Mucosal melanoma: clinical, histological and c-kit gene mutational profile of 86 French cases

Article Type : Original Article

Mucosal melanoma: clinical, histological and *c-kit* gene mutational profile of 86 French cases.

E. Cinotti[1,2], J. Chevallier[1], B. Labeille[1], F. Cambazard[1], L. Thomas[3], B. Balme[4], M.T. Leccia[5], M. D'Incan[6], P. Vercherin[7], C. Douchet[8], P. Rubegni[2] and J.L. Perrot[1]

[1]Department of Dermatology, University Hospital of Saint Etienne, France,
[2]Department of Medical, Surgical and Neurological Science, Dermatology Section, University of Siena, S.Maria alle Scotte Hospital, Siena, Italy,
[3]Dermatology Department, University Hospital of Lyon Sud, Pierre Bénite, France.
[4]Dermatopathology Department, University Hospital of Lyon Sud, Pierre Bénite, France
[5]Department of Dermatology, University Hospital of Grenoble, France
[6]Dermatology Department, University Hospital of Clermont-Ferrand, France
[7]Department of Public Health and Medical Information, University Hospital of Saint Etienne, France.
[8]Department of Pathology, University Hospital of Saint Etienne, France

Running head: **Mucosal melanoma**

Conflict of interests: None declared

Financial support: None

Corresponding author:
Elisa Cinotti. Department of Medical, Surgical and Neurological Science - Dermatology Section, University of Siena, S.Maria alle Scotte Hospital, Viale Bracci 16, 53100 Siena, Italy. Tel: 0039-0577 585484. Fax: 0039 0577 44238, elisacinotti@gmail.com

Keywords: melanoma, mucosa, epidemiology, histopathology and *c-kit*.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jdv.14353

ABSTRACT

Background: Mucosal melanomas are rare and highly aggressive tumors. Few studies evaluated mucosal melanomas of locations other than the head and neck region, and other than those of the Asian population.

Objectives: The objective of this study was to analyze the clinical and histological features, as well as the mutational status of *c-kit* and *b-raf* gene of mucosal melanoma in any localization in a French series.

Methods: We investigated clinical (sex, age, performance status, survival, treatment of the patients and lack of pigmentation of the tumors) and histopathologic features (ulceration, Breslow's index, mitotic rate), as well as the mutational status of *c-kit* and *b-raf* of 86 mucosal melanomas diagnosed in 15 years in four French University Hospitals.

Results: Most melanomas affected women (72%) and the genital region (46.5%). A fifth of melanomas were amelanotic. 81% of melanomas had a Breslow's index ≥ 1, whereas all glans melanomas and most vulvar melanomas had a Breslow index ≤ 1mm. Overall survival was 54% at 3 years. 11.6 % of the 43 tested mucosal melanomas were c-kit mutated while the 15 tested genital melanomas were not. The *c-kit* gene mutation did not influence the overall survival. Age≥50, amelanotic type and Performance status≥1 were not poor prognostic factors in our series.

Conclusion: This study confirmed that mucosal melanomas are rare and could be difficult to diagnose being often amelanotic and in hidden sites. Most melanomas were thick at the diagnosis, but glans and vulvar melanomas were thinner probably because of their greater visibility. The frequency of the c-kit mutation varied depending on the initial tumor site. In our series the prognosis was poor, independently from c-kit mutations and the patient's general health and age. The presence of metastasis at diagnosis was associated with a worse prognosis indicating the importance of an early diagnosis.

INTRODUCTION

Mucosal melanomas are rare and particularly aggressive tumors[1–3]. They develop from melanocytes, which derive from the neural crest and are distributed throughout mucosal surfaces and the skin during embryogenesis[1]. They represent 0.2-10 % of melanomas[4,5] and their proportion varies according to the ethnicities, being higher in the Asian than in the European population[6,7]. Mucosal melanomas include sinonasal, oral, genital, urinary, gastrointestinal and anorectal locations. Previous research has shown that there are distinct differences between mucosal melanoma and its cutaneous counterpart in incidence, epidemiology, etiology, genetic makeup, and prognosis[1,8].

Knowledge about incidence, clinical presentation, natural history, treatment modalities and prognosis of mucosal melanoma is limited. There is no standard of care for treatment in mucosal melanoma and, despite aggressive surgical resection and a multitude of adjuvant treatments, the prognosis remains grave[3,8,9].

