Characteristics and clinical outcomes of mucosal melanoma

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Contents

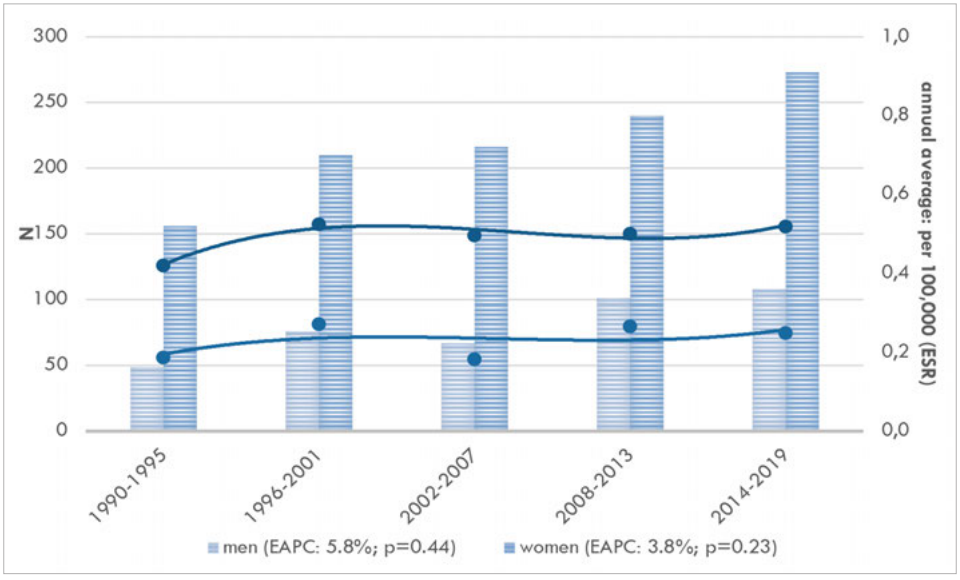
General introduction and outline of this thesis

1

Mucosal melanoma and uveal melanoma: rare types of melanoma

Mucosal melanoma (MM) is a rare type of tumour that arises from melanocytes located in the mucosal lining. [1] MM represents 1.4% of all melanomas and has an age-adjusted incidence of 2.2 cases per million, which remained stable over time (Figure 1). [2-6] The RARECARE network, a large collaboration between population-based cancer registries across Europe, estimated approximately 850 new cases per year in Europe. [7] In the Netherlands, 1496 patients were diagnosed with MM between 1990-2019, corresponding with an age adjusted incidence of 3.5 cases per million. [8]

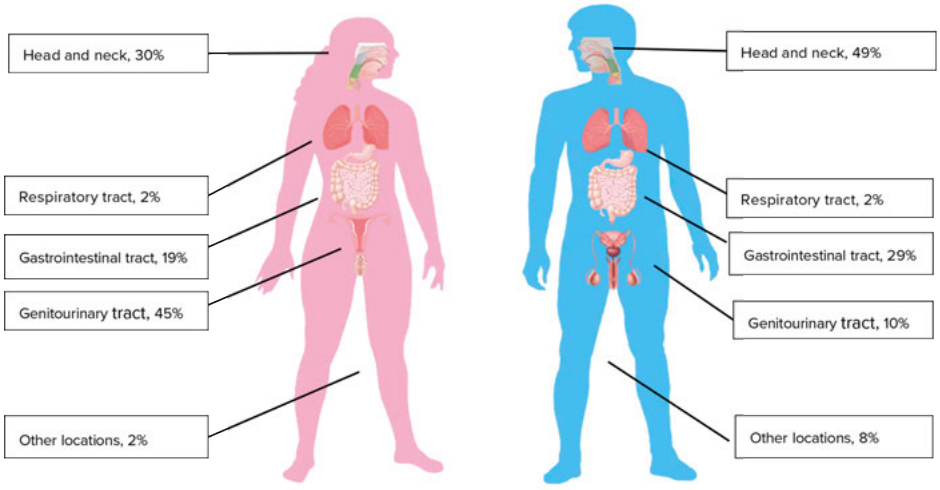
Figure 1. Crude numbers (bars, left axis) and annual averaged, age-adjusted incidence rates (line, right axis) for patients with mucosal melanoma by sex in the Netherlands.



MM is more common in females than in males, with an incidence of 2.8 cases per million and 1.5 cases per million, respectively. Among females, the genitourinary tract and head and neck region are the most commonly affected sites, whereas in males, MM is predominantly seen in the gastrointestinal tract and head and neck region (Figure 2, Table 1). [9, 10] The higher incidence in females can be attributed by the high percentage of MM located in the female genital tract, particularly the vulva and vagina. [2, 9, 11] Similar to cutaneous melanoma (CM), MM exhibits a large geographic and racial variation, but in contrast with CM has a higher prevalence in non-Caucasians. [12, 13] In particular, the Asian population has a higher proportion of melanomas located in the mucosal lining (23-38%) compared

to the Caucasian population (<1%). [2, 13, 14] Whilst in CM, geographical differences are related to the amount of UV-radiation exposure and type of skin-color, the rationale of the geographic and racial differences in MM is not clear. [13] Unfortunately, due to a low number of population-based studies, the epidemiology of this disease is poorly understood.

Figure 2. Distribution of mucosal melanoma in men and women



This figure is based on six population-based studies ([2, 6, 9, 15-17]) Other locations consists of MM located at the gallbladder, prostate, brain, spinal cord

Like MM, uveal melanoma (UM) is a rare subgroup of melanoma which develops from the iris, ciliary body, or choroid of the eye. The incidence of UM is 4.4 cases per million in Europe and comprises 3-5% of all melanomas. [7, 18, 19] Despite their common origin, both MM, UM and CM display extreme differences in their biological behavior. [20] Whilst, in all, metastatic disease is the leading cause of mortality, the metastatic pattern is distinct. Disease spreads hematogenously in UM, resulting in metastasis in the liver in 90-95% of the cases, whilst in CM disease spreads lymphatically, thus metastasis are seen in the lungs, brain, lymph nodes and soft tissue. [20, 21] In MM the metastatic pattern is not fully understood and large variations exist between anatomical locations. [22] Moreover, the rapid disease progression and concomitantly poor prognosis of UM, together with the comparable lower efficacy of immunotherapy as compared to CM, has resulted in a liver-directed treatment approach in metastatic UM. [20]

Table 1. Overview of clinical characteristics and survival and recurrence rates in total and in subgroups of mucosal melanoma.

	Proportion of total (%)	Median age at diagnosis (years)	Gender (%) Male	Female	2-year OS (%)	5-year OS (%)	Median OS (months)	Recurrence rate (%)
Total [1, 8, 15, 23, 24]	-	55-72	44	56	44.4	14-27.6	**11**	**83**
Tumour site								
Head and neck [25-32]	50-60	64	50-54	42-50	64	20-44	54	81
Oral [1, 25, 28, 29, 33]	20-24	58	55	45	55.7	28, 33.1-45	24	41
Sinonasal [5, 29, 32, 34-37]	14.5	60/70	44	56	45	21, 23-43	21	50-70
Larynx [38, 39]	4-15	59.7	77.3	20.5	46	12	-	88
Anorectal tract [8, 40-43]	24	62-75	61	39	30.2	7-17	8-19/17	65
Rectum [8, 42-44]	39.7	67	22-39 [1]	61-78	23.5	18	16-22	70
Anus [8, 42, 43]	60.3	61	39	61	38.4	11	27	59
Upper gastrointestinal tract [5, 15, 45, 46]	5/6.7-12	53	56	44	-	21	34	-
Female genital tract [8, 40, 47-51]	15-20/18	66	0	100	50-57	27.8	24	56
Vulva [8, 40, 47, 49]	68-75	62	0	100	0-63	45	45	38, 42-70
Vagina [8, 40, 49-51]	5-25/ 19	73	0	100	26.1	13-32	11-17	-
Urinary tract [8, 15, 45, 52]	3-5	57-63	32	68	63	31	25-34	71-92

This table consists of studies including a minimum of ten patients. OS: Overall survival

Clinical presentation and subtypes of mucosal melanoma

MM typically presents in the seventh decade of life, though also younger cases, particularly in the head and neck region and female genital tract, have been reported. [9, 53] As this disease is heterogenous with various primary locations, clinical presentation is diverse. Regardless of the primary location, common symptoms are a painful or itching sensation and (oral, nasal, vaginal or rectal) bleeding. [54] MM located at more visible locations, such as the vulva, vagina, penis and some sublocations of the head and neck region, may present with a brown, black or blue lesion which changes over time. However, approximately 40% of the MM are amelanotic which make them difficult to identify and hard to distinguish from benign or premalignant lesions. [55] The lack of visibility and late or aspecific symptoms result in a substantial delay in diagnosis, with patients being diagnosed at an advanced stage in approximately 40-60% of the cases. [40, 45, 56] Yet, even in patients who present with local disease, the course of disease is aggressive with eventually 79% developing regional or distant metastasis, of which many in the first year. [40, 57]

Most common are head and neck mucosal melanoma (HNMM), which predominantly arise from the sinonasal and oral cavity, and less frequently are located in the pharynx or larynx (Figure 20). [28] The vast majority of all HNMM (90-95%), do not have distant metastasis at presentation, leading to a better prognosis compared to other locations. [26, 27] Yet, oral MM still have a high risk of nodal involvement (25-43%) as compared to sinonasal MM (<10%). [27] Within the HNMM, patients generally are diagnosed at a younger age which may be attributed to the more visible and accessible location, leading to earlier detection. [27] The female genital tract is the second most prevalent location of MM, of which the vulva comprises three quarter of the cases. [58] Unlike other locations, the vulva consists of both hairy (cutaneous) skin which gradually transitions to glabrous (mucosal) skin. Due to the fact that melanoma can develop from both cutaneous and mucosal skin in the vulvar region, determining the origin of the melanoma can be challenging. [59] Like many other vulvar or vaginal issues, reluctance to get gynecological examination can often lead to a delay in seeking medical attention, leading to a more advanced disease at diagnosis. The third largest group of MM is found in the anorectal region. Like in the female genital tract, metastasized disease is common in patients with anorectal MM, resulting in lower 5-year OS rates of 47%. [60, 61]

Staging

Although various staging systems have been proposed, a universal staging system for MM does not exist (Table 2). The challenge in developing a staging system suitable for all locations lies in the heterogenous course of disease, the pathological differences and

surgical considerations which heavily depend of the site-specific anatomy. [62] Additionally, the low number of patients make it difficult to validate staging systems. The Ballantyne staging system was introduced in 1970 to stage HNMM, classifying patients based on the extent of the disease as local, regional, or distant spread (Table 2). [63] The American Joint Committee of Cancer (AJCC) developed a staging system including depth of invasion (i.e. TNM classification), which proved to be more predictive for survival in HNMM than the Ballantyne staging system (Table 2 and 3). [28, 64] In 2009, the AJCC updated the staging system for MM by removing T1 and T2 but keeping T3 and T4 as categories for tumour invasion, resulting in at least AJCC stage III disease, indicating the aggressiveness of MM (Table 2). [65] For other locations than the head and neck, there is no site-specific staging system. The AJCC staging system for CM is proven to predict OS in anorectal and vulvar MM and therefore is commonly used in clinical practice (Table 2). [45, 61]

Table 2. Staging systems for head and neck, female genital and anorectal melanoma [67]

	Staging system	Important characteristics of the current staging system	Previously proposed staging system(s)
Vulvar MM			
	AJCC for CM [66]	See Table 3 for the entire staging system.	Macrostaging: FIGO staging [67] Microstaging: Clark [68], Breslow[69], Chung staging [70]
Vaginal MM			
	Ballentyne staging system for head and neck MM (I/II/III) [63]	Stage I Local disease Stage II Regional disease Stage III Distant metastasis	
Head and Neck MM			
	AJCC MM head and neck [71]	T3: Epithelium/submucosa T4a: Deep soft tissue, bone, overlying skin T4b: Brain, dura, skull base, lower cranial nerves, masticator space, carotid artery, prevertebral space, mediastinal structures, cartilage, skeletal muscle, bone	Ballentyne [63], Modified Prasad/Ballantyne staging [72], Thompson staging [73]
Anorectal MM			
	Adapted AJCC for CM [66, 74]	T1 Thin (≤ 1 mm) T2 Intermediate (2-4 mm) T3 Thick (>4 mm)	
	Ballentyne staging system for head and neck MM [63]	Stage I Local disease Stage II Regional disease Stage III Distant metastasis	

MM: Mucosal melanoma, CM: Cutaneous melanoma, FIGO: The International Federation of Gynecology and Obstetrics, AJCC: The American Joint Committee on Cancer, T stage: the T describes the extent of the primary tumour.

Treatment

The management of local disease, irrespective of location, consists of surgical resection, with complete resection providing the highest chance for cure. [9] Though based on retrospective small studies, the existing evidence in MM, does not support wider pathological or surgical margins to benefit OS or recurrence-free survival (RFS). [75] As more extensive surgery in an often challenging location, in close relation to vital organs, is mutilating and can negatively affect quality of life, further research on this topic is warranted. [76] Also, as the course of disease is aggressive with high risks of local or distant recurrence, (extensive) surgical procedures should be weighed carefully against these risks and complications of surgical intervention.

As there is a high rate of regional spread in MM, close follow-up of clinically negative nodes is required. Although the evidence is limited, sentinel node biopsy in locally confined VMM, HNMM, and anorectal MM may be valuable for identifying patients who might benefit from adjuvant treatment. [40, 77] Elective lymph node dissection is no part of standard treatment as there is no proven survival benefit and has high complication rates. [40, 78] In HNMM and anorectal MM adjuvant radiotherapy may improve local control and reduce the risk of local recurrences but does not prolong survival, in part because of the high rate of systemic relapses. [28, 79, 80] The treatment plan of locally advanced and unresectable MM should be individualized depending on age, involvement of adjacent tissue/organs, feasibility, and the patient's preference. Whilst evidence is still awaiting, neoadjuvant therapy is of interest in bulky MM, as it may reduce tumor load ensuing complete resection. [81] An investigational approach, in which immunotherapy is given in neoadjuvant setting, is of interest in MM. Those treated with neoadjuvant/adjuvant immunotherapy in a phase II trial including resectable stage III/IV melanoma, less often had disease progression or recurrence, when compared with those treated with adjuvant immunotherapy only. [82] Yet, in this trial only four patients with MM were included, all of them received the same treatment strategy and OS was not evaluated. Given the high recurrence rates and challenges associated with achieving tumour-free surgical margins, neoadjuvant immunotherapy presents itself as a viable option in MM treatment. Currently, clinical trials (NCT03313206 and NCT02519322) are ongoing, investigating the use of neoadjuvant immunotherapy in MM.

The optimal clinical management for patients with positive lymph node(s) has not been established. In HNMM, therapeutic lymph node debulking (LND) is thought to optimize regional control without improving OS. [26, 78, 83] Some advocate LND in VMM and anorectal MM, but most studies emphasize the high complication rate of LND in the groin without survival benefit. Therefore nodal treatment should be individualized and outcomes should be monitored. [77] Despite not prolonging survival, it may identify patients who are in need of intensified treatment. Radiotherapy of (bulky) lymph nodes only has a role in improving local control and complaints, but does not improve OS. [28, 77]

For metastatic disease, guidelines are lacking, and therefore treatment choices are often made on case-by-case basis and relying on expert opinion. Radiotherapy can be offered as palliative treatment to relieve symptoms and improve quality of life, whilst chemotherapy is not part of treatment as response rate and duration are low and does not improve survival. [77] The overall response rate (ORR) of various chemotherapeutic agents in MM is slightly lower than in CM (0-20% vs 15-30%), but most importantly survival benefit in both CM and MM is very limited, whilst toxicity rates are high. [84-86] Hence, research focusses on novel systemic therapies, which, since the introduction in 2011, have drastically improved OS of advanced CM. [87] In particular the immune-checkpoint inhibitors (ICI) nivolumab, pembrolizumab and ipilimumab have revolutionized the therapeutic field in immunogenic cancers. These agents target CTLA-4 and PD-1 which are immune checkpoints located on activated T-cells, normally suppressing immune response against cancer cells. By blocking the CTLA-4 and PD-1 pathway, the suppression is released, and the cancer cells are recognized and attacked providing a boost in immune response. [88] In advanced CM, single agent ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1) both have superior efficacy over chemotherapy. The combination of both yields even better ORR, though is accompanied with concomitant toxicity. [87] The Checkmate 238, a randomized phase 3 trial assessing adjuvant nivolumab vs ipilimumab in resected stage IIIB-C and stage IV melanoma reported that nivolumab is a more efficacious drug with a higher 4-year RFS respectively 41.2% vs 51.7%. [89] Similarly, the CheckMate 067 trial, demonstrated the benefit of ipilimumab, nivolumab and combined nivolumab/ipilimumab in advanced CM, with a median OS after 6.5 years of follow-up of 19.9, 36.9 and 72.1 months. [90]

In MM anti-CTLA-4 blockage has shown only a minor survival benefit, with an ORR of 0-17% and progression-free survival (PFS) of less than 5 months. [91] As in CM, anti-PD1 blockage and in greater degree the combination of anti-CTLA-4 and anti-PD1 blockage are more promising. A pooled analysis of six clinical trials report that in advanced MM, nivolumab and nivolumab combined with ipilimumab have an ORR of 23% and 37% and a median PFS of 3 and 6 months, respectively. [92] However, whilst only little comparative data is available, MM seems less immunogenic than CM with on average 30% lower ORR of combined anti-CTLA-4 and anti-PD-1. [91] Unfortunately, patients with rare types of melanoma as MM and uveal melanoma (UM) are often excluded from clinical trials, and therefore evidence is limited. Therefore, though having distinct clinical behaviour and a different genetic profile, treatment of metastatic MM follows the insights of CM.

Pathogenesis and tumour biology

Together with acral melanoma, Spitz melanoma, and a melanoma in a blue or congenital naevus, MM is classified as a distinct entity by the World Health Organization, all lacking a

relation with chronic sun damage. [19] MM arise from melanocytes which are specialized cells derived from neural crest cells, producing melanin. Melanin is pigment that gives our skin color but most importantly protects the DNA in the cell from UV light. [93] The purpose of melanocytes located at sun shielded locations as the vulva or anus or head and neck is not clear. It is hypothesized that cells may have migrated during neural crest migration. [94] In contrast to its cutaneous counterpart, which has a high mutational load caused by UV mutagenesis, MM has a lower mutational burden, endorsing that UV light exposure does not play a role in the pathogenesis of MM. [19] In CM the UV-induced BRAF mutation and the NRAS mutation is found in 35-50% and 43% of the patients, whilst in MM this is seen in respectively 6% and 8% of the cases. [93] However, MM harbour a KIT mutation and NF-1 mutation in 13% and 20% of the cases, both being infrequent in CM. [93]. Thus, as BRAF mutations are lacking in MM, the KIT mutation is the only mutation for which targeted therapy is available (Imatinib). Furthermore, in the era of immune and targeted therapy, studies in CM have demonstrated that a higher tumor mutational load is predictive for a better and more durable response to ICI. [95, 96] The low mutation rate in MM explains that this entity seems less immunogenic and highlights the need for translational research in this field. [97] Besides unraveling the genetic landscape, efforts are made to understand the pathogenic role of well-known carcinogenic factors as smoking and human papilloma virus. Although both are risk factors for squamous cell carcinoma of the vulva and head and neck, they have not been associated to MM. [98] Up to today, no hereditable genetic predispositions for MM have been identified.

Prognosis

Survival of patients with MM is poor, and regardless of stage of disease, is worse than CM (Table 1). [99] 5-year OS of patients with MM is only 27.6% compared with 76.3% in CM. [5] Also, MM has high recurrence rates and time to recurrence is relatively short. [100] Unlike CM, in which a 6.4% annual decline in mortality was seen between 2013 and 2017, survival rates for MM have not improved over the last decades. [101] The rapid decrease in mortality in CM is largely attributed to better preventive measures and early diagnosis, leading to a higher proportion of patients with localized disease and a lower Breslow thickness at diagnosis. [87] In contrast, MM is often diagnosed at an advanced stage and effective preventive measures do not exist. Moreover, the introduction of immune and targeted therapy has improved 5-year OS in advanced CM from less than 10% to 40-50%, which has lower efficacy in MM. [87] As MM is rare, and patients have only recently been included in trials, the effect of the novel systemic therapies on OS in MM is not well studied.

The knowledge gap in MM

Whilst research in CM has accelerated, the rarity of this tumour kept MM from being investigated at the same pace. Unfortunately, studies assessing incidence and survival covering the last decades are limited. While 20% of the patients with CM develop metastasis, this percentage is rigorously higher in patients with MM, highlighting the need for better treatment strategies for advanced MM. The evaluation of novel systemic treatment on survival of MM and research focusing on the pathogenesis and tumour environment are crucial to guide the way forward.

Thesis outline

The main aim of this thesis is to give an overview of clinical behavior, incidence, survival, and predictors of survival of rare melanomas of which mucosal melanoma (MM). Second, to analyze if survival has changed against the background of recently introduced immune and targeted therapies. In this thesis we highlight an important subgroup of MM, those located at the vulva, by presenting a full-spectrum overview of a large cohort of patients with vulvar melanoma (VM).

Using data from the nationwide cancer registry, we were able to accurately evaluate incidence and survival of MM over time in the Netherlands. The Dutch Melanoma Treatment Registry (DMTR) adds specific value by registering treatment data combined with survival and recurrence data. In light of the rapidly evolving treatment landscape this gives valuable data of fairly new treatment in a real-world setting.
In **part 1** we evaluate incidence and survival of mucosal melanoma in the Netherlands. Chapter 2 gives an overview of MM over a thirty-year time period (1990-2019) in the Netherlands using data from the National Cancer Registry. Nationwide incidence rates, clinical characteristics, primary treatment strategies and survival for all stages of MM are analysed. Moreover, we evaluate if survival of MM has improved over the last decades.

The last two decades are marked by the introduction of immune- and targeted therapy, and population-based research has confirmed that the introduction of these therapies resulted in survival benefit for CM and therefore has changed perspectives for these patients dramatically. In Chapter 3 data from the DMTR is used to investigate the survival benefit of MM treated with immune and targeted therapy as compared to with those who received no treatment or conventional therapies. Furthermore, we analysed if survival of MM has improved as much as CM over the same time period. Chapter 4 includes data from the same DMTR database in which we assess the response and toxicity rates of patients with MM and UM treated with the combination of ipilimumab and nivolumab.

In **part 2** we take a detailed look at a subgroup of MM, located at the vulva. Due to the low numbers evidence is limited and there are no guidelines comprising the management of this disease. Chapter 5, presents a comprehensive overview of the literature, discussing the clinicopathological and genetic characteristics of VM. Furthermore, we evaluated the predictive value of these factors in terms of survival and recurrence. As a translation to the clinical practice, we established a flowchart including the diagnostic process and therapeutic strategies that can be used in clinical management. In Chapter 6 we describe a large international retrospective cohort of VM's and asses the clinicopathological characteristics, mutation status and treatment of 198 cases. In addition, recurrence rates, survival curves and prognostic factors of survival and recurrence are presented.

In the general discussion in **part 3** a summary of this thesis is given and implications for future research are discussed.

Table 3. Seventh edition of the AJCC staging (2009) for cutaneous melanoma [66]

T category	Thickness	Ulceration status/ mitoses	
T0: No evidence of primary tumour	Not applicable	Not applicable	
Tis (melanoma in situ)	Not applicable	Not applicable	
T1	≤1.0 mm	a: without ulceration and mitosis < 1/mm^2 b: with ulceration or mitosis > 1/mm^2	
T2	>1.0–2.0 mm	a: without ulceration and mitosis < 1/mm^2 b: with ulceration or mitosis > 1/mm^2	
T3	>2.0–4.0 mm	a: without ulceration and mitosis < 1/mm^2 b: with ulceration or mitosis > 1/mm^2	
T4	>4.0 mm	a: without ulceration and mitosis < 1/mm^2 b: with ulceration or mitosis > 1/mm^2	
N category	**Number of metastatic nodes**	**Nodal metastatic burden**	
N0	No regional metastases detected	No	
N1	One tumour-involved node	a:micrometastasis b: macrometastasis	
N2	Two or three tumour-involved nodes	a:micrometastasis b: macrometastasis c: in transit or satellite metastasis without metastatic nodes.	
N2a	Two or three clinically occult (i.e., detected by SLN biopsy)	No	
N2b	Two or three, at least one of which was clinically detected	No	
N2c	One clinically occult or clinically detected	Yes	
N3	Four or more tumour-involved nodes, or matted nodes, or in transit metastases/satellites with metastatic nodes		
M category	**Anatomic site**	**LDH level**	
M0	No evidence of distant metastasis	Not applicable	
M1a	Distant metastasis to skin, soft tissue including muscle, and/or nonregional lymph node	Not elevated	
M1b	Distant metastasis to lung	Not elevated	
M1c	Distant metastasis to non-CNS visceral sites with or without M1a or M1b sites of disease	Not elevated	
M1d	Any distant metastasis	Elevated	
Stage	**Primary tumour (T)**	**Lymph node (N)**	**Metastases (M)**
0	Tis	N0	M0
IA	T1a	N0	M0
IB	T1b T2a	N0 N0	M0 M0
IIA	T2b T3a	N0 N0	M0 M0

Table 3. (*Continued.*)

Stage	Primary tumour (T)	Lymph node (N)	Metastases (M)
IIC	T4b	N0	M0
IIIA	T1- 4a	N1a	M0
	T1- 4a	N2a	M0
IIIB	T1- 4b	N1a	M0
	T1- 4b	N2a	M0
	T1- 4a	N1b	M0
	T1- 4a	N2b	M0
	T1- 4a	N2c	M0
IIIC	T1- 4b	N1b	M0
	T1- 4b	N2b	M0
	T1- 4b	N2c	M0
	Any T	N3	M0
IV	Any T	Any N	M1

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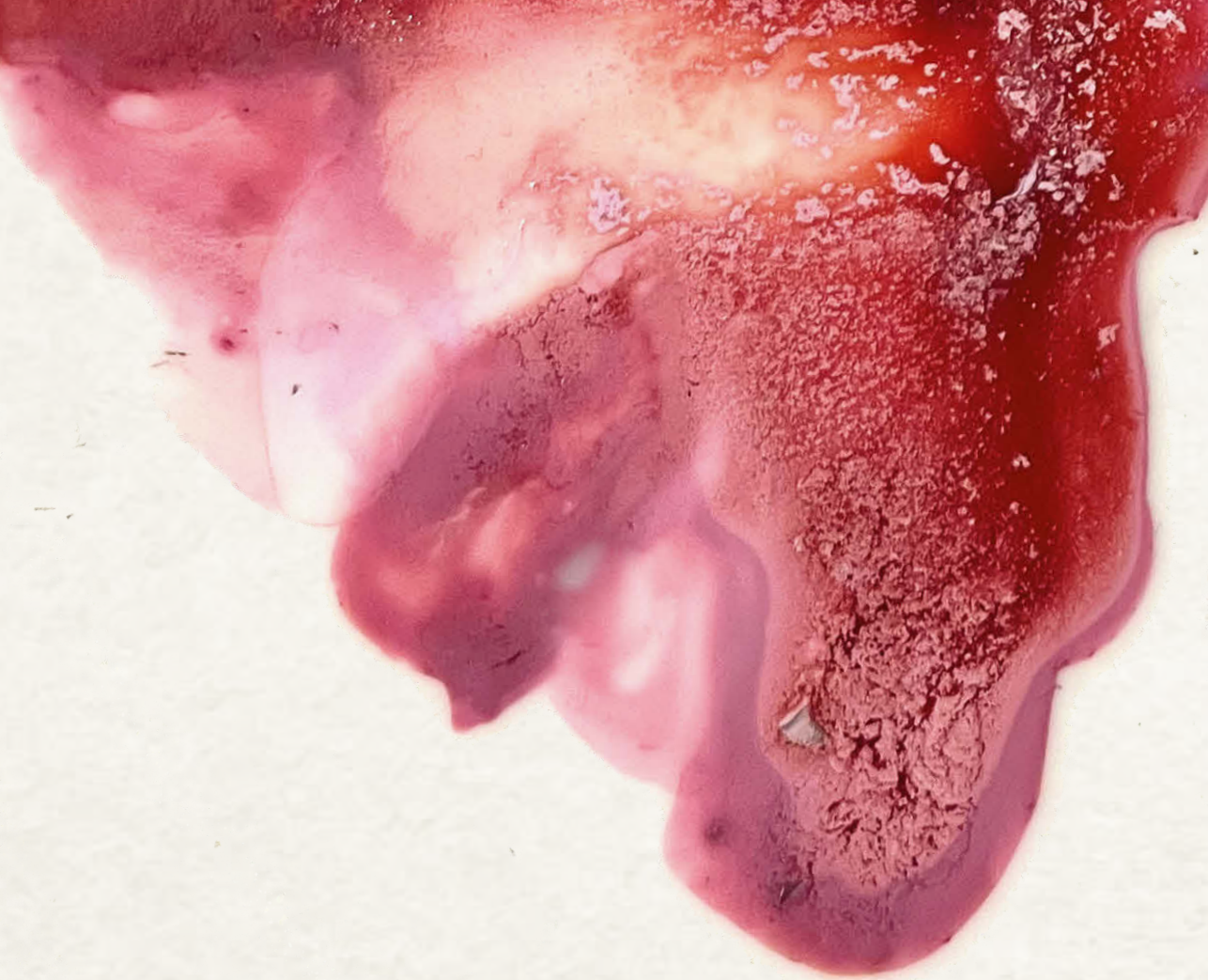

PART I

Mucosal melanoma

Trends in incidence and survival of 1496 patients with mucosal melanoma in the Netherlands (1990-2019)

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Simple summary

Mucosal melanoma (MM) is rare and entails a poor prognosis. MM is biologically different from cutaneous melanoma (CM). For advanced CM, overall survival has improved since the introduction of immune and targeted therapy. In contrast, little is known about the effect of their introduction on the survival of MM. This study presents the incidence, clinical characteristics, treatment characteristics, and survival of MM over 30 years (1990–2019) in the Netherlands. We conclude that the incidence of MM remained stable, and survival has slightly improved when comparing the timeframe 2014–2019 with previous years. However, the prognosis of MM remains poor as compared to CM. Future studies addressing the effect of immune and targeted therapy in MM are needed to improve outcomes for patients with MM.

Abstract

Background

Mucosal melanoma (MM) is a rare tumour with a poor prognosis. Over the years, immune and targeted therapy have become available and have improved overall survival (OS) for patients with advanced cutaneous melanoma (CM). This study aimed to assess trends in the incidence and survival of MM in the Netherlands against the background of new effective treatments that became available for advanced melanoma.

Methods

We obtained information on patients diagnosed with MM during 1990–2019 from the Netherlands Cancer Registry. The age-standardized incidence rate and estimated annual percentage change (EAPC) were calculated over the total study period. OS was calculated using the Kaplan–Meier method. Independent predictors for OS were assessed by applying multivariable Cox proportional hazards regression models.

Results

In total, 1496 patients were diagnosed with MM during 1990–2019, mostly in the female genital tract (43%) and the head and neck region (34%). The majority presented with local or locally advanced disease (66%). The incidence remained stable over time (EAPC 3.0%, p = 0.4). The 5-year OS was 24% (95%CI: 21.6–26.0%) with a median OS of 1.7 years (95%CI: 1.6–1.8). Age ≥70 years at diagnosis, higher stage at diagnosis, and respiratory tract location were independent predictors for worse OS. Diagnosis in the period 2014–2019, MM located in the female genital tract, and treatment with immune or targeted therapy were independent predictors for better OS.

Conclusion

Since the introduction of immune and targeted therapies, OS has improved for patients with MM. However, the prognosis of MM patients is still lower compared to CM, and the median OS of patients treated with immune and targeted therapies remains fairly short. Further studies are needed to improve outcomes for patients with MM.

1. Introduction

Mucosal melanomas (MM) are malignant tumours arising from melanocytes located in the mucosal lining of the head and neck region or the respiratory, gastrointestinal, anorectal, or genital tract. [1] MM is rare and accounts for approximately 1.4% of all melanomas in the Caucasian population. Incidence is higher in the Asian population (23% of all melanomas), boosting research on this entity in this region. [2] MM has a higher incidence in women than men (2.8 cases per million versus 1.8 cases per million). This is partly explained by the mucosal lining in the female genital tract, which comprises 15–20% of all MM. [3-7] Due to its rarity, MM is still poorly understood, and clinical management is mostly based on guidelines for cutaneous melanoma (CM) [8-10].

MM has a significantly lower 5-year overall survival (OS) compared to CM (37% versus 92%). [11] Furthermore, MM entails a lower median OS after the detection of distant spread disease (9.1 versus 11.7 months). [12] The poor prognosis of MM is assumed to be caused by aggressive tumour behaviour, higher tumour stage at diagnosis, and an often-challenging location for surgical excision, more often leading to incomplete resections. Additionally, MM has a lower tumour mutational burden and may be less immunogenic, which makes the metastatic disease less sensitive to immunotherapy. Compared to CM, MM harbour a BRAF mutation less often (40–50% in CM versus 10% in MM). However, MM more often contain a targetable KIT mutation (2–10% in CM versus 15–39% in MM), although response duration on KIT inhibitors is short. [13] More importantly, a lower PD-1 expression rate (17–29% in MM versus 34% in CM) may affect the potential benefit of immunotherapy. [14] Since its introduction in 2011, immunotherapy with CTLA-4 and PD-1 inhibitors and targeted therapy (BRAF and MEK inhibitors) have completely changed treatment strategies for stage III and IV CM. The effect of these therapies is reflected by an increase in 5-year OS between 2013 and 2016, from 81% to 92% in men and from 88% to 96% in women. This is predominantly due to improved OS in stage II, III, and IV disease. [15, 16] Furthermore, as neoadjuvant therapy in both high-risk resectable and locally advanced CM, immunotherapy can result in shrinkage of the primary tumour, facilitating R0 resections and improving surgical morbidity. [17]

In contrast to CM, the efficacy of immune and targeted therapy in MM remains unknown, as patients with MM are often excluded from clinical trials. Moreover, it is hypothesized that MM does not benefit from the introduction of immune and targeted therapy as much as CM. This is demonstrated by a recent observational study reporting that the median OS of stage III and stage IV MM did not improve in the time period 2015–2017 compared to 2013–2014 (8.7 months vs. 8.9 months, respectively). [16]

This population-based study reports on long-term trends in the incidence and survival of MM in the Netherlands. We aimed to evaluate whether survival has improved since the

introduction of immune and targeted therapies. We estimated the impact of these therapies by assessing the effectiveness of their time of introduction as a proxy for the prognosis of patients with MM. Furthermore, by analyzing all stages and all tumour sites of this disease, alternative explanations for the poorer survival of MM compared to CM may be explored.

