Long-term response to ipilimumab after nivolumab failure in a case of anorectal melanoma with an intermediate tumor mutation burden and negative for PD-L1 expression

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Abstract. Anorectal melanoma is a rare disease with a poor prognosis and its response to immunotherapy remains poorly studied. The current study reports a case of recurrent anorectal melanoma in a 60-year-old woman that has exhibited a durable response to ipilimumab for >2 years. Given that the combination of nivolumab and ipilimumab was not approved for use in unresectable or metastatic melanoma at the time of presentation, the patient was initially treated with nivolumab monotherapy and switched to ipilimumab after nivolumab failure. The tumor was microsatellite stable, had an intermediate tumor mutation burden and was negative for programmed cell death-ligand-1 expression. However, the neutrophil-to-lymphocyte ratio in peripheral blood remained at <5 throughout the disease course. Although mucosal melanoma is not caused by ultraviolet radiation and has a lower mutation burden than cutaneous melanoma, the present case responded well to immunotherapy. Further evaluation of potential biomarkers for such patients is required.

Introduction

Mucosal melanoma is subtype of melanoma which arise from melanocytes in mucosal membranes. The major primary sites of mucosal melanoma are head and neck, anorectal, and female genital tract (1). Mucosal melanoma is typically diagnosed at a more advanced stage compared to cutaneous melanoma and generally carry a worse prognosis. Genome sequencing of mucosal melanomas revealed that they are not markedly enriched in ultraviolet signature mutations, different from cutaneous melanoma (2).

Anorectal melanoma is a rare disease, accounting for only 1% of all anorectal malignancies, 1% of all melanomas and 18% of mucosal melanomas (3-5). The 5-year survival rate of anorectal melanoma was reported to be <20% in the era before immunotherapy (4,6), with this poor prognosis being related to early disease dissemination and a delay in diagnosis (7,8). Clinically, it is sometimes misdiagnosed as hemorrhoids due to rectal bleeding. Furthermore, about 30% of anorectal melanomas are amelanotic and pathological diagnosis is difficult in those cases (9).

The only potentially curative option for mucosal melanoma is complete surgical resection with negative margins. Radiotherapy after surgery may be a treatment option, but there it has not been evaluated in prospective study (10). Given the limited treatment options available for individuals with metastatic mucosal melanoma, new therapies are urgently needed to improve prognosis. Immune checkpoint inhibitors (ICIs)-including the combination of nivolumab and ipilimumab-have recently become the standard treatment option for unresectable or metastatic melanoma regardless of tumor subtype, although treatment data for mucosal melanoma are limited compared with those for cutaneous melanoma (11-13). Here we report a case of advanced anorectal melanoma that progressed during nivolumab monotherapy but subsequently showed a durable response to ipilimumab.

Case presentation

A 60-year-old woman with a history of hemorrhoids for >30 years visited her local clinic with a complaint of hemorrhoid enlargement that had persisted for >1 year. Blood analysis revealed anemia, with a hemoglobin level of 6.5 g/dl. She underwent a hemorrhoidectomy, and the resected tissue was submitted for pathological examination because it had an atypical appearance. The pathological findings were suggestive of primary malignant melanoma. She was referred to our hospital 2 months after the surgery. Her Eastern Cooperative Oncology Group performance status was 0. Digital examination and anoscopy did not detect any residual tumor. Chest

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and abdominal computed tomography (CT) as well as positron emission tomography-CT also did not reveal residual tumor tissue or distant metastasis. Blood analysis, including the level of lactate dehydrogenase, showed no abnormalities. Histopathologic reevaluation revealed that atypical cells with enlarged nuclei were gathered in the stroma with a solid or alveolar pattern (Fig. 1) as well as the presence of brown pigment granules in the tumor. Immunostaining showed the tumor to be partly positive for vimentin, S-100, HBM-45, Melan A, and D2-40 as well as negative for cytokeratin, AE1/AE3, CD56, and chromogranin A. These findings were thus consistent with melanoma. No lymphatic invasion was evident by staining for the D2-40 lymphatic marker, and no vascular invasion was detected by CD31 immunostaining. The tumor proportion score (TPS) for programmed cell death-ligand-1 (PD-L1) was <1% with PD-L1 antibody clone 28-8. The tumor was also negative for the V600E mutation of *BRAF*. Next-generation sequencing analysis with the FoundationOne CDx panel, which detects mutations in 324 genes, select gene rearrangements, and genomic signatures including microsatellite instability and tumor mutational burden, revealed the tumor to be microsatellite stable (MSS) and to have a tumor mutation burden (TMB) of 8 mutations/Mb, a deletion of exons 28 to 37 of *NF1*, an S37Y mutation of *CTNNB1*, and an R625H mutation of *SF3B1*. These findings supported a diagnosis of *BRAF* mutation-negative anorectal melanoma. Given the operation report and that hematoxylin-eosin staining of the excised tissue confirmed a surgical margin of 10 mm, no additional surgery was performed. Six months after her first visit to our hospital, CT revealed that the patient had developed multiple lung and liver metastases (Fig. 2). Nivolumab monotherapy (3 mg every 2 weeks) was initiated. After six treatment cycles, chest and abdominal CT showed progression of liver metastasis (Fig. 2). Ipilimumab monotherapy (3 mg/kg every 3 weeks) was then started. After four cycles, the maximum number allowed, chest and abdominal CT confirmed a partial response. The treatment was well tolerated, with no immune-related adverse events. The response has been persisted over 32 months at the time of this writing (november 2019) (Fig. 2). The neutrophil-to-lymphocyte ratio (NLR) in peripheral blood has remained <5 throughout the disease course (Fig. 3).