Melanomas of the head and neck account for more than half of mucosal melanomas and most studies focus on this subject[2,4–7]. However, few papers deal with melanomas of other locations and especially in European population. The objective of this study was to analyze the clinical and histological features and the mutational profile of the *c-kit* and *b-raf* gene of all types of mucosal melanoma in a French population.

MATERIALS AND METHODS

This retrospective descriptive study evaluates mucosal melanomas diagnosed between May 1998 and December 2012 in the University Hospitals of Saint- Etienne, Lyon, Grenoble and Clermont-Ferrand. Mucosal metastases from cutaneous melanoma, ocular melanoma and melanoma of unknown primary site were excluded because of the different clinical and histological features. Eighty-six sinonasal, oral, genital, gastrointestinal and anal histologically proven melanomas were included. Patients' data were collected from their clinical record.

Demographic and clinical characteristics of patients (ethnicity, gender, age at diagnosis and Eastern Cooperative Oncology Group Performance Status[10]), and the clinical (initial localization and amelanotic type) and the histopathological (ulceration, mitotic rate and Breslow's index) features of tumors were investigated. Ballantyne's clinical stage[11] and the staging system suggested by Mehra et al[12] that considers tumor thickness (T stage), lymph node involvement (N stage) and presence of metastasis (M stage) were also evaluated.

The mutational status of the *b-raf* gene (exon 15) and the *c-kit* gene (exons 11, 13, 17 and 18) was evaluated. Paraffin-embedded samples from primary melanomas were macrodissected from five 10-µm-thick unstained sections by comparison with haematoxylin–eosin-stained slides to analyse only samples for which more than 70% was tumour tissue. Nucleic acids were extracted using the automated QIAcube system (Qiagen, Germany). V600 mutation of b-raf gene was searched by PCR analysis with mini-sequencing Snap Shot using a SNaPshotMultiplex System (Life Technologies, USA) on the Applied Biosystem 3130XL DNA analyser (Life Technologies). The data were interpreted with GeneMapper Analysis Software (Life Technologies). Bi-directional sequencing of c-kit exons 11, 13, 17 and 18 was performed using BigDye V1.1 cycle sequencing kit (Life technologies) and BigDye XTerminator Purification Kit (Life Technologies). The sequence reaction was analyzed on the Applied Biosystem 3130XL DNA analyser (Life Technologies) by means of the Sequencing Analysis software and the Sequence scanner software (Life Technologies).

The data on the therapeutic management (initial surgery, sentinel lymph node biopsy, postoperative radiotherapy and chemotherapy) and disease progression (presence and location of metastases, recurrence-free survival, overall survival and prognostic factors) were also analyzed.

Statistical analysis was carried out employing the SAS statistical package (version 9.2, March 2009, Cary, USA). Mean and standard deviation were obtained for each continuous measurement. Absolute and relative frequencies of the observations were calculated. Student's t-test was used to compare quantitative variables. The chi-squared test was used to compare qualitative variables. In case of small

numbers, Fisher's exact test was used to compare proportions of qualitative dichotomous variables. The Kaplan–Meier estimator was used to study survival. The log-rank test was used to compare two survival curves. A ρ value lower than 0.05 was considered significant.

RESULTS

Demographic and clinical characteristics of the patients

86 patients were included for analysis (Table 1). 72% (n=61) of patients were female and 28% (n=25) were male. The male/female ratio was 1:2.6. In particular, genital and anal melanomas were largely predominant in women with 92% (n= 37) and 82% (n= 9) of cases in women, respectively.

The average age at diagnosis was 63 +/- 16 years (range 12-93 years). 22% of patients (n=19) was less than 50 year-old at diagnosis, with the same proportion in the genital and non-genital localization.

96.5 % (n = 82) of the study population was Caucasian and 3.5% (n = 4) came from Maghreb. At diagnosis, 84 % of patients had a Performance Status of 0, 10% of 1, and 6% of 2 or 3.

Tumors clinical characteristics

The genital location was the most frequent (n = 40, 46.5%), and included 3 melanomas of the glans, 7 vaginal melanomas and 30 vulvar melanomas, followed by tumors with gastrointestinal (n = 8, 9%), anal (n = 11,13%), sinonasal (n = 16, 18%) and oral (n = 11, 13%) localisations. 12% of genital melanomas were amelanotic against 35% of non-genital melanomas with a statistically significant difference (chi -2 test, $\rho < 0.05$).

