Gynecologic Oncology 161 (2021) 202–210

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

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

Florine L. Boer [a,*,1], Mieke L.G. ten Eikelder [b,1], Nan van Geloven [c], Ellen H. Kapiteijn [d], Katja N. Gaarenstroom [a], Geoff Hughes [e], Linda S. Nooij [f], Marta Jozwiak [g], Ming Y. Tjiong [h], Joanne M.A. de Hullu [b], Khadra Galaal [i], Mariette I.E. van Poelgeest [a]

[a] Department of Gynaecology, Leiden University Medical Centre, Leiden, the Netherlands
[b] Department of Gynaecology Oncology, Radboud University Medical Centre, the Netherlands
[c] Department of Biomedical Data Sciences, Leiden University Medical Centre, the Netherlands
[d] Department of Medical Oncology, Leiden University Medical Centre, Leiden, the Netherlands
[e] Department of Gynaecology, Derriford hospital NHS Trust, Plymouth, United Kingdom
[f] Department of Gynaecology Oncology, Centre for Gynaecologic Oncology, the Netherlands Cancer Institute, Antoni van Leeuwenhoek, the Netherlands
[g] Department of Gynaecology Oncology, Erasmus MC Cancer Institute, Erasmus Medical Centre, the Netherlands
[h] Department of Gynaecology Oncology, Amsterdam University Medical Centre, the Netherlands
[i] Department of Gynaecology, Royal Cornwall hospital NHS trust, Truro, United Kingdom

## HIGHLIGHTS

- Vulvar melanoma is a rare gynaecological cancer with poor prognosis due to its high metastatic potential.
- The only potentially curative option for localized disease is complete surgical resection with negative margins.
- Sentinel lymph node biopsy is only recommended in order to direct adjuvant treatment.
- Analysis for targetable mutations should be incorporated into routine clinical testing for vulvar melanoma.
- Immunotherapy with anti-PD1 or anti-CTLA4 should be considered in metastatic or non-resectable vulvar melanoma.

## ARTICLE INFO

*Article history:*
Received 23 December 2020
Accepted 18 January 2021
Available online 26 January 2021

*Keywords:*
Vulvar melanoma
Treatment
Prognostic factors
Survival
Recurrence

## 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.



* Corresponding author at: Department of Gynecology, Leiden University Medical Centre, P.O. Box 9600, 2300, RC, Leiden, the Netherlands.
*E-mail address:* f.l.boer@lumc.nl (F.L. Boer).
[1] Florine L. Boer and Mieke L.G. ten Eikelder contributed equally to this article.

https://doi.org/10.1016/j.ygyno.2021.01.018

## 1. 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 and 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.

## 2. Methods

### 2.1. Study design and patients

A retrospective evaluation of patients diagnosed with primary VM at five academic medical centers 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, microsatellosis, 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).

## 3. 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

**Table 1**
Clinical and histological characteristics.

| 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) |
| Lentigous | 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) |
| Microsatelossis | |
| Yes | 20 (10.1) |
| 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 (37.2) |
| Missing | 11 (6.1) |
| 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) | 8 [4,16] |

[a] Of the analysed patients.
[b] Of the surgically treated patients

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.

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 microsatellosis 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 and 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

**Table 2**
Treatment characteristics.

| 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 | 64 (35.5) |
| ≥ 10 mm | 30(16.7) |
| < 2 mm | 7 (3.9) |
| ≥ 2 mm | 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

radiotherapy or local excision of metastasis. Twenty-one of 78 patients (27%) with distant metastases received immunotherapy.

### 3.2. Clinical outcomes

Clinical follow-up ranged from 1 to 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 (Fig. 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 (Fig. 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).

### 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 (Fig. 2A). Prognostic factors for OS and RFS are presented in Table 4 and Fig. 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, microsatellosis and angiolymphatic involvement. Lymph node treatment was not significantly associated with OS (Fig. 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), microsatellosis, 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, Fig. 2 CD). T3/T4 stage was associated with worse OS and RFS compared to T1/T2 stage disease (Fig. 2 EF).

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.