2. Materials and Methods

2.1. Patient Selection

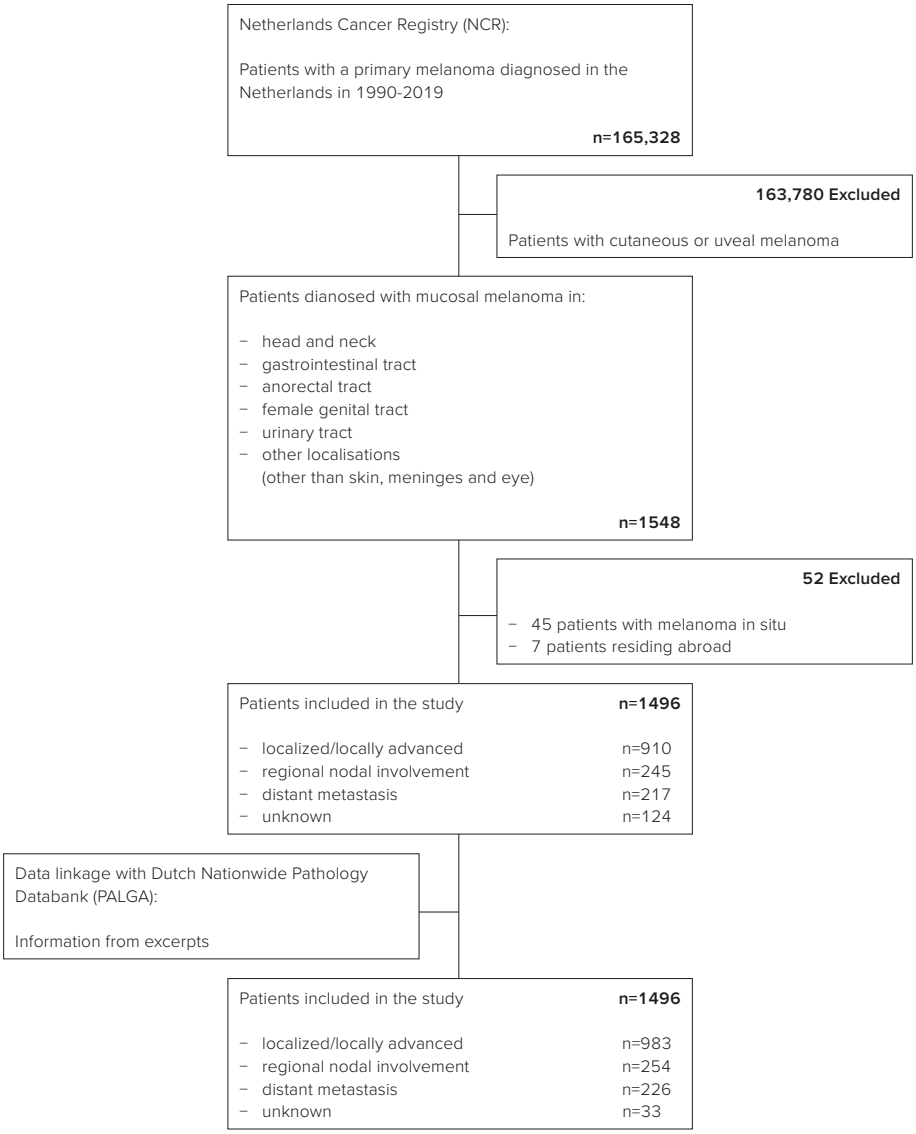
We retrieved patient records from the Netherlands Cancer Registry (NCR), hosted by the Netherlands Comprehensive Cancer Organization (IKNL). The NCR is a nationwide population-based registry containing information on patient and tumour characteristics, primary treatment, and survival of all newly diagnosed cases of cancer in the Netherlands since 1989. Follow-up information on the vital status of every patient is obtained through a yearly linkage with the Municipal Personal Records Database (Gemeentelijke Basisadministratie, GBA), with the latest update obtained on 31st January 2022. Primary treatment is registered for therapies provided as part of the initial treatment plan; no information was available on second or higher-line treatment. The study design, data abstraction process, and storage protocols were approved by the national supervisory committee of the NCR.

From the NCR database, all patients with a primary MM diagnosed during the period 1990–2019 were selected. Cases were identified based on topography and histology codes of the International Classification of Diseases for Oncology (ICD-O). Patients with melanoma in situ were excluded, as were foreign patients, as the date of death was not available for these patients (Figure 1).

Due to the different staging classifications applied to different tumour locations (e.g., TNM and Extent of Disease), and concurrent changes of the TNM staging system over time, MM were reclassified as local or locally advanced disease, locoregional spread disease, or distant spread disease. Local or locally advanced disease was defined as a disease confined to the primary tumour location and close surroundings. Locoregional spread disease entails being either pathologically or radiologically confirmed as spread to any lymph node(s). Distant spread disease is defined as a disease with either pathologically or radiologically confirmed spread to distant skin, visceral organs, or bone. Given the large proportion of cases with an initially unknown stage (n = 124; 8.3%, Table 1), the study database was matched with the Dutch Nationwide Pathology Databank (PALGA) (Figure 1). Based on the detailed information from pathology reports, most of the cases with unknown stages could be reclassified (unknown stage n = 33; 2.2%, Table 1). With respect to the tumour site, cases were classified based on the ICD-O code in the head and neck, gastrointestinal tract, anorectal tract, female genital tract, and respiratory tract. The head and neck were

subcategorized as oral, sinonasal and pharynx/glottis, female genital tract in vulva, vagina, and other, and anorectal in the anus and rectum (Table S1). As immunotherapy and targeted therapy could only be reliably distinguished from one another for the most recent years, they were grouped together for all analyses.

Figure 1. STROBE diagram for case selection for the study (STROBE: Strengthening the reporting of observational studies in epidemiology).



2.2 Statistical analysis

To assess trends over time, cases of MM were analysed according to 6-year time periods based on their year of diagnosis, with an additional focus on comparing the latest period (2014–2019) with all previous years. This cut-off was chosen since, in our population, immune and targeted therapies were introduced in clinical practice from 2014 onwards. We analysed the following variables: sex, age, tumour site, tumour stage, type of hospital at the time of diagnosis (academic center, general hospital), and primary treatment (surgery, radiotherapy, chemotherapy, and targeted therapy and immunotherapy grouped together).

Normally distributed continuous data were reported as means with standard deviations and skewed distributions as medians with interquartile ranges. Differences between descriptive variables were tested with the Chi-square test, Fisher's exact test, or the independent t-test.

Reporting on incidence, annual rates per 100,000 person-years with corresponding 95% confidence intervals (95% CI) were calculated using the average annual population provided by Statistics Netherlands (Centraal Bureau voor de Statistiek, CBS). The rates were age-adjusted through standardization to the European standard population (European Standardized Rate, ESR). Trends in incidence were evaluated through the Estimated Annual Percentage Change (EAPC)

OS was calculated using the Kaplan–Meier method. Differences in survival curves between groups were assessed with log-rank tests. Relative survival (RS) was calculated by matching observed OS in patients to expected survival in the general Dutch population summarized in annual life tables on age, gender, and calendar year (retrieved from CBS) using the Pohar-Perme estimator. Independent predictors for OS were evaluated by applying multivariable Cox proportional hazards regression models, following the selection of potential predictors based on a *p*-value of <0.1 in univariable analyses. All statistical analyses were two-sided, with a *p*-value <0.05 being considered significant. Analyses were performed using software packages IBM SPSS Statistics version 20.0 and Stata version 17.0 (StataCorp, College Station, TX, USA).

3. Results

3.1. Incidence

Between 1990 and 2019, 1496 patients were diagnosed with MM in the Netherlands (Table 1). MM was more prevalent in women than men (73.2% versus 26.8%). The median age at diagnosis was 72 years, with an interquartile range (IQR) of 62–81 years. Most MM were located in the female genital tract (*n* = 640; 42.8%) and the head and neck region (*n* = 505; 33.8%), and the majority concerned local or locally advanced disease (*n* = 983; 65.7%).

Table 1. Baseline, tumour, and treatment-related characteristics of patients with mucosal melanoma in the Netherlands.

	Total		1990–2013		2014–2019		
	N = 1496		N = 1115		N = 381		
	n	%	n	%	n	%	*p*
Sex							0.43
Male	401	26.8%	293	26.3%	108	28.3%	
Female	1095	73.2%	822	73.7%	273	71.7%	
Age at diagnosis (years)							0.32
0–59	323	21.6%	242	21.7%	81	21.3%	
60–69	297	19.9%	209	18.7%	88	23.1%	
70–79	429	28.7%	324	29.1%	105	27.6%	
≥80	447	29.9%	340	30.5%	107	28.1%	
Median (interquartile range)	72 (62–81)		73 (62–81)		71 (62–80)		
Tumour site							**0.04**
Head and neck	505	33.8%	380	34.1%	125	32.8%	
Gastrointestinal tract	76	5.1%	51	4.6%	25	6.6%	
Anorectal tract	248	16.6%	176	15.8%	72	18.9%	
Female genital tract	640	42.8%	488	43.8%	152	39.9%	
Urinary tract	16	1.1%	9	0.8%	7	1.8%	
Respiratory tract	11	0.7%	11	1.0%	0	0.0%	
Tumour stage							0.15
Local/locally advanced disease	983	65.7%	741	66.5%	242	63.5%	
Locoregional spread disease	254	17.0%	184	16.5%	70	18.4%	
Distant spread disease	226	15.1%	161	14.4%	65	17.1%	
Unknown	33	2.2%	29	2.6%	4	1.0%	
Surgery							**<0.01**
No	344	23.0%	233	20.9%	111	29.1%	
Yes	1152	77.0%	882	79.1%	270	70.9%	
Hospital of first surgery							**0.04** **
Academic center	504	43.8%	351	39.8%	153	56.7%	
General hospital	459	39.8%	347	39.3%	112	41.5%	
Unknown	189	16.4%	184	20.9%	5	1.9%	
Radiotherapy							*0.38*
No	1036	69.3%	779	69.9%	257	67.5%	
Yes	460	30.7%	336	30.1%	124	32.5%	
Systemic therapy *							*<0.01*
No	1409	94.2%	1079	96.8%	330	86.6%	
Yes	87	5.8%	36	3.2%	51	13.4%	
Chemotherapy							*<0.01*
No	1462	97.7%	1081	97.0%	381	100.0%	
Yes	34	2.3%	34	3.0%	0	0.0%	
Immune and targeted therapy							*<0.01*
No	1443	96.5%	1113	99.8%	330	86.6%	
Yes	53	3.5%	2	0.2%	51	13.4%	

Table 1. (*Continued.*)

	Total		1990–2013		2014–2019		
	N = 1496		N = 1115		N = 381		
	n	%	n	%	n	%	*p*
Hospital of first contact							*0.43* **
Academic center	202	13.5%	155	13.9%	47	12.3%	
General hospital	1291	86.3%	957	85.8%	334	87.7%	
Unknown	3	0.2%	3	0.3%	0	0.0%	

** Only primary therapy is listed; ** test academic centers versus general hospitals.*

The majority of cases in the head and neck region (79.8%), the female genital tract (67.7%), and the urinary tract (68.8%) presented as local or locally advanced diseases (Table 2). Anorectal and gastrointestinal diseases were more likely to present at a higher stage at diagnosis, i.e., locoregional spread (28.2% and 13.2%, respectively) or distant spread disease (29.4% and 51.3%, respectively). Over the total study period, the proportion of local or locally advanced diseases decreased from 73.2% in 1990–1995 to 63.5% in 2014–2019. The distribution of stage at diagnosis was not significantly different in 2014–2019 compared to all previous years.

Over three-quarters of all patients underwent surgery (n = 1152; 77.0%). Radiotherapy was part of the primary treatment in 30.7% of cases, while systemic therapy was part of the initial treatment in 5.8% (Table 1). Half of the patients who received systemic therapy did not have surgery or radiotherapy (data not shown). The majority of the patients with local or locally advanced disease underwent surgery (86.9%, n = 854) or radiotherapy (33.3%, n = 326). Systemic treatment was not often part of the initial treatment in this stage (Table S2). Surgery and radiotherapy were also the main treatment strategies in locoregional spread disease (respectively 79.9%, n = 203 and 28.7%, n = 73). Patients with distant spread disease received various types of treatment, of whom 28.3% were systemic treatments. Of these patients, only 16.8% received immune and/or targeted therapy. Compared to previous years, patients diagnosed in 2014–2019 underwent surgery less often (70.9% versus 79.1%; $p < 0.01$). This was the case for patients with local or locally advanced disease (82.6% versus 88.3%; $p = 0.03$) and those with distant spread disease (23.1% versus 37.3%; $p = 0.04$), but not for patients with locoregional spread disease (77.1% versus 81.0%; $p = 0.50$) (data not shown). Overall, the first surgery took place in one of the academic centers more often (56.7% versus 50.3%; $p = 0.04$). Systemic therapy was initially provided in 5.8% of patients, but before 2014, this mainly consisted of chemotherapy (34/36 patients). Immune and targeted therapy were more often provided as part of primary treatment in 2014–2019 compared to all years before 2014 (13.4% vs. 0.2% of cases ($p < 0.01$)). The number of MM patients increased from 205 cases in 1990–1995 to 381 in 2014–2019 (Figure 2). The age-adjusted incidence rate remained stable over time, estimated at 0.33 per 100,000 ESR in 1990–1995 and 0.39 per 100,000 ESR in 2014–2019 (EAPC 3.0%, $p = 0.38$).

Table 2. Distribution of tumour stage by site of mucosal melanoma.

	Total		Local/Locally Advanced Disease		Locoregional Spread Disease		Distant Spread Disease		Unknown	
	1496		65.7%		17.0%		15.1%		2.2%	
Tumour site	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Head and neck	505	33.8%	403	79.8%	50	9.9%	51	10.1%	1	0.2%
Oral	83	5.5%	58	69.9%	15	18.1%	10	12.0%	0	0.0%
Sinonasal	412	27.5%	342	83.0%	32	7.8%	37	9.0%	1	0.2%
Pharynx/glottis	10	0.7%	3	30.0%	3	30.0%	4	40.0%	0	0.0%
Gastrointestinal tract	76	5.1%	27	35.5%	10	13.2%	39	51.3%	0	0.0%
Anorectal tract	248	16.6%	104	41.9%	70	28.2%	73	29.4%	1	0.4%
Rectum	136	9.1%	45	33.1%	40	29.4%	51	37.5%	0	0.0%
Anus	112	7.5%	59	52.7%	30	26.8%	22	19.6%	1	0.9%
Female genital tract	640	42.8%	433	67.7%	122	19.1%	54	8.4%	31	4.8%
Vulva	458	30.6%	301	65.7%	101	22.1%	26	5.7%	30	6.6%
Vagina	157	10.5%	111	70.7%	20	12.7%	25	15.9%	1	0.6%
Other	25	1.7%	21	84.0%	1	4.0%	3	12.0%	0	0.0%
Urinary tract	16	1.1%	11	68.8%	0	0.0%	5	31.3%	0	0.0%
Respiratory tract	11	0.7%	5	45.5%	2	18.2%	4	36.4%	0	0.0%

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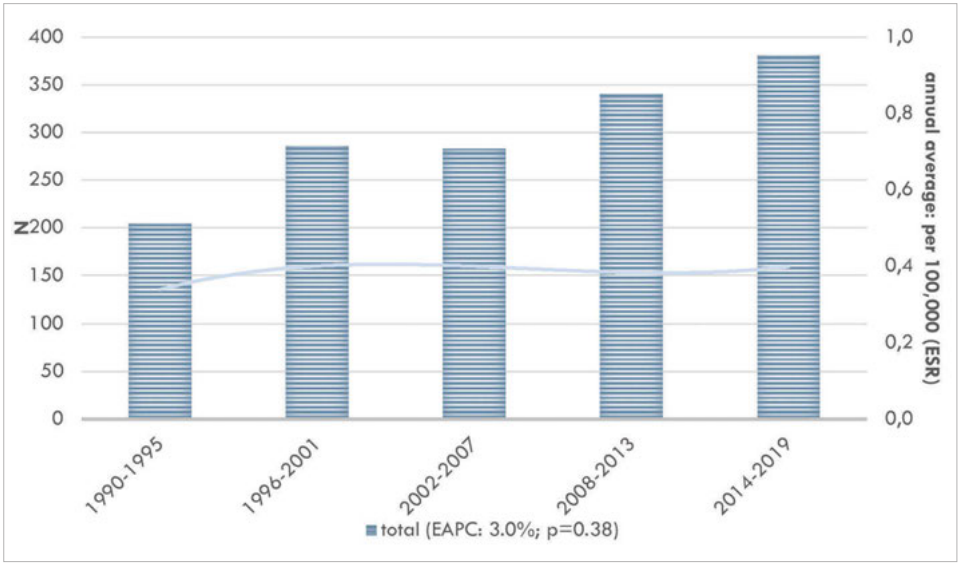
Figure 2. Crude numbers (bars, left axis) and annual averaged, age-adjusted incidence rates (line, right axis) for patients with mucosal melanoma in the Netherlands.



3.2. Survival

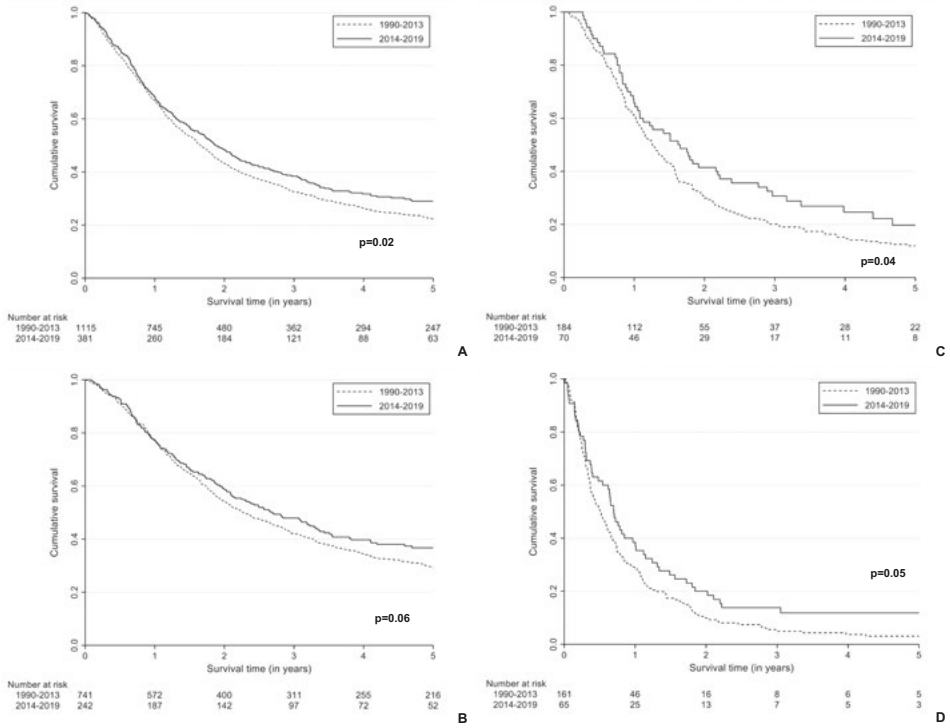
Overall, patients with MM had a 1-, 2-, and 5-year OS of 67.2% (95%CI: 64.7–69.5%), 44.4% (95%CI: 41.9–46.9%), and 23.8% (95%CI: 21.6–26.0%), respectively, with median OS of 1.7 years (95%CI: 1.6–1.8) (Table 3). OS differed across tumour stages, with 5-year OS rates of 30.8% for patients with local or locally advanced disease (95%CI: 27.9–33.7%), 14.0% for patients with locoregional spread disease (95%CI: 10.0–18.8%), and 5.2% for those with distant spread disease (95%CI: 2.8–8.8%). Accordingly, median OS was 2.4 years (95%CI: 2.1–2.7), 1.3 years (95%CI: 1.1–1.6), and 0.6 years (95%CI: 0.4–0.7), respectively. OS was relatively higher for MM of the urinary tract (5-year OS 31.3%, 95%CI: 11.4–53.6%), the head and neck region (24.7%, 95%CI: 20.9–28.6%), and the female genital tract (5-year OS 27.8%, 95%CI: 24.4–31.4%), and within the latter site, prognoses differed significantly for specific subsites. Median OS for patients with MM located at the vulva was 2.9 years (95%CI: 2.5–3.4), while this was 1.1 years (95%CI: 1.0–1.4) for those with MM located in the vagina (Table 3). The 5-year RS for all patients with MM was 29.0% (95%CI: 26.2–31.8%).

Table 3. Overall 1-, 2-, and 5-year and median survival and 5-year relative survival for patients with mucosal melanoma by stage and tumour site.

	1-Year OS		2-Year OS		5-Year OS		Median OS		5-Year RS	
	%	95%CI	%	95%CI	%	95%CI	Years	95%CI	%	95%CI
All	**67.2**	**(64.7–69.5)**	**44.4**	**(41.9–46.9)**	**23.8**	**(21.6–26.0)**	**1.7**	**(1.6–1.8)**	**29.0**	**(26.2–31.8)**
Tumour stage										
Local/locally advanced	77.2	(74.5–79.7)	55.2	(52.1–58.3)	30.8	(27.9–33.7)	2.4	(2.1–2.7)	37.5	(33.7–41.2)
Locoregional spread disease	62.2	(55.9–67.8)	33.1	(27.4–38.9)	14.0	(10.0–18.8)	1.3	(1.1–1.6)	17.4	(12.1–23.4)
Distant spread disease	31.4	(25.5–37.5)	12.8	(8.9–17.6)	5.2	(2.8–8.8)	0.6	(0.4–0.7)	6.7	(3.4–11.5)
Tumour site										
Head and neck	68.7	(64.5–72.6)	47.7	(43.3–52)	24.7	(20.9–28.6)	1.9	(1.7–2.1)	30.8	(26.0–35.7)
Oral	77.1	(66.5–84.7)	54.1	(42.8–64.1)	28.4	(18.9–38.5)	2.6	(1.7–3.5)	31.0	(20.2–42.3)
Sinonasal	67.0	(62.2–71.3)	46.4	(41.5–51.1)	23.8	(19.7–28.1)	1.8	(1.5–2.1)	30.8	(25.4–36.3)
Gastrointestinal tract	36.8	(26.2–47.5)	19.7	(11.7–29.3)	13.2	(6.7–21.8)	0.7	(0.5–0.9)	17.2	(9.1–27.4)
Anorectal tract	58.5	(52.1–64.3)	30.2	(24.6–36)	14.8	(10.6–19.6)	1.2	(1.0–1.4)	18.3	(12.9–24.4)
Rectum	52.9	(44.2–60.9)	23.5	(16.8–30.9)	11.5	(6.8–17.6)	1.0	(0.8–1.3)	13.9	(7.9–21.5)
Anus	65.2	(55.6–73.2)	38.4	(29.4–47.3)	18.9	(12.2–26.8)	1.6	(1.2–1.8)	23.9	(15.0–34.0)
Female genital tract	73.8	(70.2–77.0)	50.5	(46.5–54.3)	27.8	(24.4–31.4)	2.0	(1.8–2.5)	33.4	(28.8–38.0)
Vulva	79.0	(75.0–82.5)	59.0	(54.3–63.3)	34.5	(30.0–38.9)	2.9	(2.5–3.4)	41.4	(35.5–47.2)
Vagina	58.0	(49.8–65.2)	26.1	(19.5–33.2)	9.5	(5.6–14.7)	1.1	(1.0–1.4)	12.1	(7.0–18.8)
Urinary tract	68.8	(40.5–85.6)	56.3	(29.5–76.2)	31.3	(11.4–53.6)	2.8	(0.5–5.1)	38.0	(12.4–63.9)
Respiratory tract	18.2	(2.9–44.2)	18.2	(2.9–44.2)	9.1	(0.5–33.3)	0.4	(0.2–0.9)	14.1	(1.2–41.6)

Compared to the period 1990–2013, patients diagnosed in 2014–2019 had a better 5-year OS (p = 0.02), without significant improvement in median OS: 1.9 years (95%CI: 1.6–2.2) versus 1.6 years (95%CI: 1.5–1.8), respectively (Figure 3A). At 5 years, OS was 29.0% (95%CI: 24.2–33.9%) compared to 22.3% (95%CI: 19.9–24.8%) for the periods 1990–2013 and 2014–2019 (data not shown). OS improved across all tumour stages, but only significantly for locoregional spread disease (p = 0.04) (Figure 3B–D). For these patients, 5-year OS was 19.7% (95%CI: 10.4–31.1%) in 2014–2019 compared to 12.0% (95%CI: 7.8–17.1%) in 1990–2013. For patients with distant spread disease, the 5-year OS was 11.9% (95%CI: 5.3–21.2%) in 2014–2019 compared to 3.1% (95%CI: 1.2–6.7%) in 1990–2013 (Figure 3D).

Figure 3. Kaplan–Meier curves representing the overall survival of patients with MM according to the period of diagnosis for (**A**) the total population, (**B**) local or locally advanced disease, (**C**) locoregional spread disease, and (**D**) distant spread disease.



3.3. Predictors for Survival

Univariable analysis showed that diagnosis between 2014–2019, female sex, surgery as primary treatment, and MM located at the female genital tract were associated with better survival (Table 4). Higher age, gastrointestinal, anorectal, or respiratory location, higher stage at presentation, radiotherapy, chemotherapy, or immune and targeted therapy as primary

treatment were associated with worse survival. Multivariable analysis showed that respiratory location, higher age, and higher stage at presentation were independently associated with worse OS. Diagnosis in the period 2014–2019 was associated with better OS compared to diagnosis between 1990–2013 (Table 4) (HR 0.82 (95%CI: 0.71–0.95; $p < 0.01$). Other factors that were significantly associated with a better prognosis in multivariable analysis were patients' younger age, MM located in the female genital tract, local or locally advanced disease, and initial provision of immune or targeted therapy. Patients who received immune or targeted therapy had an HR of 0.60 (95%CI: 0.42–0.86; $p = 0.01$) compared to those who were not treated with immune or targeted therapy. Although surgery showed a significant effect in both univariable and multivariable analyses, the proportional hazards assumption was considered violated, and the estimates of the definitive model were stratified for this variable.

Table 4. Univariable and multivariable analysis on the impact of the period of diagnosis on the survival of patients with mucosal melanoma.

	Univariable Analyses			Multivariable Analyses: Complete Model			Multivariable Analyses: Definitive Model Stratified for Surgery		
	HR	95%-CI	*p*	HR	95%-CI	*p*	HR	95%-CI	*p*
Period of diagnosis									
1990–2013	ref			ref			ref		
2014–2019	0.85	(0.74–0.98)	**0.02**	0.82	(0.71–0.95)	**<0.01**	0.82	(0.71–0.95)	**<0.01**
Sex									
Male	1.32	(1.17–1.49)	**<0.01**	1.11	(0.96–1.28)	0.17			
Female	ref			ref					
Age at diagnosis (years)									
0–59	ref			ref			ref		
60–69	1.23	(1.03–1.47)	**0.02**	1.27	(1.06–1.52)	**<0.01**	1.33	(1.11–1.59)	**0.01**
70–79	1.54	(1.31–1.82)	**<0.01**	1.51	(1.28–1.78)	**<0.01**	1.59	(1.35–1.87)	**<0.01**
≥80	2.28	(1.94–2.68)	**<0.01**	2.26	(1.91–2.67)	**<0.01**	2.34	(1.98–2.76)	**<0.01**
Tumour site									
Head and neck	ref			ref			ref		
Gastrointestinal tract	1.87	(1.45–2.40)	**<0.01**	1.27	(0.97–1.65)	0.08	1.22	(0.95–1.60)	0.12
Anorectal tract	1.39	(1.18–1.63)	**<0.01**	1.15	(0.96–1.38)	0.13	1.12	(0.95–1.33)	0.17
Female genital tract	0.86	(0.76–0.97)	**0.02**	0.89	(0.76–1.05)	0.17	0.82	(0.72–0.93)	**<0.01**
Urinary tract	0.93	(0.53–1.61)	0.79	0.85	(0.49–1.50)	0.58	0.83	(0.47–1.44)	0.51
Respiratory tract	2.79	(1.53–5.08)	**<0.01**	2.42	(1.30–4.50)	**<0.01**	2.45	(1.32–4.54)	**<0.01**
Tumour stage									
Local/locally advanced disease	ref			ref			ref		
Locoregional spread disease	1.67	(1.44–1.93)	**<0.01**	1.55	(1.33–1.80)	**<0.01**	1.61	(1.38–1.87)	**<0.01**
Distant spread disease	3.56	(3.05–4.15)	**<0.01**	2.73	(2.27–3.30)	**<0.01**	2.56	(2.13–3.09)	**<0.01**
Unknown	1.98	(1.39–2.82)	**<0.01**	1.75	(1.22–2.51)	**<0.01**	1.68	(1.17–2.42)	**0.01**
Surgery									
No	ref			ref					
Yes	0.31	(0.27–0.35)	**<0.01**	0.45	(0.38–0.52)	**<0.01**			

Table 4. *(Continued.)*

	Univariable Analyses			Multivariable Analyses: Complete Model			Multivariable Analyses: Definitive Model Stratified for Surgery		
	HR	**95%-CI**	***p***	**HR**	**95%-CI**	***p***	**HR**	**95%-CI**	***p***
Hospital of first surgery									
Academic center	ref								
General hospital	0.97	(0.84–1.11)	0.63						
Unknown	1.03	(0.86–1.23)	0.77						
Radiotherapy									
No	*ref*			*ref*					
Yes	*1.19*	*(1.06–1.34)*	*<0.01*	*1.05*	*(0.92–1.21)*	*0.48*			
Chemotherapy									
No	*ref*			*ref*					
Yes	*1.94*	*(1.38–2.73)*	*<0.01*	*0.82*	*(0.57–1.18)*	*0.29*			
Immune and targeted therapy									
No	*ref*			*ref*			*ref*		
Yes	*1.39*	*(1.02–1.90)*	*<0.01*	*0.55*	*(0.38–0.79)*	*<0.01*	*0.60*	*(0.42–0.86)*	*0.01*
Hospital of first contact									
Academic center	*ref*			*ref*					
General hospital	*1.08*	*(0.92–1.27)*	*0.34*	*1.08*	*(0.92–1.27)*	*0.34*			
Unknown	*0.42*	*(0.10–1.69)*	*0.22*	*0.42*	*(0.10–1.69)*	*0.22*			

4. Discussion

This large retrospective population-based study analyzing real-world data of stage I-IV MM in the Netherlands from 1990–2019 shows that despite the introduction of immune and targeted therapies, survival of MM remains poor. The 5-year OS is 23.8%, and the indisputable aggressive course of the disease is reflected by the short median survival of 1 year and 8 months. Though survival has improved when comparing timeframes before and after the introduction of immune and targeted therapies, the absolute survival benefit seems fairly limited (1 year and 7 months vs. 1 year and 10 months for all stages). For patients with regional or distant spread disease, improvement was limited to 2 months only.

In our study, the mean age-adjusted incidence rate for MM over the total period was 0.38 per 100,000 person-years and remained stable over time. These findings are in line with a large Survival, Epidemiology, and End Results (SEER) database, which included CM and MM patients between 1973 and 2013 in the United States of America (totaling 133.996 patients, of which 1522 had MM), also showing increasing incidence and improved survival over time for CM whilst incidence for MM remained stable, and survival remained poor. [11] The same trend of increasing incidence and higher survival rates for CM, particularly for stage II, III, and IV disease, was observed in Dutch epidemiologic research with data from 2003 to 2018. The median OS of advanced CM increased from 11.3 to 16.9 months, whilst the median OS of advanced MM did not improve when comparing the same timeframes (2013–2014 vs. 2015–2017). [15, 16] As immune and targeted therapy were introduced in 2011, data on this subject should be read with caution as the absolute number of patients treated with these therapies in studies are low. Our study confirms the unfavorable prognosis of MM compared with CM. [12] However, there is a significant improvement in survival over all stages, and specifically, the locoregional spread of disease, when comparing 2014–2019 with all previous years. Moreover, a trend towards better survival was seen for local or locally advanced disease and the distant spread of disease.

Multivariable analysis showed that diagnosis during the timeframe 2014–2019 is independently associated with better OS. This may be explained by the application of immune and targeted therapy as second or later-line treatment. In this study, we only had access to the stage at initial diagnosis and first-line therapy. However, recurrence rates are high in MM and most often recur as regional or distant spread disease. [18, 19] We hypothesize that patients included in this study may have received immune and targeted therapies following disease progression or recurrence. As such, the benefit of these therapies may be expressed directly in our analysis as well as through diagnosis during 2014–2019. In addition to the timeframe 2014–2019, other independent factors associated with better survival were treatment with immune or targeted therapies and MM located in the female genital tract.

Data regarding the location of MM as a predictor of survival are inconsistent. Large studies, including 704 and 1814 MM, demonstrated no difference in survival between MM originating from various locations, even when correcting for the stage of the disease. In contrast, other studies associated MM located in the female genital tract or head and neck with better OS, while the latter was also reported to have worse survival compared to other locations. [20-25] We found that MM of the female genital tract and the head and neck more often present with localized disease, corresponding with higher survival rates compared with other locations of MM. Within MM of the female genital tract, the better prognosis of vulvar MM compared to vaginal MM is in line with the literature. [26, 27] Vulvar and vaginal MM are often classified as one entity. However, the vulva consists of both cutaneous and glabrous skin, whilst the vagina only consists of glabrous skin with a mucosal lining. MM originating from cutaneous and not mucosal lining and a more visible location allow vulvar MM to be diagnosed at an earlier stage than vaginal MM, which may contribute to a better prognosis. Moreover, a mutational analysis of 95 female genital tract melanomas showed that BRAF mutation, which is often found in CM, is more often detected in vulvar MM compared with vaginal MM, respectively, in 28% and 9% of cases. [26] These data suggest that MM located at the vulva may even have more resemblance to CM than with MM and that immune and targeted therapy may likewise be promising for advanced disease. We suggest that vulvar and vaginal melanomas should not be classified as one entity, given their distinct origin with different prognoses.

Immune and targeted therapy are the cornerstone of advanced CM treatment nowadays since they demonstrate better and more durable response rates and better long-term outcomes than chemotherapy. [28] In the Netherlands, immune and targeted therapy for advanced CM have been available since 2011 and have been used in clinical practice since 2014. [16] In contrast, data on these therapies in MM is limited, and few patients with MM have been treated with immune and targeted therapies. Additionally, studies are mostly retrospective, and in the case of a prospective set-up, follow-up is short. A retrospective multicenter international study and a multicenter Japanese study including 545 and 329 advanced or unresectable stage II MM treated with anti-PD1 (pembrolizumab) alone or combined with anti-CTLA-4 (ipilimumab) state that these therapies have lower efficacy than in CM (response rate of 30% and 26% in MM), and that response is also less durable (mean duration of response (mDoR) is 25 months). [29, 30] Moreover, the 5-year follow-up of 79 patients with MM treated with anti-PD-1, anti-CTLA-4, or a combination (ipilimumab and/or nivolumab) in Checkmate 067 showed poor long-term efficacy for either of these agents. [31] In contrast with CM, there is no difference in progression-free or overall survival when comparing combined anti-PD-1 and anti-CTLA-4 therapy or anti-PD-1 monotherapy with pembrolizumab. Evidence of anti-PD-1 therapy in advanced CM demonstrates a response rate of 42% and mDoR of 52 months. [32, 33] Data on MM treated with anti-CTLA-4, anti-PD-1, or a combination of both agents show a median OS of 9.6, 11.5, and 11 months which

is comparable with the 55 patients in our cohort who were treated with either immune or targeted therapy and had median OS of 12 months. [16, 34] Whilst we have no definite data on the type of systemic therapy, we are certain that the majority of these patients are treated with immune therapy and not with targeted therapy. This is endorsed by a Dutch paper which published treatment data of advanced MM from 2013–2017, of which 76.4% of the first-line systemic treatment consisted of ipilimumab or nivolumab. [16]

Though the role of immunotherapy in MM is still controversial, this could be beneficial in resectable or bulky MM, given the promising results of neoadjuvant immunotherapy in CM. This treatment strategy may contribute to less invasive surgery in anatomically challenging locations and possibly reduce significant morbidity. Only one retrospective study analysed neoadjuvant immunotherapy in MM and demonstrated a pathological response rate of 35% (11/31), of which three patients did not require surgical treatment and had an ongoing response. [35] Further research, including prospective data on this subject, is needed.

The observational set-up of this study warrants some caution in interpreting the results presented here. In addition, as information on recurrences, progression of the disease, and associated treatment was not available, progression-free survival could not be analysed. Unfortunately, as immune, and targeted therapy could only be reliably distinguished from one another in more recent years, we were unable to evaluate the independent efficacy of immune and targeted therapy. Despite these limitations, we presume that this study established valuable additions to current knowledge on MM by providing real-world data on incidence and survival in a large cohort over a 30-year time period.

5. Conclusions

In conclusion, the incidence of MM has remained stable over the last 30 years, whilst overall survival has slightly improved since the introduction of immune and targeted therapy. However, the median survival remains fairly short, especially as compared to CM, reflecting the poor prognosis of this aggressive cancer type. Future studies examining the effect of immune and targeted therapies in MM are highly needed. Therefore, considering the rarity of MM, we advocate international multicenter collaborations and the inclusion of patients with MM in clinical trials.