Discussion

We here report a case of anorectal melanoma that has shown a long-lasting response to ipilimumab after failure of nivolumab treatment. The tumor was found to be MSS, to have an intermediate TMB, and to be negative for PD-L1 expression. Given that the combination of nivolumab and ipilimumab had not been approved for melanoma in Japan at the time the patient presented at our hospital, we initiated nivolumab monotherapy followed by ipilimumab monotherapy. A pooled analysis of patients with cutaneous (*n*=665) or mucosal (*n*=86) melanoma revealed a longer progression-free survival (PFS) and higher objective response rate for nivolumab in combination with ipilimumab than for nivolumab monotherapy (11). Among patients who received the combination therapy, the median PFS was 5.9 months for mucosal melanoma and 11.7 months for cutaneous melanoma, with objective response rates of 37.1 and 60.4%, respectively. One reason for the poorer response

of mucosal melanoma may be a lower TMB. The TMB, the total number of somatic mutations in a defined region of a tumor genome, is currently the most reliable predictive marker for ICI treatment in melanoma (14,15). Two studies have shown that TMB as determined by whole-exome sequencing is related to the clinical benefit rate for ipilimumab monotherapy in melanoma (16,17). A study based on next-generation sequencing with FoundationOne CDx for patients with advanced melanoma revealed the TMB to be high (>23.1 mutations/Mb), intermediate (3.3-23.1 mutations/Mb), or low (<3.3 mutations/Mb) in 27 (41.5%), 24 (36.9%), and 14 (21.5%) patients, respectively, with the TMB correlating with benefit from therapy targeted to the programmed cell death-1 (PD-1)-PD-L1 checkpoint (18). Furthermore, TMB in mucosal melanoma was found to be markedly lower than that in cutaneous melanoma, likely because of the contribution of ultraviolet-induced mutagenesis to cutaneous melanoma (2,19,20).

PD-L1 expression has also been investigated as a potential biomarker for ICI therapy in melanoma. PD-L1 expression on tumor cells did not tend to be related to the response rate in melanoma patients treated with the combination of nivolumab and ipilimumab (21). As far as we are aware, the relation between PD-L1 expression and response to ipilimumab monotherapy has not been examined. With regard to PD-L1 positivity in mucosal melanoma, a small study found that, with a TPS of ≥5% as the cutoff, the proportion of tumors positive for PD-L1 was 44% (16/36), a value similar to that for cutaneous melanoma at 35% (19/54) (22,23).

The present case was found to be MSS, with an intermediate TMB, and negative for PD-L1 expression. These characteristics of an 'immunologically cold tumor' would be expected to confer a low sensitivity to ICIs. A recent report showed that many mucosal melanoma cases had PD-L1 low/negative disease, and average mutational load of 4 cases was much lower than that of cutaneous melanoma (24). Consistent with these findings, our tumor immune profile showed low PD-L1 expression as well as low TMB; however, there is a discrepancy the efficacy of ICI. The reason for the discrepancy between the biological features of the tumor and the clinical benefit conferred by ipilimumab in the current case is unclear. However, a high NLR in peripheral blood has been shown to be strongly associated with a poor outcome of ipilimumab treatment in patients with advanced melanoma (25-28). An NLR of ≥4 before initiation of ipilimumab treatment was thus associated with a worse overall survival compared with a ratio of <4 in patients with metastatic melanoma (25). An NLR of ≥5 at each time point examined was also associated with a worse overall survival, PFS, and response to ipilimumab treatment (26). In the present case, the NLR was <5 at baseline and remained so during and after ipilimumab treatment. The potential of the NLR as a biomarker for ipilimumab treatment in melanoma patients thus warrants further investigation.

