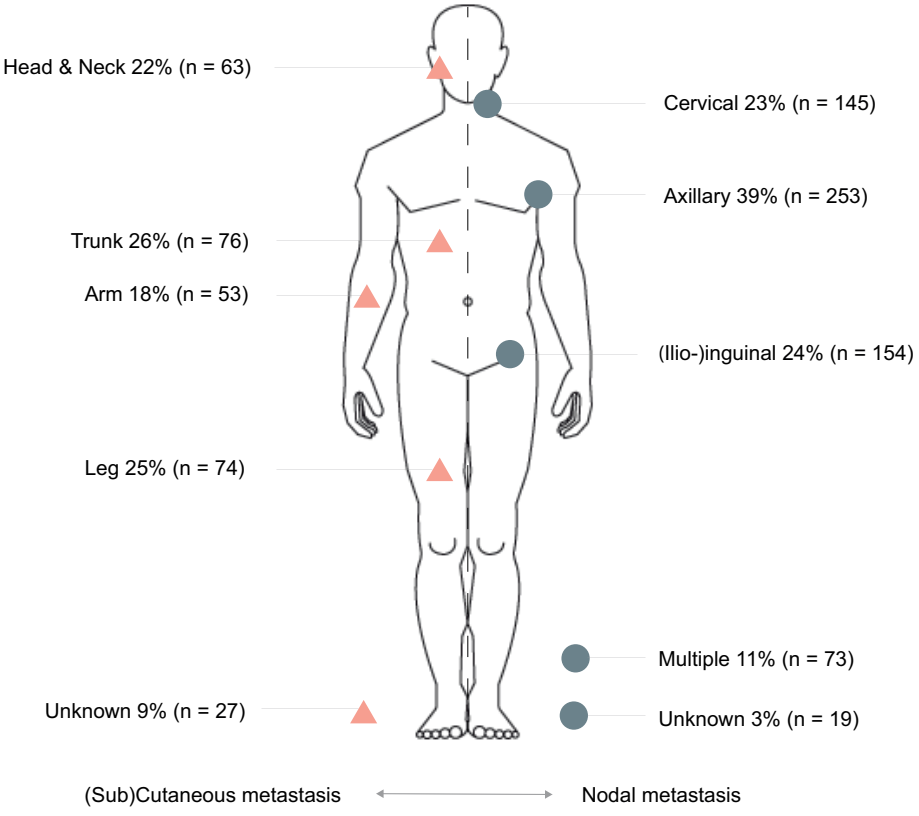
SUPPLEMENTARY MATERIAL

Supplementary Figure 1 Anatomic distribution of (sub)cutaneous tissue and lymph node involvement in patients with stage III MUP



Involved (sub)cutaneous tissue and lymph nodes are shown by pink triangles and grey circles, respectively. Abbreviations: MUP, melanoma unknown primary.

9

ANNEX 2

Author's reply to: The real-world outcome of metastatic melanoma:

Unknown primary vs. known cutaneous

Daniëlle Verver, Astrid. A.M. van der Veldt, Alexander C.J. van Akkooi, Cornelis (Kees) Verhoef, Dirk J. Grünhagen, Marieke W.J. Louwman

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Dear editor,

We thank Dr Ellebaek and colleagues for their interest in our manuscript on real-world outcomes of patients with metastatic melanoma of unknown primary (MUP) in the era of novel therapy[1].In their response, Ellebaek and colleagues address the important question whether patients with MUP and patients with melanoma of known primary (MKP) have a similar prognosis during novel therapy. To answer this question, they analysed the survival of patients with metastatic MUP and MKP from a nation-wide real-world population in Denmark in the present modern era and demonstrated similar survival between patients with MKP and MUP, with a median survival of 9.7 and 10.0 months, respectively. This result seems unexpected at first, since previous research has demonstrated superior survival for patients with MUP as compared to stage-matched patients with MKP[2]. In addition, MUP may have a favorable immunologic biology, as MUP may be associated with immune-mediated spontaneous regression of primary melanoma. In patients with regressing melanomas, immunologic surveillance mechanisms are known to be activated, which may contribute to their improved survival[3–5].For this specific analysis, cases of patients with metastatic MKP and MUP were retrieved from the Danish Metastatic Melanoma Database. One of the major advantages of this database is that it includes both patients with synchronic and metachronic metastatic disease. As a result, the Danish cohort is representative and suitable for comparing outcomes between patients with MKP and MUP. In our database, however, only patients with newly diagnosed melanoma were registered. Since approximately 50% of MUP patients and only ~3% of MKP patients present with advanced metastatic disease at initial diagnosis, patients with metachronic metastatic MKP were likely to be underrepresented and were therefore not included in our analyses[1,6,7]. A possible limitation of the current analysis by Ellebaek and colleagues is the difference in at least one of the relevant prognostic factors between the two groups. Although the proportion of "trial-like" patients in both groups was similar (41% MKP vs. 36% MUP, p= 0.46), which was defined as those patients who fulfil eligibility criteria for phase III immunotherapy trials (i.e., WHO performance status of 0 or 1, normal lactate dehydrogenase, no active brain metastasis or leptomeningeal metastases, no serious or uncontrolled medical condition, no autoimmune diseases, no previous malignancies in the last 3 years, no immunosuppressive medications and no unmeasurable disease according to the Response Evaluation Criteria in Solid Tumours, 1.1), there was an imbalance in higher disease stages according to the 7th edition of the American Joint Committee on Cancer (AJCC) staging criteria. Among patients with MUP, there was a significant higher percentage of patients with stage IV M1c disease (68% in MKP vs. 80% in MUP, p= 0.03). This imbalance in patient groups could have contributed to a relative worse survival for MUP patients, explaining the similar survival for patients with MKP and MUP during novel therapy. In summary, Ellebaek and colleagues show that there is no survival benefit for patients with MUP in the era of novel therapy. This similar survival may be explained by the fact that patients with MUP more frequently present with metastatic disease at initial diagnosis and seem to have worse baseline prognostic features.