## 4. 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.

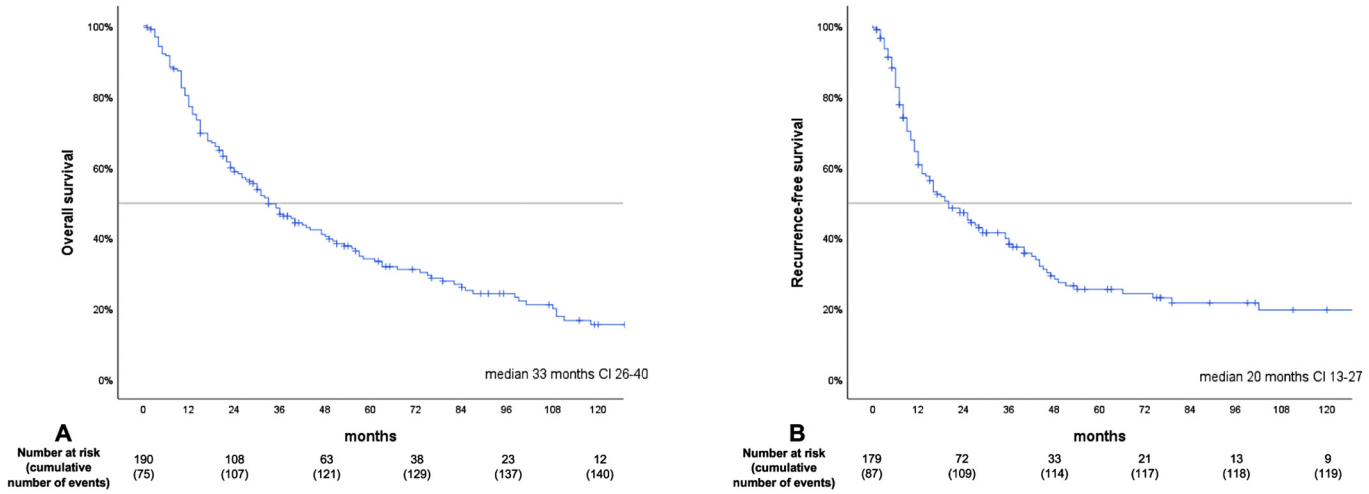


**Fig. 1.** Overall survival and recurrence-free survival. A Overall survival B Recurrence-free survival.

**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 |
| | 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 |

9 patients have been treated with two immunotherapeutic strategies of which the second one is underlined.
Drug names: DTIC: Dacarbazine, IL-2: Interleukin 2, IFNa: Interferon Alpha, GCSF: Granulocyte colony-stimulation factor. PD: progressive disease, SD: stable disease, PR: partial response, CR: complete response.

