Retracted ***CD117 immunohistochemical expression correlates with poor outcome in vulvar melanoma.*** Retracted

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Disclosure of interest: none to declare.

Keywords: melanoma, vulva, cancer, c-KIT, CD117, immunohistochemistry

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ABSTRACT

Aim. Melanoma of the vulva is the second most common vulvar cancer after epidermoide carcinoma. Patients with the disease usually present with a late stage disease with a poor prognosis. The prognostic factors reported in previous studies are not homogeneous and it is no clear the clinical or pathogenic role of c-KIT expression this neoplasm. Breslow staging currently is the most accurate predictor factor. **Materials/Method.** We performed a clinicopathological study with literature review to identify predictors of prognosis and survival in melanoma of the vulva and investigated the expression of c-KIT (by immunohistochemistry) in 10 patients from the Instituto Nacional de Cancerología (Mexico City, Mexico). **Results.** Ten patients were identified, all older women with delayed presentation, high stage disease and limited response to treatment. We identified 5 patients (50%) with c-KIT expression, four of them recurred (p=0.01) and ultimately 3 died (p=0.038). We identified a) Satellitosis and b) c-KIT expression as prognostic predictors for death. **Conclusions.** We conclude that c-KIT expression is a valuable predictor of prognosis and survival, especially in tick (> 4 mm) melanoma.

Keywords: melanoma, vulva, cancer, c-KIT, CD117, immunohistochemistry.

INTRODUCTION

Malignant melanoma of the vulva (MMV) is a very rare neoplasm representing less of 1% of all female genital tract malignancies. However, MMV is the second vulvar cancer after epidermoid carcinoma with an incidence of 0.1 / 100,000 individuals[1]. Typically, older women are affected with an average patient age of 70 years[2]. Women affected commonly present with late stage disease and have a poor overall prognosis with 5-year survival rates of 8 to 61%[3,4]. This is unlike cutaneous melanomas where, as a result of increased clinical awareness, many patients are now diagnosed at an early stage. However, nowadays there has been a substantial increase in understanding the biology of malignant melanoma, especially in .cases arising in non-sun-exposed areas, like mucosal sites. Furthermore, melanomas arising in mucosal sites have been shown to differ not only from cutaneous melanomas but also from site to site with a substantial heterogeneity of alterations in a number of genes, some of which such as BRAF or KIT may be targeted by specific drugs. Today there is no consensus in the adequate staging system and the treatment for MVV. The standard FIGO staging is not satisfactory. In MVV, lesions are usually small and prognosis is related to Breslow rather than diameter. For this reason, Breslow staging today is the best independent prognostic factor[1,5-7].

For this reason, there is a search for histopathological features that allow improve the prognosis of MVV; but the majority of studies are small case series, retrospective reviews and have showed inconclusive and inconsistent results. We performed a clinicopathological review of 10 MVV cases of Mexican patients to

identify potential predictors of outcome, and immunohistochemistry for c-KIT expression in a whole section specimen slides.

Materials and Methods

A total of 10 primary malignant melanomas of the vulva were collected from the archives of the Pathology Department at Instituto Nacional de Cancerología de México, Mexico city, Mexico, in the period between January of 2005 to November of 2014. Patients with a history of extragenital melanoma or with synchronous extragenital melanomas detected on clinical examination were excluded. The slides of each case were reviewed for tumor depth according to Breslow, the presence of ulceration, growth phase, satellitosis, tumoral lymphocytic infiltrate, regression data and surgical margins measured in mm. Follow-up information was available in all cases (median follow-up 28.6 months, range 1–89 months), within this time, 3 patients had died of disease, 2 were alive with disease , and 5 were alive without evidence of disease.

Immunohistochemistry against KIT were performed according to standard procedures. In brief, deparaffinized slides were subjected to heat-induced epitope retrieval (citrate buffer, pH 6.0) followed by incubation with a polyclonal KIT antiserum (Dako) at a dilution of 1:50. For visualization, a modified avidin-biotin-complex method was employed using the LSABþ Kit (Dako) according to the manufacturer's instructions.

Statistical analysis. The clinicopathological data were collected in an database based on EXEL (Microsoft Windows, U.S.) and were analyze with SPSS statistical software (U.S.). Mean values with standard deviation and range were generated for longitudinal datasets and nominal data were presented as percentages. Potential risk factors for mortality were assessed using Kaplan-Meier curves and Cox hazards models. As the number of cases was limited, the significance of each hazard ratio (HR) was primarily assessed by their effect size as p-values alone were likely to miss important results.

