ANTICANCER RESEARCH *41*: 999-1004 (2021)
doi:10.21873/anticanres.14854

Lower Genital Tract Melanomas: Staging, Predictors of Outcome, and New Therapeutic Options

EVA KATHARINA EGGER[1], MATTHIAS B. STOPE[1], FLORIAN RECKER[1], DOMINIQUE KONSGEN[1], JENNIFER LANDSBERG[2], ANNE FROHLICH[2], ALINA ABRAMIAN[3] and ALEXANDER MUSTEA[1]

[1]*Department of Gynecology and Gynecological Oncology, University Hospital Bonn, Bonn, Germany;*
[2]*Department of Dermatology and Allergy, University Hospital Bonn, Bonn, Germany;*
[3]*Department of Senology, University Hospital Bonn, Bonn, Germany*

Abstract. *Background/Aim: Identification of predictors of survival of patients with lower genital tract melanoma (LGTM) and evaluation of the effectiveness of immunotherapy. Patients and Methods: Data of twenty women with LGTM were retrospectively collected. Survival outcomes were evaluated using the Kaplan–Meier method. Survival distributions were analyzed using the Log rank test. Results: Twenty patients with LGTM (6 vaginal/14 vulvar) were evaluated. Factors significantly affecting Five-year OS was the stage of the American Joint Committee on Cancer (AJCC 2017) (I+II: 55.6% vs. III+IV: 25.9%; p=0.030) and the T-Stage (I+II: 100% vs. III+IV: 7.5%; p=0.280). Factors negatively affecting Five-year PFS was T-Stage >II (p=0.005), AJCC stage >II (p<0.001), depth of tumor infiltration >3 mm (p=0.008), nodal involvement (p=0.013), distant disease (p=0.002), and resection margins <10 mm (p=0.024). Nine patients received immunotherapy [median duration of response (DOR)=4 months]. Three patients received immuno- and radiation therapy (median DOR of 5 months). Two patients received T-VEC, only one responded. Conclusion: Surgery has a therapeutic effect in early stage LGTM. Advanced stages may be treated with immunotherapy, radiation therapy, a combination of both, and oncolytic viral immunotherapy.*

Lower genital tract melanomas (LGTM) are extremely rare tumors, accounting for 5.5% of all vulvar malignancies and 5.3% of all vaginal malignancies (1-3). Originally, vulvar and vaginal melanomas were considered to be mucosal melanomas, but recent studies suggest a different molecular profile. Genetically, vulvar and vaginal melanomas exhibit *c-Kit* mutations in more than 20%, while mucosal melanomas show this only in 8% and cutaneous melanomas in 3%. *NRAS* mutations are rare, but *BRAF* mutations are present in up to 26%. PD-L1 and PD-1 are expressed in over 50% and 75% of cases, respectively (4).

Pathological predictors for overall survival in vulvar melanomas are lymph node involvement and mitotic count whereas in vaginal melanomas tumor thickness and mitotic count (2-4). Median overall survival for vaginal melanomas is as low as 19 months compared to 53 months in vulvar melanomas. This may reflect the advanced stage of vaginal melanomas at initial diagnosis, however, this may also be due to a different tumor biology (2, 3). So far data regarding immunotherapy in LGTM are scarce and limited to case series (5-8). The aim of the present study was to identify clinical and pathological predictors of progression-free survival (PFS) and overall survival (OS). Furthermore, we aimed to describe our experiences with immunotherapeutic interventions in recurrent and advanced LGTMs.

Patients and Methods

This is a retrospective study on patients with LGTMs treated at the Gynecologic Oncology department and at the Oncologic Dermatology Department of the University Hospital, Bonn, Germany between January 2007 and September 2020. This study was conducted in accordance with the standards of the local ethics committee of the medical faculty of the Rheinische Friedrich Wilhelms University, Bonn, Germany (Nr: 532/20). Data of all 20 patients were retrospectively reviewed regarding age, date of surgery, type of surgery, tumor entity, tumor stage according to the American Joint Committee on Cancer Classification of 2017 (AJCC 2017), lymph node involvement, resection margins, distant metastasis, lymphovascular space invasion, depth of tumor infiltration, *BRAF, NRAS* and *c-Kit* mutations and systemic therapies. The survival analyses for PFS and OS are based on the Kaplan–Meier method. The log rank test was used to compare

Correspondence to: Eva Egger, Department of Gynecology and Gynecological Oncology, University Hospital Bonn, Venusberg-Campus 1, 53127 Bonn, Germany. Tel: +49 22828715447, Fax: +49 22828716091, e-mail: eva-katharina.egger@ukbonn.de

Key Words: Lower genital tract melanoma, immunotherapy.

