Characteristics and clinical outcomes of mucosal melanoma

Boer, F.L.

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

Florine L. Boer, Mieke L.G. ten Eikelder, Nan van Geloven, Ellen H. Kapiteijn, Katja N. Gaarenstroom, Geoff Hughes, Linda S. Nooij, Marta Jozwiak, Ming Y. Tjiong, Joanne M.A. de Hullu, Khadra Galaal, Mariëtte I.E. van Poelgeest

Gynaecologic Oncology, **2021** April; *161 202-210*

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

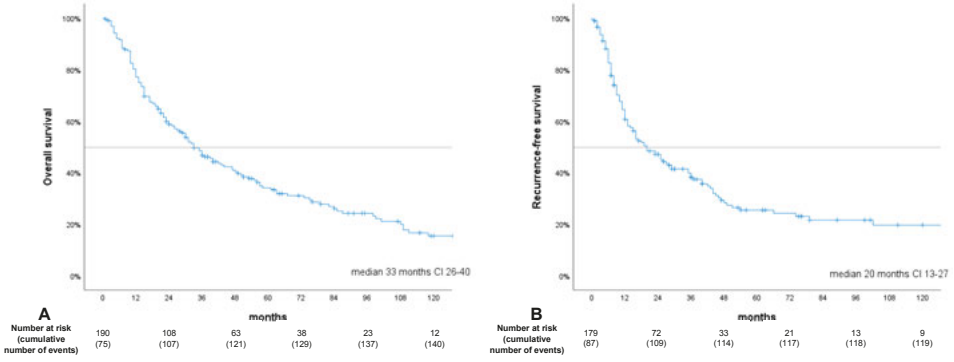


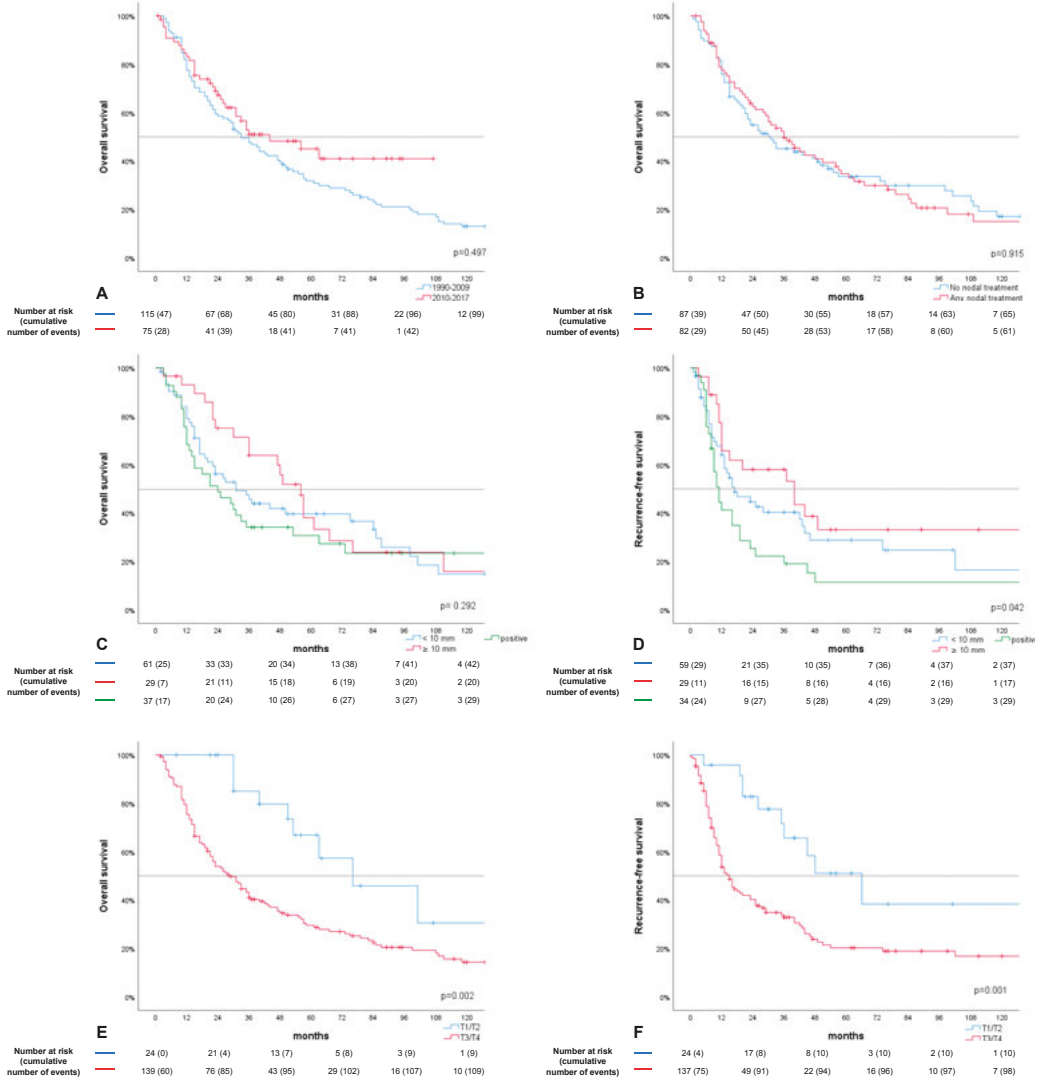
Table 3. Targeted and immunotherapy for the VM cohort