Results

Patient and tumor characteristics are summarized in Table 1. The average patient age at the time of diagnosis was 61.5 years (range, 26–81 years). The predominant growth pattern was vertical (90%) followed by superficial spreading (horizontal) (10%). Ulceration was present in 80% of cases, but interestingly all c-KIT positive cases were ulcerated. In all, six cases (60%) were deeply infiltrative (tumor depth of >4 mm) and only one case (10%) was a superficial tumor (tumor depth < 1 mm). The average Breslow tumor depth was 4.3 mm (range 0.2-11 mm). The average mitotic count form square millimeter was 13.2 (range 2-40). Three cases showed regression data. Using immunohistochemistry, moderate or strong cytoplasmic KIT expression was detected in 5 of the 10 cases (50%).

Of the patients with c-KIT expression four recurred during the follow-up (p=0.010) and merit a pelvic exenteration (p=0.010). Ultimately, three of the patients with c-KIT expression dead of the disease (p=0.038). The c-KIT expression was also

associated with the presence of satellitosis but without statistical significance (p=0.114) (Table 2).

Presence of satellitosis and the presence of immunohistochemical C-KIT expression were significantly associated with poorer patient outcome (overall survival) in univariate analysis (Table 3, Figures 1 and 2); multivariate analysis was not performed owing to the low numbers of patients.

Discussion

Patients with MVV commonly present with a late stage disease and have a poor prognosis. The MVV etiology is poorly understood and the prognostic factors reported in the literature are not fully conclusive. Research in MVV is also limited due to the low incidence of cases, but over the past years, a surprising molecular heterogeneity of malignant melanoma has emerged. Activating V600E or V600K mutations in the BRAF kinase have been observed in up to 62% of melanomas arising in sun-exposed skin[8]. Targeting BRAF using specific inhibitors like dabrafenib or vemurafenib has led to substantially increased survival rates in BRAF mutated, but not in BRAF wild-type melanoma[9]. However, in melanomas arising on mucosal surfaces or non-sun-exposed skin, BRAF mutations have only infrequently been reported. C-KIT mutations and amplifications have been observed in varying frequencies in melanomas arising from different primary sites including mucosae[10-13]. According to these exposed data, we tested our tumors in the series only for C-KIT expression. In our series we also found an increased c-KIT protein expression in 50% of the patients, suggesting a potential role of c-KIT

in MVV. In fact, c-KIT mutations have been shown to be more common in vulvar than cutaneous melanomas[14,15]. c-KIT is a receptor tyrosine kinase regulating a variety of biological responses, such as chemotaxis, cell proliferation, apoptosis and adhesion in many cell types, including melanocytes, and activating KIT mutations are integral for tumor growth and progression; however, their role in MVV is yet not known[16].

Additionally, immunohistochemical C-KIT protein expression or overexpression has been reported to correlate with C-KIT mutations or amplifications but has been insufficient to predict response to C-KIT-targeted therapy with imatinib. In a recent phase II study, response rates for metastatic melanomas treated with imatinib mesylate were 64.7% in patients with KIT exon 11 mutations, 40% for exon 17 mutations, and 33% for KIT amplifications[13]. The frequency of KIT mutations in mucosal melanomas has been reported to be as high as 39%[17]. We observed five C-KIT positive cases in our series, all with poor overcome. Some other authors had published similar results[17]. Although some authors interpret vulvar tumors as melanomas of the non-sun-exposed skin, MVV show molecular and morphological similarities to head and neck mucosal primaries that typically lack KIT mutations [18]. Over the years, several histopatological features have been shown to correlate with adverse prognosis, including Breslow's depth, ulceration, epithelioid cell type, microsatellitosis, regression, angiolymphatic involvement, high mitotic rate, amelanosis and association with an existing nevus. In our series, the average Breslow depth was very high, superior to 4 mm in most cases, a great reason for not have been demonstrated as a prognostic factor. Nevertheless, our study also

identified other predictive features. Among those was the presence of satellitosis and c-KIT expression were also identified as a strong negatives predictors of DFS and a strong positive predictor of earlier relapse, these parameters let us identified in a subset of patients with poor prognostic MVV, especially in patients with a high Breslow depth. These patients relapsed and died of disease with a prediction of 100% sensitivity, making the presence of these characteristics strong predictors for outcome, both in terms of relapse and survival. This group of patients may qualify for follow-up after surgery, particularly when an optimal adjuvant therapy is not available.