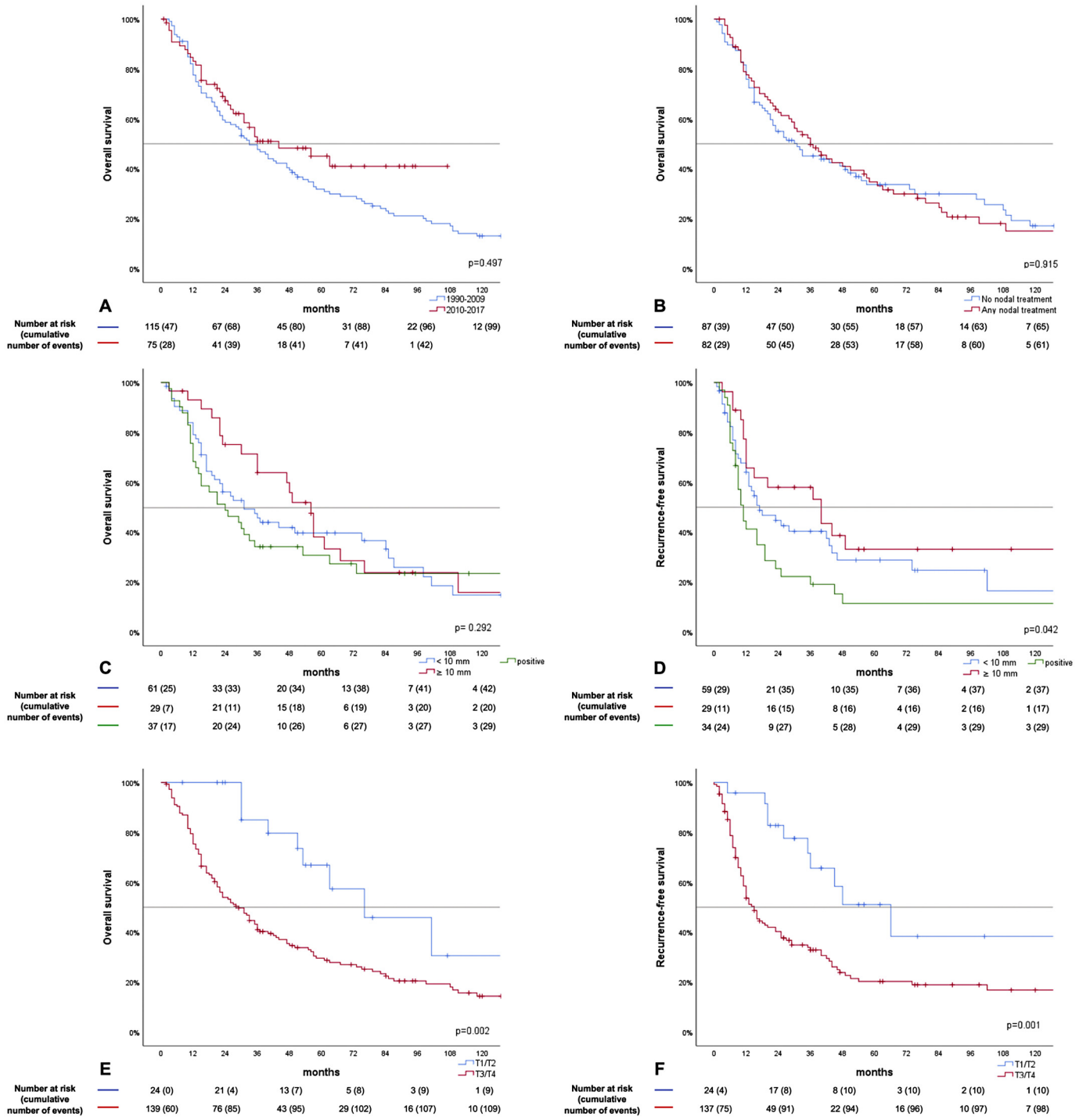


**Fig. 2.** Overall survival by timeframe and nodal treatment and overall and recurrence-free survival by margin status and T stage. A Overall survival by timeframe (1990–2009 vs 2010–2017) B Overall survival by nodal treatment (no treatment vs any type of nodal treatment) C Overall survival by margin 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).

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, Fig. 2 CD), 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

**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 (0.73–15.2) | 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** | | | |
| Microsattelosis (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 | | | |
| 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 (0.78–3.84) | 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 | | | |
| Microsattelosis (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.

and RFS compared to margins <10 mm (Table 4, Fig. 2 CD). 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, Fig. 2 EF). 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 ($p < 0.001$) 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.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2021.01.018.

## Declaration of Competing Interest

The authors declare that they have no conflict of interest. Authors have full control of all primary data. They agree to allow the journal to review the data if requested.

## Acknowledgements

We would like to thank Raj Naik and Ann Fisher, gynaecooncologists, and Wendy Mc Cormick and Lorraine Pearce, research nurses, for providing data from the Queen Elizabeth Hospital, NHS trust in Gateshead.

## References

[1] C.C. McLaughlin, X.C. Wu, A. Jemal, H.J. Martin, L.M. Roche, V.W. Chen, Incidence of noncutaneous melanomas in the U.S, Cancer 103 (5) (2005) 1000–1007.
[2] E.A. Merkel, P. Gerami, Malignant melanoma of sun-protected sites: a review of clinical, histological, and molecular features, Lab. Investig. 97 (6) (2017) 630–635.
[3] K.W. Nassar, A.C. Tan, The mutational landscape of mucosal melanoma, Semin. Cancer Biol. 61 (2020) 139–148.