	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.

References

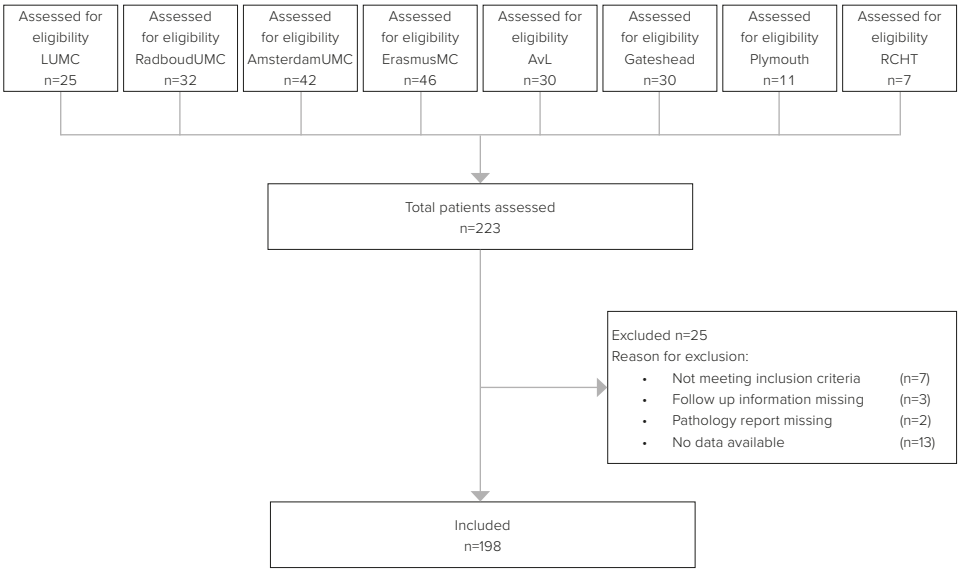
1. McLaughlin CC, Wu XC, Jemal A, Martin HJ, Roche LM, Chen VW. Incidence of noncutaneous melanomas in the U.S. Cancer. 2005;103(5):1000-7.
2. Merkel EA, Gerami P. Malignant melanoma of sun-protected sites: a review of clinical, histological, and molecular features. Lab Invest. 2017;97(6):630-5.
3. Nassar KW, Tan AC. The mutational landscape of mucosal melanoma. Semin Cancer Biol. 2020;61:139-48.
4. Sugiyama VE, Chan JK, Shin JY, Berek JS, Osann K, Kapp DS. Vulvar melanoma: a multivariable analysis of 644 patients. Obstet Gynecol. 2007;110(2 Pt 1):296-301.
5. Boer FL, Ten Eikelder MLG, Kapiteijn EH, Creutzberg CL, Galaal K, van Poelgeest MIE. Vulvar malignant melanoma: Pathogenesis, clinical behaviour and management: Review of the literature. Cancer Treat Rev. 2019;73:91-103.
6. Mihajlovic M, Vlajkovic S, Jovanovic P, Stefanovic V. Primary mucosal melanomas: a comprehensive review. Int J Clin Exp Pathol. 2012;5(8):739-53.
7. Sinasac SE, Petrella TM, Rouzbahman M, Sade S, Ghazarian D, Vicus D. Melanoma of the Vulva and Vagina: Surgical Management and Outcomes Based on a Clinicopathologic Reviewof 68 Cases. Journal of Obstetrics and Gynaecology Canada. 2019;41(6):762-71.
8. Akhtar-Danesh N, Elit L, Lytwyn A. Trends in incidence and survival of women with invasive vulvar cancer in the United States and Canada: a population-based study. Gynecol Oncol. 2014;134(2):314-8.
9. Lee AY, Berman RS. Management of Noncutaneous Melanomas. Surg Oncol Clin N Am. 2020;29(3):387-400.
10. van Zeijl MCT, Boer FL, van Poelgeest MIE, van den Eertwegh AJM, Wouters M, de Wreede LC, et al. Survival outcomes of patients with advanced mucosal melanoma diagnosed from 2013 to 2017 in the Netherlands - A nationwide population-based study. Eur J Cancer. 2020;137:127-35.
11. Tas F, Keskin S, Karadeniz A, Dağoğlu N, Sen F, Kilic L, et al. Noncutaneous melanoma have distinct features from each other and cutaneous melanoma. Oncology. 2011;81(5-6):353-8.
12. Furney SJ, Turajlic S, Stamp G, Nohadani M, Carlisle A, Thomas JM, et al. Genome sequencing of mucosal melanomas reveals that they are driven by distinct mechanisms from cutaneous melanoma. J Pathol. 2013;230(3):261-9.
13. Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, et al. Distinct sets of genetic alterations in melanoma. N Engl J Med. 2005;353(20):2135-47.
14. Hou JY, Baptiste C, Hombalegowda RB, Tergas AI, Feldman R, Jones NL, et al. Vulvar and vaginal melanoma: A unique subclass of mucosal melanoma based on a comprehensive molecular analysis of 51 cases compared with 2253 cases of nongynecologic melanoma. Cancer. 2017;123(8):1333-44.
15. Curtin JA, Busam K, Pinkel D, Bastian BC. Somatic activation of KIT in distinct subtypes of melanoma. J Clin Oncol. 2006;24(26):4340-6.
16. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228-47.
17. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, Byrd DR, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol. 2009;27(36):6199-206.
18. Seifried S, Haydu LE, Quinn MJ, Scolyer RA, Stretch JR, Thompson JF. Melanoma of the vulva and vagina: principles of staging and their relevance to management based on a clinicopathologic analysis of 85 cases. Ann Surg Oncol. 2015;22(6):1959-66.
19. Michielin O, van Akkooi ACJ, Ascierto PA, Dummer R, Keilholz U. Cutaneous melanoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Ann Oncol. 2019;30(12):1884-901.
20. Faries MB, Thompson JF, Cochran AJ, Andtbacka RH, Mozzillo N, Zager JS, et al. Completion Dissection or Observation for Sentinel-Node Metastasis in Melanoma. N Engl J Med. 2017;376(23):2211-22.
21. Morton DL, Thompson JF, Cochran AJ, Mozzillo N, Elashoff R, Essner R, et al. Sentinel-node biopsy or nodal observation in melanoma. N Engl J Med. 2006;355(13):1307-17.
22. Leiter U, Stadler R, Mauch C, Hohenberger W, Brockmeyer N, Berking C, et al. Complete lymph node dissection versus no dissection in patients with sentinel lymph node biopsy positive melanoma (DeCOG-SLT): a multicentre, randomised, phase 3 trial. Lancet Oncol. 2016;17(6):757-67.
23. Vitale SG, Valenti G, Biondi A, Rossetti D, Frigerio L. Recent trends in surgical and reconstructive management of vulvar cancer: review of literature. Updates in Surgery. 2015;67(4):367-71.
24. Kaufman HL, Kirkwood JM, Hodi FS, Agarwala