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9

GENERAL DISCUSSION AND FUTURE DIRECTIONS

EARLY STAGE MELANOMA

SENTINEL LYMPH NODE BIOPSY – INCREASE, ALTERNATIVES AND DISAPPEARANCE

With the recent introduction of effective adjuvant therapies, the sentinel lymph node biopsy (SLNB) has been upgraded from solely being a staging method to becoming an essential selection procedure. Namely, patients with stage III melanoma, including those with a positive sentinel node (SN), are now eligible to receive adjuvant immune checkpoint inhibition or targeted therapy. As only 18% of Dutch patients with T1b melanoma (described in **chapter 2**) and up to 40% of all suitable Dutch patients with melanoma actually underwent SLNB in an era where it was still only a selection method[1, 2], it can be expected that the number of performed SLNBs will increase in the upcoming years.

Routinely performing SLNB has been regularly criticized, mainly due to its absent survival benefit, potential significant morbidity and relatively high false-negative rate[3-7]. Though it can be argued whether a regional recurrence occurring beyond several months after SLNB can still be regarded a false-negative SLNB. Nonetheless, considering these criticisms and with the expected increase in performed SLNBs, it becomes highly relevant to pursue an accurate, more minimal or even preferably non-invasive alternative. The current golden standard for SLNB includes SN identification by lymphoscintigraphic imaging with technetium Tc 99m (a radioactive tracer), followed by gamma probe guided surgical excision commonly performed under general anaesthesia.

Indocyanine green, a fluorescent optical contrast agent, has already been introduced as an alternative for radiocolloid agents. Fluorescent SNs can be detected using near-infrared fluorescence imaging systems. This technique is relatively cheap, makes use of non-ionizing radiation and offers high accuracy and sensitivity in SN identification, but the limited penetration depth of optical signals is a potential drawback[8]. Pre-operative (hybrid) single-photon emission computed tomography (SPECT)/CT imaging is another novel method used for SN identification. It has been shown to be associated with an improved detection of SNs, a lower false-negative rate, superior aesthetic results due to smaller incisions, reduced operating time and feasibility to perform the excision under local anaesthesia which results in a significant cost reduction[9-11]. Gamma-probe guided ultrasonography, as previously discussed in **chapter 3**, represents another easy applicable alternative for percutaneous identification of the SN[12].

To determine whether the identified SN also harbours metastatic disease forms another challenge, especially since the size of metastatic lesions can be very small. To date,

ultrasonography alone or combined with core needle biopsy or fine needle aspiration cytology have failed to replace surgical excision[12, 13]. This suggests that the intact removal of the SN is crucial in order to provide preservation of tissue architecture and subsequent adequate pathological analysis. In breast cancer, an intact breast lesion biopsy system is used, mainly as a diagnostic method, but it also has therapeutic possibilities for patients with benign or borderline lesions that otherwise entail open operative excision[14-16]. Perhaps a similar intact biopsy system, combined with gamma-probe or SPECT/CT guided identification, could pose a more minimal invasive alternative for SLNB.

A completely non-invasive method, where a SN is being identified percutaneously while simultaneously evaluating its metastatic status, would be an even better alternative. In this light it was examined whether 18F-fluordesoxyglucose positron emission tomography/magnetic resonance could replace the SLNB. Unfortunately, this technique was unable to reliable differentiate between patients with a positive and those with a negative SN[17]. Multispectral optoacoustic imaging may be a more promising novel technique. It showed high accuracy and sensitivity in identifying cancer-free SNs in both ex vivo as in vivo analysis[18]. Though encouraging initial data, this technique still demands further investigation.

Ultimately, the SLNB and its potential less-invasive alternatives will disappear from the scene completely. Instead, new prognostic tests based on genetic profiling using material from the primary cutaneous melanoma combined with traditional markers such as Breslow thickness will become the leading method for prognostication and treatment strategy selection. Currently, a 31-gene expression profile (GEP) is already commercially available, and its classification has been demonstrated to more accurately stratify melanoma patients in high- or low-risk as compared to SN status alone[19-21]. This profile showed consistent independent prognostic value across multiple prospective and retrospective validation studies. Another profile that seems promising and is biologically well founded is the clinicopathological (CP)-GEP profile for SLNB positivity, which could reduce the number of surgical procedures[22]. It was recently validated in an independent Dutch cohort[23]. However, despite efforts in its direction, a prognostic profile has not been established in clinical routine yet. This is partially explained by difficulties in the development of GEP-based tests, as it is hampered by several technical issues[24], and also since the clinical utility and cost-effectiveness in patients with low-risk thin melanomas (who comprise the majority of patients) is still under debate[25]. Nonetheless, it is expected that more studies will emerge examining the prognostic abilities of GEP, eventually leading to valid tools.

COMPLETION LYMPH NODE DISSECTION – NO LONGER ROUTINE PRACTICE

As was discussed previously in this thesis, CLND was not associated with a survival benefit in patients with SN positive melanoma in two randomized trials[3, 26, 27]. The increase of local control by CLND and its indispensable prognostic value were initially proposed as the two remaining arguments to continue the routine practice of CLND. Indeed, the MSLT-2 trial observed a decrease in the rate of regional nodal relapses in patients who underwent immediate CLND[3], however, having a regional nodal recurrence does not necessarily equals loss of local control[28]. Patients presenting with regional nodal recurrence can still be successfully treated with therapeutic lymph node dissection (TLND). Furthermore, this delayed dissection does not seem to effect surgical morbidity since it has been demonstrated that performing a lymph node dissection either as CLND after a positive SLNB or as TLND for palpable disease with usually greater disease burden, results in merely similar complication rates[29]. The argument that CLND is necessary for adequate prognostication also no longer applies. As shown in **chapter 6**, CLND only leads to a relevant shift in staging in a minority of patients (5-6%) and it does not appear to be necessary for adequate risk stratification since patients can be properly stratified solely based on the presence or absence of ulceration and low or high SN tumour burden (≤1.0 mm or >1.0 mm)[30]. Moreover, in **chapter 7** the additional value of positive non-SNs (retrieved after CLND) in a prediction model based on solely markers from the primary melanoma and the SLNB (including Breslow thickness, ulceration, age, and SN tumour burden) was examined, and it was observed that it only caused a marginal improvement in discrimination. Thus, CLND is not required for adequate risk stratification. In conclusion, there is no place left for CLND in the routine treatment of SN positive patients.