[4] V.E. Sugiyama, J.K. Chan, J.Y. Shin, J.S. Berek, K. Osann, D.S. Kapp, Vulvar melanoma: a multivariable analysis of 644 patients, Obstet. Gynecol. 110 (2 Pt 1) (2007) 296–301.
[5] F.L. Boer, M.L.G. Ten Eikelder, E.H. Kapiteijn, C.L. Creutzberg, K. Galaal, M.I.E. van Poelgeest, Vulvar malignant melanoma: pathogenesis, clinical behaviour and management: review of the literature, Cancer Treat. Rev. 73 (2019) 91–103.
[6] M. Mihajlovic, S. Vlajkovic, P. Jovanovic, V. Stefanovic, Primary mucosal melanomas: a comprehensive review, Int. J. Clin. Exp. Pathol. 5 (8) (2012) 739–753.
[7] S.E. Sinasac, T.M. Petrella, M. Rouzbahman, S. Sade, D. Ghazarian, D. Vicus, Melanoma of the vulva and vagina: surgical management and outcomes based on a clinicopathologic reviewof 68 cases, J. Obstet. Gynaecol. Can. 41 (6) (2019) 762–771.
[8] N. Akhtar-Danesh, L. Elit, A. Lytwyn, Trends in incidence and survival of women with invasive vulvar cancer in the United States and Canada: a population-based study, Gynecol. Oncol. 134 (2) (2014) 314–318.
[9] A.Y. Lee, R.S. Berman, Management of noncutaneous melanomas, Surg. Oncol. Clin. N. Am. 29 (3) (2020) 387–400.
[10] M.C.T. van Zeijl, F.L. Boer, M.I.E. van Poelgeest, A.J.M. van den Eertwegh, M. Wouters, L.C. de Wreede, 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 137 (2020) 127–135.
[11] F. Tas, S. Keskin, A. Karadeniz, N. Dağoğlu, F. Sen, L. Kilic, et al., Noncutaneous melanoma have distinct features from each other and cutaneous melanoma, Oncology 81 (5–6) (2011) 353–358.
[12] S.J. Furney, S. Turajlic, G. Stamp, M. Nohadani, A. Carlisle, J.M. Thomas, et al., Genome sequencing of mucosal melanomas reveals that they are driven by distinct mechanisms from cutaneous melanoma, J. Pathol. 230 (3) (2013) 261–269.
[13] J.A. Curtin, J. Fridlyand, T. Kageshita, H.N. Patel, K.J. Busam, H. Kutzner, et al., Distinct sets of genetic alterations in melanoma, N. Engl. J. Med. 353 (20) (2005) 2135–2147.
[14] J.Y. Hou, C. Baptiste, R.B. Hombalegowda, A.I. Tergas, R. Feldman, N.L. Jones, 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 123 (8) (2017) 1333–1344.
[15] J.A. Curtin, K. Busam, D. Pinkel, B.C. Bastian, Somatic activation of KIT in distinct subtypes of melanoma, J. Clin. Oncol. 24 (26) (2006) 4340–4346.
[16] E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, et al., New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1), Eur. J. Cancer 45 (2) (2009) 228–247.
[17] C.M. Balch, J.E. Gershenwald, S.J. Soong, J.F. Thompson, M.B. Atkins, D.R. Byrd, et al., Final version of 2009 AJCC melanoma staging and classification, J. Clin. Oncol. 27 (36) (2009) 6199–6206.
[18] S. Seifried, L.E. Haydu, M.J. Quinn, R.A. Scolyer, J.R. Stretch, J.F. Thompson, 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. 22 (6) (2015) 1959–1966.
[19] O. Michielin, A.C.J. van Akkooi, P.A. Ascierto, R. Dummer, U. Keilholz, Cutaneous melanoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up†, Ann. Oncol. 30 (12) (2019) 1884–1901.
[20] M.B. Faries, J.F. Thompson, A.J. Cochran, R.H. Andtbacka, N. Mozzillo, J.S. Zager, et al., Completion dissection or observation for sentinel-node metastasis in melanoma, N. Engl. J. Med. 376 (23) (2017) 2211–2222.
[21] D.L. Morton, J.F. Thompson, A.J. Cochran, N. Mozzillo, R. Elashoff, R. Essner, et al., Sentinel-node biopsy or nodal observation in melanoma, N. Engl. J. Med. 355 (13) (2006) 1307–1317.
[22] U. Leiter, R. Stadler, C. Mauch, W. Hohenberger, N. Brockmeyer, C. Berking, 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. 17 (6) (2016) 757–767.