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Supplementary material

Table S1. Grouping of mucosal melanoma according to the International Classification of Diseases for Oncology (ICD-O-3) topography coding
See online: https://www.mdpi.com/2168206

Table S2. Primary treatment of mucosal melanoma by stage of disease

	total		local/locally advanced disease		locoregional spread disease		distant spread disease		unknown	
	1496		983		254		226		33	
	n	%	n	%	n	%	n	%	n	%
Surgery										
No	344	23.0%	129	13.1%	51	20.1%	151	66.8%	13	39.4%
Yes	1152	77.0%	854	86.9%	203	79.9%	75	33.2%	20	60.6%
Radiotherapy										
No	1036	69.3%	657	66.8%	181	71.3%	171	75.7%	27	81.8%
Yes	460	30.7%	326	33.2%	73	28.7%	55	24.3%	6	18.2%
Systemic therapy										
No	1409	94.2%	974	99.1%	240	94.5%	162	71.7%	33	100.0%
Yes	87	5.8%	9	0.9%	14	5.5%	64	28.3%	0	0.0%
Chemotherapy										
No	1462	97.7%	979	99.6%	250	98.4%	200	88.5%	33	100.0%
Yes	34	2.3%	4	0.4%	4	1.6%	26	11.5%	0	0.0%
Immune and targeted therapy										
No	1443	96.5%	978	99.5%	244	96.1%	188	83.2%	33	100.0%
Yes	53	3.5%	5	0.5%	10	3.9%	38	16.8%	0	0.0%

Survival outcomes of patients with advanced mucosal melanoma diagnosed from 2013-2017 in the Netherlands – a nationwide population-based study

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3

Abstract

Background

Mucosal melanoma (MM) is rare and has a poor prognosis. Since 2011, new effective treatments are available for advanced melanoma. It is unclear whether patients with mucosal melanoma equally benefit from these new treatments compared with patients with cutaneous melanoma (CM).

Methods

Patients with advanced MM and CM diagnosed between 2013 and 2017 were included from a nationwide population-based registry - the Dutch Melanoma Treatment Registry. Overall survival (OS) was estimated with the Kaplan-Meier method (also for a propensity score-matched cohort). A Cox model was used to analyse the association of possible prognostic factors with OS.

Results

In total, 120 patients with MM and 2960 patients with CM were included. Median OS was 8.7 months and 14.5 months, respectively. Patients with MM were older (median age 70 versus 65 years) and more often female (60% versus 41%), compared with CM. In total, 77% and 2% of the MM patients were treated with first-line immunotherapy and targeted therapy, respectively, compared with 49% and 33% of the CM patients. In contrast to CM, OS for MM did not improve for patients diagnosed in 2015-2017, compared with 2013-2014. ECOG performance score ≥1 (HR = 1.99 [1.26-3.15; p=0.003]) and elevated LDH level (HR = 1.63 [0.96-2.76]; p=0.069) in MM were associated with worse survival.

Conclusions

Within the era of immune and targeted therapies, prognosis for patients with advanced MM has not improved as much as for CM. Collaboration is necessary to enlarge sample size for research to improve immunotherapeutic strategies and identify targetable mutations.

1. Introduction

Primary mucosal melanoma (MM) is a rare type of cancer accounting for 1-2% of all melanomas. [1, 2] In contrast to cutaneous melanoma (CM), the incidence of MM has not increased and lies between 0.2 and 0.4 cases per 100,000 people. [3, 4] MM can originate from any mucosa-lined surface of the body, but the highest incidence is reported in the head and neck, vulvovaginal, anal, and rectal region [5,6]. Because of the rareness of MM outcome data is scarce and mainly based on retrospective studies with the limited number of cases. MM is still a poorly understood disease.

In recent years, for advanced CM, effective immune and targeted therapies have improved overall survival (OS). [7] However, it is unclear whether the prognosis of patients with advanced MM has changed in the new era of immune and targeted therapy. Melanomas arising from mucosal sites differ from CM in clinical characteristics and prognosis. From primary diagnosis of any stage melanoma, the 5-year overall survival probability for MM and CM are 37% and 92%, respectively. [3] Advanced stage of disease at presentation and high recurrence rates of mucosal melanoma are responsible for the low survival probability. Other possible explanations for poor prognosis of MM are the low tumour mutational burden (leading to a low response to checkpoint inhibitors), the absence of targetable oncogenic drivers, the alleged biological aggressiveness, and the rich lymphatic and vascular supply of the mucosa. [6, 8, 9]

The aim of this study is to report real-world outcomes of patients with advanced MM and identify prognostic factors for OS. Furthermore, we aim to explore whether OS for patients with MM has improved after the introduction of immunotherapy. We used data from a nationwide population-based registry, in which all patients with unresectable stage IIIC and stage IV melanoma in the Netherlands are registered.

2. Materials and methods

2.1 Study design

We performed a retrospective observational study analysing patients aged ≥18 years with unresectable stage IIIC or stage IV (advanced) mucosal or cutaneous melanoma diagnosed between 2013 and 2017 from the Dutch Melanoma Treatment Registry (DMTR). The DMTR prospectively collects data from all advanced melanoma patients in the Netherlands and has been described in detail in a previous publication. [10] Electronic patient records were checked again to determine if patients had an MM. Characteristics and survival outcomes of patients with advanced MM were compared with a control group of patients with advanced CM. The stage for CM and MM were determined as per the American Joint Committee

on Cancer version. [11] Patients with MM were analysed by location of the primary tumour, categorised as head and neck region, upper gastrointestinal (oesophagus and stomach), lower gastrointestinal (anus and rectum) vulvovaginal, and other locations (location not further defined). Data-set cutoff date was 01-06-2019.

2.2 Statistical analysis

Baseline characteristics were analysed with descriptive statistics. Median follow-up was estimated with the reverse Kaplan-Meier method. [12] OS, estimated with the Kaplan-Meier method, was defined as time of diagnosis of advanced MM to death from any cause. OS of MM and CM was also compared by creating a propensity score matched cohort. A matched CM cohort was created by using the propensity scores estimated based on the baseline variables age, gender, Eastern Cooperative Oncology Group Performance Score (ECOG PS), lactate dehydrogenase (LDH) level, distant metastases (<3 or ≥3 organ sites involved), brain and liver metastasis and BRAF mutational status. The algorithm of the nearest neighbour matching with 1:3 ratio was used. A Cox proportional hazards model was used to estimate the association of prognostic factors with OS; age, gender, ECOG PS, LDH level, brain and liver metastases and distant metastases (<3 or ≥3 organ sites involved) were included in the Cox models. We imputed missing covariates for the Cox model according to White and Royston (2009) using the multiple imputation by chained equation method and pooled coefficients as per Rubin's rules (100 imputations and 20 iterations). [13] Statistical software used was R (version 3.6.1: packages car, lubridate, tidyverse, survival, MatchIt and mice).

3. Results

3.1 Patient characteristics

From 2013 to 2017, 3974 patients were diagnosed with advanced melanoma. After exclusion of 894 patients with uveal, acral or melanoma of unknown origin, a total of 120 (3.0%) patients with MM and 2960 (96%) patients with CM were included in the study; supplement Fig. S1. Patients with MM were older, more often female, less often had stage IV-M1d disease and fewer distant metastases in ≥ 3 organ sites, but liver metastases were more frequent. The baseline characteristics of CM and MM are shown in Table 1.

MM was located in the head and neck region in 39 (33%) patients, in the vulvovaginal region in 29 (24%) patients and in the upper and lower gastrointestinal tract in 7 (5.8%) and 38 (32%) patients, respectively. Seven patients (5.8%) had MM located at other primary location(s). The LDH level, ECOG score, and stage of disease were similar between the different locations of MM. Median time from initial diagnosis until confirmed advanced stage disease was shorter for MM located in the upper and lower gastrointestinal tract than that in the vulvovaginal region and the head and neck region (0 and 6 months compared with 9 and 15 months, respectively).

Baseline characteristics of MM by location are shown in the supplement (Table S1). Oncogenic mutation(s) were less frequent for MM than for CM (Table 1). A BRAF mutation was found in 1649 (55.9%) patients with CM in seven of the 122 patients with MM (5.8%; five patients had V600E, one had V600R and one V600K, and one patient was classified as 'other' type BRAF mutation). NRAS and KIT mutations were found in 625 (21.1%) and 39 (1.3%) patients with CM and in 17 (14.2%) and 15 (11.7%) patients with MM, respectively (Table 1). Patients with MM originating in the head and neck region most often had NRAS mutations (eight (20.5%) patients). The KIT gene was most often mutated in MM located in the lower gastrointestinal tract and vulvovaginal region, in eight (21.1%) and four (13.8%) patients, respectively.

Table 1. Patient characteristics of patients with mucosal and cutaneous melanoma at diagnosis of unresectable stage III or stage IV disease. Missing data <2.5% are not shown in this table. Values are *n* (%) unless otherwise indicated.

	Cutaneous	Mucosal	*P*-value*
Patients; *n*	2960	120	
Median age, year (IQR)	65 [54, 73]	70 [62, 76]	
Months to advanced melanoma			
Median (IQR)	35 [14, 75]	9 [0, 21]	<0.001
Female	1212 (41.0)	72 (60.0)	<0.001
ECOG PS			
0	1448 (53.7)	57 (52.3)	0.647
1	866 (32.1)	39 (35.8)	
≥2	384 (14.2)	13 (11.9)	
Unknown	262	11	
LDH level			
Normal	1788 (63.5)	76 (69.7)	0.277
1x ULN	3.5)	24 (22.0)	
>2x ULN	366 (13.0)	9 (8.3)	
Unknown	146	11	
Stage			
Unresectable IIIC	230 (7.8)	15 (12.5)	<0.001
IV-M1a	222 (7.5)	7 (5.8)	
IV-M1b	318 (10.7)	17 (14.2)	
IV-M1c	1387 (46.9)	70 (58.3)	
IV-M1d	791 (26.7)	11 (9.2)	
Metastases in ≥3 organ sites	1330 (45.0)	36 (30.0)	0.002
Mutations			
BRAF	1649 (55.7)	7 (5.8)	<0.001
NRAS	625 (21.1)	17 (14.2)	0.042
KIT	39 (1.3)	14 (11.7)	<0.001
GNAQ	18 (0.6)	2 (1.7)	0.001
GNA11	15 (0.5)	2 (1.7)	0.001

**P*-value of statistical tests comparing characteristics of patients with cutaneous and mucosal melanoma (excluding missing values). IQR - interquartile range, ECOG PS - Eastern Cooperative Oncology Group performance status, LDH - lactate dehydrogenase, ULN - upper limit of normal.

3.2 Treatment characteristics

Fifteen (12.5%) patients with advanced MM were treated with local therapy alone (surgery, radiotherapy, hyperthermia therapy, radiofrequency, or microwave ablation), 89 (74.2%) patients with systemic (and local) therapy and 16 (13.3%) patients did not receive any treatment. First-line systemic therapy for patients with MM mostly consisted of immunotherapy; 43 (48.3%) patients received an anti-PD-1 antibody, 16 (18.0%) patients ipilimumab and nine (10.1%) patients ipilimumab plus nivolumab combination therapy. Best overall response (BOR) to immunotherapy was a complete response (CR) in four (5.9%) patients, partial response (PR) in 10 (14.7%) patients and stable disease (SD) in 16 (23.5%) patients. Of the patients with advanced CM, 271 (9.2%) patients received local therapy, 2440 (82.4%) patients were treated with systemic therapy and 249 (8.4%) patients received no treatment. First-line systemic therapy in CM consisted of anti-PD-1 antibodies in 709 (29.1%) patients, ipilimumab in 356 (14.6%) patients and ipilimumab plus nivolumab combination therapy in 133 (5.5%) patients. BOR to immunotherapy for CM was a CR in 154 (12.9%) patients, PR in 298 (24.9%) patients and SD in 335 (28.0%) patients. First-line BRAF inhibitors were used in 409 (16.8%) patients and combined BRAF plus MEK inhibitors in 401 (16.4%) patients. Distribution of all first-line systemic therapies used in CM and MM are shown in the supplement (Fig. S3).

3.3 Overall survival

Median follow-up was 38 months for MM and 34 months for CM. Median OS of all patients with advanced MM and CM was 8.9 months (95%CI: 7.3-12.7) and 14.5 months (95%CI: 13.7-15.4), respectively. The 1- and 3-year OS probabilities of patients with MM were 42% (95%CI; 34-52) and 15% (95%CI; 9.0-24; Fig. 1). For patients with CM, the 1- and 3-year OS probabilities were 55% (95%CI: 54-57) and 30% (95%CI: 29-32; Fig. 1). OS of CM in the propensity score-matched cohort (Table S2) was also associated with better OS in CM than MM (17.1 versus 10.8 months, p=0.003; supplement Fig. S2). Median OS of patients with MM diagnosed in 2013-2014 and in 2015-2017 was comparable (8.7 months [95%CI: 6.9-16.7] and 8.9 months [95%CI: 6.8-13.5], respectively), but median OS of patients with CM increased from 11.3 months (95%CI: 10.2-12.4) in 2013-2014 to 16.9 months (95%CI: 15.4-18.2) in 2015-2017 (Fig. 2a). Median OS of patients treated with systemic therapy was 11.8 months (95%CI: 8.8-16.1) for MM and 17.9 months (95%CI: 16.6-18.9) for CM (Fig. 2b). Median OS was 9.0 months (95%CI: 5.9-18.9) for lower gastrointestinal MM, 8.6 months (95%CI: 6.8-21) for vulvovaginal MM and 7.1 months (95%CI: 4.9-14) for head and neck MM (Fig. 3).

At the 3-year landmark 11 patients with MM were alive and in the follow-up. All of these 'long-term' survivors had a baseline ECOG PS of ≥1; seven patients had a normal LDH level, and three patients had a LDH level of 1 ≤ upper limit of normal. No patient had stage IV-M1d disease, and two (18.2%) patients had distant metastases in ≥3 organ sites (supplement Table S3). Seven (63.6%) patients were treated with immunotherapy; three patients received an anti-PD-1 antibody, and of the four patients who received first line ipilimumab, three patients

received an anti-PD-1 antibody as second-line treatment. Of the remaining four patients, two patients were treated with local therapy and two patients with another systemic treatment.

3.4 Prognostic factors of survival

Distant metastases in ≥3 organ sites (hazard ratio [HR]: 1.56 [95%CI: 1.02-2.40; p = 0.041]), ECOG PS of ≥ 1 (HR 1.79 [95%CI: 1.17-2.75]; p= 0.007), elevated LDH level (HR 1.53 [95%CI: 0.96-2.43]; p= 0.073) and brain metastases (HR 1.84 [95%CI 0.94-3.59]; p = 0.073]) (although the association of the latter two was not statistically significant) were associated with death in the univariable Cox model for MM. ECOG PS of ≥ 1 (HR 1.99 [95%CI: 1.26-3.15]; p = 0.003) and elevated LDH level (HR 1.63 [95%CI: 0.96-2.76]; p = 0.069) were associated with death in the multivariable Cox model for MM. Age of 70 years and older was not significantly associated with death (HR 1.43 [95% CI: 0.93-2.21]; p = 0.11). The univariable and multivariable Cox models are shown in Table 2.

Figure 1. Kaplan-Meier curves of overall survival of patients with unresectable stage III or stage IV mucosal versus cutaneous melanoma. OS = Overall survival, CI = confidence interval. (Log rank test: p < 0.001).

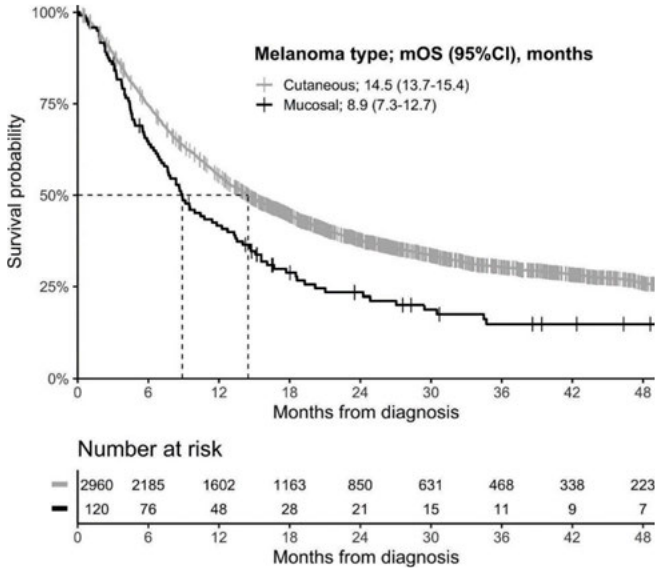

Figure. 2. Kaplan-Meier curves of overall survival for unresectable stage III or stage IV mucosal and cutaneous melanoma of A) patients diagnosed in 2013 and 2014 versus 2015, 2016 and 2017 and B) stratified by treatment modality. OS = Overall survival, CI = confidence interval.

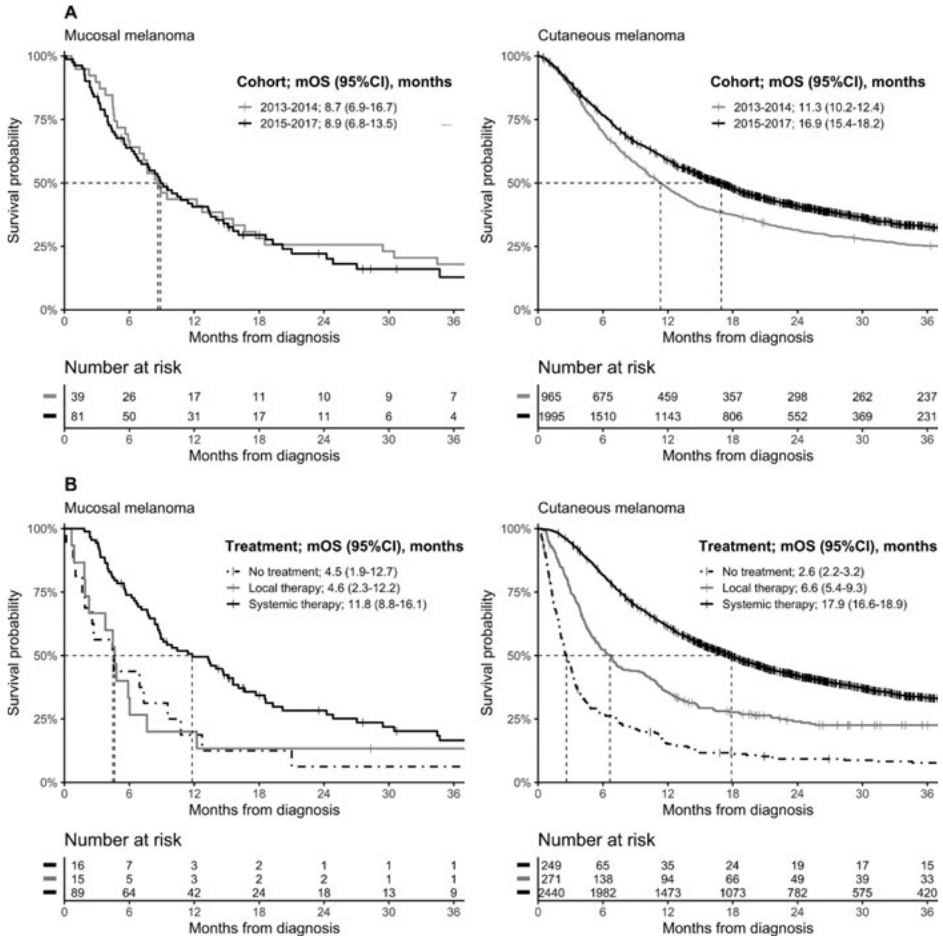

Figure 3. Kaplan-Meier curves of overall survival of patients with advanced mucosal melanoma stratified by location. Upper gastrointestinal (n=7) and 'other' (n=7) mucosal melanoma were excluded. mOS = median overall survival, CI = confidence interval.

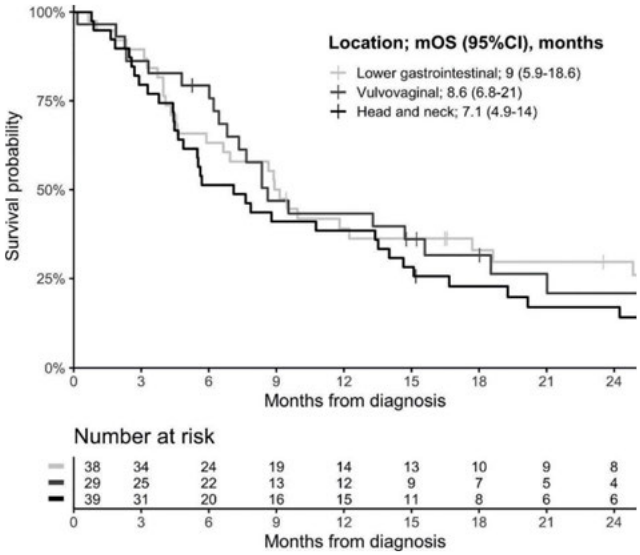

Table 2. Univariable and multivariable Cox regression model for the association of prognostic factors with overall survival for mucosal melanoma. There was a total of 100 events.

	Univariable				Multivariable*		
	n	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age							
≤69 years	59	1.08	(0.73-1.61)	0.692	1.43	(0.93-2.21)	0.107
≥70 years	61	1			1		
Gender							
Male	48	1			1		
Female	72	0.80	(0.53-1.19)	0.267	0.88	(0.58-1.34)	0.548
ECOG PS							
0	57	1			1		
≥1	52	1.79	(1.17-2.75)	0.007	1.99	(1.26-3.15)	0.003
LDH level							
Normal	76	1			1		
>1x ULN	33	1.53	(0.96-2.43)	0.073	1.63	(0.96-2.76)	0.069
Distant metastases							
<3 organ sites	84	1			1		
≥3 organ sites	36	1.56	(1.02-2.4)	0.041	1.16	(0.68-1.99)	0.577
Liver metastasis							
No	58	1			1		
Yes	44	1.41	(0.91-2.19)	0.123	1.03	(0.63-1.7)	0.895
Brain metastasis							
Absent	91	1			1		
Present	11	1.84	(0.94-3.59)	0.073	1.43	(0.68-3)	0.346

HR - hazard ratio, CI - confidence interval, ECOG PS - ECOG performance score, LDH lactate dehydrogenase, ULN - upper limit of normal. *Multivariable model are pooled results after multiple imputation.

4. Discussion

To our knowledge, this is the first nationwide population-based cohort study of patients with advanced MM, reflecting the care and outcomes in the Netherlands of patients diagnosed from 2013 to 2017. Despite comparable baseline characteristics, the survival of patients with MM was worse than that of patients with CM. This also holds for the subgroup of patients who were treated with systemic therapy. In contrast to patients with CM, OS of patients with MM did not improve between 2013 and 2017, despite the introduction of novel therapies. Elevated LDH level and ECOG PS of ≥1 were independently associated with worse OS in MM. The prognosis of MM, originating from different types of primary locations appeared to be similar. NRAS (mainly in head and neck MM) and KIT mutations (mainly in gastrointestinal and vulvovaginal MM) were most common in MM.

Results of our cohort of advanced MM and CM confirm that patients with advanced MM have a worse prognosis than patients with CM. [8, 14] Despite that patients with advanced

MM had the favourable disease stage and similar ECOG PS and LDH levels compared with patients with advanced CM, outcomes for MM were worse. This suggests that MM has an inherent worse prognosis and it is hypothesised this may be due to a different, more aggressive, biological behaviour. [8] The clinical behaviour of MM and low tumour mutation burden with distinct driver mutations advocate that MM has a different pathogenesis than CM and should be seen as a unique entity of melanomas. [2, 6, 15, 16] More studies found that MM less often metastasise to the brain and that lungs, liver and/or non-regional lymph nodes are involved most frequently. [17-21] Similar to CM and consistent with the literature for advanced MM, we found that the ECOG score of ≥1 and the elevated LDH level were independent prognostic factors for OS. [15, 17, 22]

From 2011 to 2016, immune and targeted therapies gradually became available for patients with advanced melanoma in the Netherlands. [23] Unlike for CM, we did not observe an increase in OS for MM when comparing the cohorts of 2013-2015 to 2016-2017. However, the median OS of patients with MM who received systemic therapy in our cohort is comparable with the median OS of post-hoc analysis of patients with MM in the pembrolizumab trials (respectively 11.8 months and 11.3 months), and it is higher than the historical median OS of 6-8 months for advanced melanoma in general. [11, 24, 25] It also resembles the median OS of 11.5 months in patients with MM treated with nivolumab after progression on or after ipilimumab, although OS was defined from start of nivolumab treatment. [26] From currently available treatment options for advanced MM, immunotherapy has the potential to induce durable remissions, although much less frequently compared with advanced CM. Ipilimumab monotherapy has shown to have some antitumour activity in advanced MM, but overall response rates (ORRs) were lower than those in CM (<10%). [14, 27, 28] The post-hoc analysis of the KEYNOTE 001, 002, 006 trials showed an ORR of 19% and a median OS of 11.3 months for MM patients treated with pembrolizumab. [24] A median OS of 11.5 months was found in the CHECKMATE-172 study, in which OS was defined from start of nivolumab on or after progression of ipilimumab. [26] Pooled analysis of the CheckMate studies compared effectiveness of ipilimumab, nivolumab and ipilimumab plus nivolumab in MM and observed the highest potential for the ipilimumab plus nivolumab, but no information on long-term outcomes is available (ORR of 8.3%, 23.3% and 37.1%, respectively). [29] An immunotherapeutic strategy that has shown the promising ORR in advanced cutaneous and uveal melanoma is adoptive cell therapy with tumour-infiltrating lymphocytes [30], but effectiveness data specifically for MM are lacking. MM-specific vaccine development will remain challenging given the low tumour neo-antigen burden. [31]

Patients with a BRAF-mutated MM can benefit from BRAF plus MEK inhibitors, but BRAF mutations are rare in MM. [32-35] KIT and NRAS mutations are more common, and additionally, we observed KIT mutation was more frequent in lower gastrointestinal and vulvovaginal MM. [34, 35] KIT is a targetable driver mutation, and KIT inhibitors have demonstrated clinical

activity in advanced melanoma, but ORR and survival benefit varied by the type of KIT alteration. [9] Within the DMTR, no data are captured on the use of imatinib or another c-KIT inhibitor for KIT mutated MM. It is clear that systemic treatment for MM is lagging behind its cutaneous counterpart. Research should be focused on identifying vulnerabilities specific for MM and attempt to target these with either immunotherapies or targeted therapies. Some small studies on MM, in which all stages of MM were analysed, have found that localisation of MM was predictive of survival. Head and neck and gastrointestinal MM of any stage appeared to have inferior survival compared with other MM. [15, 36] However, three large studies on prognostic factors for survival in MM found that location was not a prognostic factor for the early or advanced stage. [8, 37, 38] Cui et al. even conclude that MM can be staged as a single group irrespective of location of MM. Our results endorse that survival between the subtypes of advanced MM is comparable, but imbalances in baseline characteristics of patients between the subtypes of advanced melanoma and low number of patients do not allow a fair comparison of OS. [37]

A major limitation is the small sample size hampering analysis and adequate correction for confounding factors when comparing outcomes. Still, this is one of the largest real-world cohorts of MM that gives insight in the outcomes in recent years.

5. Conclusion

Survival of patients with advanced MM is worse than that in advanced CM. In the era of immune and targeted therapies, prognosis for patients with advanced MM has not improved as much as the prognosis of CM. The aim of future research should be to gain further knowledge on the vulnerabilities of MM to target these with novel strategies. We emphasize the need for international collaboration allowing data exchange to increase sample size and research on this rare disease.

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Supplementary material

Figure S1. Flowchart of patient inclusion of this observational study. * Mucosal melanoma confirmed, but location was unknown or uncertain.

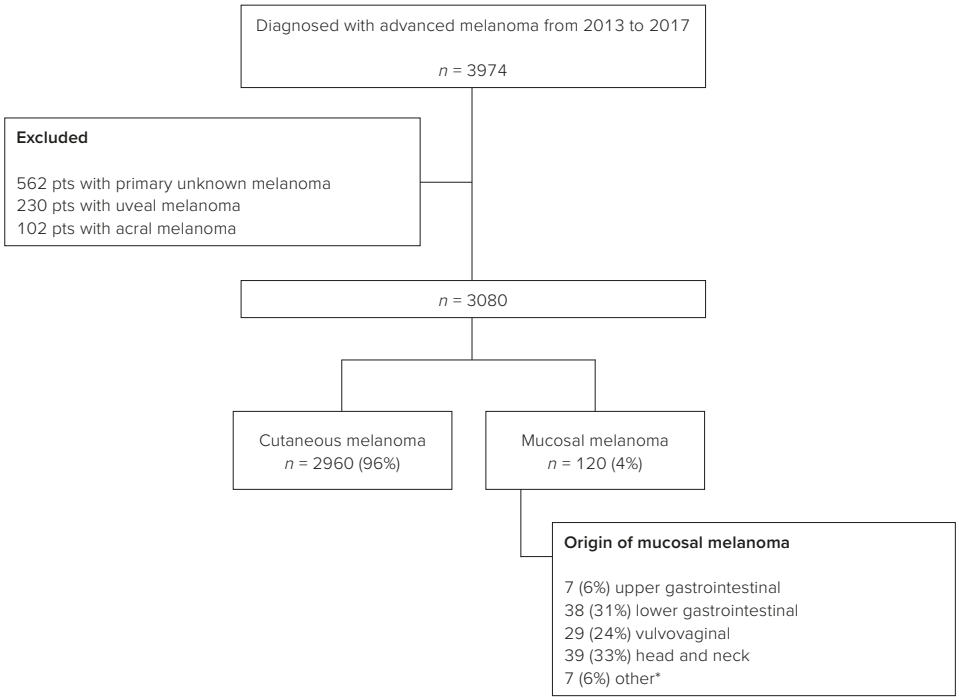

Figure S2. Kaplan Meier curves of overall survival of patients with unresectable stage III or stage IV mucosal versus cutaneous melanoma in a propensity score matched cohort (Log rank test: *p*=0.003). Cases with missing data were omitted from this analysis, leading to n=81 cases for mucosal melanoma. OS - Overall survival, CI - confidence interval. Baseline characteristics are shown in Table S2 in the supplement.

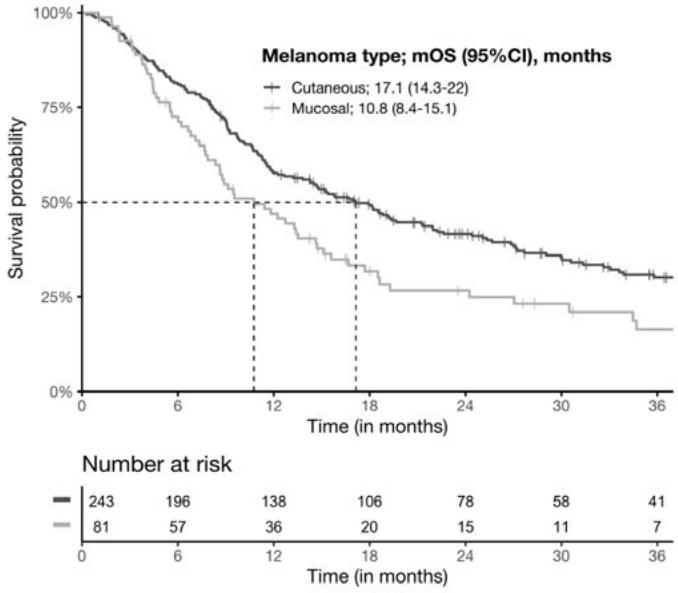

Figure S3. First-line systemic therapy of patients with cutaneous melanoma and mucosal melanoma. Category 'Other' also consists of systemic therapy given in trials. Data on KIT inhibitors were not available in the DMTR.

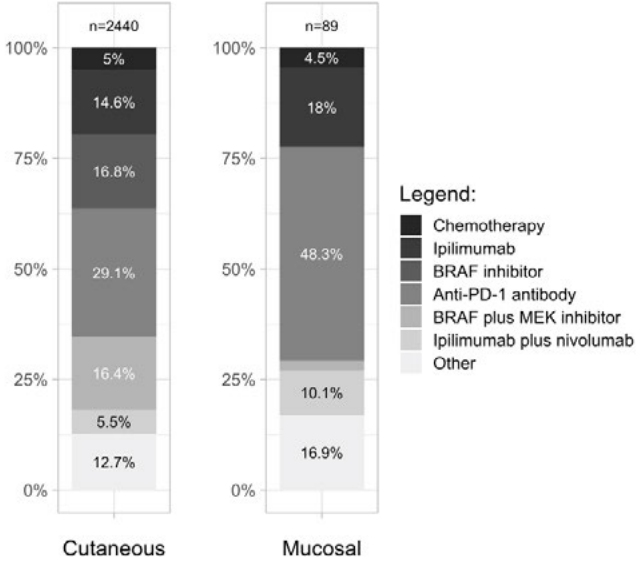

Table S1. Characteristics of patients with mucosal melanoma at diagnosis of unresectable stage III or stage IV disease stratified by location of primary site. Missing data of less than 2.5% are not shown in this table.

	Upper GI (*n=7*)	Lower GI (*n=38*)	Vulvo-vaginal (*n=29*)	Head and neck (*n=39*)	Other (*n=7*)	*P*-value*
Median age, year (IQR)	65 [54, 67]	72 [66, 76]	70 [63, 78]	64 [58, 73]	72 [66, 75]	
Months to advanced melanoma						
Median (IQR)	0 [0, 0]	6 [0, 13]	9 [0, 15]	15 [4, 35]	9 [4, 25]	0.001
Female	2 (28.6)	17 (44.7)	29 (100.0)	20 (51.3)	4 (57.1)	<0.001
ECOG score						
0	0 (0.0)	22 (64.7)	15 (53.6)	15 (42.9)	5 (71.4)	0.113
1	4 (80.0)	11 (32.4)	8 (28.6)	15 (42.9)	1 (14.3)	
≥2	1 (20.0)	1 (2.9)	5 (17.9)	5 (14.3)	1 (14.3)	
Unknown	1	4	0	4	2	
LDH level						
Normal	5 (71.4)	21 (58.3)	17 (65.4)	27 (79.4)	6 (100.0)	0.091
1x ULN	2 (28.6)	10 (27.8)	9 (34.6)	3 (8.8)	0 (0.0)	
>2x ULN	0 (0.0)	5 (13.9)	0 (0.0)	4 (11.8)	0 (0.0)	
Unknown	0	2	2	5	2	
Stage						
III (unresectable)	0 (0.0)	4 (10.5)	4 (13.8)	6 (15.4)	1 (14.3)	0.360
IV-M1a	0 (0.0)	3 (7.9)	1 (3.4)	2 (5.1)	1 (14.3)	
IV-M1b	0 (0.0)	4 (10.5)	6 (20.7)	5 (12.8)	2 (28.6)	
IV-M1c	4 (57.1)	25 (65.8)	15 (51.7)	23 (59.0)	3 (42.9)	
IV-M1d	3 (42.9)	2 (5.3)	3 (10.3)	3 (7.7)	0 (0.0)	
Metastases in ≥3 organ sites	4 (57.1)	11 (28.9)	8 (27.6)	13 (33.3)	0 (0.0)	0.218
Mutations:						
BRAF	2 (28.6)	2 (5.3)	0 (0.0)	2 (5.1)	1 (14.3)	0.032
NRAS	2 (28.6)	4 (10.5)	2 (6.9)	8 (20.5)	1 (14.3)	0.39
KIT	0 (0.0)	8 (21.1)	4 (13.8)	2 (5.1)	0 (0.0)	0.38
GNAQ	0 (0.0)	0 (0.0)	2 (6.9)	0 (0.0)	0 (0.0)	0.21
GNA11	0 (0.0)	0 (0.0)	1 (3.4)	1 (2.6)	0 (0.0)	0.68

GI - gastrointestinal, IQR - interquartile range, ECOG PS - Eastern Cooperative Oncology Group performance status, LDH - lactate dehydrogenase, ULN - upper limit of normal. * *P*-value of statistical tests comparing characteristics of patients by location of primary site of mucosal melanoma (excluding missing values). Values are *n* (%) unless otherwise indicated.