ADJUVANT THERAPY – WHO AND WHEN

Selection of patients is mainly based on risk of relapse and survival reflected by the American Joint Committee on Cancer (AJCC) staging system. To date, adjuvant therapy has been approved by the FDA for all stage III melanoma patients, although the trials on which its approval was founded applied different inclusion criteria. In addition, it has been demonstrated that patients with stage IIC disease have worse prognosis than those with stage IIIA disease[31]. As was discussed in **chapter 4**, perhaps patients with high-risk stage II disease should also be considered candidates. This is an important discussion in terms of public health since stage II melanoma comprises a large group of patients. The Keynote-716 trial, the first to compare adjuvant pembrolizumab with placebo in high-risk stage II melanoma patients, is likely to pave the way for adjuvant therapy in stage II disease in the upcoming years.

Using predictive markers might function as a different selection approach for adjuvant therapy. But, unfortunately, despite efforts in its direction, there is no known predictive factor for immune checkpoint inhibition or targeted therapy yet that would facilitate adequate selection, in contrast to ulceration in the case of adjuvant interferon therapy (discussed in **chapter 8**). Another approach would be to calculate the absolute treatment benefit for individual patients, since the estimated overall treatment effect that is provided by randomized trials is insufficient to decide which treatment is best suited for an individual patient.

Timing of initiation of therapy is also of discussion, namely right after surgery or post-relapse. This question is likely to be answered by results from the Keynote-054/ EORTC 1325 trial, which is the only adjuvant trial that offered crossover therapy at recurrence.

ADVANCED MELANOMA

MELANOMA UNKNOWN PRIMARY – SIMILAR BENEFIT

As shown in **chapter 9**, approximately 3% of newly diagnosed patients with melanoma have an undetectable primary, also known as melanoma unknown primary (MUP). These patients initially present with (presumed) locoregional or distant melanoma metastasis. The introduction of novel systemic therapies has also led to a significant increased survival in this specific population. As MUP may originate from primary melanomas with immune-mediated spontaneous regression and consequently have a different biology with immunological surveillance mechanisms, patients with MUP may have a more favourable prognosis as compared to patients with melanoma of known primary (MKP). However, as examined in **chapter 10**, both patients with MKP and MUP seem to derive similar benefit from treatment with novel agents, although patients with MUP are likely to present with poorer prognostic factors such as a higher performance status and more often central nervous system metastasis.

LOCALLY ADVANCED MELANOMA – ONCOLYTIC HERPES VIRUS

Locally advanced melanoma includes patients with satellite and/or in transit metastasis with or without locoregional lymph node involvement. In transit metastases are usually treated with (repeated) surgical excisions, but several other treatment options for locally advanced melanoma have emerged over the past decade including isolated limb perfusion[32, 33] and systemic immunotherapies and targeted therapies[34-37]. Recently, intralesional viral immunotherapy in the form of talimogene laherparepvec

(T-VEC), which is a modified herpes simplex virus type 1 (HSV-1), has been introduced as a new locoregional treatment option for locally advanced melanoma (stage IIIB to IVM1a according to the AJCC 7th edition). It was approved based on data from the OPTiM, a randomized phase III open-label trial, which demonstrated improved durable response rates, overall survival and quality of life, compared to subcutaneous GM-CSF injections[38-40]. The first real-world experiences with T-VEC monotherapy showed even more promising results with overall response rates varying between 57 and 89% and complete response rates between 44 and 62%[41, 42]. A key advantage of T-VEC is its tolerable safety profile, with a low rate of grade 3/4 adverse events[39-41]. Most patients only experience flu-like symptoms or have minor locoregional complaints. It is therefore a very suitable treatment option for many patients. It has even been shown to be a potential effective and safe treatment option in patients with a history of organ transplantation, who usually are not ideal candidates for systemic immunotherapy due to the potential risk of organ rejection and/or ineffectiveness associated with severe immunosuppressive medication[43, 44].

For the future, a broader role for T-VEC is expected. Currently the Masterkey 265 phase III clinical trial is ongoing, which investigates pembrolizumab with or without T-VEC for patients with unresected stage IIIB to IVM1c melanoma. Data from the phase 1B part of this trial suggest that oncolytic viral therapy may improve the efficacy of anti-PD-1 therapy by changing the tumour microenvironment (e.g. increased CD8+ T-cells, elevated PD-L1 protein expression and IFN-γ expression)[45]. Results from a previous phase II study investigating ipilimumab with or without T-VEC also indicated a greater anti-tumour activity[46]. Another role could be in the neoadjuvant setting. A phase II trial is currently comparing neoadjuvant T-VEC plus surgery with surgery alone for patients with completely resectable stage IIIB to IVM1a melanoma (NCT02211131). An interim-analysis recently demonstrated that more patients in the combination group were able to undergo surgery as planned, had a higher pathological complete response and a higher R0 resection margin[47]. The combination with BRAF/MEK inhibitors is also being studied in the neoadjuvant setting for advanced nodal BRAF mutant melanoma (NCT03972046). Lastly, their might also be a role for T-VEC in the treatment of primary melanomas in difficult locations such as digits or toes, which normally often require a digit or toe amputation, based on positive experiences in our own centre.