Tumor histopathological characteristics

The presence of ulceration in the primary melanoma was more common in non-genital (70%) than in genital sites (45%) (chi-squared test, $\rho < 0.05$). However, all vaginal melanomas were ulcerated (against 36% of vulvar melanomas). 57% of primitive melanomas had a high rate of mitosis (≥ 1/field). A high mitotic rate was found in 100% of vaginal melanomas and in only 26% of vulvar melanomas.

There was no significant difference in age between patients with a vulvar melanoma with Breslow's index less than 1 mm and those with a vulvar melanoma with Breslow's index more than 1mm.

Stages

At diagnosis, 76% of patients were in stage I of the Ballantyne's clinical staging system (local disease; disease confined to the primary site regardless of extent), 12% of patients were in stage II (lymph nodes invasion) and 12% in stage III (distant metastasis). There was no statistically significant difference in the Ballantyne's stage distribution between genital and non-genital locations (Student's t-test, ρ = 0, 3).

T, N and M stages are reported in table 1. The melanomas of two patients (2% of total) were classified as Tis, 18 as T_1 (21%), 5 as T_2 (6%), 11 as T_3 (13%) and 46 as T_4 (54%). The remaining 4 patients (5%) had an unknown tumor thickness at the time of diagnosis and were classified as TX. Both Tis melanomas were genital (vulva and penis). All glans melanomas were Tis or T_1, whereas 75% of gastrointestinal melanomas were T_4. At the time of diagnosis, 17 patients had an involvement of the regional lymph nodes (N_1-N_2, 20%) and 10 patients had metastatic disease (M_1, 24%).

Mutations of *c-kit* gene and *b-raf* gene

The mutation of the *c-kit* gene was investigated in 43 patients (Table 2). 11.6 % (n=5) melanomas were *c-kit* mutated (one in exon 13 and 4 in exon 11). Interestingly, none of the 15 tested genital melanomas showed a c-*kit* gene mutation, while 18% (n=5) of the tested extra genital melanomas were *c-kit* mutated. However, according to Fisher's exact test, the difference in *c-kit* mutation observed between genital and extra genital melanomas was not statistically significant. The mutation of the *b-raf* gene was investigated in 42 patients and no mutation has been identified. None of the nine tested amelanotic melanomas (two oral, two vaginal, one vulvar, three sinonasal and one gastrointestinal melanomas) was c-kit or *b-raf* mutated.

Treatment

Seventy-two patients (84 %) underwent surgery of the primary melanoma. Thirteen patients with poor classic prognostic factors (high Breslow's index, ulceration and small surgical margins) had adjuvant radiotherapy on the surgical scar of the excised

melanoma. Adjuvant post-surgery radiotherapy showed no statistically significant benefit in the rate of local recurrence at 1 year (36% for surgery plus radiotherapy against 22% for surgery alone). The average overall survival in patients treated with surgery plus radiotherapy (40 months) was even lower than in those treated with surgery alone (49 months).

Of the 14 patients who did not have initial surgery, 3 were at an inoperable stage I, were treated with radiotherapy alone, and quickly evolved into stage III. The other 11 patients were at stage III, and were treated in first line with either dacarbazine or fotemustine, with no statistically significant difference between the two treatments (Student's t, ρ <0.05) in overall survival. No patient was treated with a *c-kit* target therapy.

Disease progression

Among 86 patients, 66% (n=57) were metastatic at diagnosis or became metastatic (on a medium follow-up of 43 months) (n=36). The patients that were metastatic at diagnosis had a median survival of 16 months, whereas the patients that later developed metastasis had an average survival of 23 months from the date of the first metastasis. The most frequent metastatic locations were neighboring mucosa (27%), lymph node (30%), lung (17%) and liver (13%).

The overall survival was 90 % at 1 year, 68 % at 2 years and 54 % at 3 years, with an average follow-up of 43 months (follow-up range of 6-145 months). The median overall survival measured by the Kaplan Meier estimator was similar in the group of patients with a mutation of the *c-kit* gene and the non-mutated group. Being older than 50 years (Fig.1) at the diagnosis and being PS ≥ 1 were not poor prognostic factors for overall survival in univariate analysis.