Table S2. Characteristics at diagnosis of unresectable stage IIIC or stage IV disease of the propensity score matched cohort of patients with mucosal and cutaneous melanoma.

	Mucosal (n=81)*	Cutaneous (n=243)	P-value**
Median age, year (IQR)	67 [62, 74]	69 [60, 76]	0.903
Female	54 (66.7)	168 (69.1)	0.273
ECOG score			0.287
0	39 (48.1)	119 (49.0)	
1	33 (40.7)	98 (40.3)	
≥2	9 (11.1)	26 (10.7)	
LDH level			0.491
Normal	61 (75.3)	179 (73.7)	
1x ULN	16 (19.8)	53 (21.8)	
>2x ULN	4 (4.9)	11 (4.5)	
Stage			-
III (unresectable)	1 (1.2)	0 (0.0)	
IV-M1a	6 (7.4)	26 (10.7)	
IV-M1b	13 (16.0)	42 (17.3)	
IV-M1c	56 (69.1)	165 (67.9)	
IV-M1d	5 (6.2)	10 (4.1)	
Metastases in ≥3 organ sites	25 (30.9)	73 (30.0)	1.000
Brain metastasis			0.782
Absent	76 (93.8)	233 (95.9)	
Asymptomatic	2 (2.5)	3 (1.2)	
Symptomatic	3 (3.7)	7 (2.9)	
Liver metastasis	35 (43.2)	92 (37.9)	0.803
BRAF-mutant	5 (6.2)	19 (7.8)	0.351

*Cases with missing data were omitted from this analysis, leading to n=81 cases for mucosal melanoma. IQR - interquartile range, ECOG PS - Eastern Cooperative Oncology Group performance status, LDH - lactate dehydrogenase, ULN - upper limit of normal. ** *P*-value of statistical tests comparing characteristics of the mucosal and cutaneous melanoma cohort.

Table S3. Characteristics at diagnosis of unresectable stage IIIC or stage IV of patients with mucosal melanoma alive at the 3-year landmark.

	Alive at 3-year landmark
Patients; n	11
Median age, year (IQR)	66 [58, 73]
Median time to stage IIIC or IV	
Months (IQR)	4 [0, 44]
Female	7 (63.6)
ECOG score	
0	9 (81.8)
1	2 (18.2)
≥2	0 (0.0)
LDH level	
Normal	7 (70.0)
1x ULN	3 (30.0)
>2x ULN	0 (0.0)
Unknown	1
Stage	
III (unresectable)	1 (9.1)
IV-M1a	1 (9.1)
IV-M1b	2 (18.2)
IV-M1c	7 (63.6)
IV-M1d	0 (0.0)
Metastasis in ≥3 organ sites	2 (18.2)
Mutations:	
BRAF	1 (9.1)
NRAS	1 (9.1)
KIT	3 (27.3)
GNAQ	0 (0.0)
GNA11	0 (0.0)

IQR - interquartile range, ECOG PS - Eastern Cooperative Oncology Group performance status, LDH - lactate dehydrogenase, ULN - upper limit of normal.

Clinical outcomes and toxicity of combined ipilimumab/nivolumab in rare melanomas – a nationwide population-based study and a review of the literature

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Submitted

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Simple summary

Mucosal and uveal melanomas (MM and UM) are rare melanomas with a poor prognosis. Whilst immune checkpoint inhibitors have improved overall survival in advanced cutaneous melanoma (CM), MM and UM appear less immunogenic, which is probably the reason for lower response rates. In this study we assessed efficacy, toxicity and predictors of survival in 46 advanced MM and 13 advanced UM treated with ipilimumab/nivolumab, and provided a review of the literature. We confirmed the lower efficacy of ipilimumab/nivolumab in MM and UM as compared to CM, but found that half of the MM and UM experienced clinical benefit. However, the prognosis of advanced MM and UM remains poor and toxicity rates of ipilimumab/nivolumab are high. Therefore, future research should focus on identifying the subgroup of patients with rare melanomas who may benefit from ipilimumab/ nivolumab including clinical trials testing novel therapeutic (combination) strategies.

Abstract

Background

Immune checkpoint inhibitors, and in particular combined anti-CTLA-4/anti-PD-1, have improved outcomes for patients with advanced cutaneous melanoma (CM). Mucosal and uveal melanoma (MM and UM) seem less immunogenic compared to CM, and the benefit of anti-CTLA-4/anti-PD-1 treatment is unclear. The aim of this study is to assess clinical outcomes and toxicity of combined ipilimumab/nivolumab treatment in advanced MM and UM, using nation-wide real-world data. Moreover, we aim to identify prognostic factors for outcomes and toxicity and provide a review of the literature.

Methods

All patients diagnosed with advanced MM and UM treated with ipilimumab/nivolumab between 2013 and 2021in the Netherlands were included from the Dutch Melanoma Treatment Registry. Best overall response rate and grade ≥3 toxicity rates were calculated. Progression-free survival (PFS) and overall survival (OS) were calculated using the Kaplan-Meier method. Independent predictors of OS in MM were assessed with Cox proportional hazards models.

Results

46 patients with MM and 13 patients with UM were included. Complete response, partial response or stable disease, as best overall response was achieved in 52% and 46% of the MM and UM, respectively. Median OS and PFS were 9.7 and 4.1 months for MM and 12.4 and 5.5 months for UM. One-year OS and two-year-OS for MM were 43% and 23%. Multivariable analysis showed LDH level of ≥ two times upper limit of normal and the presence of liver metastasis to be associated with worse OS. Grade ≥3 toxicity occurred in 48% of MM and 38% of UM.

Conclusion

Our study shows that advanced MM and UM have a poor prognosis and that half of the patients with MM and UM experience clinical benefit of ipilimumab/nivolumab but that OS is short and toxicity rates are high. International collaboration and novel clinical trials are essential to improve outcomes for patients with advanced MM and UM.

Introduction

Immune-checkpoint inhibitors (ICI) have revolutionized outcomes for patients with advanced (irresectable stage III and stage IV) cutaneous melanoma (CM). ICIs block the immunologic inhibitory receptors CTLA-4 and PD-1 located on T-lymphocytes. Blockage of these receptors results in a boost of the immune response by T-cells attacking cancer cells. [1] At 6.5-year follow-up, the CheckMate 067 trial demonstrated a median overall survival (OS) and 6.5-year OS in advanced CM of ipilimumab, nivolumab and combined ipilimumab/nivolumab of 19.9, 36.9 and 72.1 months and 23%, 42%, and 49%, respectively. Combined ipilimumab/nivolumab also showed a higher response rate (RR) of 58% compared to ipilimumab monotherapy (19%) and nivolumab monotherapy (45%). [2] The favourable RR, along with long-term durable cancer control, have established the combined ICI and anti-PD1 monotherapy as the preferred treatments in advanced CM. [1, 3] However, an important problem of combining agents is the higher toxicity rate. In ipilimumab/nivolumab, grade ≥3 toxicity is experienced in 59% of the patients, leading to discontinuation of treatment in 31%, whilst toxicity rates in ipilimumab monotherapy and nivolumab monotherapy are lower (24% and 28%), and also less often lead to discontinuation of treatment (14% and 8%). [2, 4]

Mucosal melanoma (MM) and uveal melanoma (UM) represent small subgroups of melanoma. MM originates from the lining of any mucosal surface in the body and comprises 1-2% of all melanomas. [5] UM develops from the iris, ciliary body or choroid of the eye. [6, 7] Even though UM is the most common intraocular tumor, it comprises only 3-5% of all melanomas. [8, 9] Due to the unique clinical and biological characteristics and the low incidence of MM and UM, patients are often excluded from clinical trials, and the numbers of patients treated with ICI are low.

Although evidence is scarce, the limited data on the efficacy of ICI in MM and UM suggest that these may not be as promising as in CM. [10] Overall RR in MM are 0-17% for ipilimumab and 9-50% for nivolumab, respectively. [11] In UM, efficacy is even lower, with RR ranging from 0-6.5% for ipilimumab and 6-30% for nivolumab or pembrolizumab monotherapy. [12] The synergetic effect of combined ipilimumab/nivolumab treatment leading to a higher RR and better OS is evident in CM. In MM and UM, the limited data report overall RR ranging between 16-43% and 0-21%, respectively. In CM, the high tumor mutational burden (TMB) is associated with immunogenicity and therefore may in part, explain the high efficacy of ICI. It is likely that the lower TMB of both MM and UM contributes to the lower RR of ICI in these entities. [13-15]

The aim of this study is to assess efficacy and toxicity of ipilimumab/nivolumab in advanced MM and UM, using real-life data from a Dutch nationwide database in melanoma patients. In addition, we aim to identify prognostic factors for outcomes and toxicity and present a review of the literature covering anti-CTLA-4/anti-PD-1 treatment in MM and UM.

Materials and Results

Study design and data

Since 2013, systemic therapy for melanoma patients in the Netherlands is centralized in 14 melanoma centers. Data of these patients are prospectively collected in the Dutch Melanoma Treatment Registry (DMTR). We performed a retrospective observational study analyzing all patients registered in the DMTR between 2013 and 2021 with advanced (i.e. unresectable stage III or stage IV) MM or UM, treated with combined ipilimumab/nivolumab. [16] Registered information includes patient and tumor characteristics, treatment, clinical outcomes and toxicity data according to the Common Terminology Criteria for adverse events (CTCAE) 5.0. [17]

Exclusion criteria for this analysis were age under 18 years and less than six weeks of follow-up from the start of ipilimumab/nivolumab (a minimum of two courses ipilimumab/nivolumab). Patients were staged by the 8th edition of the American Joint Committee Cancer (AJCC) melanoma staging system. The data cut-off was October 2021. The study design was approved by the scientific board of the DMTR. In compliance with Dutch regulations, use of DMTR data for research was approved by the Medical Ethics Review Committee of Leiden University Medical Center.

Definitions of outcome

OS was defined as the time between start of treatment with ipilimumab/nivolumab until death. Patients not reaching the endpoint were right-censored at the date of the last contact. Progression-free survival (PFS) was calculated from start of ipilimumab/nivolumab until date of first progression according to the response evaluation, or death. In this real-world database, response is based on the evaluation by the RECIST (Response Evaluation Criteria in Solid Tumors) criteria of the treating physician at one of the melanoma expert centers. [18] Best overall response (BORR) was the best response evaluation that a patient had after initiation of treatment until start of a new melanoma therapy, last follow-up visit or death. The BOR rate was defined as the proportion of evaluable patients who achieved a complete or partial response. Based on the BOR rate at any moment during follow-up, clinical benefit was defined as either stable disease (SD), partial response (PR) or complete response (CR). One-year and two-year OS rates were calculated for MM, but due to a short median follow-up period, they were not calculated for UM.

Statistical analysis

The baseline characteristics at the start of treatment with combined ipilimumab/nivolumab were summarized using descriptive statistics. Categorical variables were summarized with counts and percentages. Age was presented as a median with a interquartile range (IQR) and was divided into two categories (<70 years or ≥70 years). Descriptive and survival

data were presented for MM and UM separately. Median follow-up was estimated with the reverse Kaplan-Meier method. [19] Median PFS and OS were estimated with the Kaplan-Meier method. For MM, a Cox proportional hazards model was used to estimate the association between prognostic factors with OS; age (<70 or ≥70) gender, WHO classification at baseline (0-1 or ≥2), number of metastatic sites at (<3 or ≥3 organ sites involved), LDH level, the presence of liver metastasis at baseline and presence of brain metastasis at baseline. Independent predictors for OS were evaluated by applying multivariable Cox proportional hazards regression models, following the selection of potential predictors based on a p-value is <0.1 in the univariable analyses. Due to the low number of patients, univariable and multivariable Cox regression analysis were not performed for UM. Statistical analyses were performed using SPSS (SPSS, version 25, IBM Corp. released 2017, Armonk, NY, USA). Values of p=0.05 or smaller were considered statistically significant and all tests were two sided.

Data sources

For the literature review, data on combined anti-CLTA-4/anti-PD-1 therapy in MM and UM were collected through the search engines PubMed, and Web of Science (date of last search June 15th, 2023. We included studies analyzing 5 or more cases, treated with any type of combined anti-CTLA-4/anti-PD-1 therapy.

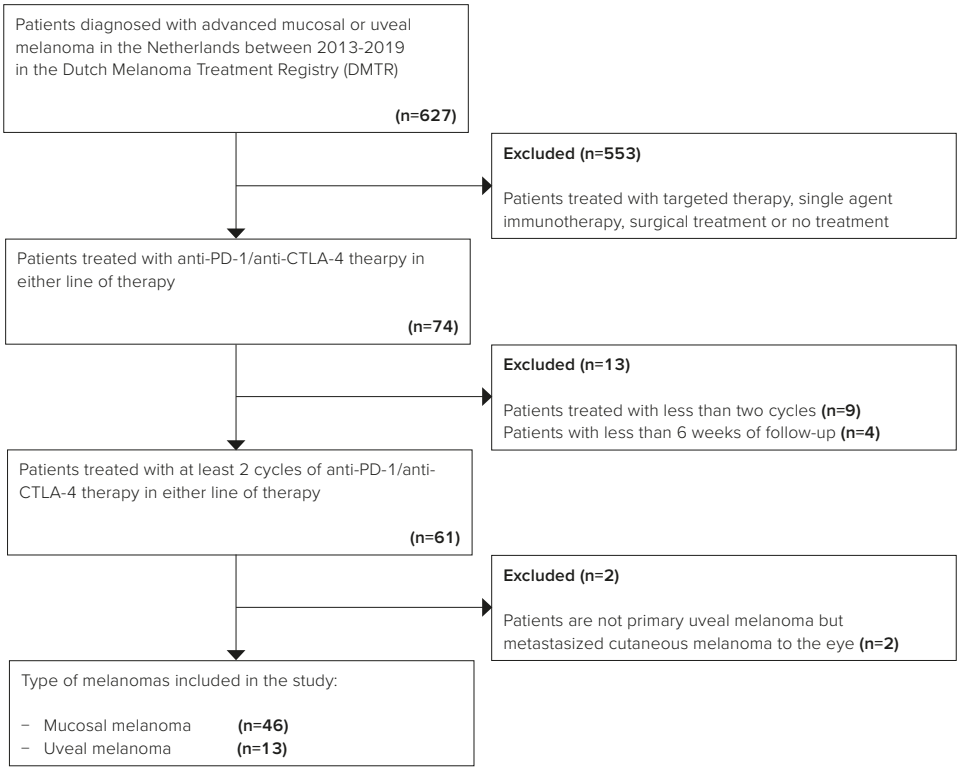
Results

Patient and treatment characteristics

From 2013 to 2021, 221 patients were diagnosed with advanced MM and 406 with advanced UM. In total, during this period, 46 patients with MM and 13 patients with UM were treated with ipilimumab/nivolumab and were included in this study (Figure 1). Thirteen patients were excluded, of which four due to inadequate follow-up, nine as they received less than two cycles of ipilimumab/nivolumab (Figure 1), and two as they were primary CM and not UM. The median follow-up was 15.2 months for MM and 4.9 months for UM.

Patients with MM had a median age of 66 years (IQR 59-73) and more often were female (n=34, 73.9%) (Table 1). Patients had stage IV M1c disease in 50% (n=20) and LDH level was elevated only in the minority of the patients (n=15, 32.6%). Treatment with ipilimumab/nivolumab was the first line of treatment in the majority of the patients (n=44, 95.7%) (Table 1). The two patients who received ipilimumab/nivolumab therapy as second-line treatment were treated with an anti-PD-1 inhibitor in the first line (n=2) (data not shown).

Figure 1. STROBE diagram for the study (STROBE: Strengthening the reporting of observational studies in epidemiology)



In UM, median age at diagnosis was 58 years (IQR 50-72) and females and males were equally affected (53.8% and 47.2%) (Table 1). Stage at diagnosis was IVc in 92.3% of the patients and liver metastasis were present in 76.9% of the patients. LDH levels were elevated in the greater part of the patients (n=9, 69.3%). In two patients, the disease had only spread to the liver (15.5%), in eight to both liver and other sites (61.3%) and three only had extra-hepatic metastasis (23.1%). Four cases (33.3% received ipilimumab/nivolumab as second-line treatment. As prior treatment strategy, one patient received radiofrequency ablation (RFA) and ipilimumab monotherapy, two patients were treated with liver perfusion and one patient underwent a surgical resection of a liver metastasis (data not shown). MM and UM both had low *BRAF*, *NRAS* and *KIT* mutation rates. Baseline characteristics are presented in Table 1.

Table 1. Baseline patient characteristics of patients with advanced mucosal melanoma and uveal melanoma

	Mucosal melanoma		Uveal melanoma	
	N= 46		N= 13	
	n	%	n	%
Median follow-up (months)	15.2		4.9	
Gender				
Male	12	26.1	6	46.2
Female	34	73.9	7	53.8
Age at diagnosis, years				
0–69	30	65.2	9	69.2
≥ 70	16	34.8	4	30.8
Median age at diagnosis (IQR)	66 (59-73)		58 (50-72)	
WHO performance status				
0-1	43	93.5	13	100.0
≥2	2	4.3	0	0.0
Unknown	1	2.2	0	0.0
Tumour stage				
Stage III (unresectable)	11	23.9	0	0.0
Stage IVa	1	2.2	0	0.0
Stage IVb	8	17.4	1	7.7
Stage IVc	23	50.0	12	92.3
Stage IVd	3	6.5	0	0.0
LDH level				
Normal	31	67.4	4	30.8
≥ 1x ULN – 2x ULN	8	17.4	6	46.2
≥ 2x ULN	7	15.2	3	23.1
Number of metastatic sites				
0	11	23.9	0	0.0
< 3	31	67.4	10	76.9
≥ 3	4	8.7	3	23.1
Brain metastasis				
No	36	78.3	13	100.0
Yes	3	6.5	0	0.0
Unknown	7	15.2	0	0.0
Liver metastasis				
No	34	73.9	3	23.1
Yes	12	26.1	10	76.9
Line of systemic treatment				
First line	44	95.7	8	66.6
Second line	2	4.3	4	33.3
BRAF mutation				
Yes	1	2.2	0	0.0
No	41	89.1	8	61.5
Not assesed	4	8.7	5	38.5

Table 1. (*Continued.*)

	Mucosal melanoma		Uveal melanoma	
	N= 46		N= 13	
	n	%	n	%
NRAS mutation				
Yes	7	15.2	0	0.0
No	32	69.6	8	61.5
Not assesed	7	15.2	5	38.5
KIT mutation				
Yes	2	4.3	0	0.0
No	35	76.1	8	61.5
Not assesed	9	19.6	5	38.5
Number of treatment cycles				
2	15	32.6	4	30.8
3	12	26.1	3	23.1
4	19	41.3	6	46.1

IQR, Interquartile range, LDH – lactate dehydrogenase, ULN Upper limit of normal

Treatment outcomes

In both MM and UM approximately half of the patients had clinical benefit from ipilimumab/nivolumab treatment (52.2% and 46.2%, Table 2). Of these patients, CR and PR was seen in 5/46 (10.9%) and 13/46 (28.3%) of the patients with MM. No patients with UM had a complete response, whilst partial response was seen in 4/13 (30.8%) patients. The median OS for all patients with clinical benefit in MM and UM were 10 and 12 months, respectively (Table 2).

Median OS for all patients with MM was 9.7 months [95% CI:5.9-13.5] and median PFS was 4.1 months [95% CI: 2.3-5.9] (Figure 2). For patients with UM, median OS was 12.4 months [95%CI: 1.4-23.4] and median PFS was 5.5 months [95% CI: 0.0-11.6] (Figure 3). Patients with MM had a 1- and 2- year OS of 43% [95%CI: 27.4-58.6] and 23% [95% CI: 5.4-40.6], respectively.

Table 2. Response rates based on best overall response and median OS in mucosal and uveal melanoma.

	Mucosal melanoma (n=46)		Uveal melanoma (n=13)	
	% of patients (n)	**Median OS in months (IQR)**	**% of patients (n)**	**Median OS in months (IQR)**
CR	10.9% (5)	15.5 (6.4-43.0)	0% (0)	-
PR	28.3% (13)	6.0 (3.6-17.3)	30.8% (4)	7.4 (3.0-14.8)
SD	13.0% (6)	10.0 (5.1-16.1)	15.4% (2)	13.9 (12.4-NR)
PD or death	47.8% (22)	3.3 (2.1-7.0)	38.5% (5)	4.1 (2.5-5.8)
Unknown	0.0% (0)	-	15.4% (2)	-
Clinical benefit	52.2% (24)	9.8 (4.1-17.4)	46.2% (6)	11.8 (3.3-15.6)
ORR	39.1% (18)	8.1 (3.9-19.2)	30.8% (4)	7.4 (3.0-14.8)

OS: overall survival, CR: complete response, PR partial response, SD Stable disease, PD progressive disease. Clinical benefit comprises CR, PR and SD.ORR: objective response rate comprises CR and PR. NR Not reached

Figure 2. Kaplan Meier curves of progression-free survival (A) and overall survival (B) of mucosal melanoma

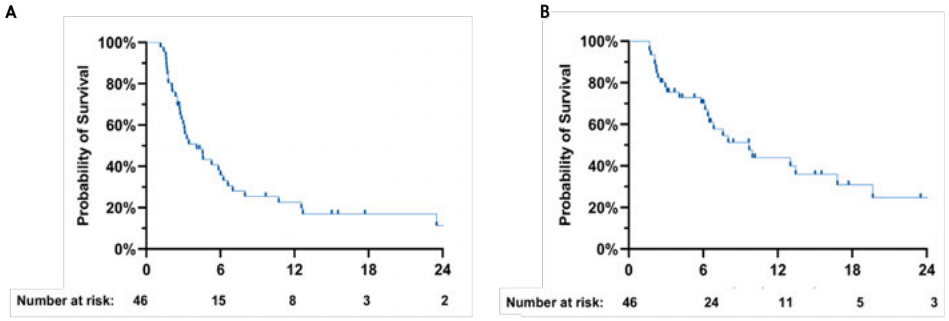
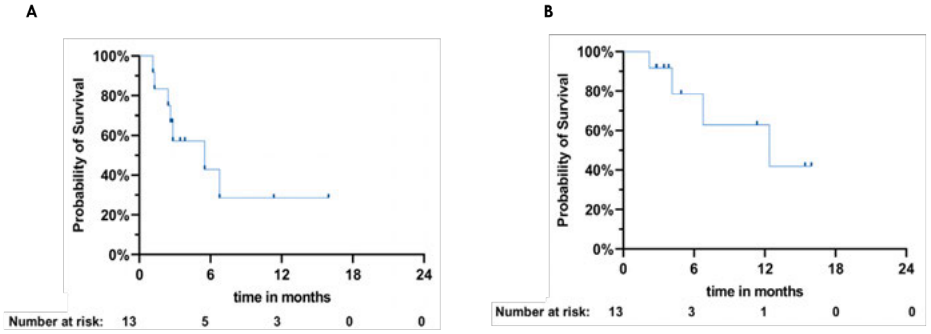


Figure 3. Kaplan Meier curves of progression-free survival (A) and overall survival (B) of uveal melanoma



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Treatment toxicity

Grade ≥3 toxicity occurred in 47.8% (n=22/46) of the patients with MM and 38.4% (n=5/13) of the patients with UM, and led to hospital admission in 12/22 patients (54.5%) in MM and 2/5 (40.0%) patients in UM (Supplementary Table 1). Toxicity led to discontinuation of treatment in 18/22 (81.2%) patients with MM and in 5/5 (100%) patients with UM. No treatment-related deaths occurred during treatment or the observation period. The most frequent reported toxicities in MM were hepatitis (19.6%) and colitis (17.4%), and in UM colitis (20.0%), rash (6.7%), hepatitis (6.7%) and pneumonitis (6.7%) (Supplementary Table 1).

Predictors of overall survival

In MM, univariable analysis showed that LDH level ≥ 2ULN, WHO status ≥2, ≥3 metastatic sites, and the presence of liver metastasis were associated with worse survival (Table 3). The multivariable analysis showed that LDH level ≥ 2ULN (HRadj: 6.57 95% CI:1.14-37.75) and the presence of liver metastasis (HRadj: 3.04 95% CI:1.13-8.16) were associated with worse survival (Table 3).

Table 3. Univariable and multivariable cox regression model for the association of determinants of overall survival for mucosal melanoma

	Mucosal melanoma					
	Univariable analysis			Multivariable analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Age						
0-69	ref		ref			
≥ 70	0.84	0.36-1.93	0.68			
Gender						
Male	ref		ref			
Female	0.59	0.25-1.41	0.23			
Stage						
Stage III (unresectable)	ref					
Stage IVa	3.33	0.34-32.40	0.30			
Stage IVb	1.59	0.34-7.37	0.55			
Stage IVc	2.94	0.84-10.24	0.09			
Stage IVd	3.16	0.63-15.83	0.16			
LDH level						
Normal	ref		ref	ref		ref
≥ ULN – 2x ULN	0.39	0.11-1.37	0.14	0.31	0.08-1.16	0.08
≥ 2x ULN	3.50	1.22-10.01	**0.02**	2.88	0.78-10.7	0.11
WHO perfomance status						
0-1	ref		ref	ref		ref
2 or higher	5.13	1.09-24.14	**0.04**	0.26	0.03-2.48	0.24
Number of metastatic sites						
< 3	ref		ref	ref		ref
≥ 3	10.19	2.89-35.95	**> 0.001**	6.57	1.14-37.75	**0.04**
Brain metastasis						
Absent	ref		ref			
Present	1.51	0.44-5.19	0.50			
Liver metastasis						
Absent	ref					
Present						
Present	2.98	1.33-6.68	**0.008**	3.04	1.13-8.16	**0.03**

LDH – lactate dehydrogenase, ULN Upper limit of normal

Discussion

MM and UM are rare melanomas in which the optimal treatment strategy has not yet been established. In this retrospective study using real-world data from 2013-2021 we found that only the minority of the patients were treated with ipilimumab/nivolumab (Figure 1). Though, it is important to note that, as combined ipilimumab/nivolumab therapy was introduced in 2016, this therapy was not available in the first years or our cohort, partly explaining these figures. In both MM and UM, approximately half of the patients experienced clinical benefit of

ipilimumab/nivolumab and median OS was 9.7 months and 12.4 months, respectively. Grade ≥3 toxicity occurred in 47.8% of the MM and in 38.4% of the UM, leading to discontinuation of treatment in most of these patients. In MM, the presence of liver metastasis and an LDH level ≥ 2 x ULN level were predictive for worse OS.

Next to our analyses, we present an overview of the studies assessing the efficacy of combined ipilimumab/nivolumab in both MM (7 studies) and UM (9 studies) (Table 4). [10, 20-23] In MM, a pooled analysis published by D'Angelo et al., a retrospective study including 197 patients by Dimitriou et al., and a phase II trial by Namikawa et al. demonstrated BORR of 37.1%, 36% and 31%, which are comparable with our findings (39.2% of the patients had CR of PR). [10, 20, 21] In all studies, including ours, only the minority responded completely. As compared to the other studies, Takahashi et al. presented a lower BORR of 16.7%. [22] However, in that study, half of the patients received ipilimumab/nivolumab as a second-line or higher treatment, whilst in the other studies this ranged between 0-7%. Moreover, when comparing the treatment-naïve group with the prior-treatment group, the BORR increased from 5.9% to 26.3%. Though not statistically significant, the lower efficacy of those receiving ipilimumab/nivolumab as second-line or higher treatment, was also reflected by lower OS and PFS (1-year OS 78% vs 43% and 1-year PFS 38.6% vs 17.6%). In CM, combined ipilimumab/nivolumab as second-line treatment is associated with lower RR, but still, Silva et al. found complete or partial response in 30% of the patients. [24-26] Our study population included only two patients (4.3%) with prior treatment (both anti-PD-1 monotherapy) hampering analysis between the treatment naïve group and the prior-treatment group.

As in CM, the pooled analysis of MM demonstrated that whilst combination therapy has a higher efficacy than anti-PD-1 monotherapy, this is at the expense of higher toxicity rates. [10, 27] ORR for nivolumab monotherapy and ipilimumab/nivolumab combination therapy were 23.3% and 37.1% and grade 3 toxicity or higher was seen in 8.1% and 40.0% of the patients. Interestingly, in CM treated with ipilimumab/nivolumab, grade 3 or higher toxicity occurred in 54.5%, whilst in MM this was only 40.0%, also leading to lower treatment discontinuation rates (31.0% vs 17.1%). [10] Yet, this pooled analysis is hampered by a short median follow-up. A post-hoc analysis of the Checkmate 067 trial has overcome this by presenting long-term outcomes of 79 MM patients with a minimum follow-up of 60 months. This study confirmed the higher efficacy of combined therapy when compared to ipilimumab or nivolumab monotherapy including more CR, respectively 14%, 4% and 0%. [28] In contrast, Dimitriou et al., Umeda et al., and Nakamura et al., concluded that anti-CTLA-4/anti-PD-1 treatment and anti-PD-1 had similar efficacy with an objective response rate (ORR) ranging between 26-29% vs 28-31%, respectively. Moreover, median PFS and median OS were similar between both treatment regimens. [20, 23, 29] Altogether, the evidence regarding the superior efficacy of combined nivolumab plus ipilimumab compared to ICI monotherapy for MM, based on non-randomized and retrospective studies, remains much less robust than in CM.

Table 4. Overview of studies describing efficacy and toxicity of combined anti-CTLA-4/ anti-PD-1 therapy in mucosal and uveal melanoma

Mucosal Melanoma							
Study (year)	**Number of patients**	**Type of study**	**Type of ICI**	**OS (months)**	**PFS (months)**	**ORR (%)**	**Grade ≥3 toxicity (%)**
D'Angelo et al (2017)	35 *86* *36*	Pooled analysis	Ipi/Nivo *Nivolumab* *Ipilimumab*	NA NA NA	5.9 3.0 2.7	37.1 23.3 8.3	40.0 8.1 12.5
Namikawa et al (2020)	12	Phase II trial	Ipi/Nivo	NR	NR	36	NA
Shoustari et al (2020)	28 *23* *28*	Phase III trial	Ipi/Nivo *Nivolumab* *Ipilimumab*	22.7 20.2 12.1	5.8 3.0 2.6	43 30 7	54 26 25
Umeda et al (2021)	42 *171*	Real world data	Anti CTLA-4/anti-PD-1, not specified *Anti-PD-1, not specified*	31.7 19.2	5.8 6.2	28 26	56.0 20.0
Nakamura et al (2021)	66 *263*	Retrospective study	Anti CTLA-4/anti-PD-1, not specified *Anti-PD-1, not specified*	20.1 20.4	6.8 5.9	29 26	53 17
Takahashi et al (2022)	36	Retrospective study	Ipi/Nivo	14	3.25	16.7	NA
Dimitriou et al (2022)	197 *348*	Retrospective study	Ipi/Anti-PD-1 Anti-PD-1, not specified	21 19	4 5	31 29	NA NA
Boer et al (2023)*	46	Real world data	Ipi/Nivo	9.7	4.1	39.1	47.8
Uveal Melanoma							
Study (year)	**Number of patients**	**Type of study**	**Type of ICI**	**OS (months)**	**PFS (months)**	**ORR (%)**	**Grade ≥3 toxicity (%)**
Shoustari et al (2016)	6	Expanded access program	Ipi/Nivo	15	2.5	0	NA
Heppt et al (2019)	64	Retrospective study	Ipi/Anti-PD-1	16.1	3.0	15.6	39.1
Bol et al (2019)	19 24 53	Retrospective study	Ipi/Nivo *Pembrolizumab* *Ipilimumab*	18.9 10.3 9.9	3.7 4.8 3.0	21.1 7.0 0.0	NA NA NA
Najjar et al (2020)	89	Retrospective study	Ipi/Nivo	15	2.7	11.6	NA
Pelster et al (2021)	30	Phase II trial	Ipi/Nivo	19.1	5.5	18	40
Piulats et al (2021)	52	Phase II trial	Ipi/Nivo	12.7	3.0	11.5	75
Koch et al (2021)	109 *53* *15*	Retrospective study	Anti CTLA-4/anti-PD-1, not specified *Anti-PD-1, not specified* *Ipilimumab*	NA NA NA	NA NA NA	13.8 8.9 0.0	58.3 24.0 NA
Salaun et al (2022)	47	Retrospective study	Ipi/Nivo	NA	2.9	4.3	15

Table 4. (*Continued.*)

Uveal Melanoma							
Study (year)	**Number of patients**	**Type of study**	**Type of ICI**	**OS (months)**	**PFS (months)**	**ORR (%)**	**Grade ≥3 toxicity (%)**
Van Aken et al (2022)	14	Retrospective study	Anti CTLA-4/anti-PD-1, not specified	NA	NA	14	NA
	27		*Anti-PD-1, not specified*	NA	NA	15	NA
	24		*Ipilimumab*	NA	NA	8	NA
Boer et al (2023)*	15	Real world data	Ipi/Nivo	12.4	5.5	30.8	38.4

ICI: Immune checkpoint inhibitor Ipi: Ipilimumab, Nivo: nivolumab, OS: Overall survival, PFS: Progression-free survival. ORR: Overall response rate, NA: not assessed, NR: not reached. For the studies assessing ipilimumab/nivolumab together with ipilimumab of nivolumab monotherapy, the outcomes for all treatment arms are presented.

* Results of the current study

Though our ORR in MM is comparable with other studies, median OS (9.7 months) fell below the range of 14.0-31.7 months, reported in the literature and presented in table 4. [10, 20-23, 29, 30] Whereas comparing baseline characteristics between studies is difficult, no large differences were found explaining the lower OS in our study. In line with the literature, in our study LDH level was elevated in 32.6% of the patients and stage at presentation most often was stage IV M1c. [23, 29] In our study, multivariable analysis demonstrates that higher LDH levels (HRadj:6.57 95% CI:1.14-37.75) and the presence of liver metastasis (HRadj:3.04 95% CI:1.13-8.16) were associated with worse OS. Yet the small sample size of our study restrains effective analysis of predictors of survival.

Toxicity is an important issue in patients treated with combined ipilimumab/nivolumab, as demonstrated in our study (grade ≥3 toxicity in 47.8%). Whilst grade ≥3 toxicity of MM is quite similar to CM, the high discontinuation rate in our study (80% of those experiencing toxicity discontinued treatment) is remarkable. [2, 31] Yet, the negative effect of discontinuing treatment is not evident, as Schadendorf et al. analysed pooled data of CM from the Checkmate 067 and 069 trials and could not link treatment discontinuation with worse outcomes. [32] Furthermore, the eleven patients with MM (15%) who started ipilimumab/nivolumab treatment, but were excluded from this study due to short follow-up, death or receiving less than two cycles, represent the aggressiveness of this disease. This should be taken into account when considering ICI on patient-level, as they may not derive benefit from these agents while they can face the potential harm of associated toxicities.

In UM, a total of nine studies evaluated safety and efficacy of anti-CTLA-4/anti-PD-1 of which all but three consisted of retrospective cohort studies. [30, 33-40] The two phase II studies including 30 and 52 patients found a median OS of 12.7 and 19.1 months and a BORR of 11.5% and 18%. [36, 39] In our study we observed an ORR of 30.8%, which is higher than the BORR of 12% calculated from all published studies in Table 4, but still is remarkably lower than in CM. [30, 33-40] Similar to the literature, no patients responded completely in our cohort. Unfortunately, the limited number of patients with UM in this study, impedes the ability to draw definitive and far-reaching conclusions.

