Multiple Phenotypic Conversion in Malignant Melanoma: Obtainment of Granulocyte Colony-Stimulating Factor-Producing Ability during the Intermission of Nivolumab Therapy

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Abstract
Malignant melanoma (MM) is one of the most aggressive, recalcitrant, and recurrence-prone skin neoplasms. Its feature is likely to be associated with phenotypic conversion due to tumor heterogeneity. The multidisciplinary assessment, including surgery, drug therapy using anti-cancer agents and immune checkpoint inhibitors, and radiotherapy, is needed for the treatment of advanced MM. Herein, we report a long-term follow-up MM, in which multiple phenotypic conversion occurred during several treatments. In particular, our case obtained granulocyte colony-stimulating factor-producing ability during the intermission of nivolumab therapy and it was successfully controlled by re-administration of nivolumab. Sharing the case having a varied clinical course is meaningful to increase the knowledge and decision branches for the treatment of melanoma.
Introduction

Malignant melanoma (MM) is one of the most severe skin cancers derived from melanocyte. MM easily relapses, metastasizes, and finally progresses to the advanced stage. Therapeutic strategy for melanoma is markedly progressed, and unresectable and distant metastatic melanomas can be treated using BRAF and MEK inhibitors, and ICI, such as anti-PD-1 and anti-CTLA-4 antibodies. However, advanced MM is still difficult to treat and control despite several therapeutic options. Melanoma usually contains a high number of clones harboring various mutations, so-called tumor heterogeneity. Each clone has distinct characteristics and behaves differently against the treatment. Tumor heterogeneity strongly refers to metastasis, recurrence, and drug resistance which may originate from different subclones of primary lesion [1–3].

Granulocyte colony-stimulating factor (G-CSF)-producing cancer has been reported in various organs such as lung, bladder, and breast, since reported in 1977 [4]. Currently, we encountered a case of MM obtaining G-CSF-producing ability during the intermission of nivolumab therapy, although G-CSF-producing MM is rare [5, 6]. This phenomenon may be also associated with tumor heterogeneity. Our case suffered from multiple relapses; however, it was well controlled by the multidisciplinary therapy for a long period, although G-CSF-producing ability in neoplasm is known to be associated with poor prognosis. Herein, we present the detail of our impressive case.

Case Report

A 52-year-old Japanese woman was referred with severe back pain in July of 2014. Computer tomography (CT) showed the metastatic retroperitoneal tumor, and then primary MM was revealed in her left first toe (stage IV, BRAF V600 mutation negative, PD-L1 <1%) (Fig. 1a). After the lesional amputation, the combination therapy of dacarbazine (DTIC) and interferon beta was performed since ICI was not approved in Japan at that time. Fortunately, partial response (PR) was temporally observed for 2 years (Fig. 1b); however, a metastasis was found in the right external obturator muscle. Thus, newly approved nivolumab therapy (240 mg/every 2 weeks) was subsequently started in August of 2016 (25 months from the first visit) and PR was achieved again (Fig. 1c). However, nivolumab therapy was discontinued in accordance with the patient’s request (nervousness against adverse events: she suffered from a hypothyroidism) after 12-time administrations.

Twelve months later (August 2018, 49 months from the first visit), the follow-up CT revealed a novel metastasis at the caudal side of right kidney. Concurrently, severe leukocytosis (WBC count: 37,400/μL) was observed and the provisional diagnosis of retroperitoneal abscess was made (Fig. 2a). However, antibiotic administration and CT-guided drainage were not effective, resulting in more remarkable elevation of WBC count (57,200/μL: neutrophil 90.5%). Low level of C-reactive protein (3.55 mg/dL), little amount of drained fluid, and negative results of bacterial culture indicated G-CSF-producing tumor rather than bacterial infection. Therefore, we examined serum G-CSF level and the result showed 614 pg/mL, much higher than normal range (<39 pg/mL) (Fig. 2b). Therefore, we considered that the tumor obtained G-CSF-producing ability. After restarting nivolumab therapy, WBC count and serum G-CSF level immediately decreased to the normal range, and metastatic lesions were re-maintained with PR for almost 1 year (Fig. 2a).

Regrettably, metastases appeared in her lung and enlarged after 13-time re-administration of nivolumab in June of 2019 (59 months from the first visit) (Fig. 2c); however, WBC count and serum G-CSF level never re-increased. Subsequent combination therapy of nivolumab
Fig. 1. Clinical and image findings in the first half of the clinical course. 

**a** Metastatic retroperitoneal tumor (right) was observed by CT scan and primary melanoma lesion was found in the left first toe (left). 

**b** The combination therapy of dacarbazine and interferon beta was effective. The volume of metastatic lesions (right: broken-line circle) was reduced (left). 