Initial stage III was associated with a worse prognosis than stage I (Student's t, p = 0.0041) and there was no significant difference in overall survival between initial stage I and initial stage II treated with lymphadenectomy (Student's t, ρ >0 .05) (Fig. 3). Moreover, there was no statistically significant difference in overall survival between genital and extragenital localisations (Fig.3), head and neck melanomas and extra-head and neck melanomas, amelanotic and non-amelanotic melanoma in univariate analysis.

DISCUSSION:

This study evaluated 86 patients with mucosal melanomas examined in four French University Hospitals over a period of 15 years. This is one of the largest series that exhaustively investigated the clinical and histological features of mucosal melanomas, as well as the presence of the *c-kit* and *b-raf* mutations, in all mucosal localisations and in Caucasian patients.[9,13] Most series of mucosal melanomas focus on the Asian population where this disease is more frequent.[6,14] Moreover, large studies on Caucasian patients are either mainly focused on melanoma of the head and neck[2] or are based on national registers and contain few clinical and histological details, especially on the mutational status of the *c-kit* gene.[12,15–17]

Concerning the demographic characteristics of our patients, 72% were women, percentage which is much higher than in the Asian population[18], but it is similar to that one reported by other studies on Caucasian patients[9,12] . In addition in our series we had a great proportion of anogenital melanoma, location that is known to be at female predominance[19], as confirmed by our series, where 92% of genital melanomas and 82% of anal melanomas affected women. The man/woman ratio in other locations (see table 1) corresponded to data of the literature.[15,20] The average age at diagnosis was 63 years, in line with the literature that reports an average age between 61 and 67.[9,15,21]

Concerning the localization of the tumors in our series, 46% of melanomas were genital, similarly to the studies of Keller et al[9] and Mehra et al[12], but differing significantly from the distribution in other studies, where genital melanomas were between 16% and 32% of all mucosal melanomas[13,18,22]. However, in our case this difference may be related to a center effect, being the dermatology department of the University Hospital of Lyon referent for vulvar diseases. Nevertheless, although the vulva represents only 1-2 % of the body surface, 3-7 % of melanomas of women are located in the vulva according to another study.[23]

Our series confirms that the amelanotic type (21%, n=19) is much more common for mucosal melanoma than for cutaneous melanoma (3-7%).[24] Nevertheless, for what concerns vulvar melanomas, we found that only 10% were amelanotic, unlike the Swedish study of Ragnarsson-Olding et al[25] that found nearly 25%.

Interestingly, a high percentage of thick melanoma was detected but the Breslow index was lower for melanomas of the glans and the vulva, probably due to their more visible localization. With regard to the vulvar localization, we studied the women age with the tumor Breslow's index < 1mm compared to those with thicker tumors, assuming that if tumors developed in younger women, they were likely to have been diagnosed earlier due to a more regular gynecological care (follow-up during pregnancy, Pap test, etc.). However, we did not observe a significant difference in age between the two groups.

The c-kit gene may be mutated in mucosal melanomas, what is unlikely in the skin (except in acral lentiginous melanoma). [26,27] We found 11.6% of the 43 tested tumors with the c-kit mutation, which is consistent with previous studies.[13,26,28] In our series anal melanomas were the most frequently mutated (25%), slightly less from what has been reported in a previous series of 31 cases (36%).[29] 18% of sinonasal melanomas had a *c-kit* mutation, differently from what has been reported by other studies that found a lower prevalence.[16,30,31] Interestingly, none of the 15 tested genital melanomas had a *c-kit* mutation, whereas other studies found *c-kit* mutations in 16% to 57% of genital melanomas.[16,27,32,33] A high prevalence of *c-kit* mutations has been found in amelanotic acral melanomas[34], whereas in our series of mucosal melanomas no amelanotic tumor was *c-kit* mutated.

The prognostic implication of the *b-raf* mutation in cutaneous melanoma is controversial[35] and it is usually too rare in mucosal melanoma[36] to assess its relation with the prognosis of mucosal melanoma. In our series none of the tested mucosal melanomas was *b-raf* mutated, in agreement with the literature where the b-raf mutations are rarely found.[13,16] We also evaluated if the presence of the *c-kit* mutation could have influenced the prognosis but we found no relation with the progression of the disease and the overall survival.

Concerning the overall survival (on a medium follow-up of 43 months) in our series, we found a 2-year survival of 68% and a 3-year survival of 54%, which is consistent with the data from literature concerning mucosal melanoma[37] and is much lower than for cutaneous melanoma[38]. The median survival of patients with metastatic disease

at diagnosis was 16 months against 23 months for patients that became metastatic secondarily, what highlights the importance of an early diagnosis. Differently from the series of Keller et al[9] there was no statistically significant difference in overall survival between head and neck melanomas and mucosal melanomas from other localisations.