UM is characterized by disease predominately spreading to the liver (90-95%). [41] Recent studies have suggested that UM which spreads exclusively to the liver has a worse prognosis than those with extra-hepatic disease (with or without liver metastasis). [40, 42] Moreover, response to dual ICI may be better in those with extra-hepatic disease when compared to patients with liver-only disease. Though not statistically significant, a study with 109 patients treated with combined anti CTLA-4/anti-PD-1 therapy of which 38 had liver only disease and 71 liver and extra-hepatic disease, reported worse ORR for those with liver-only disease (8.7% vs 16.7%, p=0.45). [40] Moreover, median OS was better for those with hepatic and extra-hepatic disease compared to liver-only spread disease (6 vs 18 months, p=0.07).

In the Netherlands, this has led to criteria for providing ipilimumab/nivolumab across the fourteen melanoma centers, in which patients are selected with a fairly good prognosis, characterized by limited tumor load, preferably extra-hepatic disease only, a good performance status and a normal LDH level. Due to these criteria, our study includes a selected population with relatively less liver metastasis as compared to the literature (73.3% vs 90-95%) and more patients with extra-hepatic disease only (26.7%). These patients, with a more favorable prognosis, may have affected our outcomes (median OS 12.4 months and 1-year OS 78%), which are promising as compared to data from a meta-analysis including 912 patients from 29 trials treated with various treatment regimens (median OS 10.2 months and 1-year OS 43%). [43] Due to the small numbers in our study, we could not compare outcomes of patients with liver-only disease compared to patients with extra-hepatic metastases. Still, the aggressive course of this disease is illustrated by the Kaplan-Meier curves (Figure 3). All deaths occurred within the first year and progression of disease occurred in 6/13 patients (46%), all within 6 months.

In CM, a thorough assessment of combined ipilimumab/nivolumab in 140 patients with in total 833 metastasis, found distinct heterogeneity in response patterns between different anatomical sites. [44] They observed that, when comparing nine locations of metastasis, site specific response of metastasis in the liver was the lowest (46%) whilst that of the lung was the highest (77%). [44] In multivariate analysis, those with liver metastasis had lower ORR, PFS and OS, whilst those with lung metastasis had better ORR and PFS. A hypothesis is that the liver possesses a distinct immunosuppressive tumor microenvironment (TME), which may hamper the function of tumor-infiltrating lymphocytes, explaining why UM has lower RR to ICI than CM. [45] In various cancer types, including CM, studies have demonstrated that the presence or abundance of specific T-cells and PD-L1 levels in the TME can strongly predict the response of ICI. [46, 47] In MM, the potential predictive value of the TME has not yet been analysed, but could be an important avenue for the future.

In this study, grade ≥3 toxicity occurred in 38.4% of the patients with UM. The six studies including 302 patients assessing toxicity in UM report grade ≥3 toxicity in 15-75% of the patients, with a calculated average of 46.4%. (Table 4). This is 15% lower than in CM, in which the largest clinical trial reported grade ≥3 toxicity in 59% the patients. [31] Bomze et al. and Kerepesi et al. found that tumours with a high TMB, such as CM and non-small cell lung cancer, are associated with a higher risk of immune-related adverse events (irAE), whilst lower TMB is associated with a lower risk of irAE. [48, 49] The comparatively low toxicity rates of UM and MM, which are characterized by a low TMB, fits this hypothesis.

A strength of this study is the use of real-world data, provided by a validated and detailed prospective registry in the Netherlands, which includes all patients since the era of immunotherapy. However, due to the low incidence of MM and UM combined with the

highly aggressive character resulting in only a minority of the patients receiving systemic therapy, we could only analyse 46 MM and 13 UM patients. Therefore our data should be seen in a larger context within the published evidence which we present in Table 4.

The lower efficacy of ICI, when compared with its cutaneous counterpart, suggests the lower immunogenicity of both MM and UM. [2] Still, in our study, approximately half of the patients with MM and UM experienced clinical benefit from ipilimumab/nivolumab. Yet, toxicity remains a constraining factor and the aggressive nature of the disease can catch up on time, diminishing the potential effect from ipilimumab/nivolumab, which can be seen in the low OS rates. Therefore, future studies should focus on identifying patients who have a high likelihood of benefitting from ipilimumab/nivolumab therapy. Moreover, studies assessing innovative (combination) treatment strategies for both MM and UM, and in particular clinical trials, are needed.

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Supplementary material

Supplementary Table 1. Overview of specific toxicities in mucosal and uveal melanoma

	Mucosal melanoma (n=46)	Uveal melanoma (n=13)
Total patients with grade ≥3 toxicity	22 (47.8)	5 (38.4)
Type of toxicity	**N (%)**	**N (%)**
Colitis	8 (17.4)	3 (23.1)
Hepatitis	9 (19.6)	1 (7.7)
Pneumonitis	0 (0)	1 (7.7)
Adrenal insufficiency	1 (2.2)	0 (0)
Hypopituitary insufficiency	1 (2.2)	0 (0)
Thyroiditis	1 (2.2)	0 (0)
Fatigue	3 (6.5)	0 (0)
Rash or pruritus	4 (8.7)	1 (7.7)

Supplementary Table 2. Toxicity determinants for mucosal and uveal melanoma

	Mucosal melanoma (n=46)				Uveal melanoma (n=13)			
	% of treated patients with toxicity	**OR**	**95% CI**	**p-value**	**% of treated patients with toxicity**	**OR**	**95% CI**	**p-value**
Age								
0-69	50 (15)	ref			44.4 (4)	ref		
≥ 70	43.8(7)	0.78	0.23-2.63	0.69	25.0 (1)	0.42	0.03-5.71	0.51
Gender								
Female	50.0 (6)	Ref			57.1(4)	ref		
Male	47.1(16)	0.90	0.24-3.32	0.86	16.7 (1)	6.6	0.49-91.3	0.16
Number of comorbidities								
0-2	41.9 (13)	ref			33.3 (3)	ref		
3 or more	57.1 (8)	1.9	0.52-6.62	0.35	50.0 (2)	2.0	0.18-22.1	0.57
WHO performance status								
0-1	46.5 (20)	Ref			5 (38.5)	Ref		
2 or higher	50.0 (1)	1.15	0.07-19.6	0.92	0 (0.0)	0.63	NA	0.41

In patients with MM there is 1 patient of which the number of comorbidities is unknown and 1 patient of which the WHO classification is not known. These patients are not presented in this table. NA: not assessable as 1 category does not include any patients.

PART II

Vulvar melanoma

Vulvar malignant melanoma: pathogenesis, clinical behaviour and management: review of the literature

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5

Abstract

Vulvar malignant melanoma (VMM) is a rare disease, accounting for 5% of all vulvar malignancies and is characterized by low survival and high recurrence rates. It is considered as a distinct entity of mucosal melanoma. Prognostic factors are higher age, advanced Breslow thickness, and lymph node involvement whilst central localization and ulceration status are still under debate. Surgery is the cornerstone for the treatment of primary VMM, however, it can be mutilating due to the anatomical location of the disease. Elective lymph node dissection is not part of standard care. The value of sentinel lymph node biopsy in VMM is still being studied. Radiation therapy and chemotherapy as adjuvant treatment do not benefit survival. Immunotherapy in cutaneous melanoma has shown promising results but clinical studies in VMM are scarce. In metastatic VMM, checkpoint inhibitors and in case of BRAF or KIT mutated metastatic VMM targeted therapy have shown clinical efficacy. In this review, we present an overview of clinical aspects, clinicopathological characteristics and its prognostic value and the latest view on (adjuvant) therapy and follow-up.

Introduction

Vulvar malignant melanoma (VMM) is a rare type of cancer responsible for 5% of all vulvar malignancies. [1-6] The incidence in the United States is 0.136 cases per 100,000, with 1059 vulvar melanomas in a 30-year period. [4] Most women with VMM are postmenopausal and diagnosis is usually delayed due to the location of the disease and lack of early symptoms. [7] Recurrence rate is high and distant metastases are commonly seen, even in apparent early stages of VMM. [8, 9] The five-year survival rates of VMM range from 10-63%. [10, 11] In addition to the high mortality rates there is a substantial decrease in the quality of life. This is due to bothersome symptoms, bleeding, foul odour, decreased sexual functionality and surgery related morbidities. [12]

Although Breslow thickness does have predictive value no consensus exists on the most accurate staging system for VMM. [13] Treatment modalities for VMM have mostly been extrapolated from cutaneous melanoma. Surgery is the cornerstone for the treatment of primary VMM. Wide local excision (WLE) is recommended while there is no clear indication for groin node dissection. [14] The value of sentinel node biopsy is still a matter of debate. [15] Radiation therapy and chemotherapy both have shown to be poorly effective in prolonging survival. [16] Therefore, there is need for new treatment strategies. Immunotherapy and in presence of KIT or BRAF mutations targeted therapy have shown promising results in cutaneous melanomas and may also be of advantage in the treatment of VMM. [17]

In this review, we present an overview of the current literature on vulvar malignant melanomas including clinicopathological characteristics, predictors of outcome, and current and future therapeutic options.

Data sources

Data on VMM has been collected through the search engines PubMed, and Web of Science (date of last search May 8th, 2018). A combination of Medial Subject Headings (MeSH), Majr terms (MeSH heading that is of major importance in an article) and free text words was established. We used the search comprising the terms vulvar melanoma, genital melanomas, vulvovaginal melanomas, mucosal melanomas, BRAF, KIT and NRAS. Furthermore, we included several articles using reference lists of articles found via electronical search. The Dutch, American and British oncological guidelines of both vulvar cancer and cutaneous melanomas have been consulted. For clinicopathological characteristics and survival rates, all studies including more than ten vulvar melanomas and published after 1990 have been sorted in tables. In total 30 articles analysing VMM cases have been included. The final search strategy has been included as appendix (Appendix 1).

Clinical features

VMM is mostly seen in Caucasian women, the mean age at diagnosis is 61.6 years (range 10-86). [4, 18-29] Though VMM is a disease mostly confined to the middle aged women, children as young as ten years have been diagnosed with VMM. [5, 19, 29] The mean age at diagnosis is similar to that of other cutaneous melanomas (63 years) and mucosal melanomas of the head, neck, anus and rectum (respectively 61 and 68 years). [30-32] Aetiology does not seem to be similar to cutaneous melanoma since the most important risk factor UV-light exposure, cannot be collaborated with the vulvar area, which is barely exposed to light. Therefore, although VMM can anatomically be located either on mucosal or cutaneous surface, the general opinion is to categorize VMM as a distinct entity of mucosal melanoma. [33, 34]

Most common presenting symptoms are pain, bleeding, pruritus and a vulvar lesion or lump. [29] Occasionally, VMM are asymptomatic, in a study including 98 genital melanomas, 85 were identified by the patient whilst 13 melanomas were asymptomatic and diagnosed through clinical examination. [16, 23] Figure 1 shows a clinical presentation of a patient with a VMM.

A delay of presentation is common, mostly due to an absence of early symptoms and low body awareness. [33] Moreover, amelanotic VMM can be mistaken for a benign or premalignant disease. [7, 35] , In a large cohort of 123 vulvar melanomas, 30% of all vulvar melanomas were reported to be macroscopically amelanotic. Slightly more melanomas are located on the labia majora than the labia minora. [36] Only 26.7% (range 10-62.5%) of the VMM are multifocal at presentation. [3, 23, 26, 28, 29]

Diagnosis

At first visit, detailed medical history including presenting complaints and family history should be taken. Clinically, vulvar melanomas are assessed using the same ABCDE rule as in cutaneous melanomas. [37] These letters stand for Asymmetry, Border irregularity, Colour, Diameter and Evolving (in size, shape or colour) of which the latter one is the most important in melanomas. [38] Also a blue-black colour, a raised lesion, a mole >6mm and a raged, notched or fuzzy border should raise suspicion. [38] Physical examination of the vulvar lesion and groins should be performed. Of special importance is the exact location of the lesion in relation to adjacent structures such as the urethra, anus and clitoris since surgery is the primary treatment. [14] The impact on social, sexual and psychological health should not be underestimated and is very much recommended to be part of counselling and assessment. [38-40]

Pigmented lesions should be differentiated from benign vulvar and vulvar melanosis, however this is difficult by clinical assessment. [41, 42] For a final diagnosis histological confirmation should be done through a full-thickness biopsy reaching up to subcutaneous tissue. [28] To prevent difficulty in confirming diagnosis excisional biopsy (excision of entire lesion) is recommended. In case of the possible harm of near structures with excisional biopsy, incisional biopsy should be considered. [43, 44] Biopsies should be reviewed by a pathologist, and immunohistochemical staining with HMB-45 and S-100 protein and Melan-A and MART-1 antibodies can be used to confirm diagnosis and differentiate from other vulvar conditions. [10, 42] Because of the rare entity of the disease, VMM biopsies are recommended to be assessed by either experienced pathology teams specialized in vulvar or gynaecologic pathology or teams specialized in melanomas.

Clinical work-up for VMM is identical to cutaneous melanomas in which Computed Tomography (CT), Positron Emission Tomography (PET) or CT/PET scans of head, abdomen, and pelvis is advised for clinically suspected stage IIIB, IIIC or stage IV disease. [43] Due to the metastasizing nature of VMM, some advocate imaging as part of standard work-up for all cases. CT, MRI or ultrasound of the groin and pelvis for locoregional spread and PET/CT for distant spread is recommended. [45]

VMM staging

For VMM, micro staging systems of Breslow, Clark and Chung, evaluating pathological characteristics of the primary melanoma, and macro staging systems (AJCC, FIGO), evaluating both primary melanomas and spread of disease, are used. [45] Table 1 summarizes studies that assessed the survival outcomes using the different staging systems in VMM. [3, 7, 13, 16, 18, 20–22, 24–27, 46–51] Based on these studies, Clark and Breslow staging were found more predictive for survival and recurrence than FIGO staging (1988) (Table 1). This can be explained by the fact that survival of VMM predominantly depends on tumour depth and in lesser extent on the diameter of the tumour, which is used in FIGO staging. Clark micro staging, which measures depth of invasion to papillary dermis, reticular dermis, and subcutaneous fat, was found to be predictive for both recurrence-free survival and overall survival. The two studies which did not support the predictive value of Clark staging for survival also could not do so for Breslow thickness. [13,16]

Half of all studies addressing AJCC as possible staging system for VMM found a correlation with either survival or recurrence-free survival (Appendix 2, Table 1). [13, 21, 47, 51] Two studies favoured AJCC above Breslow, Clark and FIGO (1988) staging in predicting recurrence-free survival. [21, 47] In conclusion, many staging systems are used without accurate predictive value for survival.

Whereas no staging system exists for VMM, mucosal melanomas of vaginal and anorectal origin by the Ballantyne's staging as either local, regional, or distant. For head and neck mucosal melanomas an adapted Union for International Cancer Control staging system (2017) has been designed (Appendix 3). [31, 52] This system however cannot be applied to VMM due to a different anatomical location.

Table 1. Prognostic value of staging systems

Study	Number of patients (location/ of which vulvar)	FIGO staging 5YS per stage	AJCC staging 5YS per stage	Breslow staging 5YS per stage	Clark staging 5YS per stage
Bradgate et al. [27]	50 (vu)	FIGO 1971:I 58%, II 55%, III 0% IV 0% - FIGO only predictive I/ II vs III/IV		Breslow is not predictive for 5YS	
Tasseron et al. [46]	30 (vu)			Breslow is not predictive for OS	
Trimble et al. [20]	80 (vu)			Breslow is predictive for OS (p=0.006)	
Phillips et al. [47]	71 (vu)		AJCC 1992: AJCC is related to RFI (p<0.0001)	Breslow is related to risk of recurrence (p=0.0003)	Clark level related to risk of recurrence (p=0.0003)
Scheistroen et al. [48]	75 (vu)	FIGO 1988: I 63%, II 44%, III 0%, IV 0% - FIGO only predictive I/ II vs III/IV		Breslow is only predictive for survival in cases >5mm	
Raber et al. [24]	89 (vu)			Breslow is predictive for 5YS (p=0.0007)	Level I-III 64.6% Level IV-V 9%. Clark is predictive for 5YS
De Matos et al. [25]	43 (ge/30 vu)	FIGO 1988: I 50%, I/II 60% II 50%, III 63% - FIGO not predictive for survival			
Creasman et al. [49]	569 (vu)				Level I 77%, II 70%, III 50%, IV 48%, V 24%
Raspagliesi et al. [26]	40 (vu)			Breslow is predictive for local recurrence (p=0.018)	
Verschraegen et al. [21]	51 (vu)		AJCC 1992: I 91%, ≥ IIA 31% RR 2.85 of OS (p=0.0001) AJCC predictive for survival	Breslow is predictive for overall survival RR 1.25 (p=0.0001)	Level < III 91%, level ≥III 27%
Moxley et al. [13]	77 (vu)		AJCC 2002: Not significant but a trend between higher AJCC stage and recurrence. Advanced AJCC stage correlates with survival significantly (p=0.006)	Breslow is not predictive for OS, whilst being predictive for recurrence.	Clark is not predictive for survival

Table 1. (*Continued.*)

Study	Number of patients (location/ of which vulvar)	FIGO staging 5YS per stage	AJCC staging 5YS per stage	Breslow staging 5YS per stage	Clark staging 5YS per stage
Tcheung et al. [50]	85 (ge/43 vu)			Breslow is predictive for MSS (p<0.01)	
Heinzelmann et al. [7]	33 (vu)			Breslow is related to RFS: HR=1.08 (p=0.049)	
*Seifried et al. [51]**	85 (ge/62 vu)		AJCC 2009: 5Y MSS stage 0-II 0%, stage III 63.6% AJCC only predictive for 0-II vs III (p<0.0012)	Breslow is predictive for 5YS HR 1.16 (p=0.007)	
Ditto et al. [16]	98 (vuva/67 vu)		AJCC 2009: AJCC staging not related to survival	Breslow is not predictive for OS	Clark is not predictive for survival
Nagarajan et al. [3]	100 (vu)		AJCC 2009:AJCC is only predictive in VMM > 2mm thick	Breslow is predictive for OS [MA] (p=0.03)	
Iacoponi et al. [22]	42 (vu)		AJCC 2002: AJCC staging not related to survival. AJCC staging is related to distant recurrence/metastasis		
Udager et al. [18]	59 (ge/48 vu + 6 vuva)			Breslow is predictive for MSS HR 1.043 (p=0.001)	

Only studies published after 1990 and that reported a minimum of 10 patients were included. vu: vulvar melanomas, ge: female genital melanomas, vuva: vulvovaginal melanomas, 5YS: 5-year overall survival

*These outcomes have not been subdivided for different types of melanomas included in this study.

Predictors of outcome

Clinical Characteristics

Age at diagnosis is found to be an independent prognostic factor of 5-year -, disease-free -, and overall survival in most studies. [3, 4, 6, 19, 20, 22, 27, 36, 48, 53]

Melanomas located centrally on the vulva have been correlated with reduced short-term and long-term survival and with shorter recurrence-free interval. [3, 28, 48, 54] An hypothesis is that in central lesions priority was given to avoid urethral injury which may have been at the expense of the surgical margin. [55] Hypotheses for worse prognosis in centrally located vulvar squamous cell carcinoma (VSCC), which could also apply to VMM, is the rich lymphovascular supply of the clitoris. [56] In a study combining vulvar and genito-urinary melanomas the centrally localized lesions (bilateral, clitoral, urethral, vaginal, perineal and anal) were associated with a higher risk of nodal involvement in the groin than lateral lesions (p=0.003). Furthermore, nodal involvement was an independent factor for recurrence and survival. [47]

Evidently, multifocal spread and involvement of the urethra, vagina, perineum, or anus of the vulvar melanomas leads to a worse prognosis. [48]

Histological characteristics

Lymph node (LN) status in VMM as a predictor of survival has extensively been studied. Positive LN status is prognostic for distant recurrence, yet, local recurrence is not predicted by the involvement of nodes. [26] Table 2 summarizes the studies evaluating LN status as possible prognostic factor in VMM. Seven studies stated the 5-year survival rate in LN positive patients with an average of 23.4% (range 0-68%) (table 2). [20, 24-26, 46, 48, 51] 10- year survival rates are 43.8% in the LN negative cases and 11.5% in the LN positive cases (table 2). [20, 24, 25, 48]

Also the extent of LN involvement is shown to be prognostic for survival. [6, 19, 26, 50] With multivariate analysis both LN status ($p < 0.002$) as extent of LN involvement ($p < 0.0003$) were significantly associated with survival. Survival rates for VMM with 0, 1 or more than 2 positive lymph nodes were respectively 65%, 20% and 0%. The four cases with more than two positive nodes passed away within two years of follow up. [26]

Breslow defined tumour thickness as the distance from the top of the epidermal granular layer to the deepest point of invasion. Table 3 summarizes studies on Breslow thickness and other pathological characteristics (ulceration, mitotic rate, and histological type) in relation to clinical outcome.

In VMM the majority of the studies support increasing Breslow thickness as negative predictor of survival (Table 3). [3, 21, 24, 25, 27, 48, 50, 51, 53] Most studies propose a minimum cut-off value for high-risk melanomas of 1.5 mm tumour thickness for the prediction of survival (Table 3). [21, 24, 25, 46, 54] Few studies failed to correlate tumour thickness as prognostic factor. [16, 20, 26] Higher tumour depth of VMM is also associated with higher rates of nodal involvement and with higher rates of recurrence. [3, 7, 13, 16, 20, 21, 46, 47, 51]

Ulceration is defined by the AJCC staging system as the absence of an intact epidermis overlying a major portion of the primary melanoma based on microscopic examination of histological sections. [57] In most studies ulceration is a prognostic factor for 5-year survival (Table 2). [3, 16, 24, 25, 27, 46, 48, 51] An association between ulceration with higher tumour thickness and mitotic rates may explain why ulceration has not been identified as independent predictor of survival. [27, 46]

Studies on the prognostic relevance of mitotic rate in VMM show varying results. Two recent studies showed that the mitotic rate was independently associated with disease-specific and disease-free survival (Table 3). [3, 16] A higher risk of dying due to progression of VMM was found in those with a mitotic rate of > 2 mm^2 compared to those with a mitotic rate of < 2 mm^2 (HR 3.36, p = 0.03, multivariate analysis (Table 3)). [16] Vulvar melanomas can be classified based on their growth pattern as superficial spreading melanoma (SSM), nodular malignant melanoma (NMM) and acral lentiginous melanoma (ALM). To distinguish the different variants the histopathological identification of the radial and vertical growth phase is the most important. The majority of the VMM's are classified as SSM (47%, range 33–56% (Table 3)). [16, 21, 26, 27] Efforts to correlate histological type with survival generally have been unsuccessful due to scarce and inconsistent results (Table 3). [27, 48 16, 21, 26, 27, 48]

In summary, LN status and Breslow thickness are the strongest predictors for survival in VMM. LN status is also prognostic for recurrence whilst for Breslow thickness more evidence is needed. The cut-off value of tumour thickness and the predictive value of the extent of nodal disease still remains unclear. Ulceration should be considered as risk factor for survival but validation in larger studies is needed.

Table 2. Prognostic value of lymph node status

Study	Number of patients (location/ of which vulvar)	Survival according to LN status of the groin	Significance	# of positive lymph nodes predictive?
Bradgate et al. [27]	50 (vu)	5YS LN total 35% LN- 55% 10YS LN total 22% LN- 35%	Not significant	
Tasseron et al. [46]	30 (vu)	5YS LN total 57% LN + 33%	Not significant	
Piura et al. [118]	18 (vu)	LN positivity is not related to worse survival	Not significant	
Trimble et al. [20]	80 (vu)	5YS LN- 70% LN+27% 10YS LN- 43% LN+ 20%	p<0.001	
Phillips et al. [47]	71 (vu)	LN positivity is related to worse survival	p<0.006	
Scheistroen et al. [48]	75 (vu)	5YS LN- 56% LN+ 0% [MA] 10YS LN- 44% LN+ 0%	p<0.016	
Raber et al. [24]	89 (vu)	5YS LN- 57% LN+ 9.2% 10YS LN- 45% LN+ 11%	p=0.0007	
De Matos et al. [25]	43 (ge /30 vu)	5YS LN- 60% LN+ 68% 10YS LN- 43% LN+ 15%	Not significant	
Raspagliesi et al. [26]	40 (vu)	5YS LN- 65.2% LN+ 26.8% [MA]	p<0.002	Yes, 5YS 0 nodes 65.2%, 1-3 nodes 37.5%, >3 nodes, 0% (p<0.0003)
Sugiyama et al. [19]	644 (vu)	5YMSS LN- 68.3% LN+ 24.0%	p<0.01	Yes, 5YSMSS 0 nodes 68.3%, 1 node 29%, >1 node 19.5% (p<0.01)
Baiocchi et al. [11]	11 (vu)	LN positivity is related to worse survival	p=not stated	
*Tcheung et al. [50] **	85 (ge/43 vu)			Yes, extent of nodal involvement is inversely related with survival (p<0.01)
Tran Janco et al. [17]	50 (vuva/36 vu)	LN positivity is related to worse survival	p=0.02	
*Mert et al. [6] **	769 (vuva/597 vu)	LN positivity is related to worse survival	p=0.0043	Yes, extent of nodal involvement is inversely related with survival
*Heinzelmann et al. [7]**	33 (vu)	LN positivity is not related to worse survival [MA]	Not significant	
Seifried et al. [51]	85 (ge/62 vu)	5YS LN - 63.6% LN+ 0%	p<0.001	

Table 2. (*Continued.*)

Study	Number of patients (location/ of which vulvar)	Survival according to LN status of the groin	Significance	# of positive lymph nodes predictive?
Ditto et al. [16]	98 (vuva/67 vu)	HR for 5YOS LN + vs LN- 1.76 [MA]	p=0.02	
Sanchez et al. [4] *	1586 (genitourinary/1059 vu)	5YMSS LN- 63% LN+ 24% 10YMSS LN- 49% LN+ 17%	p<0.001	
Udager et al. [18] *	59 (ge/48 Vu+ 6 vuva)	MSS was lower for LN+ vs LN- group	p=0.008	

Only studies published after 1990 and that reported a minimum of 10 patients were included. vu: vulvar melanomas, ge: female genital melanomas, vuva: vulvovaginal melanomas, LN: lymph node, LN total: survival for all patients included in their review , LN +: survival for all patients with positive LN status, LN-: survival for all patients with negative LN status MA: multivariate analysis, MSS: melanoma specific survival, DFS: disease free survival, OS: overall survival *These outcomes have not been subdivided for different types of melanomas included in this study.

Table 3. Prognostic value of histological parameters

Study	Number of patients (location/of which vulvar)	Breslow thickness Survival	Breslow thickness Risk of recurrence/RFS	Ulceration status 5YS/DFS ulcerated/non-ulcerated	Mitotic rate 5YS/ DFS/ association with survival	Histological type Association with survival
Bradgate et al. [27]	50 (vu)	5YS 0-4 mm 59% (p<0.05) 5YS 4.1-8.0 mm 38% 5YS >8.0mm 10%		5YS 27%/62% (p<0.05)	5YS 0-4 HPF 57% (p<0.05) 5YS >4 HPF 24 %	histological type of tumour is not related to survival
Tasseron et al. [46]	30 (vu)	Cum. S. 0-3 mm 100% (p=0.99) Cum. S. 3-6 mm 60% Cum. S. > 6mm 22%	5YDFS 0-3 mm 80% (p= 0.27) 5YDFS 4-6 mm 63% 5YDFS ≥ 6mm 30%	DFS 40%/70% [MA] (p=0.004)	mitotic rate is not related to survival	
Trimble et al. [20]	80 (vu)	5YS <0.75 mm 48% (p<0.001) 5YS 0.75-1.5 mm 79% 5YS 1.51-3.0 mm 56% 5YS >3.0 mm 44%			mitotic rate is related to survival [UA] (p<0.001) mitotic rate not related to survival [MA] (p=ns)	
Phillips et al. [47]	71 (vu)		Breslow thickness related to DFS [MA] (p =0.0003)			
Scheistroen et al. [48]	75 (vu)	5YS <0.75 mm 80.0% (p= ns) 5YS 0.75-1.5 mm 88.9% 5YS 1.51-3.0mm 54.5% 5YS 3.0-5.0 mm 54.5% 5YS >5.0 mm 27.4%		5YS 40.5%/62.7% (p<0.118) ulceration is related to DFS (p=0.027)		histological type of tumour is not related to survival
Raber et al. [24]	89 (vu)	Median survival ≤ 1.5 mm 112 months (p=0.0007) > 1.5 mm 25 months		5YS 14.3%/20.4% (p=0.082)		
De Matos et al. [25]	43 (ge/30 vu)	6YS ≤ 1.5 mm 100% (p= ns) 6YS > 1.5 mm 29%		5YS 75%/57% (p= ns)		
Ragnarsson et al. [53]	219 (vu)	thickness related to survival [MA] (p=0.009)		all stages: HR 2.17 for MSS [MA] (p=0.069) ulceration predictive only for clinical stage I VMM (p<0.001)		

Table 3. (*Continued.*)

Study	Number of patients (location/of which vulvar)	Breslow thickness Survival	Breslow thickness Risk of recurrence/RFS	Ulceration status 5YS/DFS ulcerated/non-ulcerated	Mitotic rate 5YS/ DFS/ association with survival	Histological type Association with survival
Raspagliesi et al. [26]	40 (vu)	thickness significant related to survival [UA] (p=ns) thickness not significant related to survival [MA] (p=0.07)		ulceration is not related to survival [MA] (p=0.1)		histological type of tumour is not related to survival
Verschraegen et al. [21]	51 (vu)	RR 1.25 for OS [UA] (p= 0.0001) 5YS ≤0.75mm 75% 5YS 0.75-1.5mm 57% 5YS >1.5 mm 11%	RR 1.19 for DFS [UA] (p=0.0004)			RR for OS = 13.71 SSM vs NMM (p=0.0006)
*Tcheung et al. [50]**	85 (ge/43 vu)	5YS <1.0 mm 60% (p<0.01) 5YS 1.1-2.0 mm 50% 5YS 2.1-4.0 mm 30% 5YS >4.0mm 10%		ulceration is not related to survival	mitotic rate is not related to survival	
Heinzelmann et al. [7]	33 (vu)		HR 1.08 for DFS [MA] (p= 0.049)			
Seifried et al. [51]*	85 (ge/62 vu)	HR for MSS=1.16 [UA] (p=0.007)	HR 1.19 for DFS [UA] (p=0.0011)	5YS 51.6%/ 81.1% (p=0.049)	HR 1.04 for MSS [UA] (p=0.013)	
Ditto et al. [16]	98 (vuva/67 vu)	thickness not related to 5YS (p=0.75) [UA]		ulceration is not related to survival [UA] (p= 0.28)	HR 1.24 for DFS [MA] (p<0.001)	histological type of tumour is not related to survival [MA]
*Udager et al. [18]**	59 (ge /48 vu+ 6 vuva)	HR 1.04 per mm for MSS [UA] (p=0.0001)				
Nagarajan et al. [3]	100 (vu)	Thickness related to OS [MA] (p=0.03)	Breslow thickness related to DFS [MA] (p<0.02)	ulceration related to OS [UA] (p=0.01) ulceration related to MSS [UA] (p<0.01) not significant for MSS and OS [MA]	mitotic rate of <2mm2 vs ≥2mm2 HR 3.36 for OS [MA] p<0.03) HR of 4.44 for DFS[MA] (p<0.001)	

Treatment

Surgical treatment

For many years radical vulvectomy defined as "the removal of the entire vulva until the deep facia of the thigh, the periosteum of the pubis and the inferior fascia of the urogenital diaphragm" was the standard treatment for VSCC and was adopted for VMM. [5,58] Extensive surgery however is associated with serious and long term morbidity, sexual dysfunction, and psychological burden. [59] Therefore, wide local excision (WLE), defined as the excision of the malignancy with wide tumour free surgical margins, has been proposed as alternative. WLE has shown similar survival rates compared to radical vulvectomy. [13, 19, 20, 23, 25] Studies addressing recurrence rates of those either treated with radical vulvectomy or WLE are ambivalent in their results. [22, 25] Since survival is not better in cases treated with radical vulvectomy; WLE is the preferred primary surgical treatment. [2, 16] Knowledge about the optimal surgical margins of the WLE for VMM is lacking. Though in head and neck melanomas some studies found clear margins to be related with better survival there is also conflicting data not finding a significant difference in survival for patients with either tumour negative or positive margins. [60–62] In the National Comprehensive Cancer Network guidelines for cutaneous melanoma smaller surgical resection margins (0.5 cm for in situ melanomas, 1 cm for lesions up to 2 mm thick and 2 cm margins for melanomas more than 2 mm thick) have been proposed finding no survival benefit favouring wider margins. [43] Irvin et al proposed identical margins for VMM and neither found margins wider than 2 cm to improve survival. [28]

Inguinal lymph node treatment

In VMM, only one prospective trial of 71 cases compared ELND with LN treatment when clinically manifested nodal disease, concluding no survival benefit for those treated with ELND. [47] In two retrospective studies consisting of 17 and 18 VMM's electively treated with lymph node dissection only 12% and 33% had nodal involvement, respectively. [15, 25]

Sentinel lymph node biopsy (SLNB) can help to obtain information on regional involvement whilst sparing ELND. Since in VMM positive pelvic nodes are rarely encountered in case of negative inguinal nodes, sentinel lymph node (SLN) status is thought to predict the status of the further nodes. [20, 25, 54, 63] However, evidence on this subject is scarce. Of the 59 documented VMM cases treated with a sentinel node procedure, 98% successfully identified the sentinel node. [11, 22, 29, 63–68]

De Hullu et al, found 2 out of 9 cases treated with a sentinel node procedure to recur in the groin whilst 0 of the 24 cases treated with ELND recurred ($p = 0.006$). The authors hypothesize that the SLN procedure and maybe the tumour thickness in these cases (both more than 4 mm thick) could explain these recurrences. [15] In no other studies SLNB

procedure has been related to a higher recurrence rate although in another cohort of 11 cases, two of the three (< 12 month) recurrences occurred in those treated with SLN [11]. In cutaneous melanomas of less than 1 mm thick SLNB is not indicated due to the rare occurrence of regional metastasis in these cases. De Hullu has extrapolated this to VMM and advised SLNB in VMM only to be considered in melanomas between the 1 and 4 mm. [15] This is derived from their own experience as also from the NCCN and EMSO guidelines for cutaneous melanoma. [43,69] In SLN positive cutaneous melanoma LN dissection does not affect overall survival but does improve disease-free survival and therefore should be discussed with the patient. [70, 71] For VMM conclusions on this subject are lacking.

Non-surgical treatment

Currently, non-surgical treatments aimed at prolonging disease-free or overall survival are not routinely used in VMM. Whilst checkpoint inhibitors and targeted therapies in BRAF and KIT mutated metastatic cutaneous melanomas have shown clinical benefit, data in mucosal melanomas is limited. Clinical studies of therapeutic vaccination or adoptive cell transfer in mucosal or vulvar melanomas have not been performed.

Radiotherapy

In general, radiotherapy (RT) in cutaneous and mucosal melanomas of any location has a limited response. [72] Adjuvant local RT for mucosal head and neck melanomas which have a high risk of recurrence and adjuvant RT for VMM have shown to improve local control without benefiting overall survival. [16, 22, 73, 74] Difficulty in appropriate resection margins is common in both head and neck and vulvar melanomas. Hence, in case of tumour positive or narrow margins, adjuvant RT may be justified. [75]

Neoadjuvant RT has been proposed in surgically irresectable head and neck melanomas. [76] The use of neoadjuvant radiotherapy alone in VMM has not yet been described. The anti-CTLA4 antibody ipilimumab with concomitant RT has been described in four female lower genital tract melanomas after which three underwent surgical treatment. Impressive results were obtained with 1 stable disease, 2 partial remissions and 1 complete remission. The combination of RT and immunotherapy as neoadjuvant treatment should only be given in trial setting. [77]

RT of the groin following LND has not been studied in VMM. In cutaneous melanomas, lacking effect on overall survival together with complication rates of 50% have withheld RT to become part of standard treatment. [73, 78] Regional RT can be considered in case of lymph node involvement when LND is contraindicated. [79] RT in these cases could be used to prevent locoregional recurrences or progression of disease with local complications [79,80]. Radiotherapy of the groin in VMM has not been investigated in the elective setting, but is sometimes used in case of macroscopic, unresectable disease.