S, Amatruda T, Bines SD, et al. The Society for Immunotherapy of Cancer consensus statement on tumour immunotherapy for the treatment of cutaneous melanoma. Nat Rev Clin Oncol. 2013;10(10):588-98.
25. Sullivan RJ, Atkins MB, Kirkwood JM, Agarwala SS, Clark JI, Ernstoff MS, et al. An update on the Society for Immunotherapy of Cancer consensus statement on tumor immunotherapy for the treatment of cutaneous melanoma: version 2.0. J Immunother Cancer. 2018;6(1):44.
26. Leitao MM, Jr., Cheng X, Hamilton AL, Siddiqui NA, Jurgenliemk-Schulz I, Mahner S, et al. Gynecologic Cancer InterGroup (GCIG) consensus review for vulvovaginal melanomas. Int J Gynecol Cancer. 2014;24(9 Suppl 3):S117-22.
27. Kaufman HL, Atkins MB, Subedi P, Wu J, Chambers J, Joseph Mattingly T, 2nd, et al. The promise of Immuno-oncology: implications for defining the value of cancer treatment. J Immunother Cancer. 2019;7(1):129.
28. Petrella TM, Fletcher GG, Knight G, McWhirter E, Rajagopal S, Song X, et al. Systemic adjuvant therapy for adult patients at high risk for recurrent cutaneous or mucosal melanoma: an Ontario Health (Cancer Care Ontario) clinical practice guideline. Curr Oncol. 2020;27(1):e43-e52.
29. Weber J, Mandala M, Del Vecchio M, Gogas HJ, Arance AM, Cowey CL, et al. Adjuvant Nivolumab versus Ipilimumab in Resected Stage III or IV Melanoma. N Engl J Med. 2017;377(19):1824-35.
30. Eggermont AMM, Blank CU, Mandala M, Long GV, Atkinson V, Dalle S, et al. Adjuvant Pembrolizumab versus Placebo in Resected Stage III Melanoma. N Engl J Med. 2018;378(19):1789-801.
31. Del Vecchio M, Di Guardo L, Ascierto PA, Grimaldi AM, Sileni VC, Pigozzo J, et al. Efficacy and safety of ipilimumab 3mg/kg in patients with pretreated, metastatic, mucosal melanoma. European Journal of Cancer. 2014;50(1):121-7.
32. D'Angelo SP, Larkin J, Sosman JA, Lebbé C, Brady B, Neyns B, et al. Efficacy and Safety of Nivolumab Alone or in Combination With Ipilimumab in Patients With Mucosal Melanoma: A Pooled Analysis. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2017;35(2):226-35.
33. Hayward NK, Wilmott JS, Waddell N, Johansson PA, Field MA, Nones K, et al. Whole-genome landscapes of major melanoma subtypes. Nature. 2017;545(7653):175-80.
34. Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet. 2019;51(2):202-6.
35. Chłopik A, Selim MA, Peng Y, Wu CL, Tell-Marti G, Paral KM, et al. Prognostic role of tumoral PDL1 expression and peritumoral FoxP3+ lymphocytes in vulvar melanomas. Hum Pathol. 2018;73:176-83.
36. Filippi AR, Fava P, Badellino S, Astrua C, Ricardi U, Quaglino P. Radiotherapy and immune checkpoints inhibitors for advanced melanoma. Radiother Oncol. 2016;120(1):1-12.
37. Kim HJ, Chang JS, Roh MR, Oh BH, Chung KY, Shin SJ, et al. Effect of Radiotherapy Combined With Pembrolizumab on Local Tumor Control in Mucosal Melanoma Patients. Front Oncol. 2019;9:835.
38. Conry RM, Westbrook B, McKee S, Norwood TG. Talimogene laherparepvec: First in class oncolytic virotherapy. Hum Vaccin Immunother. 2018;14(4):839-46.
39. Fröhlich A, Hoffmann F, Niebel D, Egger E, Kukuk GM, Toma M, et al. Talimogene Laherparepvec in Advanced Mucosal Melanoma of the Urethra Upon Primary Resistance on Immune Checkpoint Inhibition: A Case Report. Front Oncol. 2020;10:611.
40. Kalinsky K, Lee S, Rubin KM, Lawrence DP, Iafrarte AJ, Borger DR, et al. A phase 2 trial of dasatinib in patients with locally advanced or stage IV mucosal, acral, or vulvovaginal melanoma: A trial of the ECOG-ACRIN Cancer Research Group (E2607). Cancer. 2017;123(14):2688-97.

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