In our series, the adjuvant radiotherapy provided no benefit in the recurrence rate at one year and in overall survival compared with surgery alone. These data coincide with other studies of mucosal melanoma of the head and neck.[37,39–41] However, Owens et al[42] and Schaefer et al[13] found a benefit of post-operative radiotherapy in term of local recurrence rate and it is possible that it is difficult to demonstrate the benefit of radiotherapy due to selection bias, as adjuvant radiotherapy is more often proposed for poor prognosis patients with more advanced disease.[9] In our series patients treated with surgery and radiotherapy had worse classic prognostic factors (high Breslow thickness, small surgical margins, presence of ulceration, etc.), which may explain their higher local recurrence rate at one year compared to patients treated with surgery alone. Future controlled studies to elucidate the outcome improvements in patients who receive adjuvant radiotherapy are needed.

Previous studies had conflicting reports on variables that impact survival.[9,37,42–44] In our review, the presence of metastasis at diagnosis (initial stage III) was associated with a worse prognosis than stage I (Student's t, $p = 0.0041$) and interestingly there was no significant difference in overall survival between initial stage I and initial stage II treated with lymphadenectomy (Student's t, $\rho > 0.05$), adding evidence that sentinel node biopsy and lymphadenectomy could be useful in mucosal melanoma. Performance status ≥ 1, or being older than 50 years at the diagnosis, was not a poor prognostic factor. The same data concerning age and prognosis have been previously reported.[9,43,45] It seems likely that mucosal melanoma has a poor prognosis and being young and in good general health does not affect survival, which is largely dependent on the intrinsic characteristics of the tumor. We studied the prognosis of amelanotic tumors and we could not find any difference in outcome compared to pigmented tumors.

In conclusion, this study confirmed that mucosal melanomas are rare, as we found only 86 cases diagnosed in 15 years at four University hospitals, and have a worse prognosis than cutaneous melanoma. Demographic data corresponded to the literature data, except for our large proportion of women, the difference being possibly due to the fact that our study included more than 50% of anogenital melanomas that are at female predominance. Our series confirmed that mucosal melanoma can be clinically challenging to diagnose being more often amelanotic than in the skin. Most melanomas were thick at the diagnosis, but glans and vulvar melanomas were thinner probably because of their greater visibility. We confirmed that the frequency of mutation of the *c-kit* gene could vary depending on the initial tumor site, and in particular any of our tested genital melanomas was *c-kit* mutated. Unfortunately, since the study is retrospective *c-kit* and *b-raf* mutational status has been evaluated only in a part of the tumors and mutational status of *n-ras* has not been evaluated. In our series the prognosis was poor independently from *c-kit* mutations and the patient's general health and age. The presence of metastasis at diagnosis was associated with a worse prognosis indicating the importance of an early diagnosis

REFERENCES:

1 Seetharamu N, Ott PA, Pavlick AC. Mucosal melanomas: a case-based review of the literature. *The oncologist* 2010; **15**:772–81.

2 Konuthula N, Khan MN, Parasher A, *et al.* The presentation and outcomes of mucosal melanoma in 695 patients. *Int Forum Allergy Rhinol* 2017; **7**:99–105.

3 Kirchoff DD, Deutsch GB, Foshag LJ, Lee JH, Sim M-S, Faries MB. Evolving therapeutic strategies in mucosal melanoma have not improved survival over five decades. *Am Surg* 2016; **82**:1–5.

4 Berthelsen A, Andersen AP, Jensen TS, Hansen HS. Melanomas of the mucosa in the oral cavity and the upper respiratory passages. *Cancer* 1984; **54**:907–12.

5 Rapini RP, Golitz LE, Greer RO Jr, Krekorian EA, Poulson T. Primary malignant melanoma of the oral cavity. A review of 177 cases. *Cancer* 1985; **55**:1543–51.

6 Omura K, Takemiya S, Shimada F, *et al.* Malignant mucosal melanomas of the head and neck--collective review from six cancer hospitals. *Gan No Rinsho Jpn J Cancer Clin* 1986; **32**:1511–8.

7 Chaudhry AP, Hampel A, Gorlin RJ. Primary malignant melanoma of the oral cavity: a review of 105 cases. *Cancer* 1958; **11**:923–8.