Table I. *Pathological parameters.*

Parameter	Patients	%
Vulvar melanoma	14	70
Vaginal melanoma	6	30
M0 at initial diagnosis	18	90
M1 at initial diagnosis	2	10
N- at initial diagnosis	10	50
N+ at initial diagnosis	10	50
T1 at initial diagnosis	2	10
T2 at initial diagnosis	2	10
T3 at initial diagnosis	3	15
T4 at initial diagnosis	13	65
R0 at first surgery	17	85
R1 at first surgery	2	10
Tumor thickness >3 mm	9	45
Tumor thickness < 3 mm	10	50
Stage (AJCC 2017) I	3	15
Stage (AJCC 2017) II	7	35
Stage (2017) III	8	40
Stage (AJCC 2017) IV	2	10
Resection margin 0-5 mm	9	45
Resection margin =10 mm	6	30
Resection margin >10	2	10
Resection margin unknown	2	10
No Resection	1	5

survival distributions to various clinical factors. All analyses were performed using Minitab Version 18, Minitab LLC., State College, Pennsylvania, USA.

Results

Patient characteristics. Between 2007 and 2020, 20 patients with a median age of 70.5 years (range=47-82 years) were diagnosed with LGTM. In detail, 14 vulvar melanomas and 6 vaginal melanomas were diagnosed. Two of the 6 vaginal melanomas also infiltrated into the vulva, 1 melanoma had grown into the anal mucosa, and 1 patient initially presented with a urethral melanoma which later metastasized into the vagina (Table I), 1 patient had a *BRAF* mutation, 1 patient had a *NRAS* mutation and 4 patients had *c-Kit* mutations.

Recurrence and survival data. Table II shows the clinical parameters that significantly affected the 5-year DFS and 5-year OS. Table III shows the 3 factors that significantly affected the 5-year DFS only. No significant influence on DFS and OS was found for the resection status (R1 *vs*. R0), the age of the patients, the location (vulvar *vs*. vaginal), the time to first recurrence, and whether the patients were treated adjuvantly. Median OS was 56 months and median PFS was 12 months. A total of 15 patients relapsed, 1 patient experienced 7 relapses, 1 patient experienced 5 relapses, 2 patients experienced 4 relapses, 1 patient experienced 3 relapses, 5 patients experienced 2 relapses, and 5 patients experienced one relapse. Only 5 of 15 relapsed patients are still alive. Five patients are alive without relapse (range=14-76 months). All 5 had a tumor infiltration of less than 3 mm. Figure 1 shows the number of recurrences and the time intervals between recurrences of all 15 recurrent patients. Figure 2 shows the 5-year OS and PFS.

Table II. *Factors significantly influencing the 5-year progression-free survival (PFS) and the 5-year overall survival (OS).*

Factor	5-year PFS	*p*-Value
Entire study population	26.67%	
T-Stage (T1/T2)	100%	0.005
T-stage (T3/T4)	18.7%	
Stage (AJCC 2017) I+II	48%	0.000
Stage (AJCC 2017) III+IV	0%	
Factor	**5 year OS**	***p*-Value**
Entire study population	41.48%	
T-Stage (T1/T2)	100%	0.028
T-stage (T3/T4)	7.5%	
Stage (AJCC 2017) I+II	55.56%	0.030
Stage (AJCC 2017) III+IV	25.93%	

Table III. *Factors significantly influencing the 5-year progression-free survival (PFS) only.*

Factor	5 year PFS	*p*-Value
Depth of infiltration <3 mm	64.81%	0.008
Depth of infiltration >3 mm	0%	
N0	50%	0.013
N1,N2,N3	0%	
M0	29.63%	0.002
M1	0%	
Resection margin<10 mm	11%	0.024
Resection margin >10 mm	60%	