NEOADJUVANT SYSTEMIC THERAPY – NEW STRATEGIES

As briefly mentioned in the above paragraph, the neoadjuvant approach has been introduced in the management of melanoma. Possible advantages of such an approach are reduction of tumour burden and facilitating surgical resection, providing

potentially valuable information regarding pathological response, and potentially providing prognostic and toxicity information which may for example help guide adjuvant therapy decision making[48]. Several studies have already been conducted with promising results[49-52]. In the OpACIN trial, patients with palpable stage III disease were randomized to receive ipilimumab 3mg/kg and nivolumab 1mg/kg either in an adjuvant or neoadjuvant schedule and it was observed that the neoadjuvant schedule resulted in a high percentage of pathological responses and was capable to expand more tumour-resident T cell clones than the adjuvant schedule, though at the cost of high toxicity[53]. In order to reduce toxicity and preserve efficacy the OpACIN-neo trial was initiated. This trial evaluated three schedules of neoadjuvant checkpoint blockade with ipilimumab and nivolumab and demonstrated a high proportion of patients (57%) with a complete response in the identified tolerable neoadjuvant dosing schedule: two cycles of ipilimumab 1 mg/kg plus nivolumab 3 mg/kg, and interestingly, no relapses were noted in this subgroup[54]. Currently this schedule is evaluated in another phase 2 study (PRADO trial) aiming to confirm the previous results and to test whether a TLND can be safely omitted in patients with a (near) complete response[55]. Targeted therapy in this setting has also been investigated. In a phase 2 study where patients were randomized to receive standard of care or neoadjuvant plus adjuvant dabrafenib and trametinib, it was observed that patients randomized to the latter showed significantly increased event-free survival, which caused the study to terminate accrual early[56]. The promising schedule was further examined in a single arm study, the NeoCombi trial, and a high proportion of patients with a complete response (46%) was observed, though still almost half of these patients relapsed[57]. In conclusion, a number of studies have been completed and yield promising results, and long-term data is eagerly awaited. Currently several studies with other (combinations of) targeted and immunotherapeutic agents are ongoing. It is expected that neoadjuvant therapy will be implemented in the near future with the goal to reduce tumour burden and allow for definitive surgical resection or even to minimize the extent of surgery needed. In addition, biochemical studies will provide important insights that will guide further treatment decisions.

SURGERY IN STAGE IV MELANOMA – NOVEL ROLES

In light of the rapidly evolving improvements in systemic treatment for stage IV melanoma it becomes relevant to re-evaluate the role of surgical treatment in this setting. Before this era, metastectomy was performed in appropriately selected patients, usually with oligometastatic disease, and sometimes resulted in extended survival. To date, surgical resection in combination with novel systemic treatment has

not been prospectively evaluated yet. A recent retrospective matched-pair analysis demonstrated a significant improvement in melanoma-specific survival for patients who received upfront surgery followed by modern systemic therapy compared to those only receiving modern systemic therapy[58]. A similar observation was described in a retrospective cohort study of patients with brain metastases, those patients receiving upfront (radio)surgery followed by novel therapy showed superior survival as compared to those solely receiving novel therapy[59]. Another study demonstrated that patients undergoing metastectomy showed prolonged survival even when corrected for factors such as disease stage and immunotherapy[60]. The rationale behind this may be found in the observations that modern systemic therapies seem to be more effective amongst patients with less tumour burden, i.e. lower baseline lactate dehydrogenase (LDH) or lower number of involved organ sites[61]. In addition, the surgical removal of more aggressive or potentially resistant sites, might cause the more responsive sites to remain. Based on these findings, combining surgery with modern systemic therapies seems to be a promising approach. In chapter 11 a study protocol was presented that prospectively examines this rationale. Other roles for surgery in the advanced setting are resection of symptomatic lesions, salvage surgery for those experiencing disease progression after systemic management or treating residual disease after novel therapy.

CONCLUSION

During the upcoming years, the surgical oncologist will continue to play a key role in the management of melanoma, although the main focus is likely to shift from early stage melanoma to (locally) advanced melanoma. In early stage melanoma, an increase in the number of performed SLNBs is expected initially, but numbers will decrease when more minimal invasive techniques are introduced. Even more so, the SLNB is likely to disappear completely when valid gene expression tools that solely use material from the primary melanoma are implemented. However, in the meantime, the SLNB remains of crucial importance as it selects patients for effective adjuvant therapy. The number and extent of lymph node dissections will decrease as well, as CLND after a positive SN is no longer routine practice and since TLND in the metastatic setting will become less extensive (or even obsolete) due to the introduction of effective neoadjuvant therapies. On the other hand, new roles are emerging with the introduction of local oncolytic therapies in (locally) advanced melanoma. In addition, surgical management is likely to increase in the advanced metastatic setting, either as a diagnostic method

or as salvage treatment to increase response to novel systemic therapy. Altogether, the advances in melanoma management will require an even more holistic approach than before and reflects a complicated interplay between dermatologists, pathologists, radiation therapists, surgical oncologists and medical oncologists.

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APPENDIX

SUMMARY

The research described in this thesis focuses on the highly evolving therapeutic landscape of melanoma, with special focus on the role of surgery. Part I addresses several aspects of the sentinel node (SN) in early stage melanoma and part II concentrates on the treatment and outcomes of patients with advanced stage melanoma.

PART I – EARLY STAGE MELANOMA

In 2018, the 8th American Joint Committee on Cancer (AJCC) staging edition was introduced. This edition includes revisions regarding the subdivision of pT1 melanoma; pT1a, Breslow thickness <0.8 mm without ulceration, or pT1b, Breslow thickness ≥0.8 mm, or <0.8 mm with ulceration. In **chapter 2** the expected impact of these novel criteria were evaluated, which is relevant as patients with pT1b melanoma are eligible to undergo sentinel lymph node biopsy (SLNB). For this study, data from all newly diagnosed pT1 melanomas between 2003 and 2015 were retrieved from the Netherlands Cancer Registry (NCR), which concerned over 30.000 patients. It was concluded that implementation of this new edition would not have major impact on the total number of patients classified pT1b and consequently the number of patients eligible for SLNB. In addition, as a trend for a higher SN positivity rate in pT1b melanomas classified according to the 8th edition was observed, selection of high-risk pT1 patients may improve.

Currently, the surgical SLNB is still golden standard for regional lymph node staging. In **chapter 3** a more minimally invasive alternative, fine needle aspiration cytology (FNAC) with combined gamma probe and ultrasound guidance, was prospectively studied in patients with newly diagnosed melanoma or breast cancer. The trial was terminated early, as the prespecified sensitivity of at least 80% could not be achieved. It was concluded that this new technique was not able to correctly detect metastases in the SN. Interestingly, gamma probe guided ultrasound was observed to be a highly accurate method for percutaneous identification of the SN. This method could be incorporated in future studies pursuing less invasive alternatives for regional lymph node staging.