8 Kuk D, Shoushtari AN, Barker CA, *et al.* Prognosis of mucosal, uveal, acral, nonacral cutaneous, and unknown primary melanoma from the time of first metastasis. *The Oncologist* 2016; **21**:848–54.

9 Keller DS, Thomay AA, Gaughan J, *et al.* Outcomes in patients with mucosal melanomas. *J Surg Oncol*. 2013;**108**:516-20.

10 Oken MM, Creech RH, Tormey DC, *et al.* Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol* 1982; **5**:649–55.

11 Ballantyne AJ. Malignant melanoma of the skin of the head and neck. An analysis of 405 cases. *Am J Surg* 1970; **120**:425–31.

12 Mehra T, Grözinger G, Mann S, *et al.* Primary localization and tumor thickness as prognostic factors of survival in patients with mucosal melanoma. *PloS One* 2014; **9**:e112535.

13 Schaefer T, Satzger I, Gutzmer R. Clinics, prognosis and new therapeutic options in patients with mucosal melanoma: A retrospective analysis of 75 patients. *Medicine (Baltimore)* 2017; **96**:e5753.

14 Lian B, Cui CL, Zhou L, *et al.* The natural history and patterns of metastases from mucosal melanoma: an analysis of 706 prospectively-followed patients. *Ann Oncol* 2016. doi:10.1093/annonc/mdw694.

15 Khademi B, Bahranifard H, Nasrollahi H, Mohammadianpanah M. Primary mucosal melanoma of the sinonasal tract: report of 18 patients and analysis of 1077 patients in the literature. *Braz J Otorhinolaryngol* 2011; **77**:58–64.

16 Del Prete V, Chaloupka K, Holzmann D, *et al.* Noncutaneous Melanomas: A Single-Center Analysis. *Dermatol Basel Switz* 2016; **232**:22–9.

17 Seifried S, Haydu LE, Quinn MJ, *et al.* 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**:1959–66.

18 Kim HS, Kim EK, Jun HJ, *et al.* Noncutaneous malignant melanoma: a prognostic model from a retrospective multicenter study. *BMC Cancer* 2010; **10**:167.

19 Mihajlovic M, Vlajkovic S, Jovanovic P, Stefanovic V. Primary mucosal melanomas: a comprehensive review. *Int J Clin Exp Pathol* 2012; **5**:739–53.

20 Cheung MC, Perez EA, Molina MA, *et al.* Defining the role of surgery for primary gastrointestinal tract melanoma. *J Gastrointest Surg* 2008; **12**:731–8.

21 Tasseron EW, van der Esch EP, Hart AA, Brutel de la Rivière G, Aartsen EJ. A clinicopathological study of 30 melanomas of the vulva. *Gynecol Oncol* 1992; **46**:170–5.

22 Chang AE, Karnell LH, Menck HR. The National Cancer Data Base report on cutaneous and noncutaneous melanoma: a summary of 84,836 cases from the past decade. The American College of Surgeons Commission on Cancer and the American Cancer Society. *Cancer* 1998; **83**:1664–78.

23 Stang A, Streller B, Eisinger B, Jöckel KH. Population-based incidence rates of malignant melanoma of the vulva in Germany. *Gynecol Oncol* 2005; **96**:216–21.

24 Rahbari H, Nabai H, Mehregan AH, Mehregan DA, Mehregan DR, Lipinski J. Amelanotic lentigo maligna melanoma: a diagnostic conundrum - presentation of four new cases. *Cancer* 1996; **77**:2052–7.

25 Ragnarsson-Olding BK, Kanter-Lewensohn LR, Lagerlöf B, Nilsson BR, Ringborg UK. Malignant melanoma of the vulva in a nationwide, 25-year study of 219 Swedish females: clinical observations and histopathologic features. *Cancer* 1999; **86**:1273–84.

26 Beadling C, Jacobson-Dunlop E, Hodi FS, *et al.* KIT gene mutations and copy number in melanoma subtypes. *Clin Cancer Res* 2008; **14**:6821–8.

27 Schoenewolf NL, Bull C, Belloni B, *et al.* Sinonasal, genital and acrolentiginous melanomas show distinct characteristics of KIT expression and mutations. *Eur J Cancer 1990* 2012; **48**:1842–52.

