Received: 6 April 2020 | Revised: 24 July 2020 | Accepted: 27 July 2020
DOI: 10.1111/dth.14115

DERMATOLOGIC THERAPY WILEY

LETTER

Evaluation of two different mutations in codon 12 of NRAS gene in ulcerated penile mucosal nodular malignant melanoma pT4b of the 90-year-old man in perspective of targeted therapy of NRAS-mutated advanced melanomas

Dear Editor,

Melanoma constitutes less than 2% of penile malignancies to account for less than 0.1% of all melanoma cases and the patients are characterized by a 28-month-long median survival.[1,2] Hereby, we report NRAS mutations in penile mucosal melanoma of the uncircumcised 90-year-old man. An ulcerated, irregular, solid, brown-whitish tumor of 3.5 cm in diameter destructed non-keratinizing squamous multilayered epithelium of glans penis and coronal sulcus. It consisted of mostly amelanotic, dyscohesive cells of epithelioid type and plasmacytoid appearance with amphophilic cytoplasm around vesicular nuclei with large, eosinophilic nucleoli in melanoma cells in preserved nested architecture (Figure 1). The cells were HMB-45 positive (monoclonal mouse antibody, catalog number: M0634/10072941, DAKO dilution: 1:40, incubation time 30 minutes at 37°C) (Figure 2A, B) and Ki-67 labeled 65% of their population (M7240/00075880, DAKO dilution: 1:50, incubation time: 32 minutes at 37°C) (Figure 2C). They were CKAE1/AE3 negative (monoclonal mouse anti-human cytokeratin, clone: AE1/AE3, isotype: IgG1, kappa. M3515, DAKO dilution: 1:50, incubation time: 60 minutes at room temperature) (Figure 2D). A diagnosis of invasive mucosal nodular malignant melanoma of the penis was given (pT4b [eighth edition of pTNM], Breslow's tumor thickness: 12.5 mm corresponding to Clark level V in case of skin tumors, histologic nodular type, vertical growth phase, 21 mitoses/mm^2, lymphovascular invasion present, microscopic satellite focuses present, no tumor regression, non-brisk tumor-infiltrating lymphocytes).

Maxwell 16 MDx (Promega) was used to isolate DNA from samples of formalin-fixed paraffin-embedded tissue for evaluation by the next generation sequencing method. The libraries were prepared using the Ion AmpliSeq Library Kit 2.0 and the Ion AmpliSeq Cancer Hotspot Panel v2 Kit (covers 50 genes) according to the manufacturer's instructions (Thermo Fisher Scientific). Sequencing was performed on an Ion S5 sequencer and the raw data generated during sequencing were processed using the Torrent Server Suite 5.12 TSS (Thermo Fisher Scientific). Searching for different variants (single nucleotide polymorphism mutations) was carried out using the Variant