The SN is negative in approximately 70-80% of patients with melanoma. These patients are usually only offered regular surveillance examinations, though their prognosis varies greatly. **Chapter 4** describes the development and validation of a nomogram predicting 5-year recurrence and melanoma-specific mortality (MSM) in patients with negative SNs. The study was based on data from more than 3000 patients from four European Organization for Research and Treatment of Cancer (EORTC) Melanoma

Group centres. The prediction models included three independent prognostic factors, ulceration, anatomical location and Breslow thickness, and showed good model performance with a c-index of 0.74 for the recurrence model and 0.76 for the calibrated MSM model. They also showed reasonable performance across internal-external cross-validation. These prediction models could provide accurate personalized estimates useful for personalized treatment strategies, e.g. less-intensified surveillance strategies or even consideration for adjuvant therapy.

Chapters 5, **6** and **7** focus on patients with SN-positive melanoma. In **chapter 5**, the optimal extent of groin completion lymph node dissection (CLND) was studied. Retrospective data from patients with SN-positive melanoma from four tertiary Dutch melanoma referral centres was used, and concerned 137 and 118 patients that underwent inguinal or ilioinguinal dissection, respectively. No significant differences in recurrence pattern and survival rates were observed between these two approaches. Recently, two randomized trials have confirmed that CLND in general, regardless the extent and location, is not associated with any survival benefit. In **chapter 6** consequences of omitting CLND were evaluated and it was explored whether adequate stratification is possible by solely using information from the primary melanoma and SLNB. A retrospective cohort from nine EORTC melanoma group centres was used including more than 1000 SN-positive patients. CLND identified additional positive lymph nodes in 19%, but only resulted in upstaging in AJCC stage 8th edition in 5%. Dividing patients solely on the presence or absence of ulceration and low or high SN tumour burden (≤1.0 mm or >1.0 mm) resulted in relatively adequate risk stratification in comparison with the AJCC classification. Thus, CLND only leads to a relevant shift in staging in a minority of patients and also appears not to be necessary for adequate risk stratification. In **chapter 7** risk stratification in patients with SN-positive melanoma was further examined. A nomogram predicting 5-year recurrence, distant metastasis (DM) and overall mortality (OM) was developed using the previous used retrospective cohort described in chapter 6. For external validation, a cohort consisting of 473 patients who participated in the DeCOG randomized trial complemented with 232 patients from a single centre was used. The prediction models included five independent prognostic factors, gender, age, ulceration, Breslow thickness and SN tumour burden, and showed good model performance with a c-index of 0.68 for the recurrence model, 0.70 for the calibrated DM model and 0.70 for the calibrated OM model. They were successful in external validation. A model including information on the non-SN status, retrieved after CLND, showed only marginal improvement in discrimination. In conclusion, the EORTC-DeCOG nomogram provides an adequate prognostic-tool without the need for CLND, and this model can be used for patient counselling and might aid in adjuvant

therapy decision-making. In **chapter 8** immunohistochemical analysis of various molecules was conducted in order to better understand the possibility of ulceration being a biological entity, as ulceration seems to be the overriding determinant of activity of adjuvant interferon (IFN) therapy. Two retrospective cohorts were used, one consisting of primary melanomas from the Institute of Gustave Roussy and the other of positive lymph nodes from the EORTC 18952 and 18991 trials. It was demonstrated that both primary and metastatic ulcerated melanoma exhibit higher basal expression of MHC class I molecules, independently from Breslow thickness, histology and lymphocytic infiltration. It was also found that primary ulcerated melanomas harboured higher constitutive levels of the antiviral protein Mx1 at the epidermal border of the tumour. These findings suggest that ulcerated melanomas expand in a tumour microenvironment where the chronic exposure to type 1 IFN could favor a response to exogenous IFNs.

PART II – ADVANCED MELANOMA

Approximately 3% of patients present with metastatic melanoma of unknown primary (MUP). **Chapters 9** and **10** focus on these subgroup of patients. In **chapter 9** the impact of the introduction of novel systemic therapies was explored in patients with MUP. For this study, data from all newly diagnosed MUPs between 2003 and 2016 were retrieved from the NCR, which concerned over 2000 patients. It was demonstrated that a little more than half of the patients are primarily diagnosed with stage IV disease. Median overall survival in patients with stage IV disease diagnosed in the pre-novel therapy era (2003-2010) and those diagnosed in the post-novel therapy era (2011-2016) not receiving novel therapy was 4 months. It significantly improved to 11 months in patients who were treated with novel therapy in the post-novel therapy era.

Chapter 10 tackles the relevant question whether there is a survival difference between patients with melanoma of known primary (MKP) and those with MUP on novel therapies. Data from all melanomas registered between 2012 and 2017 were retrieved from the Dutch Melanoma Treatment Registry (DMTR). A total of 2706 patients were included; 2321 with MKP and 385 with MUP. In comparative analysis, patients with MUP harboured worse prognostic factors, but median overall survival in crude analysis was similar (12 months for MKP and 14 months for MUP, P=0.278). In adjusted analysis, OS was better for MUP patients. These results support the hypothesis that patients with MUP may also have (unknown) favourable prognostic factors. However, these factors were not affected by novel therapies. In conclusion, patients with MUP and

MKP seem to similarly benefit from treatment with novel agents. In **chapter 11** a study protocol for a multicentre randomized trial is presented, where patients with advanced melanoma and stable disease on immunotherapy will be included. The primary aim of this study, the UNLOAD trial, is to compare treatment failure-free survival (proxy for progression free-survival) between patients randomized to either continuing first-line immunotherapy or continuing first-line immunotherapy with additional local treatment such as surgical resection. It is hypothesized that tumour reduction might improve response to immunotherapy and thereby leading to improved survival.

NEDERLANDSE SAMENVATTING

Het onderzoek dat wordt beschreven in dit proefschrift spitst zich toe op het sterk veranderde landschap van de behandeling van het melanoom, met speciale aandacht voor de rol van chirurgie. Deel I richt zich op de verschillende aspecten van de schildwachtklier (SWK) bij vroeg stadium melanomen en deel II concentreert zich op de behandeling en uitkomsten van patiënten met gevorderd melanoom.