28 Si L, Guo J. C-kit-mutated melanomas: the Chinese experience. *Curr Opin Oncol* 2013; **25**:160–5.

29 Santi R, Simi L, Fucci R, *et al.* KIT genetic alterations in anorectal melanomas. *J Clin Pathol* 2015; **68**:130–4.

30 Colombino M, Lissia A, Franco R, *et al.* Unexpected distribution of cKIT and BRAF mutations among southern Italian patients with sinonasal melanoma. *Dermatol Basel Switz* 2013; **226**:279–84.

31 Zebary A, Jangard M, Omholt K, Ragnarsson-Olding B, Hansson J. KIT, NRAS and BRAF mutations in sinonasal mucosal melanoma: a study of 56 cases. *Br J Cancer* 2013; **109**:559–64.

32 Tseng D, Kim J, Warrick A, *et al.* Oncogenic mutations in melanomas and benign melanocytic nevi of the female genital tract. *J Am Acad Dermatol* 2014; **71**:229–36.

33 van Engen-van Grunsven ACH, Küsters-Vandevelde HVN, De Hullu J, *et al.* NRAS mutations are more prevalent than KIT mutations in melanoma of the female urogenital tract--a study of 24 cases from the Netherlands. *Gynecol Oncol* 2014; **134**:10–4.

34 Choi YD, Chun SM, Jin SA, Lee JB, Yun SJ. Amelanotic acral melanomas: clinicopathological, BRAF mutation, and KIT aberration analyses. *J Am Acad Dermatol* 2013; **69**:700–7.

35 Bhatia P, Friedlander P, Zakaria EA, Kandil E. Impact of BRAF mutation status in the prognosis of cutaneous melanoma: an area of ongoing research. *Ann Transl Med* 2015; **3**:24.

36 Greaves WO, Verma S, Patel KP, *et al.* Frequency and spectrum of BRAF mutations in a retrospective, single-institution study of 1112 cases of melanoma. *J Mol Diagn JMD* 2013; **15**:220–6.

37 Jethanamest D, Vila PM, Sikora AG, Morris LGT. Predictors of survival in mucosal melanoma of the head and neck. *Ann Surg Oncol* 2011; **18**:2748–56.

38 Dickson PV, Gershenwald JE. Staging and prognosis of cutaneous melanoma. *Surg Oncol Clin N Am* 2011; **20**:1–17.

39 Moreno MA, Roberts DB, Kupferman ME, *et al.* Mucosal melanoma of the nose and paranasal sinuses, a contemporary experience from the M. D. Anderson Cancer Center. *Cancer* 2010; **116**:2215–23.

40 Lawaetz M, Birch-Johansen F, Friis S, *et al.* Primary mucosal melanoma of the head and neck in Denmark, 1982-2012: Demographic and clinical aspects. A retrospective DAHANCA study. *Acta Oncol Stockh Swed* 2016; **55**:1001–8.

41 Shuman AG, Light E, Olsen SH, *et al.* Mucosal melanoma of the head and neck: predictors of prognosis. *Arch Otolaryngol Head Neck Surg* 2011; **137**:331–7.

42 Owens JM, Roberts DB, Myers JN. The role of postoperative adjuvant radiation therapy in the treatment of mucosal melanomas of the head and neck region. *Arch Otolaryngol Head Neck Surg* 2003; **129**:864–8.

43 Tas F, Keskin S. Mucosal melanoma in the head and neck region: different clinical features and same outcome to cutaneous melanoma. *ISRN Dermatol* 2013; **2013**:586915.

44 Song H, Wu Y, Ren G, Guo W, Wang L. Prognostic factors of oral mucosal melanoma: histopathological analysis in a retrospective cohort of 82 cases. *Histopathology* 2015; **67**:548–56.

45 Tcheung WJ, Selim MA, Herndon JE 2nd, Abernethy AP, Nelson KC. Clinicopathologic study of 85 cases of melanoma of the female genitalia. *J Am Acad Dermatol* 2012; **67**:598–605.

Legends for figures:

Figure 1

Kaplan–Meier survival curves (number of subjects and confident band at 95%) showing survival according to the age (<50 years and ≥50 years).

Figure 2

Kaplan–Meier survival curves (number of subjects and confident band at 95%) showing survival according to the initial stage (I, II or III).

Figure 3

Kaplan–Meier survival curves (number of subjects and confident band at 95%) showing survival according to the melanoma localization (genital or extra-genital).