Immunotherapy. Of 15 patients with relapsed disease, 9 were treated with immunotherapy in different lines. Two out of these patients additionally received Talimogene laherparepvec (T-VEC). Table IV shows the details of local and systemic treatment of the 9 patients who also received immunotherapy. The observed duration of response (DOR) to immunotherapy varied widely in our cohort. In first line the DOR was 3 and 4 months in 2 patients, in the second line 5 to 11 months in 4 patients, in the third line 1 to 38 months in 4 patients, in the fourth line 2 and 4 months in 2 patients, and in the 5th line 1 month in 1 patient. Four of the 9 patients

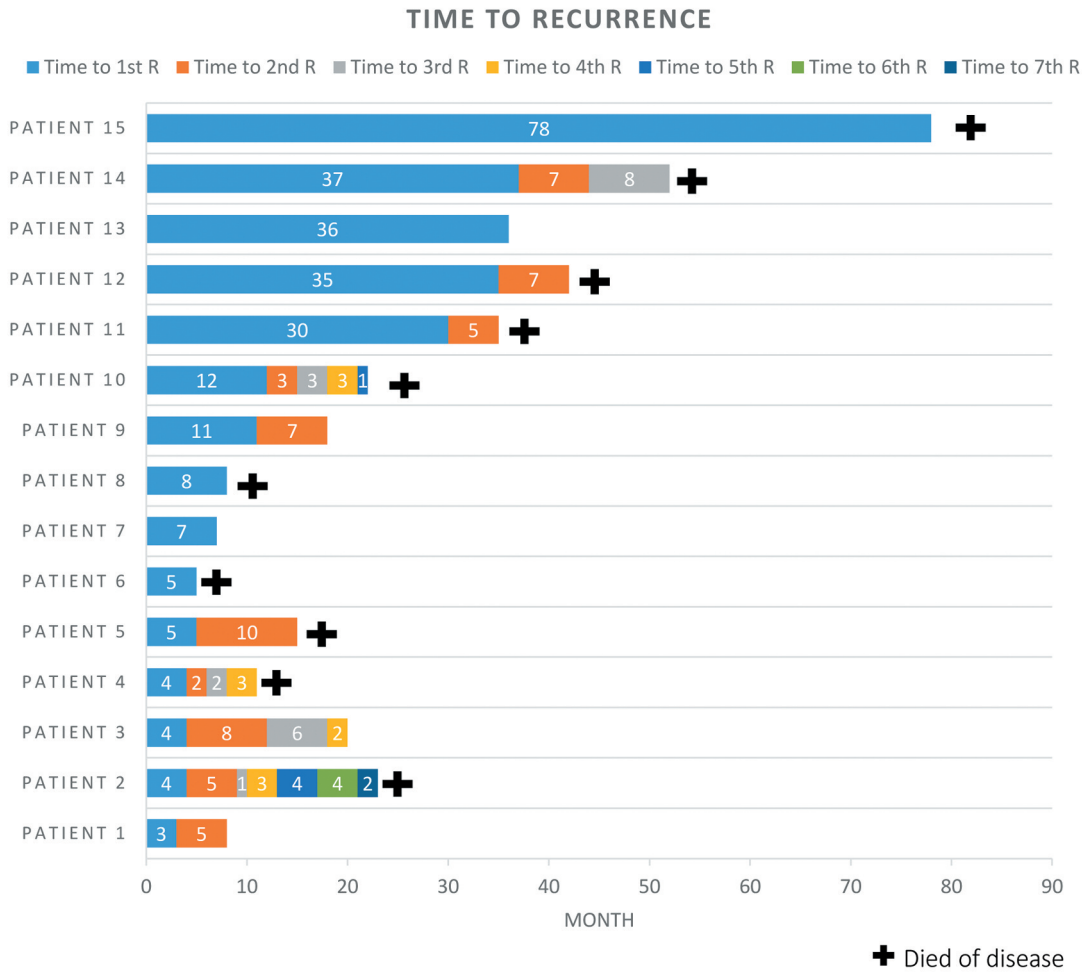

Figure 1. *Survival time and number of recurrence of all relapsed patients.*

are still alive. The survival benefit of immunotherapy ranged from 1 to 43 months in various lines. Of particular interest were patient no. 5 and 7. Patient 5 had a superficially spreading vulvar melanoma with ulcerations according to a stage IIIC disease (AJCC 2017) and an infiltration depth of 6 mm. At initial diagnosis, she received a dorsal hemivulvectomy, partial colpectomy and inguinal sentinel lymphadenectomy. After 11 months she relapsed for the first time and was treated with a pelvic exenteration. Due to systemic metastases in the liver, lung, and pleura only 7 months later she was treated with 1 cycle of pembrolizumab and 3 cycles of ipilimumab/nivolumab. Due to an immunogenic hypophysitis and a pneumonitis, therapy was discontinued. The follow-up showed a prolonged response despite