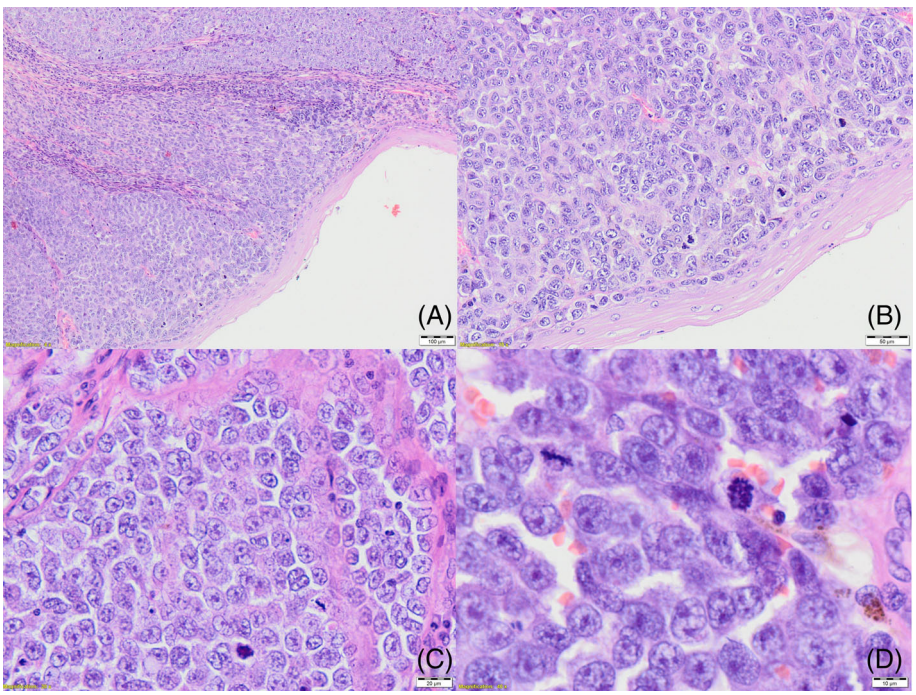

FIGURE 1 Morphology of penile melanoma. A, Mostly subepithelial densely cellular tumor of the top of the penis (magnification: ×40). B, Amphophilic appearance of melanoma cells (magnification: ×100). C, Nested pattern of growth of melanoma cells (magnification: ×200). D, Distinct mitoses and vesicular appearance of nuclei with prominent eosinophilic nucleoli and chromatin accumulation at the periphery of nuclei or in the form of clumps across the whole nuclear area. The cytoplasm is amphophilic and cells are characterized by plasmacytoid appearance (magnification: ×400)

Caller 5.12 program, which is part of Torrent Server Suite 5.12. To annotate the detected variants with the TSS, the wANNOVAR program (www.wannovar.usc.edu) was used. In result of sequencing, two different mutations were detected in the *NRAS* gene in codon 12, namely p.Gly12Ala and p.Gly12Asp (Figure 3) with an allelic frequency of 31% and 21%, respectively. No mutation was detected in the other genes analyzed.

Novel target therapeutics, which are not devoid of side effects, are administered in melanomas.[3-5] 15-20% of cutaneous melanomas harbor oncogenic driver activating NRAS mutations on average, while that rate was 33% in 15 BRAF mutation negative mucosal esophageal melanomas and 22% in 72 sinonasal melanomas to add that the exact mutation of NRAS as p.Q61L occurred in only one melanoma of scalp in a group of 18 NRAS wild-type tumors of head and neck.[5-8] NRAS mutations result in worse survival, relapse rate and far more unsatisfactory therapy response than patients with wild-type NRAS or with BRAF mutations.[5] However, NRASG12/13 mutations were less tumorigenic than NRASQ61 mutations which could execute even greater transformative potential via STAT3 recruitment.[5] Omholt et al reported on NRAS mutation c. 38G>A, p.G13D in penile melanoma.[9] In our described

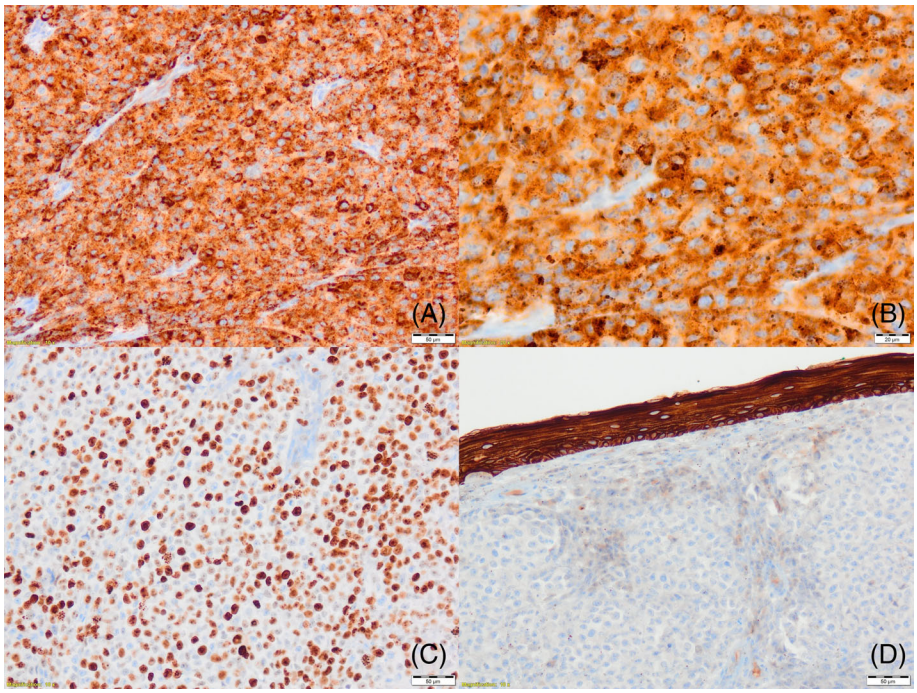

FIGURE 2 Immunohistochemistry of penile tumor. A, Strong, coarsely granular cytoplasmatic immunoreactivity to HMB-45 (magnification: ×100). B, Strong, coarsely granular cytoplasmatic immunoreactivity to HMB-45 (magnification: ×200). C, Strong nuclear immunoreactivity of Ki-67 that labeled 65% of melanoma cells (magnification: ×100). D, Negative immunoreactivity to CKAE1/AE3 in malignant cells in comparison to strong positive immunoreactivity in covering epithelial layer (magnification: ×100)