DEEL I – VROEG STADIUM MELANOOM

In 2018 werd de 8e editie van het American Joint Committee on Cancer (AJCC) stadiëringssyteem geïntroduceerd. Deze editie bevatte wijzigingen in de onderverdeling van pT1 melanomen; pT1a, Breslow dikte <0.8 mm zonder ulceratie of pT1b, Breslow dikte ≥0.8 mm of <0.8 mm met ulceratie. In **hoofdstuk 2** worden de verwachtte gevolgen van deze nieuwe criteria geëvalueerd, wat relevant is aangezien patiënten met een pT1b melanoom in aanmerking komen voor een SWK procedure. Voor deze studie werd data van alle patiënten met een nieuw gediagnosticeerd pT1 melanoom tussen 2003 en 2015 verzameld uit de Nederlandse Kanker Registratie (NKR), wat resulteerde in meer dan 30.000 patiënten. Er werd geconcludeerd dat implementatie van deze nieuwe editie waarschijnlijk geen grote impact heeft op het totaal aantal melanomen dat als pT1b zal worden beschouwd, en dus ook niet op het totaal aantal patiënten geschikt voor de SWK procedure. Bovendien wordt verondersteld dat de 8e editie tot iets betere selectie van hoog risico patiënten leidt, gebaseerd op de gevonden hogere trend voor SWK positiviteit in pT1b melanomen die geclassificeerd zijn volgens de nieuwe criteria.
Momenteel is de chirurgische SWK procedure nog steeds de gouden standaard voor de stadiëring van regionale lymfeklieren. In **hoofdstuk 3** wordt een meer minimaal invasief alternatief, dunne naald aspiratie cytologie met gecombineerde begeleiding van gamma probe en echografie, prospectief bestudeerd in patiënten met nieuw gediagnosticeerd melanoom of borstkanker. De studie werd vroegtijdig afgebroken omdat de vooraf vastgestelde beoogde sensitiviteit van minstens 80% niet meer gehaald zou kunnen worden. Er werd geconcludeerd dat deze nieuwe techniek niet geschikt was om correct metastasen in de SWK vast te stellen. Echter werd er wel waargenomen dat gamma probe geleide echografie een zeer accurate methode was om percutaan de SWK te identificeren. Deze methode kan worden gebruikt in toekomstige studies die opzoek gaan naar minder invasieve methoden voor stadiëring van regionale lymfeklieren.

De SWK is negatief in ongeveer 70-80% van de patiënten met minus de melanoom. Deze patiënten krijgen vaak alleen regelmatig poliklinische controles aangeboden, ondanks dat de prognose binnen de groep sterk varieert. **Hoofdstuk 4** beschrijft de ontwikkeling en validatie van een nomogram dat de kans op 5-jaars recidief en melanoom-specifieke mortaliteit (MSM) kan voorspellen in patiënten met een negatieve SWK. De studie was gebaseerd op data van meer dan 3000 patiënten uit vier European Organization for Research and Treatment of Cancer (EORTC) Melanoma Group centra. De predictie modellen omvatten drie onafhankelijke variabelen, ulceratie, anatomische locatie en Breslow dikte, en lieten een goede prestatie zien met een c-index van 0.74 voor het recidief model en 0.76 voor het gekalibreerde MSM model. Tevens lieten ze een redelijke prestatie zien in interne-externe crossvalidatie. De accurate individuele uitkomsten kunnen zeer waardevol zijn om gepersonaliseerde behandelstrategieën toe te passen zoals minder frequente poliklinische controles of juist selectie voor adjuvante therapie.

Hoofdstukken 5, **6** en **7** richten zich op patiënten met een positieve SWK. In **hoofdstuk 5** wordt de optimale mate van een completerende lymfeklierdissectie (CLKD) in de lies onderzocht. Retrospectieve data van patiënten uit vier tertiaire verwijs centra werd gebruikt. In totaal ondergingen 137 patiënten een inguinale klierdissectie en 118 een ilioinguinale klierdissectie. Er werd geen significant verschil gevonden in recidief patroon en overleving tussen beide technieken. Twee gerandomiseerde studies hebben inmiddels aangetoond dat CLKD op zichzelf, ongeacht de mate en locatie, niet geassocieerd is met een winst in overleving.

In **hoofdstuk 6** worden de consequenties onderzocht van het achterwege laten van CLKD en wordt er bekeken of adequate stratificatie mogelijk is op basis van alleen informatie van het primaire melanoom en de SWK. Een retrospectief cohort bestaande uit data van patiënten uit negen EORTC melanoma group centra werd hiervoor gebruikt, wat resulteerde in meer dan 1000 patiënten. CLKD identificeerde additionele positieve klieren in 19% van de patiënten, echter slechts 5% van de patiënten kreeg hierdoor een hoger stadium toegewezen (AJCC 8e editie). Het onderverdelen van patiënten alleen op basis van de aan- of afwezigheid van ulceratie en laag of hoog SWK tumour burden resulteerde in relatief adequate risico stratificatie in vergelijking met de AJCC classificatie. Dus, CLKD leidt alleen in een zeer beperkt aantal patiënten tot relevante upstaging en lijkt niet nodig te zijn voor adequate risico stratificatie.