therapy discontinuation and so far, the patient has not received any further therapy and has now remained stable for 38 months. Patient 7 had a superficial spreading melanoma of the vulva in stage IIC (AJCC 2017) with an infiltration depth of only 1.2 mm. Despite multiple surgeries, no clear

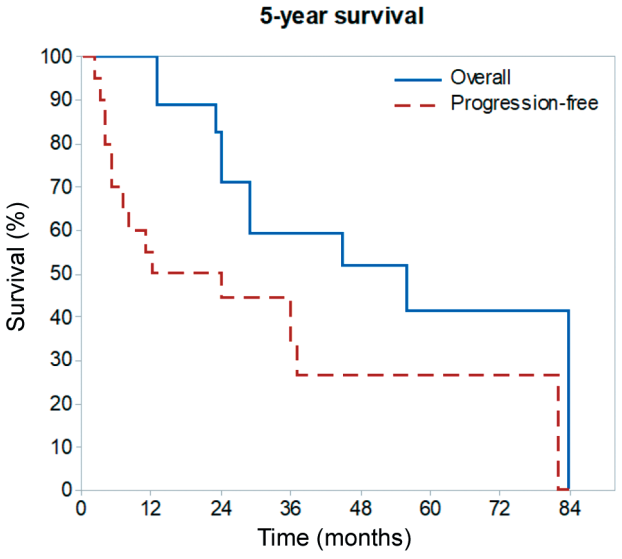

Figure 2. *Five-year overall and progression-free survival.*

Table IV. *Details of local and systemic treatments and times to recurrences.*

Pat.	1st T	Time to 1st R (months)	2nd T	Time to 2nd R (months)	3rd T	Time to 3rd R (months)	4th T	Time to 4th R (months)	5th T	Time to 5th R (months)	Follow-up status 1…alive 2…died of disease (month since initial diagnosis)
1	S/5xN	3	7x P/R	5	2xDac						1 (12)
2	4xI+N	4	7x P/R	5	1xN	1	5x T-VEC	3	7P+Embo	4	2 (24)
3	S	4	LNE+8xP	8	12xT-VEC	6	3xP	2	4xIma 3xT-VEC	3	1 (48)
4	S+INF	4	Dac+LNE	2	3xI	2	3xC/Pac	3	R		2 (24)
5	S	11	S	7	1xP-> 3xI/N						1 (56)
6	S+INF	12	S	3	INF	3	S	3	1xI	1	2 (29)
7	S+Aldara	32	7x P								1 (43)
8	S	37	None	7	2xDac-> 3xI	8	2xEmbo /2xP				2 (56)
9	S+IFN	78	S+3xI+R								2 (84)

S: Surgery; N: nivolumab, I: ipililumab; INF: interferon alpha; Dac: dacarbazin; P: pembrolizumab; Ima: imatinib; C/Pac: carboplatin/paclitaxel; Embo: embolisation; R: radiation; T-Vec: talimogene laherparepvec.

microscopic margins could be achieved. The patient refused exenterative procedures. Aldara was applied for several weeks. Only after 32 months the patient relapsed. After 7 doses of pembrolizumab, the patient remained alive until now without further progression or therapy.