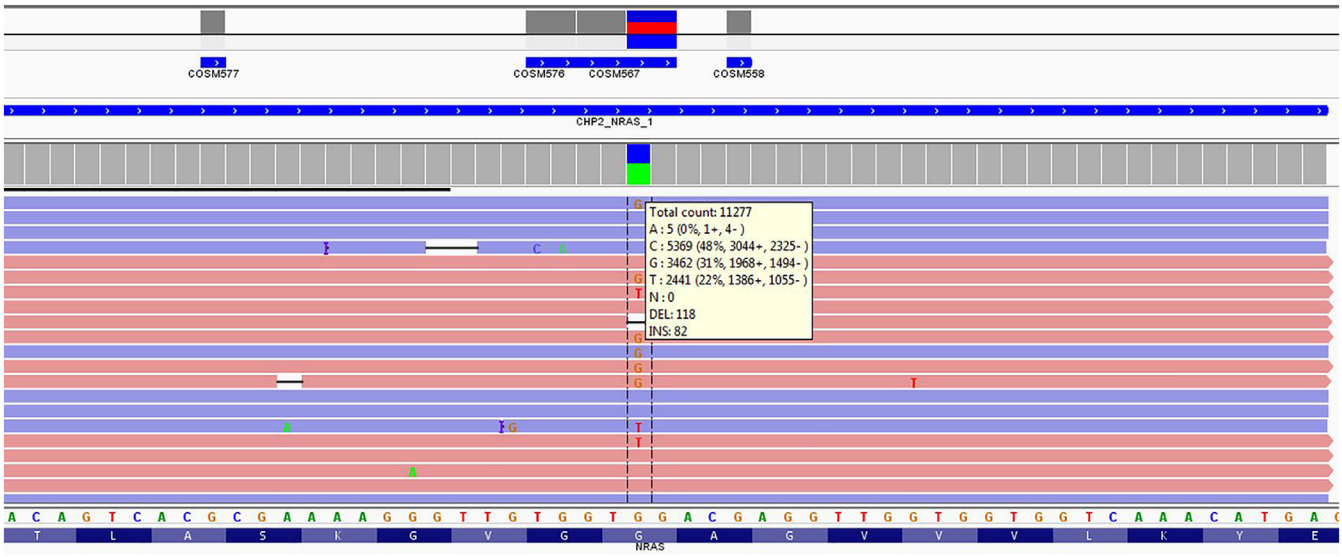

FIGURE 3 Sequencing results detected two mutations in codon 12 in NRAS gene c.35G>C (p.Gly12Ala) and c.35G>A (p.Gly12Asp). Next generation sequencing data showing reads (– strand designated in blue, + strand in red) with the reference sequences shown below the panel boxes. Variant bases are indicated by red and orange characters. As NRAS is located in the reverse orientation on chromosomes, the substituted bases appear on the figure in IGV as G and T instead of C and A, respectively

case, the mutational heterogeneity in the structure of NRAS that involved p.Gly12Ala and p.Gly12Asp is striking and raises the question of whether it is capable of induction of variegated response to therapy of NRAS with slightly differently mutated cells in the same tumor. However, it is reasonable to try trametinib (inhibitor of MEK [mitogen-activated protein, MAP, extracellular signal-regulated kinase, ERK, kinase], is also known MAP2K), because MEK is a downstream agent in relation to NRAS in signal transduction of cell survival and proliferation.[10]

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Artur Kowalik, Andrzej Wincewicz and Krzysztof Ossoliński conceived the presented idea. Krzysztof Ossoliński, Anna Ossolińska and Tadeusz Ossoliński surgically removed the tumor and provided the clinical data. Artur Kowalik and Andrzej Wincewicz designed the study. Andrzej Wincewicz preformed histopathological report and stated diagnosis. Janusz Kopczynski consulted the case. Kinga Hińcza and Artur Kowalik preformed molecular investigations and verified the analytical methods. Tadeusz Ossoliński and Stanisław Góźdź encouraged Artur Kowalik and Andrzej Wincewicz to investigate and supervise the findings of this work. All authors discussed the results and contributed to the final manuscript.