In **hoofdstuk 7** werd de risico stratificatie in patiënten met een positieve SWK verder onderzocht. De ontwikkeling en validatie van een nomogram dat de kans op 5-jaars recidief, afstandsmetastasen en overall mortaliteit kan voorspellen in patiënten met een positieve SWK wordt in dit hoofdstuk beschreven. Hiervoor werd het cohort dat

ook in hoofdstuk 6 is beschreven gebruikt. Voor externe validatie werden patiënten uit de gerandomiseerde DeCOG studie aangevuld met patiënten uit een enkel centrum gebruikt. De predictie modellen omvatten vijf onafhankelijke variabelen, geslacht, leeftijd, ulceratie, Breslow dikte en SWK tumour burden, en lieten een goede prestatie zien met een c-index van 0.68 voor het recidief model, 0.70 voor het gekalibreerde afstandsmetastasen model en 0.70 voor het gekalibreerde overall mortaliteit model. Tevens waren ze succesvol in externe validatie. Een model met ook informatie over de status van overige klieren, verkregen na een aanvullende klierdissectie, liet slechts een beperkte verbetering zien in discriminatie. Concluderend, het EORTC-DeCOG nomogram betreft een adequate prognostische tool, die geen informatie behoeft van een aanvullende klierdissectie, en die gebruikt kan worden bij het counselen van de patiënt en mogelijk kan bijdragen aan de beslissingen omtrent adjuvante therapie.
In hoofdstuk 8 worden de immunohistochemische analyses van verschillende moleculen beschreven om zodoende meer inzicht te krijgen in de mogelijkheid dat ulceratie een aparte biologische entiteit is, vooral aangezien ulceratie de belangrijkste determinant is van de werking van adjuvante interferon (IFN) therapie. Hiervoor werd gebruik gemaakt van twee retrospectieve cohorten, een bestaande uit primaire melanomen van het Instituut Gustave Roussy en de ander uit positieve lymfeklieren van de EORTC 18952 en 18991 studies. Er werd aangetoond dat zowel geulcereerde primaire melanomen en positieve lymfklieren van geulcereerde melanomen een hogere basale expressie hebben van MHC class I moleculen, onafhankelijk van Breslow dikte, histologie en lymfocyten infiltratie. Tevens werd gezien dat geulcereerde primaire melanomen een hogere peri-tumorale expressie hadden van het antivirale eiwit Mx1. Deze bevindingen suggereren dat geulcereerde melanomen expanderen in een tumor micro-omgeving waar de chronische blootstelling aan type I IFN kan leiden tot een gunstige respons op exogene IFNs.

DEEL II – GEVORDERD MELANOOM

Ongeveer 3% van de patiënten met gemetastaseerd melanoom presenteert zich met een melanoom van onbekende oorsprong. **Hoofdstukken 9** en **10** richten zich op deze subgroep. In **hoofdstuk 9** wordt de impact van de introductie van de nieuwe systemische middelen onderzocht in patiënten met melanoom van onbekende oorsprong. Voor deze studie werd data van alle patiënten met een nieuw gediagnosticeerd melanoom van onbekende oorsprong verzameld uit de NKR, wat resulteerde in meer dan 2000 patiënten. Er werd aangetoond dat iets meer dan de helft van de patiënten zich

primair presenteert met stadium IV ziekte. De mediane overleving was 4 maanden voor patiënten gediagnosticeerd in de tijd voor de introductie van de nieuwe middelen (2003-2010) en voor de patiënten gediagnosticeerd in 2011-2016 maar die geen nieuwe therapie hadden gekregen. Dit steeg naar 11 maanden voor de patiënten gediagnosticeerd in 2011-2016 die wel nieuwe middelen hadden gekregen.

Hoofstuk 10 concentreert zich op het relevante vraagstuk of er een verschil is in overleving tussen patiënten met melanoom van bekende en onbekende oorsprong die beide behandeld zijn met de nieuwe middelen. Data van alle patiënten die geregistreerd zijn met gevorderd melanoom tussen 2012 en 2017 werd verzameld uit de Dutch Melanoma Treatment Registry (DMTR). Er werden in totaal 2706 patiënten geïncludeerd; 2321 met bekende origine en 385 met onbekende origine. Uit de analyses kwam naar voren dat patiënten met een melanoom van onbekende origine vaker slechtere prognostische kenmerken hebben maar dat de mediane overleving in ongecorrigeerde analyse vergelijkbaar is (12 maanden voor patiënten met bekende origine en 14 maanden voor onbekende origine, P=0.278). In gecorrigeerde analyse is de overleving wel beter voor patiënten met een melanoom van onbekende oorsprong. Dit suggereert dat patiënten met een melanoom van onbekende origine ook over (onbekende) gunstige factoren beschikken, hoewel deze niet lijken te worden beïnvloedt door de nieuwe middelen. Concluderend hebben patiënten met een melanoom van onbekende origine en bekende origine evenveel profijt van de nieuwe middelen.

In **hoofdstuk 11** wordt een studie protocol voor een multi-centrum gerandomiseerde studie gepresenteerd, waarin patiënten met gevorderd melanoom en een stabiele respons op immunotherapie kunnen worden geïncludeerd. Het primaire doel van deze UNLOAD studie is om de treatment failure-vrije overleving (proxy voor progressie-vrije overleving) te vergelijken tussen patiënten gerandomiseerd voor continueren van immunotherapie met patiënten gerandomiseerd voor continueren van immunotherapie inclusief additionele lokale therapieën zoals chirurgische resectie. De hypothese is dat reductie in hoeveelheid tumor de respons op immunotherapie verbetert en daardoor ook de overleving.

PORTFOLIO

Name PhD student:	PhD period:
Daniëlle Verver, MD	October 2016 – December 2018
Erasmus MC department:	Promotor:
Surgery, Division of Surgical Oncology	prof. dr. C. Verhoef, MD PhD
Research school:	Copromoter:
Medicine	dr. D.J. Grünhagen, MD PhD

1. PhD training

	Year	Workload (ECTS)
General Academic Skills		
- Research Integrity	2017	0.3
- BROK (Good Clinical Practice)	2017	1.5
- 'IGZ-dag: kijken met andere ogen'	2017	0.3
Research Skills		
- ECCO-AACR-EORTC-ESMO Workshop on Methods in Clinical Cancer Research (MCCR)	2018	3.0
- Biostatistical Methods I: Basic Principles Part A (NIHES)	2017	2.0
- Open Clinica Course	2017	0.3
Oral Presentations		
- Najaarsdag 2018 NVVH, Ede, the Netherlands	2018	1.0
- 38th ESSO congress, Budapest, Hungary	2018	2.0
- EORTC MG fall meeting, Mallorca, Spain (x 2)	2018	1.0
- Chirurgendagen 2018 NVVH, Veldhoven, the Netherlands	2018	1.0
- EORTC MG spring meeting, Amsterdam, the Netherlands	2018	1.0
- SSO congress, Chicago, USA	2018	1.0
- TFGM vergadering, Utrecht, the Netherlands	2017	1.0
- EORTC MG fall meeting, Warsaw, Poland (x 2)	2017	2.0
- Chirurgendagen 2017 NVVH, Veldhoven, the Netherlands	2017	1.0
- Wetenschapsdag Heelkunde EMC, Rotterdam, the Netherlands	2017	1.0
Poster Presentations		
- 38th ESSO congress, Budapest, Hungary	2018	0.5
- Chirurgendagen 2018, NVVH, Veldhoven, the Netherlands (2x)	2018	1.0
- European Cancer Congres (ECCO), Amsterdam, the Netherlands	2017	0.5