Talimogene Laherparepvec. Two patients were treated with T-VEC. In detail, patient 2 was diagnosed with an anovaginal melanoma with liver metastases according to a stage IV disease (AJCC 2017). First line immunotherapy with 4 cycles of ipililumab and nivolumab resulted in immune-mediated hepatitis. As there was only locoregional disease progression and severe dyschezia, palliative radiotherapy with 5×5 Gray for the anovaginal region was started and 7 cycles of pembrolizumab were added. Due to a hepatic progression, ipililumab/nivolumab was reinduced but had to be discontinued due to immune-mediated hepatitis. While hepatic metastases remained stable, the patient progressed locally. To overcome this local resistance T-VEC was injected intratumorally 5 times every other week without remarkable tumor regression. Due to further local and hepatic progression after the 5th time of T-VEC, Pembrolizmab was reinduced and tumor embolization for palliation was started. Finally, the patient was selected for the Lipo-Merit trial (NCT02410733).

Patient 3 was treated with 12 intratumoral T-Vec injections due to local vaginal progression, but with stable nodal disease after 8 months of pembrolizumab (12, 13). With 12 intratumoral T-Vec injections every other week, the local tumor decreased significantly. The third and fourth progression was treated with pembrolizumab and imatinib, respectively, for a druggable *c-Kit* mutation. The reintroduction of T-VEC due to a new local progression was without benefit. Ipililumab and nivolumab as well as palliative percutaneous radiotherapy successfully stopped local progression. Immunotherapy had to be discontinued after 4 cycles due to immune mediated colitis, hepatitis and Addison disease. Currently, the immunotherapy is paused.

Safety. With regard to safety, there were no adverse events during treatment with T-VEC. Dose-limiting immune-related adverse events were observed in 4 patients. One patient with hepatitis, one patient with colitis, hepatitis, and hypophysitis, one patient with pneumonitis, and hypophysitis, and one patient with colitis. Two out of those 4 patients had received a combination therapy.

Discussion

Data on LGTMs are scarce. About one third of the patients will present with regional lymph node metastasis or even distant metastasis at initial diagnosis (2). Predictors for PFS are supposed to be *c-Kit* mutations, lymphovascular space invasion, Breslows thickness, and mitosis. To date, the GOG-71 study is the only prospective study on vulvar melanoma that concluded that AJCC-stage is the best predictor for OS (9). The AJCC stage and the T- stage represented the only predictors for the 5-year survival in our cohort. Depth of tumor infiltration, nodal involvement, distant metastasis, and resection margins were the only predictors of PFS. A *c-Kit*

mutation was observed in 4 patients without impact on OS and PFS. All 5 patients without relapse were characterized by an early AJCC stage, an early T-stage, no adjuvant therapy, and an infiltration depth of less than 3 mm.

Surgery remains the first choice in early melanomas. However, in advanced melanomas surgical margins of 1-2 cm are difficult to achieve in this region (2, 3).

The exact role of pelvic exenterations remains inconclusive. In our cohort 3 patients had received a pelvic exenteration and relapsed after 3 to 6 months. Two more patients would have been eligible for pelvic exenteration but declined this option. Both patients survived so far and relapsed after 8 months and 36 months, respectively. Due to the lack of data about long term survivorship after pelvic exenteration it seems reasonable to consider alternative options (10, 11).

In one patient local resistance to systemic immunotherapy was overcome by T-VEC injections (12, 13) Unfortunately, in another patient almost no response to T-VEC was observed. The phase III OPTIM trial in patients with unresectable cutaneous and subcutaneous melanoma stage IIIB-IV M1c showed a complete responses to T-VEC in 16% of patients with an objective response rate of 31.5% and a median time to complete response of 8.6 months (14). This fits well with our patient who had 6 months to complete local response to treatment. The combination of pembrolizumab and T-VEC due to the lack of effects of T-VEC on visceral metastases demonstrated promising results in the phase Ib/III MASTERKEY-265 trial (NCT02263508) without dose limitation or additional toxicity (15). So far, there are no further data on T-VEC injections for mucosal melanoma or LGTMs.