[23] S.G. Vitale, G. Valenti, A. Biondi, D. Rossetti, L. Frigerio, Recent trends in surgical and reconstructive management of vulvar cancer: review of literature, Updat. Surg. 67 (4) (2015) 367–371.
[24] H.L. Kaufman, J.M. Kirkwood, F.S. Hodi, S. Agarwala, T. Amatruda, S.D. Bines, et al., The Society for Immunotherapy of Cancer consensus statement on tumour immunotherapy for the treatment of cutaneous melanoma, Nat. Rev. Clin. Oncol. 10 (10) (2013) 588–598.
[25] R.J. Sullivan, M.B. Atkins, J.M. Kirkwood, S.S. Agarwala, J.I. Clark, M.S. Ernstoff, 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 6 (1) (2018) 44.
[26] M.M. Leitao Jr., X. Cheng, A.L. Hamilton, N.A. Siddiqui, I. Jurgenliemk-Schulz, S. Mahner, et al., Gynecologic Cancer InterGroup (GCIG) consensus review for vulvovaginal melanomas, Int. J. Gynecol. Cancer 24 (9 Suppl 3) (2014) S117–S122.
[27] H.L. Kaufman, M.B. Atkins, P. Subedi, J. Wu, J. Chambers, T. Joseph Mattingly 2nd, et al., The promise of Immuno-oncology: implications for defining the value of cancer treatment, J. Immunother. Cancer 7 (1) (2019) 129.
[28] T.M. Petrella, G.G. Fletcher, G. Knight, E. McWhirter, S. Rajagopal, X. Song, 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. 27 (1) (2020) e43–e52.
[29] J. Weber, M. Mandala, M. Del Vecchio, H.J. Gogas, A.M. Arance, C.L. Cowey, et al., Adjuvant nivolumab versus Ipilimumab in resected stage III or IV melanoma, N. Engl. J. Med. 377 (19) (2017) 1824–1835.
[30] A.M.M. Eggermont, C.U. Blank, M. Mandala, G.V. Long, V. Atkinson, S. Dalle, et al., Adjuvant pembrolizumab versus placebo in resected stage III melanoma, N. Engl. J. Med. 378 (19) (2018) 1789–1801.
[31] M. Del Vecchio, L. Di Guardo, P.A. Ascierto, A.M. Grimaldi, V.C. Sileni, J. Pigozzo, et al., Efficacy and safety of ipilimumab 3mg/kg in patients with pretreated, metastatic, mucosal melanoma, Eur. J. Cancer 50 (1) (2014) 121–127.
[32] S.P. D'Angelo, J. Larkin, J.A. Sosman, C. Lebbé, B. Brady, B. Neyns, et al., Efficacy and safety of nivolumab alone or in combination with Ipilimumab in patients with mucosal melanoma: a pooled analysis, J. Clin. Oncol. 35 (2) (2017) 226–235.
[33] N.K. Hayward, J.S. Wilmott, N. Waddell, P.A. Johansson, M.A. Field, K. Nones, et al., Whole-genome landscapes of major melanoma subtypes, Nature 545 (7653) (2017) 175–180.
[34] R.M. Samstein, C.H. Lee, A.N. Shoushtari, M.D. Hellmann, R. Shen, Y.Y. Janjigian, et al., Tumor mutational load predicts survival after immunotherapy across multiple cancer types, Nat. Genet. 51 (2) (2019) 202–206.
[35] A. Chłopik, M.A. Selim, Y. Peng, C.L. Wu, G. Tell-Marti, K.M. Paral, et al., Prognostic role of tumoral PDL1 expression and peritumoral FoxP3+ lymphocytes in vulvar melanomas, Hum. Pathol. 73 (2018) 176–183.
[36] A.R. Filippi, P. Fava, S. Badellino, C. Astrua, U. Ricardi, P. Quaglino, Radiotherapy and immune checkpoints inhibitors for advanced melanoma, Radiother. Oncol. 120 (1) (2016) 1–12.
[37] H.J. Kim, J.S. Chang, M.R. Roh, B.H. Oh, K.Y. Chung, S.J. Shin, et al., Effect of radiotherapy combined with pembrolizumab on local tumor control in mucosal melanoma patients, Front. Oncol. 9 (2019) 835.
[38] R.M. Conry, B. Westbrook, S. McKee, T.G. Norwood, Talimogene laherparepvec: first in class oncolytic virotherapy, Hum. Vacc. Immunother. 14 (4) (2018) 839–846.
[39] A. Fröhlich, F. Hoffmann, D. Niebel, E. Egger, G.M. Kukuk, M. Toma, et al., Talimogene laherparepvec in advanced mucosal melanoma of the urethra upon primary resistance on immune checkpoint inhibition: a case report, Front. Oncol. 10 (2020) 611.
[40] K. Kalinsky, S. Lee, K.M. Rubin, D.P. Lawrence, A.J. Iafrarte, D.R. Borger, 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 123 (14) (2017) 2688–2697.