Table 1. Main clinical and histological features of the 86 mucosal melanomas

	All sites	Vaginal	Vulvar	Glans	Gastrointestinal	Oral	Sinonasal	Anal
Patients'number (%)	86 (100)	7 (8.1)	30 (34.9)	3 (3.5)	8 (9.3)	11 (12.8)	16 (18.5)	11 (12.8)
Sex (M/F)	25/61	0/7	0/30	3/0	6/2	5/6	9/7	2/9
Mean age at diagnosis (years)	63	67	59	54	69	56	67	67
Amelanotic melanomas (%)	15 (17)	2 (28.6)	3 (10)	0	1 (12.5)	4 (36.4)	5 (31.2)	0
Thickness (%)								
TX	4 (4.7)	0	0	0	1 (12.5)	1 (9.1)	2 (12.5)	0
Tis	2 (2.3)	0	1 (3.3)	1(33.3)	0	0	0	0
T_1	18 (20.9)	0	11 (36.6)	2(66.6)	1 (12.5)	1 (9.1)	0	3 (27.2)
T_2	5 (5.8)	0	4 (13.3)	0	0	1 (9.1)	0	0
T_3	11 (12.8)	1 (14.3)	5 (16.6)	0	0	3 (27.2)	2 (12.5)	0
T_4	46 (53.5)	6 (85.7)	9 (30)	0	6 (75)	5 (45.5)	12 (75)	8 (72.7)
Lymph Node status(%)								
NX	3 (3.5)	0	0	0	0	2 (18.2)	1 (6.3)	0
N_0	66 (76.7)	6 (85.7)	23 (76.7)	3 (100)	3 (37.5)	8 (72.7)	15 (93.8)	8 (72.7)
N_{1-3}	17 (19.8)	1 (14.3)	7 (23.3)	0	5 (62.5)	1 (9.1)	0	3 (27.2)
Metastasis (%)								
M_0	76 (75.6)	5 (71.4)	28 (93.3)	3 (100)	5 (62.5)	11 (100)	14 (87.5)	10 (90.9)

M_1	10 (24.4)	2 (28.6)	2 (6.6)	0	3 (37.5)	0	2 (13)	1 (9.1)
Thickness in N_0M_0 (%)								
Total N_0M_0	66 (69.8)	5 (71.4)	23 (76.7)	3 (100)	3 (37.5)	10 (90.9)	14 (87.5)	8 (72.7)
TX N_0M_0	4 (4.7)	0	0	0	1 (12.5)	1 (9.1)	2 (12.5)	0
$TisN_0M_0$	2 (2.3)	0	1 (3.3)	1(33.3)	0	0	0	0
$T1N_0M_0$	17(19.8)	0	11 (36.6)	2(66.6)	1 (12.5)	1 (9.1)	0	2 (18.1)
$T2N_0M_0$	4(4.7)	0	3 (10)	0	0	1 (9.1)	0	0
$T3N_0M_0$	10	1 (14.3)	5 (16.6)	0	0	2 (18.2)	2 (12.5)	0
$T4N_0M_0$	29	4 (42.9)	3 (10)	0	1 (12.5)	5 (45.5)	10 (56.2)	6 (54.5)
Sentinel lymph node biopsy (%)	19 (22.1)	2(28.6)	11(36.6)	0	0	2 (18.2)	0	4 (36.4)
Positive sentinel lymph node (%)	6 (31.6)	0	4 (36.4)	0	0	1 (50)	0	1 (25)
Mean overall survival (months)	23	21	45	60	16	63	40	52

Tis: in situ, T1 : ≤1.0 mm, T2 : 1.01–2.0 mm, T3: 2.01–4.0 mm, T4 >4.0 mm, TX : Primary tumor thickness cannot be assessed ; NX: patients in whom the regional nodes cannot be assessed; N0: no regional metastases detected; N1: 1 metastatic lymph node; N2: 2-3 nodes, N3: 4 or more metastatic nodes

Table 2. Presence of *c-kit* mutation in the 43 tested mucosal melanomas

Localisation	Tested melanomas	Mutated melanomas (%)
GENITAL	15	0
Vulvar	11	0
Vaginal	4	0
Glans	0	0
EXTRAGENITAL	28	5 (18)
Oral	3	0 (0)
Sinonasal	11	2(18)
Anal	8	2(25)
Gastrointestinal	6	1(17)
ALL SITES	43	5 (11.6)