In addition, 3 patients were treated with the combination of immunotherapy and local percutaneous radiation. In 2 patients the DOR was 5 months and 1 patient died after 6 months. All 3 were treated with this combined therapy within their second relapse. Schiavone presented 4 cases of combined immunotherapy and radiotherapy with a DOR of 9 months, 16 months, 20 months, and 38 months. Only 1 patient died after 16 months (7). Response rates to immune checkpoint inhibitors in mucosal melanoma are considered as low as 23%-37.1% (8, 16). So far, there is no study that examines the role of immunotherapy specifically in LGTM. The pooled data from 6 studies identified 86 patients with mucosal melanoma receiving nivolumab and 35 patients with mucosal melanoma receiving ipililumab and nivolumab. While the combination increased efficacy, it also increased toxicity (8). Therapy-limiting toxicity was observed in our cohort in 4 out of 9 patients treated with immunotherapy. Only 2 of these 4 patients had received a combination therapy. Nevertheless, it remains doubtful whether this pooled analysis of mucosal melanomas also represents LGTM, since different molecular profiles of vulvovaginal melanomas with high PD-L1 and PD-1 expression are being proposed (4). Data on the role of immunotherapy within the adjuvant setting is limited to case reports and case series of mostly unresectable melanomas (6, 7). The DOR in our 9 patients varied widely even in different lines, which probably reflects a large tumor biological diversity of LGTMs. Despite the proposed decreased response to immunotherapy for mucosal melanoma, one patient with a vaginal melanoma with a DOR of more than 38 months could be observed beyond her treatment period. This is especially noteworthy as vaginal melanomas are not considered to be chemosensitive with overall survival rates of 22.2 month without the use of immunotherapy (17). Limitations of the present study are its retrospective nature, the small sample cohort and the heterogeneity of treatment lines.

Conclusion

LGTMs represent aggressive tumors with a worsening prognosis as T-stage and stage according to AJCC 2017 increase. In early stages the surgery has a therapeutic effect. In advanced stages, immunotherapy, radiotherapy, the combination of both, and oncolytic viral immunotherapy are therapeutic options in place of pelvic exenteration. Immunotherapy has the potential of a long DOR, even in the metastatic situation. An international register would be helpful to gain more understanding of this rare disease.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study. There was no source of financial or material support.

Authors' Contributions

Conception and design: E.K. Egger, A. Mustea; Data analysis and interpretation: E.K. Egger, F. Recker; Investigation: E.K. Egger, A. Abramian; Writing – original draft preparation: E.K. Egger, D. Könsgen, M.B. Stope; Writing – review and editing: J. Landsberg, A. Fröhlich; Supervision: A. Mustea.

References

1 Stang A, Streller B, Eisinger B and Jöckel KH: Population-based incidence rates of malignant melanoma of the vulva in Germany. Gynecol Oncol *96(1)*: 216-221, 2005. PMID: 15589604. DOI: 10.1016/j.ygyno.2004.09.052

2 Wohlmuth C, Wohlmuth-Wieser I, May T, Vicus D, Gien LT and Laframboise S: Malignant melanoma of the vulva and vagina: A US population-based study of 1863 patients. Am J Clin Dermatol *21(2)*: 285-295, 2019. PMID: 31784896. DOI: 10.1007/s40257-019-00487-x

3 Wohlmuth C and Wohlmuth-Wieser I: Vulvar malignancies: an interdisciplinary perspective, J Dtsch Dermatol Ges *17(12)*: 1257-1276, 2019. PMID: 31829526. DOI: 10.1111/ddg.13995

4 Hou JY, Baptiste C, Hombalegowda RB, Tergas AI, Feldman R, Jones NJ, Chatterjee-Paer S, Bus-Kwolfski A, Wright JD and Burke WM: 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)*: 1333-1344, 2017. PMID: 28026870. DOI: 10.1002/cncr.30473

5 Del Vecchio M, Di Guardo L, Ascierto PA, Grimaldi MA, Sileni VC, Pigozzo J, Ferraresi V, Nuzzo C, Rinaldi G, Testori G, Ferrucci PF, Marchetti P, De Galitiis F, Queirolo P, Tornari E, Marconcini R, Calabrò L and Maio M: Efficacy and safety of ipilimumab 3 mg/kg in patients with pretreated, metastatic, mucosal melanoma. Eur J Cancer *50(1)*: 121-127, 2014. PMID: 24100024. DOI: 10.1016/j.ejca.2013.09.007

6 Chanal J, Kramkimel N, Guegan S, Moguelet P, Fourchotte V and Avril MF: Locally advanced unresectable vaginal melanoma: response with anti-programmed death receptor 1. J Low Genit Tract Dis *20(1)*: 4-5, 2016. PMID: 26704337. DOI: 10.1097/LGT.0000000000000168.