(Inter)national Conferences		
- Symposium Behandeling St III Melanoom, Breda, the Netherlands	2019	0.1
- Najaarsdag 2018 NVVH, Ede, the Netherlands	2018	0.3
- 8e WIN-O symposium, Ede, the Netherlands	2018	0.3
- 38th ESSO Oncology congress, Budapest, Hungary	2018	0.5
- EORTC MG fall meeting, Mallorca, Spain	2018	0.5
- Meeting Centers of Excellence Imlygic, Eemnes, The Netherlands	2018	0.1
- Chirurgendagen 2018, NVVH, Veldhoven, the Netherlands	2018	0.5
- EORTC MG spring meeting, Amsterdam, the Netherlands	2018	0.3
- Meeting Centers of Excellence Imlygic, Utrecht, the Netherlands	2018	0.1
- SSO congress, Chicago, USA	2018	1.5
- 7e WIN-O symposium & 2e BeNe Meet, Rotterdam, the Netherlands	2017	0.3
- Post-ESMO/WCM meeting Melanoom, Utrecht, the Netherlands	2017	0.1
- EORTC MG fall meeting, Warsaw, Poland	2017	0.5
- 1st Cells to Surgery Congress, Rotterdam, the Netherlands	2017	0.3
- Chirurgendagen 2017 NVVH, Veldhoven, the Netherlands	2017	0.5
- Wetenschapsdag Heelkunde EMC, Rotterdam, the Netherlands	2017	0.5
- ECCO congress, Amsterdam, the Netherlands	2017	0.5
- 17e Wondcongres, Rotterdam, the Netherlands	2016	0.3
Other		
- Working visit Prof. Eggermont, Villejuif, France	2018	1.5
- Working visit Prof. Leiter, Tübingen, Germany	2018	1.5
Awards		
- Zilveren Kreeft Bokaal, Wetenschapsdag Heelkunde Erasmus MC, Rotterdam, the Netherlands (best abstract and oral presentation)	2017	

2. Teaching

	Year	Workload (ECTS)
Coaching		
- Basiscursus coaching Bachelor studenten	2017	0.2
- Vervolgtraining 'Talentinterview'	2017	0.1
- Intervisie/informatie bijeenkomsten coaching Bachelor studenten	2017	0.2
- Gesprekken jaar 1	2017	0.4
- Gesprekken jaar 2	'17-'18	0.4
- Gesprekken jaar 3	'18-'19	0.4
Supervising practicals and excursions		
- Basic Life Support examinator medical students	'17-'18	1.0

LIST OF PUBLICATIONS

THIS THESIS

Verver D, Grunhagen DJ, van Akkooi ACJ, Aarts MJB, van den Berkmortel FWPJ, van den Eertwegh AJM, de Groot JW, Boers-Sonderen MJ, Haanen JBAG, Hospers GAP, Kapiteijn E, Piersma D, van Rijn RS, SuijkerBuijk KPM, ten Tije AJ, Vreugdenhil G, Verhoef C, van der Veldt AAM. Clinical outcome of patients with metastatic melanoma of unknown primary in the era of novel therapy.
Submitted.

Verver D, Rekkas A, Garbe C, van Klaveren D, van Akkooi ACJ, Rutkowski P, Powell BWEM, Robert C, Testori A, van Leeuwen BL, van der Veldt AAM, Keilholz U, Stadler R, Eggermont AMM, Verhoef C, Leiter U, Grünhagen DJ. The EORTC-DeCOG nomogram adequately predicts outcomes of patients with sentinel node-positive melanoma without the need for completion lymph node dissection.
Eur J Cancer. 2020 May 23;134:9-18.

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ABOUT THE AUTHOR

Daniëlle Verver was born on the 13th of May, 1990, in Nieuwegein and grew up in IJsselstein. In 2008 she graduated secondary school at the Anna van Rijn College in Nieuwegein, where she participated in a bilingual education program (Dutch and English). In 2008 she followed the higher professional degree course in nursing and received her propaedeutic diploma. Daniëlle started medical school at Erasmus University in Rotterdam in 2009. Her interest in surgery and conducting research started early during her medical training, when she worked as a medical student at the department of Trauma Surgery at Erasmus University Medical Center and assisted PhD students at the department of Transplant Surgery, under supervision of dr. F.J.M.F. Dor. In her last year of medical training, she was selected to participate in the Excellence Program ("dedicated schakeljaar") of the department of Surgery and followed internships at the Erasmus University Medical Center and Maasstad Hospital in Rotterdam. During that time she conducted research on hepatic angiomyolipomas under supervision of prof. dr. J.N.M. IJzermans and prof. dr. R.A. de Man (Erasmus University Medical Center), and on extra-articular phalangeal fractures under supervision of dr. N.W.M. Schep (Maasstad Hospital). After she obtained her medical degree in 2016, she started working as a surgical resident not in training at Reinier de Graaf Gasthuis in Delft. In October 2016 she started to work on the research described in this thesis at the department of Surgical Oncology at Erasmus University Medical Center, under the supervision of prof. dr. C. Verhoef and dr. D.J. Grünhagen. From 2019 onwards, Danielle combined the research with clinical work at the department of Surgery at Franciscus Gasthuis & Vlietland in Rotterdam and started her surgical residency training per January 2020 (dr. T.M.A.L Klem).