**c** Recurrent lesion was found in the right external obturator muscle (right: broken-line circle). PR was achieved by nivolumab therapy (left).
and ipilimumab also achieved PR, and nivolumab monotherapy was continued to suppress the recurrence (Fig. 2c). Again, the incidence of anemia led to detect small intestinal and multiple peritoneal metastases in March of 2020 (68 months from the first visit). To prevent persisting blood loss and intestinal obstruction, the palliative resection of small intestinal metastasis was done. Subsequently, radiation therapy (60 Gy to the metastasis of external iliac lymph node) was performed in parallel with nivolumab administration in hopes of

Fig. 2. a Retroperitoneal abscess was speculated by CT scan; however, it was recurrent melanoma obtaining G-CSF-producing ability (right: broken-line circle). Re-administration of nivolumab achieved PR again (left). b Time course of WBC count and G-CSF level. Both parameters were remarkably decreased by nivolumab therapy. c Although the metastatic lesions appeared in her lung (right: broken-line circle), these were maintained by the combination therapy of nivolumab and ipilimumab (left).
occurring abscopal effect. Although she was maintained with SD and the abrupt progression was not observed for a year, novel multiple metastases were finally appeared in April of 2021 (70 months from the first visit). She currently decided to receive palliative care for the rest of her life.

**Discussion**

MM is liable to cause a relapse and obtain drug resistance. This characteristic is related to a high tumor heterogeneity of melanoma. Even if one clone in melanoma is driven out by the treatment, another clone obtaining the tolerance against the treatment might proliferate, resulting in metastasis and recurrence.

Here, we report a long-term follow-up case of advanced MM having at least 4-time rubrical phenotypic conversions: the appearance of (1) DTIC-resistant clone, (2) the clone obtaining G-CSF-producing ability during the intermission of nivolumab therapy, (3) nivolumab-resistant clone, and (4) the clone developing the tolerance against both nivolumab and ipilimumab. In particular, we observed a dramatic change at the second phenotypic conversion in the intermission of nivolumab. Although the report of GSC-F-producing MM is limited, our case fulfilled 3 out of 4 diagnostic criteria of G-CSF-producing tumor proposed in the first report: (a) marked increase in WBC count (particularly in mature neutrophils) without any infection or other diseases, (b) elevation of serum G-CSF level, (c) decrease in WBC count and a normal serum G-CSF level after tumor resection, and (d) G-CSF activity in the tumor specimens (not confirmed in this case) [4], indicating that our case was compatible with G-CSF-producing melanoma. Although the mechanism of such dramatic change has not been clearly defined, the others have also reported gain of G-CSF-producing ability in various neoplasms during treatment [7, 8], and G-CSF-producing melanoma cells may have gained growth advantage among the heterogeneous tumor cells [1, 2] during intermission of nivolumab therapy.

The prognosis of G-CSF-producing tumor is basically poor since G-CSF may suppress T-cell function and activate tumor invasion in an autocrine manner [9]. Fortunately, our case was maintained with PR by nivolumab re-administration. Therefore, it is presumably true and intriguing that tumor antigenicity recognized by nivolumab-activated T cells was not lost and maintained even after obtaining G-CSF-producing ability. In addition, after the third phenotypic conversion, WBC count and serum G-CSF level were stable, proving that the second phenotype secreting G-CSF was swept away by nivolumab. Interestingly, the combination therapy of nivolumab and ipilimumab was also effective for the third phenotype, indicating that it may acquire the different tumor antigens recognized by ipilimumab-activated T cells, probably due to tumor heterogeneity.

**Conclusion**

In conclusion, our case demonstrated that clinical phenotype of MM may be easily converted due to its heterogeneity during the therapeutic process. Thus, we need to discuss too carefully when choosing the intermission/discontinuation/switch of chemotherapy/ICI against MM. Although our case has unfortunately arrived at palliative care, sharing such highly suggestive case is of significance in understanding the characteristics, therapeutic strategy, and heterogeneity of MM.

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Statement of Ethics

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. This study protocol was reviewed and approved by the Ethics Committee of The Jikei University School of Medicine for Biomedical Research (Approval number 33-479 [11106]).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Satomi Chujo: clinical follow-up, data collection and assembly, data analysis and interpretation, and manuscript writing. Yoshimasa Nobeyama: clinical follow-up, data collection and assembly, data analysis and interpretation, and final manuscript approval. Akihiko Asahina: data analysis and interpretation, final manuscript approval, and accountable for all aspects of the work. Munenari Itoh: clinical follow-up, data collection and assembly, data analysis and interpretation, manuscript writing, final manuscript approval, and accountable for all aspects of the work.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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