ETHICS STATEMENT

The study adhered to guidelines of Declaration of Helsinki adopted by the World Medical Association (WMA) in Helsinki, Finland, June 1964, and amended in subsequent, next revisions with inclusion of latest version issued at 64th WMA General Assembly, Fortaleza, Brazil, October 2013. An informed consent was obtained from the patient who was included in the study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Andrzej Wincewicz[1]
Kinga Hińcza[2]
Krzysztof Ossoliński[3]
Anna Ossolińska[3]
Tadeusz Ossoliński[3]
Janusz Kopczynski[4]
Stanisław Góźdź[5,6]
Artur Kowalik[2,7,8]

[1]*Specialist Medical Practice Pathologist, Non Public Health Care Unit Department of Pathology, Kielce, Poland*
[2]*Department of Molecular Diagnostics, Holy Cross Oncology Centre, Kielce, Poland*
[3]*Department of Urology, Hospital of Independent Public Health Care Unit in Kolbuszowa, Kolbuszowa, Poland*
[4]*Department of Pathology, Holy Cross Oncology Centre, Kielce, Poland*
[5]*Department of Clinical Oncology, Holy Cross Oncology Centre, Kielce, Poland*
[6]*Department of Prophylaxis and Cancer Epidemiology, Collegium Medicum, Jan Kochanowski University, Kielce, Poland*
[7]*Division of Medical Biology, Institute of Biology Jan Kochanowski University, Kielce, Poland*
[8]*Department of Tumor Pathology, Maria Sklodowska-Curie – Memorial National Institute of Oncology, Krakow, Poland*

Correspondence
Associate Professor Andrzej Wincewicz, Specialist Medical Practice Pathologist, (NZOZ Zakład Patologii Spółka z o.o.), Non Public Health Care Department of Pathology, Unit Warsaw Marymont, Kielce, ul. Jagiellońska 70, 25-734 Kielce, Poland.
Email: ruahpolin@yahoo.com

ORCID

Andrzej Wincewicz https://orcid.org/0000-0003-0836-2199

REFERENCES

1. De Giorgi V, Grazzini M, Massi D, et al. Melanoma of the penis: a clinical dermoscopic case study. *Acta Derm Venereol*. 2010;90:87-88.
2. Papeš D, Altarac S, Arslani N, Rajković Z, Antabak A, Ćaćić M. Melanoma of the glans penis and urethra. *Urology*. 2014;83:6-11.
3. Poduje S, Brozić JM, Prkačin I, Delaš Aždajić M, Goren A. Vemurafenib and cobimetinib-induced toxic epidermal necrolysis in a patient with metastatic melanoma. *Dermatol Ther*. 2020;33:e13174.
4. Tas F, Erturk K. BRAF V600E mutation as a prognostic factor in cutaneous melanoma patients. BRAF V600E mutation as a prognostic factor in cutaneous melanoma patients. *Dermatol Ther*. 2020;33:e13270.
5. Kim J, Novak D, Sachpekidis C, Utikal J, Larribère L. STAT3 relays a differential response to melanoma-associated NRAS mutations. *Cancers (Basel)*. 2020;12:119.
6. Lasota J, Kowalik A, Felisiak-Golabek A, et al. Primary malignant melanoma of esophagus: clinicopathologic characterization of 20 cases including molecular genetic profiling of 15 tumors. *Mod Pathol*. 2019;32:957-966.
7. Wroblewska JP, Mull J, Wu CL, et al. SF3B1, NRAS, KIT, and BRAF mutation; CD117 and cMYC expression; and tumoral pigmentation in sinonasal melanomas: an analysis with newly found molecular alterations and some population-based molecular differences. *Am J Surg Pathol*. 2019;43:168-177.
8. Dika E, Lambertini M, Patrizi A, et al. Frequency of somatic mutations in head and neck melanoma: a single institution experience. *Pigment Cell Melanoma Res*. 2020;33:515-517.
9. Omholt K, Grafström E, Kanter-Lewensohn L, Hansson J, Ragnarsson-Olding BK. KIT pathway alterations in mucosal melanomas of the vulva and other sites. *Clin Cancer Res*. 2011;17:3933-3942.
10. Gorry C, McCullagh L, Barry M. Economic evaluation of systemic treatments for advanced melanoma: a systematic review. *Value Health*. 2020;23:52-60.