Chemotherapy

Adjuvant chemotherapy does not show a survival benefit in VMM. [17, 22, 50] In mucosal melanomas one randomized trial compared high-dose IFN (HDI) with temozolomide + cisplatin in an adjuvant setting and concluded chemotherapy to be more effective in prolonging recurrence-free survival and overall survival than HDI. [81]

Neoadjuvant use of (bio) chemotherapy aiming at reduction of tumour bulk has been reported in one vulvar and two vaginal cases. In the vulvar case carboplatin and paclitaxel in combination with the anti-angiogenetic agent bevacizumab led to considerable reduction of the 5 cm large melanoma, making resection possible whilst omitting skin graft [17].

In advanced VMM the only study addressing adjuvant (bio)chemotherapy is a case series of 11 vulvar and vaginal melanomas. They used combinations of cisplatin, vinblastine, dacarbazine, temozolomide, tamoxifen, IL-2, and IFN-A as therapy for advanced vulvovaginal melanomas. Of all cases the median survival was 10 months and 36% had a partial response, which is similar to the normally less aggressive cutaneous melanoma. [82]They propose chemotherapy to be promising in advanced disease whilst keeping the many side effects in mind.

Immunotherapy and targeted therapy

Immunotherapy and targeted therapy have shown promising results in the treatment of cutaneous melanomas. Immunotherapy is divided in non-specific stimulation of the entire immune system with cytokines and in specific stimulation using either vaccines, adoptive cell therapy or checkpoint inhibitors. Targeted therapy in melanoma focuses on targeting melanoma cells with specific gene changes on the BRAF, KIT or NRAS gene.

Cytokines

High-dose IFN has been reported to prolong overall survival and disease-free survival in radically resected stage I-II cutaneous melanomas on expense of many serious side-effects. [83]

Interferon-α-2b (IFN α-2b) or interleukin-2 (IL-2) have been administered as adjuvant treatment in mucosal melanomas including a couple of VMM cases, not finding a survival benefit for those treated with either of both. [25, 32, 50, 84] Conclusions are hard to draw since the type of immunotherapy and the stage of cases in the treatment groups are either not specified or stated.

IFN-α or IL-2 in advanced VMM have not been studied. As IFN-α and IL-2 as adjuvant treatment in metastatic cutaneous melanomas are not as effective as immune- and targeted therapies, they have been replaced by the emerging checkpoint inhibitors and targeted agents. [85, 86]

Checkpoint inhibitors

In cutaneous melanomas, blocking programmed cell death protein 1 (PD-1) expression with nivolumab or pembrolizumab and blocking cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) expression with ipilimumab has been FDA approved in unresected high-risk stage III and stage IV melanomas. Nivolumab and pembrolizumab are favoured over ipilimumab due to better recurrence-free survival rates and less treatment related toxicity. [87, 88] This is based on a randomized double-blind phase III trial of which 3.2% of the cohort were mucosal melanoma's. [89, 90] One study exclusively analysed vulvar and vaginal melanomas and found 50% of those treated with nivolumab to response partially and 50% of those to have progression of disease. [91] Ipilimumab had worse results with 66% of the treated cases to have progression of disease, 16% to have stable disease and 16% to respond with regression of disease. Median progression-free survival and overall survival in VMM, respectively 3.0 months and 2.7 months was lower than that in cutaneous melanomas, respectively 11.7 months and 5.8 months. [92]

Targeted therapy

KIT, NRAS and BRAF mutations in oncogenic pathways are identified as inhibitable targets in cutaneous melanomas. In VMM still little is known, for which this subject is of great interest. [93]

In VMM only 3.9% harbours a mutation in the BRAF gene [18,94–106]. KIT mutations are found in 31.4% (range 18.2–40%) of the VMM's and most often are located at exon 11. [7, 18, 97–109] In as study combining 8 reports 9.8% (range 0–27.6%) of the VMM's were mutated in the NRAS gene. [18, 96–104, 106, 110] Table 4 summarizes all studies with analysing mutational status of the KIT, BRAF or NRAS gene. [7, 18, 94–110]

Three large melanoma case series including mucosal melanomas have been published showing response rates of 30%, 50% and 73,8% to KIT inhibition. [111–113] A cohort of 22 oral metastatic mucosal melanomas either treated with chemotherapy or imatinib showed better overall survival for the last mentioned. [114] KIT inhibition in VMM has not been studied. Up to today, no studies report on targeted therapy in NRAS or BRAF positive VMM's.

Table 4. Mutational status of VMM

Study	Number melanomas (location/ of which vulvar)	% BRAF mutation	% NRAS mutation	% KIT mutation
*Jiveskog et al. [110] **	28 (non-exposed vs exposed to sun)		11% (3/28) exon 2 codon 61 (2) exon 1 codon 12	
*Edwards et al. [94]**	13 (mucosal/8 vu)	0% (0/13)		
Cohen et al. [95]	8 (vu)	0% (0/8)		
Wong et al. [96]	7 (vuva/ 3 vu)	33.3% (1/3) exon15, N581I	0% (0/3)	
Torres Cabala et al. [107]	61 (mucosal/11 vu)			27.1% (3/11) exon 11 L576P, exon 13 K642E(2), exon 17 N8221
Omholt et al. [97]	71(mucosal/23 vu)	8.6% (2/23) exon 15 V600E (2)	0% (0/23)	35% (8/23) exon 11 W557R, exon 11,V559D, exon 11 V560D, exon 11 P573L exon 11 L576P (2) exon 17, D820Y
Carvajal et al. [98]	13 (vuva)	0% (0/13)	8% (1/13) exon 2 Q61L, exon 1 G12D, exon 1 G13V	46%(6/13) exon 11 L576P (5), exon 11 Y553C, exon 18 V852I, exon 13 K642E
*Schoenewolf et al. [108] **	16 (vuva)			45% (5/11) exon unknown
*Abu-Abed et al. [109]) **	17 (vuva)			5.3% (1/19) exon 11 L576P
Van Engen van Grunsven et al. [99]	14 (vuva/1 vu)	0% (0/1)	0% (0/1)	0% (0/1)
Tseng et al. [100]	24 (vuva/11 vu)	0% (0/11)	27% (3/11) exon1, G13D, exon2, Q61K exon2, A59T	18% (2/11) exon 11 L576P 17, exon 11 W557R and L576P
*Aulmann et al. [102] **	65 (vuva/50 vu)	0% (0/39)	12% (5/42) G12A+ (2), G13D (2), G12V	18% (7/39) exon 11 W557R, exon 11 V559D, exon 11 V560D, Exon 11 R5861, exon 11 insertion Y578-H580dup (2), exon 17 D820V
Heinzelmann et al. [7]	50 (vuva/33 vu)			44% (12/27) exon unknown
Rouzbahaman et al. [102]	44 (vuva/13 vu)	7.6% (1/13) exon unknown	27.6% (3/13) exon unknown	27.6% (3/13) exon unknown

Table 4. (*Continued.*)

Study	Number melanomas (location/ of which vulvar)	% BRAF mutation	% NRAS mutation	% KIT mutation
Hou et al. [103] *	51 (vuva/37 vu)	27.3% (9/33) exon15 V600E (4), exon 15 T599V600E deletion	0% (0/19)	26.5% (9/34) exon 11 L576P (4), L576R(1), A736V, N822K, V654A, D816V
Dias-Santagata et al. [104]	95 (vu)	25%(10/40) exon 15 V600E(7), G469R, L597Q, A581S	9% (3/35) Q61R(2), G12V	44% (15/34) exon 11 L576P (8), D816V(2), A829P, Y646D(2), V560D
Cinotti et al. [105]	43 (mucosal/15 vu)	0% (0/15)		0% (0/15)
Udager et al. [18] *	59 (ge/48 vu+ 6 vuva)	0 % (0/59)	3.7% (1/59) exon 2	22% (6/59) exon 11 (3), exon 13, exon 18
Wylomanski et al. [106]	22 (vuva/15 vu)	33.3% (5/15) exon 15 V600E (5)	0/0% (0/15)	6.7% (1/15) exon 11 L576P

Vu: Vulvar melanomas, ge: genital melanomas, vuva: vulvovaginal melanomas *These outcomes have not been subdivided for different types of melanomas included in this study

Prognosis

Patients with VMM have a poor prognosis, reported 5-year survival rates range between 10 and 63%. [7, 48] Late stage at diagnosis and high recurrence rates contribute to low survival rates. [2, 7, 11 , 13, 16] Pleunis et al compared a cohort of VMM with a cohort of cutaneous melanomas and found a 5-year survival in the VMM group of 35% compared to 85% in the cutaneous melanoma group. Yet, when matched to the VMM cases for age at diagnosis, Breslow thickness, nodal status, presence of distant metastases, tumour ulceration and time of diagnosis, 5-year survival difference between cutaneous melanoma and VMM was only 15% ($p < 0.002$). [115] This reflects that poor prognosis in VMM is partly explained by biological aggressiveness but also unfavourable characteristics at presentation may contribute to the poor prognosis. The 5-year survival rates have been investigated by stage. As there is no consensus on the appropriate staging system, data remains heterogeneous.

VMM recurrence rates vary between 42 and 70%. [21, 22, 28, 54] In a cohort of 51 VMM, 32 recurred of which most recur locoregional (53%), less recur at distant site (28%) or at both distant and locoregional sites (19%). [21] The average time to recurrence is only 1 year (range 1 month to 14 years). [28, 54, 116] This outcome could be biased due to short follow-up. This is questioned by a recent cohort which found a mean time to local recurrence of 5 years and 3 months which suggests that a substantial number of recurrences occur late (> 5 years). [22] Late recurrences may explain the difference between 5-year survival rates and 10-year survival rates for which 10-year survival rates may be more valuable than 5-year survival rate. [3, 22, 50]

Follow-up

Follow-up of any type of cancer, including melanomas, has the primary aim of detecting locoregional or distant recurrences in an early stage to improve the long-term survival. [117] Thus far, there are no guidelines on VMM follow up, and schedules are based on the clinical experience and custom practice rather than on evidence. To date, evaluation of these current follow-up regimes has not been undertaken. [14] For vulvar cancer the most often used follow-up scheme consists of appointments 6–8 weeks postoperative, every 3–4 months in the first two years post-diagnosis and twice a year in the 3rd and 4th year. [14] This has been adopted for vulvar melanoma. [14, 44] However, since recurrence rates are higher and late recurrences(> 5 years) are common a long-term follow-up plan is needed. [28, 30] The value of PET-CT in the follow-up of cutaneous melanoma is still unclear.

The first post-operative appointment aims to inspect the wounds and evaluate the occurrence of complications of surgical or adjuvant therapy. The leading thought is that lab

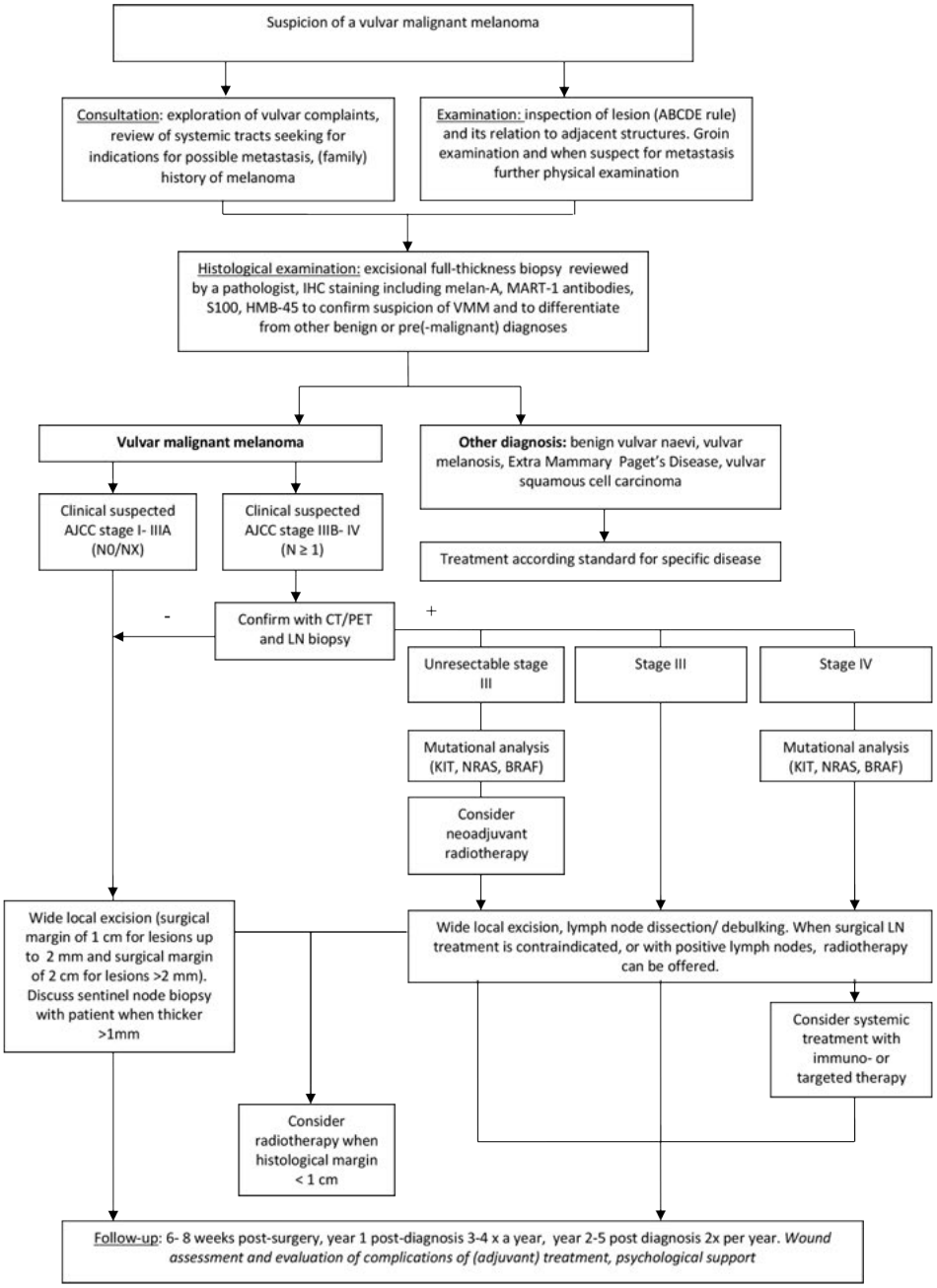
and imaging should only be done on indication when suspicion is raised for a recurrence or unidentified metastasis. Furthermore, during follow-up appointments there should be special attention for any need of psychological support as a substantial decrease in quality of life due to emotional, physical, and social functioning, sexuality, and body image in patients with any type of vulvar cancer. [39, 40]

Recommendations

- Higher age, Breslow thickness and lymph node involvement all are clear predictors of survival in VMM whilst for central localization and ulceration status this is less clear.
- For diagnosis of VMM histological evaluation through an excisional, and in case of possible damage to surrounding structures, incisional biopsy is recommended.
- We recommend imaging only in case of clinically suspected nodal involvement (AJCC stage III) with PET/CT of at least the chest, abdomen, and pelvic and inguinal regions. In case of a planned large surgery imaging can be considered since in case of distant metastasis, mutilating surgery should be reconsidered.
- When systemic treatment for stage IV disease or unresectable stage III disease is considered, mutational analysis of the KIT, BRAF and NRAS gene should be done.
- For locally confined disease treatment should consist of WLE with a surgical margin of 1 cm for lesions up to 2 mm thick and 2 cm for lesions of more than 2 mm thick.
- Neoadjuvant radiotherapy can be considered to reduce tumour bulk in case of large tumours or in case of proximity to vital structures like urethra of anus.
- For chemotherapy or immunotherapy as adjuvant or palliative treatment evidence is very scarce and treatment should only be considered in study setting and after thorough deliberation with patient and doctor.
- A sentinel lymph node biopsy can be discussed with the patient in case of a melanoma thicker than 1 mm. This should be performed by a specialized team using SLNB routinely for VSCC. In case of a negative sentinel node no further treatment is needed. The value of a lymph node dissection in case of a positive sentinel node in VMM is not known.
- In advanced melanoma with regional involvement, surgical treatment is identical to the treatment in early stage disease. Elective lymph node dissection in case of palpable nodal involvement has not shown a survival benefit but may, similar to cutaneous melanoma, prolong (distant) disease-free and melanoma-specific survival and for that reason can be managed. The advantages and disadvantages of lymph node dissection and adjuvant treatment should be weighed carefully in every individual case by both patient and doctor.
- Postoperative radiotherapy for better local control can be considered in case of histologically close of positive margins, or after lymph node dissection of positive nodes

- In recurrent or metastatic VMM treatment needs to be individualized. Local recurrences can be surgically removed in an attempt to prolong disease-free survival or local complaints. Systemic therapy can be considered to reduce complaints due to nodal or distant metastasis and to prolong survival. The checkpoint inhibitors nivolumab and pembrolizumab have shown positive results in studies including cutaneous melanomas and a small subset of mucosal melanomas. Moreover, targeted therapy, specifically imatinib in KIT-positive and BRAF-inhibitors in BRAF-positive mucosal melanomas, have shown improvement in survival Adjuvant treatment in metastatic VMM should be considered in study design. Future studies should be aimed at molecular profiling for identification of novel treatment strategies and further development of immunotherapies in VMM.
- A proposed flowchart for the management of VMM is given in Fig. 2.

Figure 2. Flowchart management of vulvar malignant melanoma



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Appendix

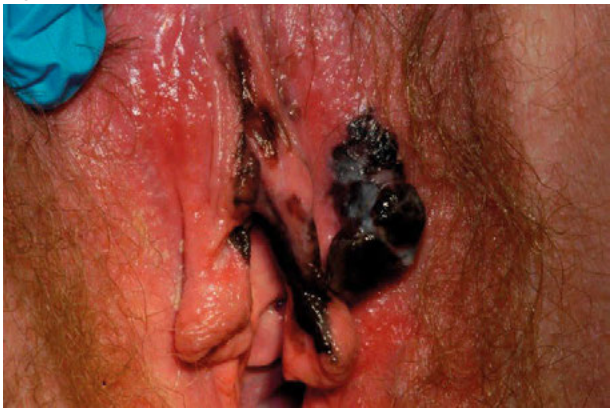
Appendix 1- definitive search strategy Pubmed
See online: https://www.cancertreatmentreviews.com/article/S0305-7372(18)30209-3/fulltext#supplementaryMaterial

Appendix 2 - AJCC staging (2009) for cutaneous melanoma
See online: https://www.cancertreatmentreviews.com/article/S0305-7372(18)30209-3/fulltext#supplementaryMaterial

Appendix 3 - UICC TNM staging for head and neck mucosal melanomas (2017)

Primary tumour (T)	
T category	**T criteria**
T3	Tumors limited to the mucosa and immediately underlying soft tissue, regardless of thickness or greatest dimension; for example, polypoid nasal disease, pigmented or nonpigmented lesions of the oral cavity, pharynx, or larynx
T4	Moderately advanced or very advanced
T4a	Moderately advanced disease. Tumor involving deep soft tissue, cartilage, bone, or overlying skin
T4b	Very advanced disease. Tumor involving brain, dura, skull base, lower cranial nerves (IX, X, XI, XII), masticator space, carotid artery, prevertebral space, or mediastinal structures.
Regional lymph nodes (N)	
N category	**N criteria**
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	Regional lymph node metastases present
Distant metastasis (M)	
M category	**M criteria**
M0	No distant metastasis
M1	Distant metastasis present

Figure 1. Clinical presentation of vulvar melanoma



Evaluation of treatment, prognostic factors and survival of 198 vulvar melanoma patients: implications for clinical practice

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6

Abstract

Objective

To identify clinicopathological characteristics, treatment patterns, clinical outcomes and prognostic factors in patients with vulvar melanoma (VM).

Materials & methods

This retrospective multicentre cohort study included 198 women with VM treated in eight cancer centres in the Netherlands and UK between 1990 and 2017. Clinicopathological features, treatment, recurrence, and survival data were collected. Overall and recurrence-free survival was estimated with the Kaplan-Meier method. Prognostic parameters were identified with multivariable Cox regression analysis.

Results

The majority of patients (75.8%) had localized disease at diagnosis. VM was significantly associated With high-risk clinicopathological features, including age, tumour thickness, ulceration, positive resection margins and involved lymph nodes. Overall survival was 48% (95% CI 40–56%) and 31% (95% CI 23–39%) after 2 and 5 years respectively and did not improve in patients diagnosed after 2010 compared to patients diagnosed between 1990 and 2009.Recurrence occurred in 66.7% of patients, of which two-third was non-local. In multivariable analysis, age and tumour size were independent prognostic factors for worse survival. Prognostic factors for recurrence were tumour size and tumour type. Only the minority of patients were treated with immuno- or targeted therapy.

Conclusion

Our results show that even clinically early-stage VM is an aggressive disease associated with poor clinical outcome due to distant metastases. Further investigation into the genomic landscape and the immune microenvironment in VM may pave the way to novel therapies to improve clinical outcomes in these aggressive tumours. Clinical trials with immunotherapy or targeted therapy in patients with high-risk, advanced, or metastatic disease are highly needed.

Introduction

Mucosal melanomas (MM) are a rare clinical entity and comprise less than 2% of total melanomas. [1] Primary MM arise from melanocytes located in mucosal membranes lining the respiratory, gastrointestinal, and urogenital tract. Compared with cutaneous melanomas (CM) (80%), MM have a poor five-year survival of only 25%. [2] About 18-40% of MM originate from the vulvar region. [3] Vulvar melanoma (VM) is the second most common malignancy of the vulva, after squamous cell carcinoma, but is still rare with an incidence of 0.1 per 100,000 females per year. [4] Although VM arises on the hairy and glabrous skin of the vulva, it is mostly described as MM due to its location and continuity with vaginal mucosa. [5,6] Because of the low incidence of VM, large studies are scarce, and treatment of the disease remains difficult. Recurrence rates lie between 42-70%, with a reported disease-free survival ranging between 12 and 63 months. [5] The reported 5-year survival rates vary between 24% and 79%. [5] Most women diagnosed with VM are postmenopausal and presentation is usually delayed due to the anatomic location which contributes to the poor prognosis. [5, 7]

Surgical treatment in the vulvar area and a high risk of recurrent disease present major clinical challenges in the treatment of patients with VM. [8] Clinical guidelines for VM have been based on evidence and recommendations for CM. [9] In addition, gynaecologic oncologists who treat VM, are influenced by the surgical management principles for the more common squamous cell carcinoma of the vulva. Therefore, consensus guidelines regarding type of surgery, optimal surgical margins, groin treatment and adjuvant therapy for VM, do not exist.

The introduction of effective immune- and targeted therapies in 2011 has significantly improved survival in advanced CM, however, the prognosis of patients with advanced MM has not changed. [10] A possible explanation might be the pathogenesis of MM, which seems to differ from that of cutaneous melanoma. [11, 12] It has been shown that MM have a different molecular signature than CM by lacking BRAF and NRAS mutations and harbouring KIT mutations. [13-15] KIT mutations were shown to be the highest in VM (22%) compared with other MM subtypes (8.8%). [14] So far only a few studies describe treatment outcomes of immune- and targeted therapy in VM.

The identification of clinicopathological characteristics and prognostic factors is important to develop clinical guidelines and define patients who may benefit from adjuvant or novel treatments. It remains uncertain whether the poor prognosis of VM is due to the usually more progressed disease at initial diagnosis or to the biologically more aggressive behaviour. Until now, prognostic factors in VM are not well established and most studies included small patient numbers.

The aim of this study was to investigate the clinicopathological characteristics in relation to clinical outcome, survival and recurrence rates in a large cohort of patients with VM treated in melanoma referral centres in the Netherlands and UK over a 27-year period. Furthermore, we summarized treatment outcomes in patients who received immune- and targeted therapies.

Methods

2.1. Study design and patients

A retrospective evaluation of patients diagnosed with primary VM at five academic medical centres in the Netherlands and three melanoma treatment hospitals in the UK was performed. Clinical, histopathological, and treatment data of all patients diagnosed between January 1990 and December 2017 in the Netherlands and between January 2000 and December 2017 in the UK were obtained from the medical records. This study was approved by the Dutch medical ethics committee (reference number G18.046) and HRA (Health Research Authority) in the UK (REC reference 19/HRA/0070). Data collection and storage was carried out according to the guidelines of the ethics committees of the corresponding hospitals.

2.2. Clinical and histopathological characteristics and treatment outcomes

Inclusion criteria were pathologically confirmed primary VM and age ≥18 years. Patients of whom clinical data or pathology reports were missing were excluded from this study. Patient demographics including age at diagnosis, primary tumour characteristics, treatment details, adjuvant therapy, the site and date of any recurrences or metastases, and follow up data were obtained from all patients. Adjuvant treatment included re-excision, radiotherapy, chemotherapy, immunotherapy or targeted therapy. For patients treated with immune- or targeted therapy, the best overall response rate (BORR) was defined following the RECIST 1.1 guideline. [16] Recurrence was defined as a pathologically or radiologically confirmed recurrence after a disease-free period. Local recurrence was defined as any recurrence on the vulva and a regional recurrence was defined as lymph node metastasis in the groin(s). Locoregional recurrence refers to concurrent local and groin recurrence. Distant recurrence was defined as any recurrent disease beyond the vulva or the groins with or without the presence of a local or regional recurrence. Date of last follow-up was defined as the last contact with a gynaecologist or oncologist or the date of death. Follow-up was completed until December 2019.

Histopathological data that were collected from the pathology reports included tumour type, tumour size, tumour thickness (Breslow), ulceration, mitotic activity, microsatellitosis, regressive changes, angiolymphatic involvement, margin status, lymph node involvement and mutation status (BRAF, cKIT, NRAS, GNAQ). All patients were classified according to the AJCC version 2009 (7th edition) staging system (S1). [17] Since this is a retrospective study,

all cases before 2009 have been re-classified according to this staging system.

2.3. Statistical analysis

Normally distributed continuous data were reported as means with standard deviations and skewed distributions as medians with interquartile ranges. Percentage calculation was based on the number of available observations. Differences between descriptive variables were tested with the Chi-square test, the Fisher's exact test, the independent T-test or the Mann-Whitney U test.

Overall survival (OS) percentages were derived from the analysis of the time in months from the date of initial diagnosis until death or last follow-up. Recurrence-free survival (RFS) percentages were derived from the analysis of the time in months from the date of initial diagnosis until recurrence or last follow-up. OS and RFS were calculated and plotted using Kaplan Meier analysis. The log rank test was used to compare OS and RFS between the groups. Prognostic factors for OS and RFS were identified with univariable and multivariable analysis using Cox regression analysis. Univariate preselection of variables was used to build a multivariable model for overall and recurrence-free survival. To deal with missing data of possible predictors, we imputed for data used in the multivariable cox regression analysis, which were assumed to be missing 'at random'. Missing covariates for the Cox regression model were imputed and summary estimation was done according to Rubin's rules. [13] An imputation model was built with age, location on the vulva, lymph node involvement, Breslow thickness and diameter of the tumour. All p-values were two-sided, and a p-value of < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 25.0 (IBM).

Results

3.1 Patients and tumour characteristics

Two-hundred twenty-three cases were assessed for eligibility and 198 cases were included in this study (S2). The clinical and histopathological characteristics are presented in Table 1. Median age at diagnosis was 72 years (IQR 61-78). In most cases (156 of 198, 78.8%), the main symptoms were bleeding, pain, or pruritis. The interval between first signs and diagnosis ranged from 1 to 55 months, with a median of 4 months. Of the overall study group, 150 (75.8%) patients were diagnosed with clinically localized disease (AJCC stage IA-IIC), 24 (12.1%) with regional disease (AJCC stage III), and 16 (8.1%) with distant disease (AJCC stage IV), and in 8 (4.0%) the stage of disease was undetermined.

Table 1. Clinical and histological characteristics of VMM

Clinical characteristics	N = 198 (%)
Age at diagnosis [years, IQR]	72 [61;78]
Symptoms at presentation	
Yes	156 (78.8)
No	25 (12.6)
Unknown	17 (8.6)
Location on the vulva	
Unilateral	140 (70.1)
Clitoris	33 (16.7)
Multifocal	22 (11.1)
Missing	3 (1.5)
Pathologic T stage	
T1	14 (7.0)
T2	10 (5.1)
T3	39 (19.7)
T4	116 (58.6)
Tx	19 (9.6)
AJCC stage (2009)	
Stage IA	7 (3.5)
Stage IB	11 (5.6)
Stage IIA	11 (5.6)
Stage IIB	43 (21.7)
Stage IIC	78 (39.4)
Stage III	24 (12.1)
Stage IV	16 (8.1)
Unknown	8 (4.0)
Breslow thickness (median) [mm, IQR]	7.0 [3;14]
Tumour size (median) [mm, IQR]	20.0 [10;30]
Melanoma subtype	
Superficial spreading	73 (36.9)
Lentiginous	8 (4.0)
Nodular	71 (35.9)
Unclassified	8 (4.0)
Missing	38 (19.2)
Ulceration	
Yes	132 (66.7)
No	30 (15.2)
Missing	36 (18.2)
Mitotic activity	
Yes	120 (60.6)
No	11 (5.6)
Missing	67 (33.8)
Microsatellitosis	
Yes	20 (10.1)

Table 1. (*Continued.*)

Clinical characteristics	N = 198 (%)
No	81 (40.9)
Missing	97 (49.0)
Angiolymphatic involvement	
Yes	41 (20.7)
No	63 (31.8)
Missing	94 (47.5)
Regressive changes	
Yes	20 (10.1)
No	48 (24.2)
Missing	130 (60.1)
Mutation status	
Not analysed	155 (78.3)
Analysed	43 (21.7)
No mutation	29 (67.4) [a]
BRAF	2 (4.7) [a]
KIT	7 (16.3) [a]
BRAF+ KIT	1 (2.3) [a]
NRAS	2 (4.7) [a]
GNAQ	1 (2.3) [a]
Tp53	1 (2.3) [a]
Recurrence[b]	
Yes	120 (66.7)
No	67 (33.3)
Missing	11 (5.6)
Location of first recurrence (n=114)	
Local	40 (35.1)
Locoregional	16 (14.0)
Regional	25 (21.9)
Distant	33 (29.0)
Missing	6 (5.0)
Median time to first recurrence [months, IQR]	11 [6,25]
Location of second recurrence (n=57)	
Local	7 (12.3)
Locoregional	2 (3.5)
Regional	3 (5.3)
Distant	45 (78.9)
Median time from first to second recurrence [months, IQR]	8 [4,16]

[a] of the analysed patients
[b] of the surgically treated patients

The majority of the patients (58.6%) presented with stage T4 (i.e., thickness > 4 mm) tumours. The most common tumour types were superficial spreading melanoma (SSM) (n=73; 36.9%) and nodular malignant (NM) melanoma (n=71; 35.9%). The median tumour thickness was 7 mm (IQR 3-14) and the median tumour size 20 mm (IQR 10-30). Ulceration and mitosis were present in 132 (66.7%) and 120 (60.7%) of the cases. Angiolymphatic involvement, regressive changes, and microsatellitosis were reported in the minority of the tumours. Mutational analysis was performed in only 43 of the 198 patients (22%). The frequency increased from 8% to 42% in patients diagnosed between 1990-2009 and 2010-2017 (Table 1, S3). In 67.4% of the tumours analysed, no potentially targetable mutation was found. KIT mutations were most frequently detected (18.6%), followed by mutations in BRAF (7%) and NRAS (4.7%).

The majority of patients (n=180; 90.9%) underwent primary surgical resection with curative intent (Table 2). 128 of 180 (71.1%) of these patients had negative histological margins whereas in 37 (20.6%) patients the resection margins were positive; in the remaining 15 (8.3%) the margin status was unknown. Re-excision was performed in 65 (36.1%) of the patients of which 18 had positive margins and 47 had close margins (data not shown).

In 74 patients (37.4%) nodal surgery was performed at the same time of the local treatment. Sentinel lymph node (SLN) biopsy was performed in 49 patients (27.2%), and 10 (5.6%) patients had a SLN subsequently followed by a full inguinofemoral lymphadenectomy (IFL). Twenty-one patients (11.7%) underwent an elective IFL and 4 (2.2%) patients had lymph node dissection.

Adjuvant treatment was given in 15 of 180 (8.3%) patients after primary surgery. Seven women received local radiotherapy on the vulva, three women radiotherapy on the groin(s) and three women both local and groin radiotherapy. Two patients were treated with systemic therapy of which one with chemotherapy and one with immunotherapy (Pembrolizumab). The clinical and histopathological characteristics of patients diagnosed between 1990 and 2009 did not significantly differ compared to patients diagnosed between 2010 and 2017, although the latter had slightly more patients with stage III/IV disease (S3). In addition, patients diagnosed between 2010 and 2017 underwent more often a SLN biopsy and palliative treatment (S4).

Recurrences were treated with many different treatment modalities (S5). Local recurrences were primarily treated with local surgery, either alone or combined with local radiotherapy. The most common treatment of a regional recurrence was either an IFL alone or combination of IFL with radiotherapy. Treatment of locoregional recurrences varied greatly and were often a combination of therapies. The most common treatment of distant metastatic disease was symptomatic treatment, with palliative radiotherapy or local excision of metastasis. Twenty-one of 78 patients (27%) with distant metastases received immunotherapy.

Table 2. Treatment characteristics of VMM

Treatment characteristics	N = 198 (%)
Treatment modality	
Surgery	165 (83.3)
Surgery plus adjuvant therapy	15(7.6)
Other	9 (4.5)
Radiotherapy of vulva	3 (1.5)
Radiotherapy of vulva + immunotherapy	1 (0.5)
Radiotherapy of metastasis	1 (0.5)
Neoadjuvant immunotherapy + palliative resection	1 (0.5)
Elective lymph node dissection	1 (0.5)
Immunotherapy	2 (1.0)
Unknown	3 (1.5)
No treatment	6 (3.0)
Type of surgical treatment of primary tumour (n=180)	
Wide local excision	156 (78.8)
Hemivulvectomy	11 (5.6)
Radical vulvectomy	8 (4.1)
Radical vulvectomy and vaginectomy	5 (2.5)
LN involvement [a]	
Positive	29 (14.6)
Negative	76 (38.4)
Not assessed	93 (47.0)
Lymph node treatment	
Not conducted	88 (48.9)
SLN	49 (27.2)
SLN + IFL	10 (5.6)
IFL	21 (11.7)
Lymph node debulking	4 (2.2)
Radiotherapy	5 (2.8)
Unknown	3 (1.6)
Resection margins	
Negative	128 (71.1)
< 10 mm margin	64 (35.5)
≥ 10 mm margin	30 (16.7)
< 2 mm margin	7 (3.9)
≥ 2 mm margin	87 (48.3)
Not specified	34 (18.9)
Positive	37 (20.6)
Unknown	15 (8.3)
Re-excision	
Yes	65 (36.1)
No	113 (62.8)
Unknown	2 (1.1)

[a] *pathologically or radiologically confirmed SLN: Sentinel lymph node biopsy, IFL: inguinofemoral lymphadenectomy*

3.2 Clinical outcomes

Clinical follow-up ranged from 1 – 272 months (median 31 months), with 141 deaths at the time of data collection. Three patients were lost to follow up. A recurrence occurred in 120 (66.7%) of the surgically treated patients, at a median of 11 months (IQR 6-25 months) (Table 1). Location of the first recurrence was local, regional, locoregional or distant in respectively 35.1%, 14%, 21.9% and 29%, suggesting occult metastasis at time of primary surgery in the majority of the patients. A second recurrence occurred in 57 of 120 patients at a median of 8 months. The second recurrence was local in 7 patients, regional in 3, locoregional in 2 and distant in 45 patients (78.9%; 95% CI 68.4-89.5).