In conclusion, the present case shows that ipilimumab is a potentially effective treatment option for patients with metastatic mucosal melanoma including anorectal melanoma, even though mucosal melanoma in general has been found to have a less favorable outcome during ipilimumab treatment compared with cutaneous melanoma. Even if tumor histology, past treatment history, and certain biomarkers suggest that a tumor is immunologically cold, it might still respond to ICI
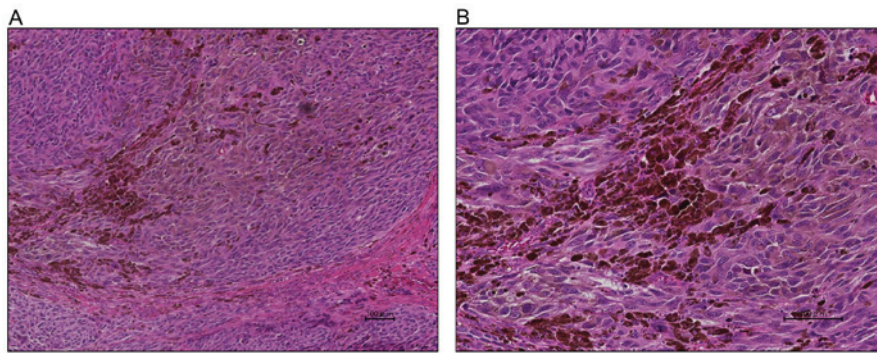
Figure 1. Histopathologic analysis of the surgical specimen. (A) Hematoxylin-eosin staining of the tumor revealed a solid or alveolar pattern. (B) Higher magnification revealed that the tumor was composed of atypical cells with enlarged nuclei and high mitotic activity as well as the presence of brown pigment granules. Scale bars, 100 μm.
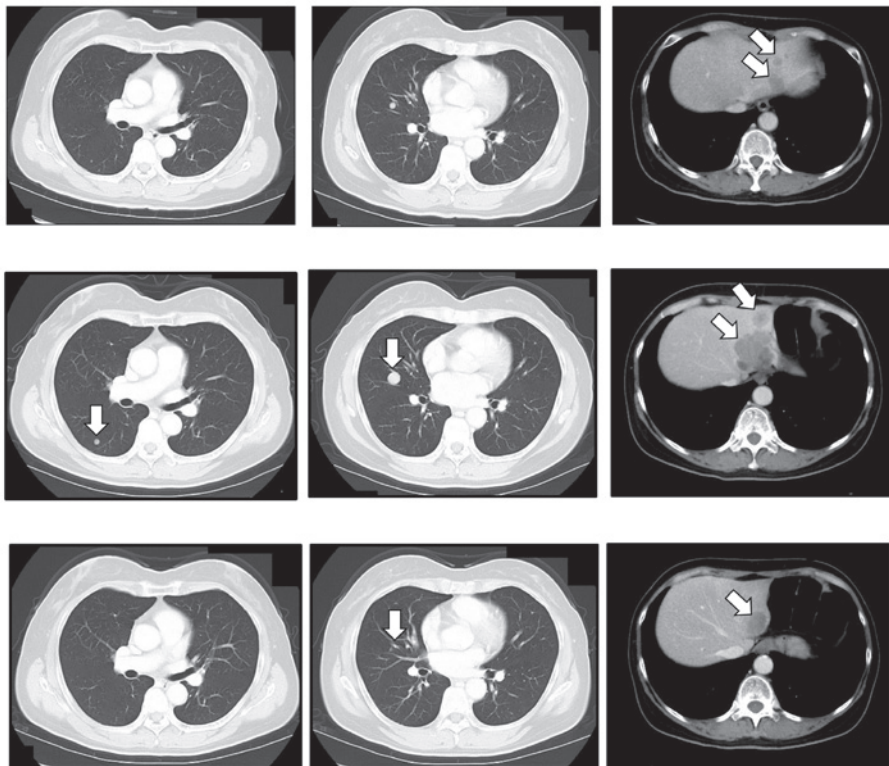

Figure 2. Computed tomography images of the patient obtained before initiation of nivolumab treatment (upper), before initiation of ipilimumab treatment (middle) and 2 years after the initiation of ipilimumab treatment (lower). Arrows indicate metastases from anorectal melanoma.
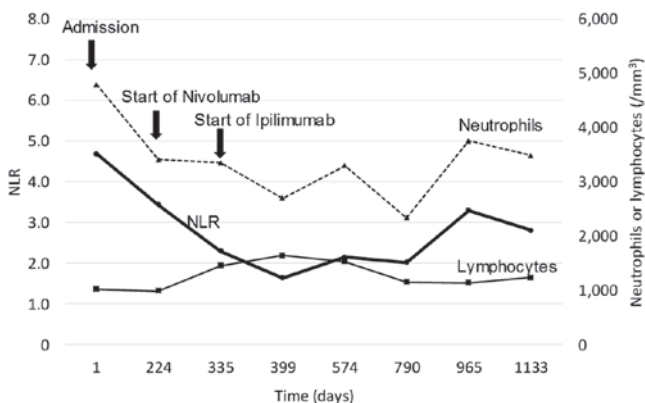

Figure 3. Time course of neutrophil and lymphocyte counts in peripheral blood as well as the NLR for the patient. NLR, neutrophil-to-lymphocyte ratio.

treatment. Evaluation of the NLR should be considered before excluding ICI therapy as an option.

Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

HS, KS, KNa, KNi and MT conceived the current study. HS drafted the current study HS, MT, KS, KNa and KNi acquired and analyzed the data. HS, MT, KS, KNi and KNa wrote, reviewed and edited the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from the patient.

Patient consent for publication

Written informed consent was obtained from the patient for publication of the present case report and accompanying images prior to receiving chemotherapy.

Competing interests

The authors declare that they have no competing interests.

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