In conclusion, MMV are aggressive tumors associated with poor overall survival. This tumors commonly are with a high Breslow's depth at the diagnosis, highlighting the need for novel adjuvant treatment approaches. As overall survival in patients with MVV is very poor, our data provide a rationale for C-KIT mutation testing and targeted treatment with specific inhibitors that may be beneficial in this neoplasm.

References

1. Heinzelmann-Schwarz VA, Nixdorf S, Valadan M et al. A clinicopathological review of 33 patients with MVV identifies c-KIT as a prognostic marker. Int J Mol Med. 2014;33(4):784-94.
2. Stang A, Streller B, Eisinger B, et al. Population-based incidence rates of malignant melanoma of the vulva in Germany. Gynecol Oncol 2005;96:216–21.

3. Ragnarsson-Olding BK, Nilsson BR, Kanter-Lewensohn LR, et al. Ringborg UK.Malignant melanoma of the vulva in a nationwide, 25-year study of 219 Swedish females: predictors of survival. Cancer 1999;86:1273–84.
4. Bradgate MG, Rollason TP, McConkey CC, et al: Malignant melanoma of the vulva: a clinicopathological study of 50 women. Br J Obstet Gynaecol 1990;97: 124-33.
5. Breslow A: Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. Ann Surg 1970; 172: 902-8.
6. Kim CJ, Reintgen DS and Balch CM: The new melanoma staging system. Cancer Control 2002;9: 9-15.
7. Zambo K, Szabo Z, Schmidt E, et al: Is the clinical staging system a good choice in the staging of vulvar malignancies? Eur J Nucl Med Mol Imaging 2007;34:1878-9
8. Cohen Y, Rosenbaum E, Begum S, et al. Exon 15 BRAF mutations are uncommon in melanomas arising in non-sun-exposed sites. Clin Cancer Res 2004;10: 3444–7.
9. Menzies AM, Haydu LE, Visintin L, et al. Distinguishing clinicopathologic features of patients with V600E and V600K BRAF-mutant metastatic melanoma. Clin Cancer Res 2012;18:3242–9.
10. Curtin JA, Busam K, Pinkel D, et al. Somatic activation of KIT in distinct subtypes of melanoma. J Clin Oncol 2006;24:4340–6.
11. Satzger I, Schaefer T, Kuettler U, et al. Analysis of c-KIT expression and KIT gene mutation in human mucosal melanomas. Br J Cancer 2008;99:2065–9.

12. Omholt K, Grafstro¨m E, Kanter-Lewensohn L, et al. KIT pathway alterations in mucosal melanomas of the vulva and other sites. Clin Cancer Res 2011;17: 3933–42.
13. Guo J, Si L, Kong Y, et al. Phase II, open-label, single arm trial of imatinib mesylate in patients with metastatic melanoma harboring c-Kit mutation or amplification. J Clin Oncol 2011;29:2904–9.
14. Omholt K, Grafstrom E, Kanter-Lewensohn L, et al: KIT pathway alterations in mucosal melanomas of the vulva and other sites. Clin Cancer Res 2011;17: 3933-42.
15. Schoenewolf NL, Bull C, Belloni B, et al: Sinonasal, genital and acrolentiginous melanomas show distinct characteristics of KIT expression and mutations. Eur J Cancer 2012; 48: 1842-52.
16. Mehnert JM and Kluger HM: Driver mutations in melanoma: lessons learned from bench-to-bedside studies. Curr Oncol Rep 2012;14: 449-57.
17. Aulmann S, Sinn HP, Penzel R, et al. Comparison of molecular abnormalities in vulvar and vaginal melanomas. Mod Pathol. 2014;27(10):1386-93.
18. Gru AA, Becker N, Dehner LP, Pfeifer JD. Mucosal melanoma: correlation of clinicopathologic, prognostic, and molecular features. Melanoma Res. 2014;24(4):360-70.

Figure legends

Figure 1. Kaplan-Meier survival curves for survival over a specific time frame (months) for patients who had satellite lesions (lower line) versus the ones who had not (upper line).

Figure 2. Kaplan-Meier survival curves for survival over a specific time frame (months) for patients who had c-KIT expression by immunohistochemistry (lower line) versus the ones who had not (upper line).