The estimated median OS for patients diagnosed with VM was 33 months (95% CI 25-40). Estimated cumulative OS was 48% (95% CI 40-56%) at 2 years, 31% (95% CI 23-39%) at 5 years and continued to fall, to 9% (95% CI 3-15%), at 10 years (Figure. 1A). The estimated RFS for the overall cohort was 41% (95% CI 33-49%), 26% (95% CI 18-34%) and 16% (95% CI 6-26%) at respectively 2, 5 and 10 years (Figure. 1B). The estimated median survival from recurrence to death for patients with any recurrence was 10 months (local 15 months, locoregional 16 months, distant 6 months).

Figure 1. Overall survival and recurrence-free survival. **A** Overall survival **B** Recurrence-free survival

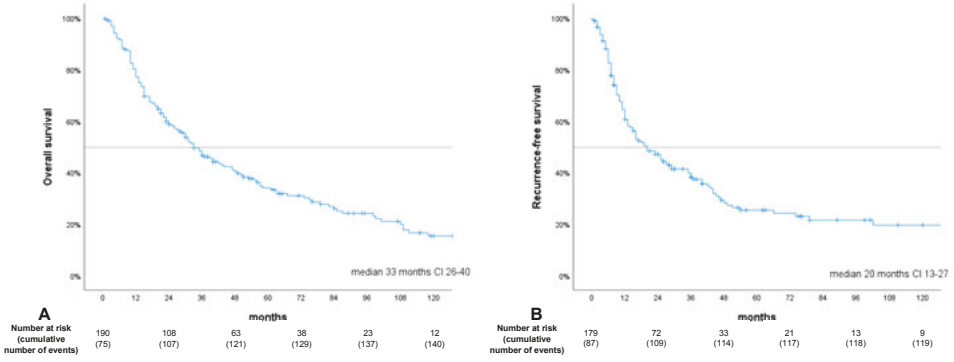


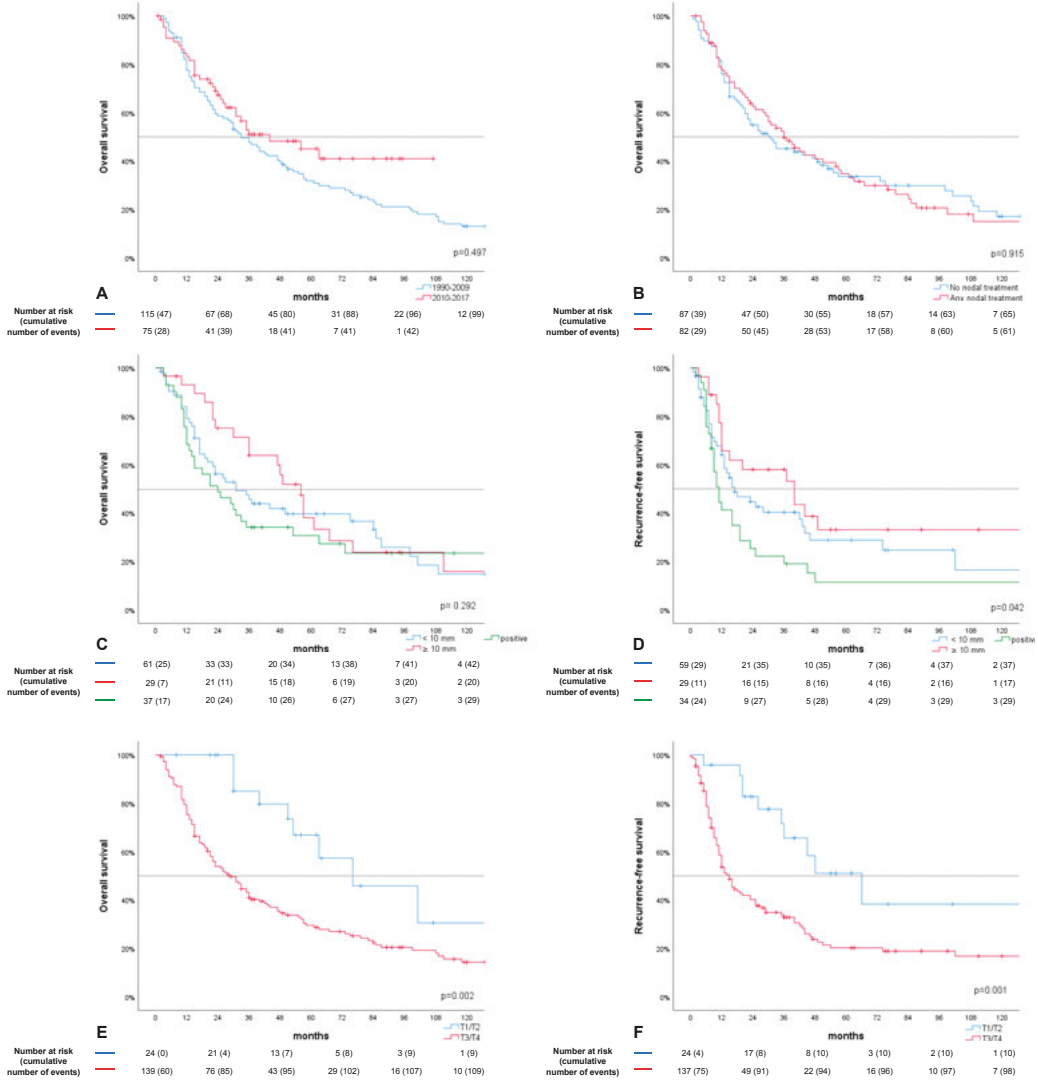
Table 3. Targeted and immunotherapy for the VM cohort

	Patient ID	Primary/ recurrent	Recurrence type	Therapy	Other treatment	BORR	OS (months)	Vital status	Post treatment survival
Targeted therapy	64	Recurrence	distant	AZD6244		SD	52	dead	5
	65	Primary		Vemurafenib		PR	10	dead	9
	93	Primary		Imatinib	local radiotherapy	SD	8	dead	6
	116	Recurrence	distant	Imatinib		PD	21	dead	4
	141	Recurrence	distant	Imatinib	local radiotherapy	PR	28	dead	16
	202	Recurrence	distant	Dabrafenib + Trametinib		SD	36	dead	14
Immune therapy	19	Primary		Pembrolizumab	wide local excision	SD	37	alive	30
	22	Recurrence	distant	Pembrolizumab		PD	12	dead	3
	35	Recurrence	locoregional	IFNa		PD	83	dead	12
	60	Primary		Pembrolizumab		PD	8	dead	6
	61	Recurrence	distant	Ipilimumab		SD	110	dead	25
	61	Recurrence	distant	Nivolumab	local radiotherapy	SD	110	dead	19
	64	Recurrence	distant	Ticilimumab		PD	52	dead	13
	65	Primary		Ipilimumab		unknown	10	dead	2
	70	Primary		Pembrolizumab	palliative resection	PD	18	dead	17
	70	Primary		Pembrolizumab + Ipilimumab		PD	18	dead	10
	83	Recurrence	regional	Nivolumab		PD	42	alive	8
	100	Recurrence	distant	Nivolumab		PR	94	alive	48
	124	Recurrence	distant	Ipilimumab	local radiotherapy	PD	159	dead	60
	124	Recurrence	distant	Pembrolizumab	local radiotherapy	CR	159	dead	unknown
	150	Recurrence	distant	Cisplatin/DTIC/IL-2/IFNa		PD	53	dead	10
	188	Recurrence	distant	Ipilimumab	local radiotherapy	PD	58	dead	unknown
	190	Recurrence	regional	Ipilimumab + Nivolumab	groin radiotherapy	SD	92	alive	21
	198	Recurrence	distant	Pembrolizumab	local radiotherapy	PD	31	dead	5
	198	Recurrence	distant	Ipilimumab		PD	31	dead	1
	58	Recurrence	distant	Ipilimumab + Nivolumab		SD	35	dead	16

Table 3. (*Continued.*)

	Patient ID	Primary/ recurrent	Recurrence type	Therapy	Other treatment	BORR	OS (months)	Vital status	Post treatment survival
	58	Recurrence	distant	Ipilimumab		SD	35	dead	9
	67	Recurrence	distant	Ipilimumab + Nivolumab	radiotherapy of distant metastasis	SD	14	unknown	1
	133	Recurrence	distant	IFNa + IL-2		PD	21	dead	6
	133	Recurrence	distant	IFNa, Leiomycin pincrestin + DTIC		PD	21	dead	3
	175	Recurrence	distant	Temzolomide, GCSF, IL-2 + IFNa	local excision metastasis	SD	43	dead	6
	199	Recurrence	distant	Ipilimumab + Nivolumab		PR	39	alive	4
	200	Recurrence	distant	Nivolumab	local radiotherapy	SD	38	alive	7
	202	Recurrence	distant	Pembrolizumab		PD	36	dead	16
	162	Recurrence	locoregional	IFNa	unilateral IFL + radiotherapy of the groin	PD	10	dead	5
	189	Recurrence	distant	Ipilimumab		PD	24	dead	7
	153	Recurrence	distant	IFNa + IL-2 + DTIC + cisplatin	radiotherapy of distant metastasis	unknown	50	dead	0

Figure 2. Overall survival by timeframe and nodal treatment and overall and recurrence-free survival by margin status and T stage. **A** Overall survival by timeframe (1990–2009 vs 2010–2017) **B** Overall survival by nodal treatment (no treatment vs any type of nodal treatment) **C** Overall survival by margin status (positive vs < 10 vs ≥ 10) **D** Recurrence-free survival by margin status (positive vs < 10 vs ≥ 10) **E** Overall survival by T stage (T1/2 vs T3/4) **F** Recurrence-free survival by T stage (T1/2 vs T3/4).



3.3 Treatment with targeted therapy and checkpoint inhibitors

Twenty-eight patients were treated with immune- or targeted therapy. (Table 3). Five patients with stage IV disease or irresectable stage III disease received immunotherapy as primary treatment and 23 patients were treated with immunotherapy for recurrent disease.

Twenty-four of 28 patients received checkpoint inhibitors of which eleven (45.8%) had anti PD-1, eight (33.3%) had anti-CTLA-4 and five (20.9%) had a combination of both. Seven patients were treated with interferon-alpha or interleukin-2 of which 4 combined with chemotherapy. Six patients received targeted therapy of whom three a KIT inhibitor, one a BRAF inhibitor, one with a MEK inhibitor (AZD6244) and one with a combination of a BRAF and MEK inhibitor.

The estimated median survival after start of immune- or targeted therapy was 16 months (95% CI 9-23) for patients with immune therapy, 6 months (95% CI 1-10) for targeted therapy and 6 months (95% CI 5-7) for cytokine therapy with or without chemotherapy.

The outcomes of these therapies have been depicted as Best Overall Response Rate (BORR, Table 3). Of the 11 patients who received anti-PD-1 therapy, six had progressive disease (PD), three had stable disease (SD), one had partial response (PR), and one complete response (CR). Patients treated with anti-CTLA-4 had PD in 5/8 and SD in 2/8 cases, in one patient the BORR was missing. Of the 5 patients who received combination therapy consisting of anti CTLA-4 and anti PD-1, one had PD, one had PR, and three had SD. Two patients who were treated with ipilimumab discontinued their therapy due to toxicity. Of the six patients treated with targeted therapy, one had PD, two had PR and three patients had SD.

3.4 Prognostic factors of overall and recurrence-free survival

Survival for patients diagnosed between 2010 and 2017 did not significantly differ from patients diagnosed between 1990 and 2009 (Figure 2A). Prognostic factors for OS and RFS are presented in Table 4 and Figure 2. Univariable analysis showed that tumour size, T stage, lymph node involvement, and age were associated with worse OS (Table 4) as well as the histological variables including mitosis, ulceration, microsatellitosis and angiolymphatic involvement. Lymph node treatment was not significantly associated with OS (Figure 2B). Tumour size, T stage, lymph node involvement and positive resection margins were univariably associated with worse RFS, as well as the histological variables including ulceration, tumour type (other vs SSM), microsatellitosis, regressive changes and angiolymphatic involvement. Patients with positive margins had a significantly worse RFS compared to patients with negative margins. There was a trend seen for the association between these factors with OS, however this was not statistically significant. (Table 4, Figure 2CD). T3/T4 stage was associated with worse OS and RFS compared to T1/T2 stage disease (Figure 2EF). Multivariable analysis showed that tumour size and tumour type (other vs SSM) were significant predictive factors for RFS, whereas age and tumour size were predictive factors for OS.

Table 4. Univariable and Multivariable analysis of overall and recurrence-free survival [a]

Overall survival	n	HR (95% CI)	p	n	HR (95% CI)	p
Age at diagnosis (per increase of 10 years)	*190*	**1.26 (1.11-1.44)**	**0.001**	*171*	**1.23 (1.06-1.43)**	**0.005**
Location on the vulva						
midline vs unilateral	*190*	1.16 (0.74-1.82)	0.509			
multifocal vs unilateral	*190*	1.32 (0.79-2.21)	0.282			
Tumour size (per increase of 1 mm)	*190*	**1.02 (1.01-1.03)**	**<0.001**	*171*	**1.02 (1.01-1.03)**	**0.001**
Breslow thickness (per increase of 1 mm)	*190*	0.99 (0.99-1.01)	0.449			
LN involvement (yes vs no)	*190*	**2.10 (1.26-3.48)**	**0.004**	*171*	1.46 (0.78-2.72)	0.234
Treatment period (2010-2017 vs 1990-2009)	*190*	0.88 (0.61-1.28)	0.499			
Mitosis						
yes vs no	*190*	**6.33 (1.56-25.75)**	**0.010**	171	3.29 (0.71-15.12)	0.125
missing vs no	*190*	**5.49 (1.33-22.60)**	**0.018**	171	3.32 (0.73-15.20)	0.122
Ulceration						
yes vs no	*190*	**2.46 (1.37-4.38)**	**0.002**	*171*	1.36 (0.72-2.57)	0.341
missing vs no	*190*	1.72 (0.88-3.39)	0.114	171	1.07 (0.50-2.32)	0.858
T stage (T3+T4 vs T1+T2)	*171*	**2.80 (1.42-5.53)**	**0.003**	*171*	1.41 (0.65-3.07)	0.381
LN treatment (yes vs no)	*169*	0.98 (0.69-1.39)	0.904			
Tumour type						
NM vs SSM	*156*	1.22 (0.83-1.80)	0.316			
other vs SSM	*156*	1.23 (0.66-2.32)	0.517			
Margins (pos vs neg)	*165*	1.33 (0.87-2.02)	0.190			
Margin < 2 mm vs ≥ 2 mm	*90*	1.20 (0.48-3.03)	0.692			
Margin < 10 mm vs ≥ 10 mm	*90*	1.24 (0.73-2.12)	0.430			
Angiolymphatic involvement (yes vs no)	*102*	**1.91 (1.20-3.04)**	**0.006**			
Microsatellitosis (yes vs no)	*96*	**3.21 (1.82-5.67)**	**<0.001**			
Regressive changes (yes vs no)	*65*	1.17 (0.59-2.31)	0.656			

Table 4. (*Continued.*)

Recurrence-free survival	n	HR (95% CI)	p	n	HR (95% CI)	p
Age at diagnosis (per increase of 10 years)	*179*	1.03 (0.90-1.17)	0.708			
Location on the vulva						
midline vs unilateral	*179*	0.79 (0.47-1.33)	0.370			
multifocal vs unilateral	*179*	1.33 (0.80-2.21)	0.278			
Tumour size (per increase of 1 mm)	*179*	**1.02 (1.01-1.03)**	**0.002**	139	**1.02 (1.00-1.04)**	**0.018**
Breslow thickness (per increase of 1 mm)	*179*	1.00 (0.10-1.01)	0.358			
LN involvement (yes vs no)	*179*	**1.87 (1.11- 3.16)**	**0.019**	139	1.44 (0.73-2.85)	0.290
Treatment period (2010-2017 vs 1990-2009)	*179*	0.93 (0.63-1.37)	0.698			
Ulceration						
yes vs no	*179*	**2.71 (1.40-5.25)**	**0.003**	139	1.86 (0.87-3.97)	0.111
missing vs no	*179*	**2.41 (1.16-5.00)**	**0.019**	139	1.99 (0.83-4.77)	0.123
Mitosis						
yes vs no	*179*	2.08 (0.83-5.20)	0.117			
missing vs no	*179*	2.23 (0.88-5.66)	0.093			
LN treatment (yes vs no)	*164*	1.13 (0.77-1.65)	0.496			
T stage (T3+T4 vs T1+T2)	*161*	**2.75 (1.43-5.29)**	**0.002**	139	1.73 (0.78-2.28)	0.178
Tumour type						
NM vs SSM	*145*	1.53 (0.99-2.37)	0.054	139	1.42 (0.88-2.28)	0.149
other vs SSM	*145*	**1.98 (1.05-3.74)**	**0.035**	139	**3.15 (1.58-6.31)**	**0.001**
Margins (pos vs neg)	*158*	**1.71 (1.11-2.61)**	**0.014**			
Margin < 2 mm vs ≥ 2 mm	*85*	1.30 (0.50-3.35)	0.592			
Margin < 10 mm vs ≥ 10 mm	*85*	1.31 (0.73-2.32)	0.363			
Microsatellitosis (yes vs no)	*95*	**2.10 (1.13-3.87)**	**0.018**			
Angiolymphatic involvement (yes vs no)	*92*	**2.60 (1.55-4.36)**	**<0.001**			
Regressive changes (yes vs no)	*59*	**3.47 (1.20-5.11)**	**0.015**			

Due to more than 50% missing values the variables under the dashed line have only been used for univariable analysis. Bold values denote statistical significance.[a] Univariable analysis and multivariable analysis for OS included respectively 190 and 171 cases with 140 and 125 events. Univariable and multivariable analysis for RFS included respectively 179 and 139 cases with 119 and 92 events. The lower count in in OS and RFS multivariable analysis is due to T stage and tumour type which have not been included in the imputation.

Discussion

To our knowledge this is the largest series of patients with primary VM. In this study we show that the prognosis of VM is associated with high-risk clinicopathological features, including age, tumour thickness, ulceration, positive resection margins and lymph node involvement. The 5-year OS and RFS in our cohort was 31% (95% CI 23-39%) and 26% (95% CI 18-34%), respectively. Survival did not improve for patients diagnosed between 2010 and 2017 compared to patients diagnosed between 1990 and 2009. Although the majority of patients (75.8%) had localized disease at diagnosis, two-third of the patients had recurrent disease with a median survival (from recurrence to death) of 10 months. Overall, the mutation rate in VM was low, although KIT mutations were relatively frequently found.

The primary treatment for resectable VM without known metastasis is wide local excision (WLE) in order to obtain complete resection with negative margins. [18] Current guidelines for CM recommend surgical margins of 1-2 cm depending on the tumour thickness. [19] Achieving these margins is often a challenge in VM because of anatomical position close to the clitoris, urethra or anus, and a large proportion of patients presenting late with locally advanced tumours (i.e., tumour thickness > 4 mm). In our study, 78% of patients presented with T3/T4 tumours, and median thickness was 7 mm (Table 1). The majority (71%) of surgical resections resulted in negative margins, whereas 21% of the specimens had positive margins reflecting the challenges surgeons meet during surgery for VM. Our data showed a statistically significant difference in RFS but not in OS for patients with positive margins compared to patients with negative margins on primary excision (Table 4, Figure 2CD), as was shown by others. [7] A possible explanation for this is the increased local recurrence risk with involved margins, which may not affect the risk for distant recurrence. Importantly, histological margins of >=10 mm were not statistically associated with better OS and RFS compared to margins <10 mm (Table 4, Figure 2CD). Also, a histological margin of < 2 mm was not statistically associated with worse OS or RFS (Table 4). Therefore, we recommend that obtaining tumour-free margins is the primary goal in VM surgery although we did not find a clear effect of wide negative margins on long-term patient outcome. This might be due to the highly aggressive nature of the disease, although a lower available sample sizes for these variables might have attributed as well.

SLN biopsy is currently considered the standard nodal assessment for CM. Since 2005, the preferred approach in patients with CM regarding SLN procedure has very much changed from complete lymphadenectomy in case of positive sentinel node to only intervene at the time positive nodal disease presents clinically. [20-22] No prospective studies of SLN in VM have been performed and are unlikely to become available because of the rarity of the disease. In our study, 49% of the surgically treated patients underwent groin treatment at the time of primary diagnosis, and 27% had SLN procedure whereas 17% underwent complete

full IFL. Lymph node treatment was not associated with better clinical outcomes. This study also shows that despite aggressive primary surgery in patients with clinically localized disease, still 60% of patients with VM develop metastatic disease with survival of less than 1 year (Table 1). Together, these data suggest complete local resection is preferable to radical surgical treatment in VM as vulvar cancer surgery is associated with serious functional and psychosexual impairment. [23]

As in CM, SLN biopsy in VM may be used to direct adjuvant therapy with high-risk disease. Adjuvant treatment is recommended for CM patients with T4 tumours (with or without ulceration), T3 tumours with ulceration, or positive lymph nodes because these patients are at high risk for recurrence. [24, 25] Our study shows that most VM patients have high-risk disease with the majority of patients presenting with T3 of T4 tumours and/or ulceration (Table 1, Table 4, Figure 2EF). Primary surgery followed by adjuvant radiation therapy has been used to maximize locoregional control in VM. [26] In our study, only 10 of 180 of patients received adjuvant radiotherapy. Therefore, we were unable to unravel the associations of local control and adjuvant radiotherapy, and thus the use of radiotherapy alongside conservative surgical approaches requires further study.

Immune checkpoint inhibition (ICI) with anti-CTLA-4 and anti-PD-1 have improved survival for unresectable or metastatic CM and are now standard of care for patients with high-risk (i.e., AJCC stage III and resected stage IV) and advanced (i.e., irresectable stage IIIC and IV) CM. [27-30]. The efficacy of anti-CTLA-4 and anti-PD-1 antibodies has not been specifically evaluated in larger cohorts of patients with MM and prospective trials in VM have not been performed. Although some studies have suggested clinical benefit in MM, response rates seem to be lower than in CM. [10] Subgroup analysis of large melanoma studies have demonstrated that ipilimumab (anti-CTLA4) has shown anti-tumour response in 12% of the advanced MM. [31] A pooled analysis by d'Angelo et al. evaluated nivolumab (anti-PD1) alone (86 patients) or in combination with ipilimumab (35 patients) in unresectable stage III and stage IV MM patients. [32] The objective response rate (CR or PR) for anti-PD-1 monotherapy was 23.3% with a progression-free survival (PFS) of 3.0 months. For combination of nivolumab with ipilimumab the response rate was 37.1% with a PFS of 5.9 months. The Checkmate 238 trial included patients with MM (29 patients, 3.2% of total) and suggests RFS may be better with ipilimumab than nivolumab; however, this result was not statistically significant due to the small number of patients and events. [29] In our study, the response rate for anti-PD-1 therapy or combination therapy of anti-PD-1 and anti-CTLA-4 was 2/11 (18%) and 1/5 (20%), however, patient numbers are too small to draw definite conclusions. The suggested lower response rate of MM in comparison to CM might be explained by the different genomic landscape of MM. Whole genome sequencing data from MM demonstrated a low mutational burden without any evidence of UV signature, but numerous large-scale copy number changes and whole chromosome gains and losses. [3,

33] A high mutational burden is associated with improved survival in patients receiving ICI across a wide variety of cancers, including melanoma. [34] Furthermore, density of tumour infiltrating lymphocytes seems to be decreased in MM compared to CM, supporting the hypothesis that MM is less immunogenic and consequently frequently primarily resistant to ICI. A recent study has demonstrated a survival benefit of high T-cell infiltration in a subgroup of patients with VM. [35] To improve the results of ICI in MM, future alternative or additional treatment strategies aimed at enhancing the immunogenicity of MM may be of interest. For example, combined radiotherapy and ICI bear the potential to create a synergistic anti-tumour response. [36,37]In addition, the use of oncolytic viruses has been shown a promising treatment modality in MM. Talimogene laherparepvec (T-VEC) is a genetically modified herpes simplex virus type 1 and augments the immunogenicity of melanomas by direct oncolytic effects. [38] T-VEC was recently shown to be effective and well-tolerated in a patient with advanced MM of the urethra after resistance to ICI. [39]

The analysis of advanced or metastatic melanomas for alterations in KIT, NRAS, and BRAF has become standard of care. [19] A recent study showed that the KIT mutation rate was the highest in VM (22%) compared with 3% in CM ($p<.001$) and 8.8% in other MM subtypes ($p=0.05$). [14] In our study, mutations were found in 14 of 43 (32.6%) of analysed tumours with KIT mutations being the most frequent (18.6%) whereas BRAF, NRAS, GNAQ and Tp53 mutations were rare. (Table 1). A recent study in 73 patients with unresectable MM, including 8 patients with VM, showed that patients with KIT-positive tumours had a PFS and OS of 2.7 months and 11.8 months, compared with 0 and 6.9 months for KIT-negative tumours, respectively. [40] The differences were not significant due to small patient numbers.

The main strength of our study is that this is one of the largest series that extensively describes the clinical, histopathological and treatment characteristics in relation to clinical outcome in patients with VM. Of course, this study has limitations besides its retrospective design. First, no central histopathologic revision was performed limiting the reliability of the histopathological characteristics. Second, our cohort over 27 years in eight different medical centres has resulted in a large but also heterogeneous dataset.

In summary, VM is an extremely rare malignancy with aggressive behaviour, which represents a challenge for gynaecological oncologists and medical oncologists in terms of early diagnosis, clinical and genetic characterization, and treatment. We would like to emphasise that all pigmented and nodular vulvar lesions should be considered potentially harmful in postmenopausal women and deserve to be biopsied in order to obtain correct diagnosis and implement early treatment. While complete surgical excision with negative margins offers the only prospect of cure, the challenging anatomical site in VM presents a high risk of surgical morbidity and most patients still develop incurable metastatic disease with survival of less than one year. In contrast to CM, survival did not show any

improvement over the last decade. Increased knowledge of tumour biology, genetics, and immune microenvironment may result in future VM-specific clinical trials focusing on adjuvant therapy in and therapy for metastatic disease. Specifically, insights into the primary and metastatic VM immune microenvironment and mechanisms driving tumour progression, will pave the way for the identification of targets for future therapies. Therefore, research should be focused on testing novel promising therapies, and international collaboration in clinical trials to increase patient numbers is highly needed. This will hopefully increase the survival benefit of VM patients similarly to what has recently been observed for CM.

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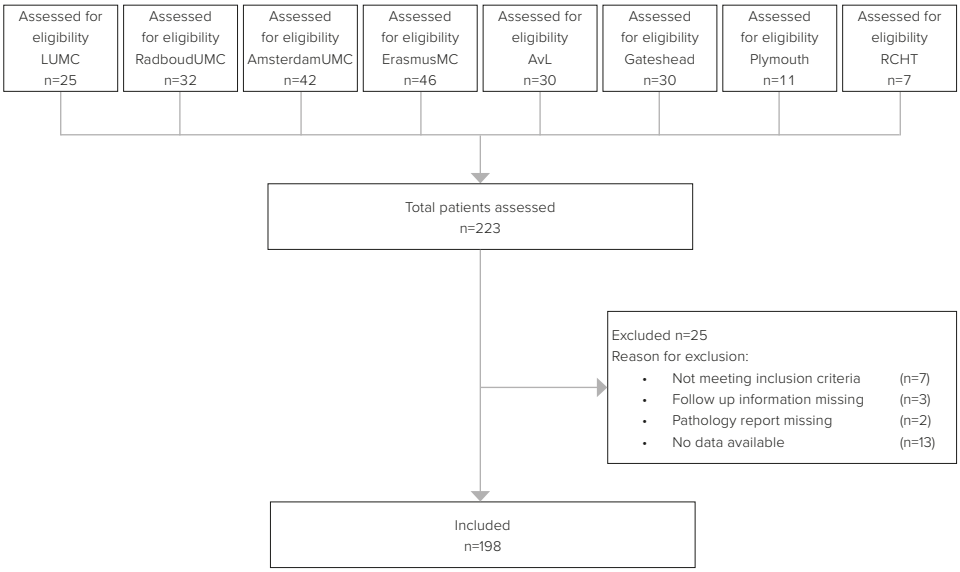
Supplementary material

S1. AJCC staging (2009) for cutaneous melanoma (2009(
See online: https://www.gynecologiconcology-online.net/article/S0090-8258(21)00078-0/fulltext

S2. Flowchart of patient inclusion and exclusion in this study



LUMC: Leiden University Medical Centre
Radboud UMC: Radboud University Medical Centre
Amsterdam UMC: Amsterdam University Medical Centre
Erasmus MC: Erasmus Medical Centre
AvL: Antoni van Leeuwenhoek
RCHT: Royal Cornwall Hospital NHS Trust

S3. Clinical and histological characteristics for 1990-2009 and 2010-2017

Clinical characteristics	Diagnosed 1990-2009 (N=120)	Diagnosed 2010-2017 (n=78)	p value
Age at diagnosis [years, median, IQR]	70 (58;80)	73 (64; 82)	0.309
Symptoms at presentation			0.622
Yes	12 (12.5)	8 (13.3)	
No	2 (2.1)	1 (1.7)	
unknown	41 (42.7)	19 (31.6)	
Location on the vulva			0.271
unilateral	80 (66.7)	52 (66.7)	
clitoris	20 (16.7)	13 (16.7)	
multifocal	15 (12.5)	9 (11.5)	
missing	5 (4.2)	4 (5.1)	
Pathologic T stage			0.098
T1	6 (5.0)	8 (10.3)	
T2	7 (5.8)	3 (3.8)	
T3	30 (25.0)	9 (11.5)	
T4	67 (55.8)	49 (62.8)	
Tx	10 (8.3)	9 (10.3)	
AJCC stage (2009)			0.250
Stage IA	2 (1.7)	5 (6.4)	
Stage IB	6 (5.0)	5 (6.4)	
Stage IIA	8 (6.7)	3 (3.8)	
Stage IIB	30 (25.0)	13 (16.7)	
Stage IIC	49 (40.8)	29 (37.2)	
Stage III	13 (10.8)	11 (14.1)	
Stage IV	7 (5.8)	9 (11.5)	
Unknown	5 (4.2)	3 (3.8)	
Breslow thickness (median) [mm, IQR]	7.0 (3.0;12.0)	7 (3.4; 15.0)	0.935
Tumour size (median) [mm, IQR]	19.0 (9.5; 26.5)	20.0 (11.0; 35.0)	0.480
Melanoma subtype			0.539
superficial spreading	45 (37.5)	28 (35.9)	
lentigous	3 (2.5)	5 (6.4)	
nodular	42 (35.0)	29 (37.2)	
unclassified	4 (3.3)	4 (5.1)	
missing	26 (21.6)	12 (15.4)	
Ulceration			**0.024**
yes	80 (66.7)	52 (66.7)	
no	11 (9.2)	19 (24.4)	
missing	29 (24.2)	7 (9.0)	
Mitotic activity			0.173
yes	62 (51.7)	53 (68.0)	
no	3 (2.5)	8 (10.3)	
missing	50 (41.7)	17 (21.8)	

S3. (*Continued.*)

Clinical characteristics	Diagnosed 1990-2009 (N=120)	Diagnosed 2010-2017 (n=78)	p value
Microsatelossis			0.455
yes	12 (10.0)	8 (10.3)	
no	39 (32.5)	42 (53.8)	
missing	69 (57.5)	17 (21.8)	
Angiolymphatic involvement			0.422
yes	23 (19.2)	18 (23.1)	
no	29 (24.2)	34 (43.6)	
missing	68 (56.7)	26 (33.3)	
Regressive changes			
yes	16 (13.0)	32 (41.0)	0.083
no	11 (9.2)	9 (11.6)	
missing	73 (77.8)	37 (47.4)	
Mutation status			0.949
Not analysed	110 (91.7)	45 (57.7)	
Analysed			
No mutation	8 (6.7)	21 (26.9)	
BRAF	0 (0.0)	2(2.6)	
KIT	1 (0.8)	6 (7.7)	
BRAF+ KIT	0 (0.0)	1 (1.3)	
NRAS	1 (0.8)	0 (0.0)	
GNAQ	0 (0.0)	2 (2.6)	
Tp53	0 (0.0)	1 (1.3)	
Recurrence*			**0.003**
yes	83 (69.2)	37 (47.4)	
no	31 (25.8)	36 (46.2)	
missing	6 (5.0)	5 (6.4)	
Location of first recurrence (n=114)			0.327
local	25 (30.9)	15 (45.5)	
locoregional	14(17.3)	2 (6.0)	
regional	18 (22.2)	7 (21.2)	
distant	24 (29.6)	9 (27.3)	
missing	2 (2.4)	4 (12.0)	
Median time to first recurrence [months, IQR]	12 [6,27]	10 [6,22]	0.346
Location of second recurrence (n=57)			0.087
local	4 (10.0)	3 (17.6)	
locoregional	0 (0.0)	2 (11.8)	
regional	3 (7.5)	0 (0.0)	
distant	33 (82.5)	12 (70.6)	
Median time from first to second recurrence (months)	7 (2.75-13.25)	9 (5.5-20.0)	0.651

* of the surgically treated patients

S4. Treatment characteristics for 1990-2009 and 2010-2017

Treatment characteristics	Diagnosed 1990-2009 (N=120)	Diagnosed 2010-2017 (N=78)	p value
Treatment modality			
surgery	105 (87.5)	60 (76.9)	0.042
surgery plus adjuvant therapy	9 (7.5)	6 (7.7)	
other	3 (2.5)	6 (7.7)	
radiotherapy of vulva	2 (1.7)	1 (1.3)	
radiotherapy of vulva + immunotherapy	0 (0.0)	1 (1.3)	
radiotherapy of metastasis	0 (0.0)	1 (1.3)	
neoadjuvant immunotherapy + palliative resection	0 (0.0)	1 (1.3)	
elective lymph node dissection	1 (0.8)	0 (0.0)	
immunotherapy	0 (0.0)	2 (2.6)	
unknown	1 (0.8)	2 (2.6)	
no treatment	2 (1.7)	4 (5.1)	
Type of surgical treatment of primary tumour			0.222
wide local excision	98 (81.7)	58 (74.4)	
hemivulvectomy	8 (6.7)	3 (3.8)	
radical vulvectomy	6 (5.0)	2 (2.6)	
radical vulvectomy and vaginectomy	2 (1.7)	3 (3.8)	
Lymph node involvement *			
positive	14 (11.7)	15 (19.2)	0.236
negative	42 (35.0)	34 (43.6)	
not assessed	64 (53.3)	29 (37.2)	
Lymph node treatment			
not conducted	56 (49.1)	34 (51.5)	0.040
SLN	29 (25.4)	20 (30.3)	
SLN + IFL	7 (6.1)	3 (4.6)	
IFL	13 (11.4)	8 (12.1)	
lymph node debulking	2 (1.8)	0 (0.0)	
radiotherapy	4 (3.5)	1 (1.5)	
unknown	3 (2.6)	0 (0.0)	
Resection margins			
negative	81 (67.5)	47 (60.2)	0.060
< 10 mm	37 (30.8)	27 (34.6)	
≥ 10 mm	18 (15.0)	12(15.4)	
< 2 mm	5 (4.2)	2 (2.6)	
≥ 2 mm	50 (41.6)	37 (47.4)	
not specified	26(21.6)	8 (10.2)	
positive	19(15.8)	18 (23.0)	
unknown	14 (11.6)	1 (1.3)	
Reexcision performed			
yes	43 (37.7)	22 (33.3)	0.375
no	69 (60.5)	44 (66.6)	
unknown	2 (1.8)	0 (0)	
* pathologically or radiologically confirmed			

SLN sentinel lymph node, IFL inguinal- femoral lymphadenectomy

S5. Treatment of local, locoregional, regional and distant recurrences

Treatment modality	Local (n=47)	Locoregional (n=18)	Regional (n=28)	Distant (n=78)
WLE	16 (34.0)	1 (5.6)		
WLE + iLND	1 (2.1)	1 (5.6)		
WLE + iLND + groin RT		2 (11.0)		
WLE+ iLND + local RT		1 (5.6)		
WLE + local RT	4 (8.5)			
WLE + iLND + groin RT + CT		1 (5.6)		
WLE + LN debulking		1 (5.6)		
HV	4 (8.5)	1 (5.6)		
RV	3 (6.4)			
RV+ AUE + vaginectomy		2 (11.0)		
RV + local RT	1 (2.1)			
iLND		1 (5.6)	14 (50.0)	
iLND + groin RT		1 (5.6)	7 (25.0)	
Bilateral iLND			2 (7.2)	
LN debulking + RT groin + interferon			1 (3.6)	
Groin RT			1 (3.6)	1 (1.3)
Local RT	6 (12.8)	2 (11.0)		
IT + groin RT		1 (5.6)		
IT		1 (5.6)	1 (3.6)	9 (11.6)
CT				6 (7.7)
CT + IT				3 (3.8)
CT + pelvis RT		1 (5.6)		3 (3.8)
CT + IT + RT local				2 (2.6)
CT + IT + excision metastasis				1 (1.3)
Symptomatic treatment	3 (6.4)	1 (5.6)	1 (3.6)	25 (32.0)
Palliative excision + local RT				2 (2.6)
Palliative RT				14 (17.9)
Palliative excision metastasis	2 (4.3)			
Laparatomy with excision of metastasis				1 (1.3)
Lasertreatment of the vulva	1 (2.1)			
Craniotomy				1 (1.3)
IT + RT metastasis				6 (7.7)
Unknown	6 (12.8)		1 (3.6)	4 (5.1)

WLE: wide local excision, SLN: sentinel lymph node, iLND: inguinal lymph node dissection, RT: radiotherapy, CT: chemotherapy, HV: hemivulvectomy, RV: radical vulvectomy, AUE: abdominal uterus extirpation, IT: immunotherapy

General discussion and future perspectives

7

Mucosal melanoma (MM) is a rare melanoma subtype characterized by its unique tumour biology, aggressive course of disease and poor prognosis. [1] Whilst in CM significant progress in preventive and therapeutic strategies has been made, in MM progress has been slow and lags behind that of its cutaneous counterpart. [2-6] For unresectable stage III and stage IV CM, the introduction of immune- and targeted therapy in 2011, has revolutionized the therapeutic landscape. Both have resulted in improvement of overall survival (OS) for advanced CM. [6] Unfortunately, the prognosis of MM is still lagging behind of CM and has not improved over the past decades.