7 Schiavone MB, Broach V, Shoushtari AS, Carvajal RD, Alektiar RD, Kollmeier MA, Abu-Rustum NR and Leitao Jr MM: Combined immunotherapy and radiation for treatment of mucosal melanomas of the lower genital tract. Gynecol Oncol Rep *14(16)*: 42-46, 2016. PMID: 27331137. DOI: 10.1016/j.gore.2016.04.001

8 D'Angelo SP, Larkin J, Sosman JA, Lebbé C, Brady B, Neyns B, Schmidt H, Hassel JC, Hodi FS, Lorigan P, Savage KJ, Miller Jr WH, Mohr P, Marquez-Rodas I, Charles J, Kaatz M, Sznol M, Weber JS, Shoushtari AS, Ruisi M, Jiang J and Wolchok JD: Efficacy and safety of nivolumab alone or in combination with ipilimumab in patients with mucosal melanoma: A pooled analysis. J Clin Oncol *35(2)*: 226-235, 2017. PMID: 28056206. DOI: 0.1200/JCO.2016.67.9258

9 Phillips GL, Bundy BN, Okagaki T, Kucera PR and Stehman FB: Malignant melanoma of the vulva treated by radical hemivulvectomy. A prospective study of the gynecologic oncology group. Cancer *73(10)*: 2626-2632, 1994. PMID: 8174062. DOI: 10.1002/1097-0142(19940515)73:10<2626::AID-CNCR2820731026>3.0.CO;2-U

10 DeMatos P, Tyler DS and Seigler HF: Malignant melanoma of the mucous membranes: A review of 119 cases. Ann Surg Oncol *5(8)*: 733-742, 1998. PMID: 9869521. DOI: 10.1007/BF02303485

11 Leitao MM: Management of vulvar and vaginal melanomas: current and future strategies. Am Soc Clin Oncol Educational Book *(34)*: e277-e281, 2014. PMID: 24857113. DOI: 10.14694/edbook_am.2014.34.e277

12 Fröhlich A, Hoffmann F, Niebel D, Egger E, Kukuk GM, Toma M, Sirokay J, Bieber T and Landsberg J: Talimogene laherparepvec in advanced mucosal melanoma of the urethra upon primary resistance on immune checkpoint inhibition: A case report. Front Oncol *8(10)*: 611, 2020. PMID: 32457834. DOI: 10.3389/fonc.2020.00611

13 Fröhlich A, Niebel D, Fietz S, Egger E, Buchner A, Sirokay J and Landsberg J: Talimogene laherparepvec treatment to overcome loco-regional acquired resistance to immune checkpoint blockade in tumor stage IIIB-IV M1c melanoma patients. Cancer Immunol Immunother *69(5)*: 759-769, 2020. PMID: 32052079. DOI: 10.1007/s00262-020-02487-x

14 Andtbacka RHI, Collichio F, Harrington KJ, Middleton MR, Downey G, Öhrling K and Kaufman H: Final analyses of OPTiM: A randomized phase III trial of talimogene laherparepvec *versus* granulocyte-macrophage colony-stimulating factor in unresectable stage III-IV melanoma. J Immunother Cancer *7(1)*: 145, 2019. PMID: 31171039. DOI: 10.1186/s40425-019-0623-z

15 Conry RM, Westbrook B, McKee S and Norwood TG: Talimogene laherparepvec: First in class oncolytic virotherapy. Hum Vaccin Immunother *14(4)*: 839-846, 2018. PMID: 29420123. DOI: 10.1080/21645515.2017.1412896

16 Indini A, Di Guardo L, Cimminiello C, Lorusso D, Raspagliesi F and Del Vecchio M: Investigating the role of immunotherapy in advanced/ recurrent female genital tract melanoma: A preliminary experience. J Gynecol Oncol *30(6)*: 94, 2019. PMID: 31576688. DOI: 10.3802/jgo.2019.30.e94

17 Rapi V, Dogan A, Schultheis B, Hartmann F, Rezniczek GA and Tempfer CB: Melanoma of the vagina: Case report and systematic review of the literature. Anticancer Res *37(12)*: 6911-6920, 2017. PMID: 29187473. DOI: 10.21873/anticanres.12155

Received December 13, 2020
Revised December 28, 2020
Accepted December 29, 2020