The worse prognosis of MM is partly ascribed to the low incidence of this disease, impeding large studies and clinical trials. Moreover, historically, treatment of localized MM is done by the doctor who is specialized in cancer at the primary tumor site. For instance, MM located at the female genital tract is treated by a gynaeco-oncologist whilst MM located at the head and neck is treated by an otolaryngologist. Consequently, for a long time, diagnostic approach, staging, treatment of local disease and most important research, did not cross the borders set by the doctor's field of interest. Fortunately, within national healthcare systems, there has been a significant improvement in collaboration leading to the concentration of care for rare diseases like MM. Within these hospitals, multidisciplinary teams work together to evaluate diagnostic and treatment strategies using the expertise of doctors across multiple disciplines. Still, to improve the care of MM, it is crucial to gain a deeper understanding of the biological behaviour and course of disease, in which international collaboration can be a powerful tool.

In this thesis, by collaborating with national and international hospitals, we analyzed clinical and histopathological characteristics and survival of a large cohort of vulvar melanomas (VM). Moreover, we reviewed the literature of VM resulting in a flow-chart In part 2 of this thesis, we assessed incidence of and analyzed trends of survival over time against the background of the new era of immune- and targeted therapy. Therefore, we used the well-functioning national cancer registry (NCR) and Dutch Melanoma Treatment Registry (DMTR), both characterized by their high coverage of patients diagnosed with any tumor or type of melanoma in the Netherlands. The DMTR includes clinical, pathological and treatment characteristics, making it a valuable database which can be used to evaluate the effect of the quickly accelerating landscape of systemic therapies in melanoma. The use of NCR data provided insights in incidence and survival over more than thirty years. This final discussion will summarize the key points of this thesis and will focus on the future by addressing new perspectives and possible new treatment options.

Understanding the course of disease

In **Chapter 2** we observed that whilst in the Netherlands, incidence of CM has increased, the incidence of MM has remained stable with approximately 50 new cases every year. [6] Moreover, we found that whilst in CM there has been a shift to more patients with localized disease at presentation, in MM stage at presentation has not changed and patients often present with advanced disease. [6] In total, MM presents with distant metastasis in 15.1-23.6% of the cases whilst in CM this is the case in only 4% of the cases. [7, 8]

In CM, detection at lower stage of disease through preventive measures, as effective screening programs has been essential for improving survival rates. However, a screening program is often driven by the prevalence of the disease being screened for. Therefore rare diseases are not part of these programs. For less common cancers, creating awareness is the best preventive measure. Apart from MM located at the exterior mucosal lining of the vulva and the penis, the lack of visibility of MM makes it difficult to detect and monitor, and doctors delay and patients delay is not uncommon. As in most cancers, stage at diagnosis largely impacts survival. We found a median OS and 1-year OS of 2.4 years and 77.2% whilst in distant spread disease this is 0.6 years and 31.4% (**Chapter 2**). As in other types of cancer, metastatic disease is the leading cause of cancer-related mortality in MM and creating awareness for these cancer types in both patients and doctors may help to minimize delay in diagnosis and treatment and thus lower stage at diagnosis.

Yet, even localized MM harbour a poor prognosis and therefore, preventive strategies may not even be the way to go. We found that 5-year OS rates between stage I MM and stage I CM differed significantly (30.8% vs 71%-100%) (**Chapter 2)**. [9, 10] This striking 40-70% survival gap is mainly due to the high recurrence rates and almost half of the patients developing regional or distant metastases. Understanding the course of disease and metastatic pattern may help to optimize the frequency and method of imaging in the follow-up, identifying spread of disease in an earlier phase.

The largest analysis of metastatic spread includes 706 patients with MM, of which 152 (21.5%) with nodal spread disease (stage III) and 163 (23.0%) with distant spread disease (stage IV). Of those who were diagnosed with, or developed, stage IV disease, disease most often spread to the lung, liver, distant lymph nodes or to both lung and liver, in respectively 21%, 19%, 9% and 7% of the patients. [8] An important note is that in the majority of the liver only or lung only metastasized cases, there were multiple metastasis (87.0% and 78.5%). Results of a smaller cohort were similar, though patients presented with disseminated metastatic disease in 19% of the cases. [7] Given the relatively low efficacy of systemic treatments for MM, local treatment of metastatic disease may represent a viable approach to delay the use of systemic treatments accompanied with high toxicity levels, and to improve OS.

Furthermore, gaining knowledge of the course of disease may allow early identification of patients who have a high risk of rapid disease progression and who may not benefit from (local) surgical treatment of the primary tumor. Though, surgeons aim for minimal resection margins, local excision often still consists of extensive surgery affecting quality of life.

In the literature, vulvar and vaginal melanomas are frequently grouped together and analysed as one entity **(Chapter 5).** However, studies that have specifically focused on vulvar and vaginal melanomas as separate entities have demonstrated a distinct course of disease. [11] Noticeable, is the worse prognosis of vaginal melanoma when compared to vulvar melanoma. In **Chapter 6,** we analysed 198 patients with vulvar melanoma and found a median OS of 33 months whilst the literature reports a median OS of 10-24 months for vaginal melanoma. [11, 12] The worse prognosis of vaginal MM may be explained by a delay in diagnosis, as the MM may be less visible, resulting in a higher stage at diagnosis. [13] Moreover, whilst the vagina exists of solely mucosal lining, the vulva exhibits both hairy skin (cutaneous lining) and glabrous skin (mucosal lining). Thus, some vulvar melanomas arise from cutaneous skin and therefore these can may also have more resemblance with CM than with MM. As CM is known for the better prognosis, this could explain better outcomes in vulvar melanomas as compared to vaginal melanomas. [14]

Unraveling the tumour biology: towards novel treatment strategies in MM

The tremendous developments in systemic treatment, particularly immune- and targeted therapy, have improved the survival of advanced CM. [15, 16] This rising tide, however, has not lifted all the boats. In **Chapter 3**, we found that since 2015, immune- and targeted therapy more often were part of treatment in MM, but that survival of advanced disease did not improve during the same time period. Moreover immunotherapy had a lower objective response rate (ORR) as compared to CM (20.6% vs 37.8%) and median OS of patients treated with systemic therapy, was 6 months lower in MM when compared to CM (11.8 months vs 17.9 months). Clinical trials have demonstrated that combined anti-CTLA-4/anti-PD-1 treatment in CM has a higher ORR as compared to anti-CTLA-4 and anti-PD-1 monotherapy (58% vs 45% and 19%) [17] In **Chapter 4** we analysed 46 patients with MM who received combined ipilimumab/nivolumab and ORR was 39.2% (n=18/46) of which five responded completely. Though the efficacy seems to be higher than single agent immunotherapy, median OS is only 9.7 months and approximately 60% of the patients does not respond, thus leaving the majority without an effective treatment strategy.

It has long been recognized that MM is biologically different than CM. A lower tumor mutational burden (TMB) and distinct oncogenic mutations are reported. [18-20] In particular

MM harbour a lower rate of BRAF mutations as compared to CM, 5.9% and 55.9% respectively **(Chapter 3).** Consequently, the highly efficacious BRAF/MEK inhibitors (vemurafenib/cobimetinib dabrafenib/trametinib and encorafenib/binimetinib), ensuing durable responses, are of less use in MM. [19-21] MM however is characterized by a relatively higher rate of KIT mutations (13-23% vs 3% in CM). [19-21] A systematic review reported an ORR of 14% of in MM treated with (any) KIT-inhibitor, though durability of response was reported inconsistently making a meta-analysis not feasible. [22] Median time to progression in a study evaluating 25 melanoma, of which the majority MM, was 3.7 months and eventually all but one had progressive disease. [23] Thus, though (targetable) KIT mutations are seen in a subset of MM, the low efficacy of KIT inhibitors, underscore the urgence for different systemic agents or new combinations of existing agents. However, as seen in **Chapter 6**, genetic analysis is only performed in the minority of the cases, resulting in incomplete understanding of the genetic landscape.

It is widely accepted that, tumour infiltrating lymphocytes (TIL's), represent the local anti-tumour immune response in pathological assessed tumours. [24] In various types of cancer, including CM, the presence of CD8+ cytotoxic T-cells, CD4+ helper T-cells and memory T-cells, is associated with better survival and a better response to immune checkpoint inhibitors (ICI) whilst the presence of regulatory T-cells (T-reg's) around the tumour is associated with worse prognosis and a lower response to ICI. [25-29] Though based on small studies, MM seem to have a lower level of TIL's and a higher level of T-reg's, compared to CM. [27, 30-33] The immune microenvironment of MM in relation to survival and ICI response has not been studied, thus far reaching conclusions cannot be made. If it follows the pattern of CM, the lower number of TIL's, may explain the relative resistance to ICI in MM.

Exploring the immune microenvironment of MM can give valuable insights in the potential drivers of the lower response to ICI. These, then can provide an avenue by minimizing the number of patients treated with ICI without gaining survival advantage, though being at risk of ICI related toxicity. [34] Moreover, the presence of TIL's may be of future benefit when considering TIL therapy, which already has demonstrated to improve PFS and OS in patients with advanced CM. [35]

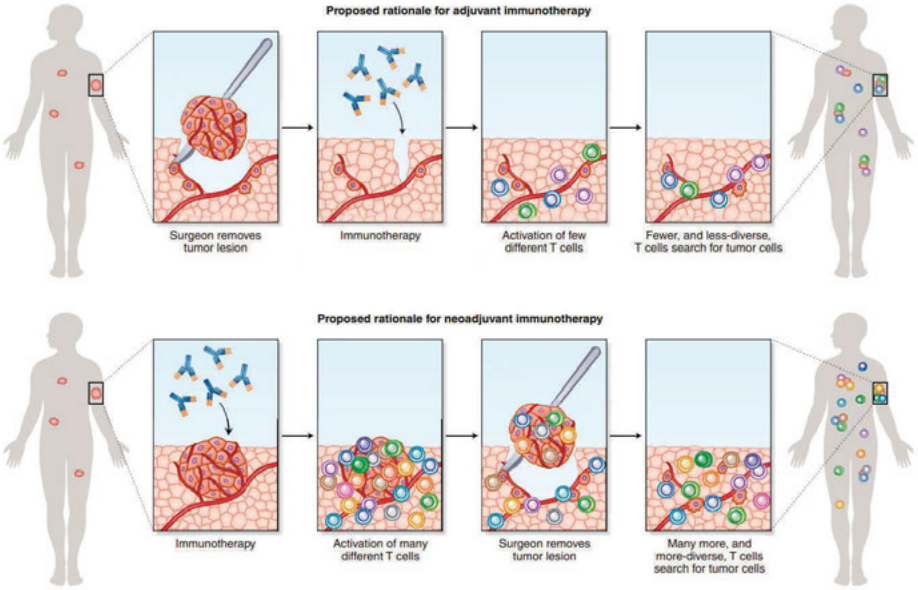
(Neo)-adjuvant immunotherapy

The striking evidence regarding immunotherapy improving OS and progression-free survival (PFS) in metastatic CM is indisputable. Furthermore, in adjuvant setting, immunotherapy lowers the risk of recurrence in stage IIB, IIC and high-risk clinical stage III CM. Still, despite the use of adjuvant immunotherapy clinical stage III CM has suboptimal outcomes, can give

life-lasting side-effects and often recurs, sometimes even before the patient has started with adjuvant therapy [36]. Therefore, there is a high need for new (combinations of) therapeutic agents, better timing of therapy and improved treatment regimes.

Preclinical trials studying mice with breast cancer, demonstrated that neoadjuvant immunotherapy improved OS and can tackle occult distant metastasis when compared with the same immunotherapy in adjuvant setting. [37] The hypothesis for better outcomes of those who received ICI before surgery when compared with those who received it following surgery, is that the tumor load in situ can establish a higher immune response when the immune microenvironment of the tumor is still intact (Figure 1). [38, 39] This neoadjuvant approach is emerging and many clinical trials evaluating efficacy and safety of different (combinations of) ICI in CM, are ongoing.

Figure 1. Rationale for neoadjuvant immunotherapy



Adapted from "Learning from clinical trials of neoadjuvant checkpoint blockade" Versluis et al. Nature Medicine, 2020. Reprinted with permission of the publisher Springer Nature

The OpACIN (phase I) trial and OpACIN-Neo (phase II) trial, assessed the pathological response (PR), safety and dosing schedule of neoadjuvant immunotherapy in resectable stage III CM. [40, 41] In the OpACIN study, PR was seen in 78% (7/9) of the cases of which, at a median follow-up of 4 years, none relapsed. [40, 42] The OpACIN-neo trial demonstrated a PR of 74% (64/86), of which at 2 years recurrence-free survival (RFS) was 97% for those with

PR and 36% for those with without PR, suggesting that PR can be predictive for RFS. [41, 42] In advanced CM (resectable stage III and stage IV), a phase II clinical trial demonstrated that neoadjuvant-adjuvant pembrolizumab compared with adjuvant pembrolizumab, provides a higher event-free survival (EFS) and that treatment related adverse events are similar. [43] In MM, two phase II clinical trials assessing neoadjuvant anti-PD-1 therapy with or without lenvatininb (a multi-targeted tyrosine kinase inhibitor) are currently open (NCT05545969 and NCT03313206), but results are awaiting. Up to today, only few studies assessing neoadjuvant therapy in MM are published. A retrospective assessment of 21 stage II/III resectable MM treated with toripalimab and axitinib by Cui et al found PR in 28.6% of the cases and median RFS of 56 weeks. [44] A second study assessed 36 stage II/III MM treated with neoadjuvant anti-PD-1, anti-CTLA-4 or combined anti-PD-1/anti-CTLA-4. PR was seen in 35% of the cases and median EFS, defined as time to progression, recurrence or death, was 40 weeks. [45] As in the adjuvant setting, the efficacy of neoadjuvant immunotherapy in MM is lower than in CM. [41]

Besides a stronger and broader T-cell response leading to longer PFS, neoadjuvant therapy can also reduce the size of bulky tumors. As surgery, with R0 resections improving RFS and OS, is the mainstay of treatment in MM, less extensive surgery has high priority. [46] In the study by Ho et al, 36 patients with resectable MM were treated with neoadjuvant immunotherapy, of which 3 patients (8%) had complete response and did not require surgery. However, in 6 cases (17%) the primary tumor progressed and was unresectable. This reveals the possible downside of neoadjuvant immunotherapy as delaying surgery may negatively impact the window of opportunity of resection of the primary tumour. However, a different perspective, is that the identification of non-responders who disseminate rapidly, is not a lost chance but a way to prevent those with an unfavorable prognosis, to undergo surgical treatment without survival benefit. [47] Though no studies are available assessing neoadjuvant immunotherapy in unresectable CM or MM the REDUCTOR trial studied BRAF/MEK inhibitors in unresectable regionally advanced CM. This resulted in shrinkage of the tumour leading to a resectable tumour in 18/21 cases of which 17 had an R0 resection. [48] If PR can become an established early endpoint, it may help to accelerate approval of neoadjuvant strategies in MM.

Lastly, an important benefit of neoadjuvant strategies is the opportunity to personalize treatment based on PR. In many types of cancer major pathologic response (MPR), defined as 10% or less residual viable tumour cells after neoadjuvant therapy, is used as outcome marker. Similarly in CM, the international neoadjuvant melanoma consortium conducted a pooled analysis of six neoadjuvant trials and demonstrated that in CM PR is associated with RFS and OS and is proposed as standard. [47] Thus, complete PR brings the opportunity to de-escalate (surgical) treatment, whilst poor response can encourage medical doctors to adjust or to add components to the treatment strategy. This individualized type of care

has been studied in the PRADO trial in which patients with clinical stage III nodal melanoma were treated with neoadjuvant immunotherapy. Nodal dissection with or without adjuvant therapy was performed in those with no or partial response, whilst in responders these were omitted, without affecting OS. [49]

Future perspectives

Experimental therapies in MM, follow the footsteps of CM. Here, we will shine the light on more experimental therapies. One of those, which currently is investigated, are antiangiogenic agents as axitinib. Vascular endothelial growth factor (VEGF) inhibition can hamper the ability of VEGF to sustain tumour growth and enhance tumour survival and therefore may be limit progression of disease in the highly vascularized MM. [50, 51] After 3-year follow-up of a phase 1B study including 33 advanced MM treated with axitinib combined with toripalimab (a humanized immunoglobulin G_4 monoclonal antibody against PD-1) demonstrated a ORR of 48.7% and a median PFS of 7.5 and median OS of 20.7 months. [52, 53] Real-world data of MM treated with anti-VEGF combined with anti-PD-1 therapy (of which half was treatment naïve and half received this as first line treatment) demonstrated a lower ORR of 24.5% but disease control was seen in 72.7% and a ORR of 30.0% in treatment naïve patients. [54] These less convincing results in a real-world setting ask a phase III or randomized trial to evaluate the effect of anti-VEGF in combination with immunotherapy.
As already mentioned earlier, adoptive cell therapy (of which T-cell receptor therapy and tumour infiltrating lymphocytes therapy) is one of these empirical treatment strategies. In CM, this has shown promising results in terms of antitumor activity and survival, whilst in MM this topic needs more evidence. [55] Moreover, local interleukin-12 combined with ICI have recently been evaluated in phase I and II trials including metastatic CM, and are promising. IL-12 is a pivotal immune regulator and has major anti-tumor effects as it inhibits tumor growth by increasing infiltration of CD8-T cells and decreasing T-regs's, but is related with alarming toxicity when administered systemically. [56, 57] Local IL-12 in stage IV CM yielded an ORR of 35.7%, also affecting distant sites other than the location of administration, and an acceptable safety profile. [58] Moreover, even in non-immune infiltrated tumours (cold tumours) which are known to be ICI resistant, IL-12 combined with anti-PD-1 showed promising results. ORR was 41%, of which 42% of the responders were anti-PD-1 refractory and ORR was 30% in a cohort consisting of solely anti-PD-1-refractory advanced CM. [59] IL-12 in combination with anti-PD-1 therapy is worth evaluating in MM, as they generally are poor responders to ICI.

Final conclusion

Over the past decade, rare cancers, including MM, have been studied at a higher pace. Still, cohorts are small and patients with MM are often excluded from clinical trials. Therefore only little evidence regarding novel therapeutic agents in MM is available. While small studies are providing some guidance, the development of effective strategies for advanced MM is progressing slowly. To improve outcomes in MM, there is a critical need for clinical trials specifically designed for this disease. Additionally, translational research can play a pivotal role in improving our knowledge of tumor biology and immune response in MM. To address the challenges ahead, there should be a focus on new combinations of existing therapies and shifting the timing and sequence of existing and novel therapeutic agents. Lastly, treating patients in the neoadjuvant setting, aiming to overcome occult metastasis, holds significant promise as a potential breakthrough.

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Appendices

Summary

Part I Mucosal melanoma

In Chapter 1 of this thesis, a comprehensive overview of the epidemiology, biology, treatment, and clinical outcomes of mucosal melanoma (MM) is provided. This rare tumour comprises 1.4% of all melanomas and has an incidence of 2.2 cases per million with approximately 850 new cases per year in Europe. MM arise from the mucosal lining of which the majority is located at the head and neck region, gastrointestinal tract, and female genital tract. The latter comprises 15-20% of all MM, explaining the higher incidence in females as compared to males. For local spread MM, wide local excision aiming for R0 resection is the primary treatment. For higher stages of disease, optimal treatment strategy is not well-established. Studies assessing the treatment strategy for nodal disease are limited and the reported outcomes of advanced MM treated with any type of systemic treatment are disappointing. Therefore, whilst MM is distinct from cutaneous melanoma, treatment for regional and distant spread MM follows the guidelines of its well-studied cutaneous counterpart.

In Chapter 2 we analyzed incidence and survival of MM over a thirty-year period (1990-2019) in the Netherlands. We emphasized on assessing trends in treatment and survival over time, by comparing the timeperiod 2014-2019 with all other years. The Dutch population was analysed using the nationwide population-based database registering all clinical, tumour and treatment characteristics together with survival of all newly diagnosed cases with cancer in the Netherlands. In this thirty-year period 1496 patients were diagnosed with MM and incidence over time remained stable. We confirmed the poor prognosis with 5-year overall survival (OS) of 24% and a median OS of 1.7 years. OS improved significantly when comparing patients diagnosed between 2014-2019 with all other years. We identified, diagnosis in 2014-2019, MM located at the female genital tract and primary treatment with immune- or targeted therapy as independent predictors for better survival and MM located at the respiratory tract, higher age, and higher stage at diagnosis as predictors for worse survival.

Next, Chapter 3 focusses on patients with advanced stage mucosal and cutaneous melanoma. Using data from the Dutch Melanoma Treatment Registry (DMTR), we investigated clinicopathological characteristics and survival of 120 MM and 2960 CM diagnosed between 2013-2017. In this cohort the median OS in advanced stage MM was lower than CM (8.7 months vs 14.5 months). Whilst OS improved for patients with CM when comparing those diagnosed between 2013-2014 with those diagnosed between 2015-2017, for MM survival did not improve when comparing the same time periods.

To take a closer look to the efficacy of immunotherapeutic agents in rare melanomas, in Chapter 4 we assessed 46 patients with MM and 13 patients with UM treated with combined

ipilimumab/nivolumab treatment. Approximately half of the patients had clinical benefit, but median OS was short. Moreover, as seen in CM, toxicity rates were high leading to discontinuation in most of the cases. The results of Chapter 3 and Chapter 4 demonstrate, that whilst immunotherapeutic agents have revolutionized the therapeutic landscape in CM, in MM, the efficacy is low and survival has not improved. This emphasizes the need for trials specifically focusing on novel (combination) of treatment strategies in MM.

Part II Vulvar melanoma

Part 2 of this thesis shifts toward an important subgroup of MM, those located at the vulva. Vulvar melanomas (VM) account for 60% of the female genital tract MM, which together with the head and neck region and the gastrointestinal tract are the most common locations of MM. Chapter 5 provides a general review including clinical characteristics, pathological characteristics, treatment, and survival of VM. This disease presents with an itching or bleeding pigmented lesion at the labia majora, labia minora or clitoris and is characterized by low survival rates and high recurrence rates. As in CM higher age, advanced Breslow stage and lymph node involvement are predictors for survival. Due to the anatomical challenging location in close relation to vital organs, local treatment of VM can be mutilating and affect quality of life. Unfortunately, as in MM, the efficacy of radiotherapy, chemotherapy, and immunotherapy are low, and the optimal management of regional and distant spread disease is still being investigated.

In Chapter 6 an international cohort of 198 VMM's was retrospectively analysed. Median age at diagnosis was 72 years, and most of the patients were symptomatic. Still, median time from symptoms to diagnosis was four months, which is partly ascribed to patient's delay. At presentation, 75.8% had had locally spread disease, 12% had regionally disease, 8% had distant spread disease and in 4% stage at presentation was unknown. However, pathological analysis demonstrated that more than half of the patients had a Breslow thickness > 4 mm (i.e. highest T stage). The aggressive course of VM was demonstrated by 2 and 5-year OS of respectively 48% and 31%. Moreover, recurrence occurred in two third of the patients of which the majority were regional or distant recurrences with a median time to recurrence of 11 months. We found that higher age and larger tumour diameter were independent predictors for survival. In conclusion, this study shows that even whilst the majority of the patients presents with early stage disease, recurrence rates are high and prognosis is poor.

Nederlandse samenvatting

Deel 1: mucosaal melanoom

In dit proefschrift presenteren we studies over een zeldzaam subtype van het melanoom, het mucosaal melanoom (MM). Net als alle andere varianten van het melanoom ontstaat MM vanuit de maligne proliferatie van melanocyten, welke in dit specifieke subtype gelegen zijn in het slijmvlies. Het merendeel van de MM is gelokaliseerd in het hoofd-halsgebied, het maag-darmkanaal en de vrouwelijke geslachtsorganen. De laatstgenoemde omvat 15-20% van alle gevallen van MM, en verklaart daarmee de hogere incidentie van MM bij vrouwen. In Hoofdstuk 1 geven we een uitgebreid overzicht van de epidemiologie, pathogenese, behandeling en klinische uitkomsten van het MM. Het MM beslaat ongeveer 1.4% van alle melanomen en heeft een incidentie van 2.2 gevallen per één miljoen personen, met ongeveer 850 nieuwe gevallen per jaar in Europa. De hoeksteen van de behandeling van lokaal beperkte ziekte is chirurgie. Dit bestaat uit een ruime lokale excisie, waarbij wordt gestreefd naar tumor-vrije resectiemarges. Er is geen wetenschappelijke consensus over de optimale behandeling voor patiënten met regionale of afstandsmetastasen. De behandeling van regionaal gemetastaseerde ziekte is weinig onderzocht en de uitkomsten van systemische behandelingen (chemotherapie, immunotherapie en doelgerichte therapie) in op afstand gemetastaseerde MM, zijn teleurstellend. Hoewel de etiologie en het ziektebeloop van MM evident verschilt van het cutane melanoom (CM), wordt tot op heden de behandeling van regionaal of op afstand gemetastaseerde MM gebaseerd behandelingen die ook worden toegepast bij CM.

In Hoofdstuk 2 is de incidentie en overleving van MM over een periode van dertig jaar (1990-2019) in Nederland geanalyseerd. In Nederland zijn vanaf 2014 immunotherapie en doelgerichte therapie voor melanoom geïntroduceerd. In deze studie is de 5-jaars overleving en mediane overleving over de tijd geëvalueerd door de periode van 2014-2019 te vergelijken met de voorgaande jaren. Met behulp van een landelijke database die alle klinische en tumor karakteristieken, behandeling en overleving van alle nieuw gediagnosticeerde kankerpatiënten in Nederland registreert, zijn alle patiënten met MM tussen 1990—2019 geanalyseerd. In deze dertig jaar werden 1496 patiënten gediagnosticeerd met MM. In tegenstelling tot het steeds vaker voorkomende CM, bleef de incidentie van MM over de decennia gelijk. De 5-jaars overleving was 24% en de mediane overleving 1.7 jaar. De mediane overleving van patiënten die gediagnosticeerd werden tussen 2014-2019 was aanzienlijk langer in vergelijking met de voorgaande jaren. Wij concluderen dat MM ontstaan vanuit het vrouwelijke geslachtsorgaan, een primaire behandeling met immuun- of doelgerichte therapie en het krijgen van de diagnose MM tussen 2014-2019, onafhankelijke voorspellers zijn voor een betere overleving, en MM gelokaliseerd in de luchtwegen, een hogere leeftijd bij diagnose en een hoger stadium bij diagnose, voorspellers zijn voor een slechtere overleving.

Hoofdstuk 3 richt zich op het vergelijken van uitkomsten van gemetastaseerd MM in vergelijking met CM. Met behulp van gegevens uit de Dutch Melanoma Treatment Registry (DMTR) hebben we de klinisch-pathologische kenmerken en overleving van 120 patiënten met MM en 2960 patiënten met CM die tussen 2013 en 2017 zijn gediagnosticeerd, geanalyseerd. De mediane overleving in gemetastaseerd MM was lager dan die van CM (8.7 maanden versus 14.5 maanden). In dit cohort werden vanaf 2015 de eerste patiënten met immuun- en doelgerichte therapieën behandeld. Hoewel de overleving van patiënten met gemetastaseerde CM gediagnosticeerd in 2015-2017 verbeterde in vergelijking met die van patiënten gediagnosticeerd in 2013-2014, verbeterde gedurende dezelfde tijdsperiode de overleving voor patiënten met MM niet. Dit illustreert dat, hoewel de introductie van immuuntherapie en doelgerichte therapie heeft geresulteerd in een betere overleving van patiënten met CM, dit in MM niet het geval is, en er voor gemetastaseerd MM nog vooruitgang moet worden geboekt.

In Hoofdstuk 4 focussen we op de effectiviteit van immunotherapie in zeldzame varianten van melanomen. In deze studie zijn 46 patiënten met MM en 13 patiënten met uveaal melanoom (UM) die behandeld zijn met ipilimumab/nivolumab geanalyseerd. In ongeveer de helft van de patiënten was er sprake van een radiologisch bevestigde complete respons, partiële respons of stabiele ziekte. Desondanks was de mediane overleving van patiënten met zowel UM als MM kort. Net als in CM, ervaarde een groot gedeelte van de patiënten toxiciteit door de behandeling met ipilimumab/nivolumab, wat bij het merendeel van de patiënten ook tot het voortijdig beëindigen van de behandeling leidde. De resultaten gepresenteerd in Hoofdstuk 3 en Hoofdstuk 4 laten zien dat hoewel de introductie van immunotherapie de behandeling en overleving van CM drastisch heeft veranderd, de effectiviteit ervan in MM laag is en de overleving niet is verbeterd. Dit benadrukt het belang van klinische studies die zich specifiek richten op nieuwe (combinatie-)behandelingen voor MM.

Deel 2: vulva melanoom

Deel 2 van dit proefschrift richt zich op een belangrijke subgroep van MM, namelijk het vulva-melanoom (VM). Van alle MM is 15-20% gelokaliseerd in de vrouwelijke geslachtsorganen, waarvan 60% gelokaliseerd in de vulva. Hoofdstuk 5 geeft een algemeen overzicht van klinische en pathologische kenmerken, behandeling en prognose van VM. Het VM presenteert zich met een jeukende of bloedende gepigmenteerde laesie in de labia majora, labia minora of de clitoris en wordt gekenmerkt door een slechte prognose met een hoge recidiefkans. Net als in CM zijn hogere leeftijd, hogere Breslow dikte en lymfeklierbetrokkenheid voorspellers voor een slechtere overleving in VM. Vanwege de anatomisch uitdagende locatie, met regelmatig nauwe betrokkenheid van de vagina, urethra en clitoris, kan lokale behandeling mutilerend zijn en daarmee invloed hebben op de kwaliteit van leven. Voor regionaal en gemetastaseerde ziekte, is er nog geen

goede behandeling. Net als MM gelokaliseerd elders in het lichaam, zijn de resultaten van radiotherapie, chemotherapie, doelgerichte therapie en immunotherapie teleurstellend.

In Hoofdstuk 6 zijn de klinische en tumor karakteristieken, overleving en voorspellers voor overleving van een internationaal cohort bestaande uit 198 patiënten met VM, geanalyseerd. In dit cohort was de mediane leeftijd bij diagnose 72 jaar. De meeste patiënten presenteerden zich met klachten van een jeukende, bloedende of veranderende vulvaire afwijking. Toch duurde het gemiddeld vier maanden vanaf het optreden van symptomen tot de diagnose, wat grotendeels te wijten is aan een patient-delay. Bij 76% van de patiënten was er sprake van lokaal beperkte ziekte, in 12% regionaal verspreide ziekte, in 8% op afstand gemetastaseerde ziekte en in 4% van de patiënten was het stadium bij presentatie onbekend. Ondanks overwegend lokaal beperkte ziekte bij presentatie, was er in meer dan de helft van de patiënten sprake van een tumor met een Breslow-dikte van meer dan4 mm (het hoogste T-stadium). De twee- en vijf-jaars overlevingspercentages van respectievelijk 48% en 31% illustreren de agressiviteit van de ziekte. Bovendien recidiveerde de ziekte in twee derde van de patiënten. Het merendeel hiervan bestond dit uit regionale en afstandsmetastasen, met een mediane tijd tot recidiveren van de ziekte van 11 maanden. In deze studie waren hogere leeftijd en een grotere tumordiameter onafhankelijke voorspellers voor overleving. Deze studie laat zien dat ondanks dat de meerderheid van de patiënten zich presenteert met lokaal beperkte ziekte, VM een agressieve ziekte is met een hoog risico op het recidiveren van de ziekte en een slechte prognose.

List of publications

2023 Florine L. Boer, Olivier J van Not , Manja Bloem, Marion Stevense-den Boer, Alfonsus J.M van den Eertwegh, Astrid A.M. van der Veldt , Jan-Willem B de Groot, Gerard Vreugdenhil, Rozemarijn S. van Rijn, Djura Piersma, Maureen J.B Aarts, Christian U. Blank, Marye J. Boers-Sonderen, Geke A.P. Hospers, Karijn P.M. Suijkerbuijk, Franchette W.P.J van den Berkmortel, Willeke A.M. Blokx, John B.A.G Haanen, Michel W.J.M Wouters, Mariëtte I.E. van Poelgeest and Ellen H.W. Kapiteijn Clinical outcomes and toxicity of combined ipilimumab/nivolumab in rare melanomas – a nationwide population based study and a review of the literature, submitted

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Curriculum vitae

Florine Levina Boer was born in Wageningen on 30th March, 1992. In 2010, she graduated from College Hageveld in Heemstede. After taking a gap year in which she fulfilled a three month leadership course in India, she started medical school at the Leiden University Medical Centre.

During medical school, Florine participated in several committees of which she was a representative of the master program of medical school from 2015-2019. Moreover, she worked as a volunteer at a hospice providing end of life and supportive care for young adults (18-30 years). During her bachelor and master degree, Florine went abroad to teach English at an orphanage in Sri Lanka, followed a public health program in Cuba and was a medical intern at the department of pediatrics in the Greater Accra Regional Hospital in Accra, Ghana.

Florine started her scientific career under the supervision of dr. M.I.E. van Poelgeest. The review of vulvar melanomas and analysis of a large cohort of vulvar melanomas, has become the foundation of this thesis. After her graduation in 2019, she started working as a resident (ANIOS) at the department of obstetrics and gynaecology at the Reinier de Graaf Gasthuis in Delft. As from 2020 on, she switched specialization and started working as a resident (ANIOS) at the urology department at the Medisch Centrum Haaglanden in the Hague and later at the Amphia hospital in Breda. Besides her job as medical doctor, she completed her PhD under supervision of dr. M.I.E. van Poelgeest, dr. H.W. Kapiteijn and prof. dr. C.B. Blank. As part of her residency training in urology, Florine started as resident at the department of surgery (AIOS) at the Sint Franciscus Gasthuis in Rotterdam on July 1st 2023.

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