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A case of non–small cell lung cancer with danazol-dependent aplastic anemia induced by pembrolizumab

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ABSTRACT

Programmed cell death protein 1 immune checkpoint inhibitor is an effective treatment for non-small cell lung cancer. Although hematological immune-related adverse events induced by antiprogrammed-cell-death-protein-1 immunotherapy have been reported, they are rare, and there remain many unknowns. We report the case of a 77-year-old woman with non–small cell lung cancer and pembrolizumab-induced danazol-dependent aplastic anemia. Sixteen days after she received pembrolizumab with carboplatin and pemetrexed as first-line treatments, she developed pancytopenia, including severe thrombocytopenia (1×10^9/L) with oral bleeding, epistaxis, and systemic purpura. We initially diagnosed immune-related thrombocytopenia based on an elevated level of platelet-associated immunoglobulin G (922ng/10^7 cells), but her thrombocytopenia was refractory to prednisolone (1mg/kg) and thrombopoietin receptor agonists.

Abbreviations: PD-1, programmed cell death protein 1; ICI, immune checkpoint inhibitor; NSCLC, non–small cell lung cancer; irAE, immune-related adverse event; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; BRAF, v-raf murine sarcoma viral oncogene homolog B1; TKI, tyrosine kinase inhibitors; PD-L1, programmed death-ligand 1; AIHA, autoimmune hemolytic anemia; PRCA, pure red cell aplasia; ITP, immune thrombocytopenia; TTP, thrombotic thrombocytopenic purpura; CT, chest computed tomography; PET, positron emission tomography; TPO-RAs, thrombopoietin receptor agonists; G-CSF, granulocyte-colony stimulating factor; TNF-α, tumor necrosis factor -α.

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We eventually diagnosed aplastic anemia based on the findings of bone marrow hypoplasia. Treatment with cyclosporine and danazol 300mg (7.5mg/kg) was initiated. Eighteen days later, her blood cell count increased, and we reduced danazol to 100mg. Twenty-four days after the reduction of danazol, her platelet count dropped again to 14×10^9/L; subsequently, increasing danazol improved her platelet count in a few days. Although aplastic anemia was recovered, she died owing to lung cancer progression. In this case, the thrombocytopenia was noticeable initially; however, pancytopenia appeared a month later, and we diagnosed her with aplastic anemia. Platelet counts improved rapidly with the use of danazol. No effective treatment has yet been established for aplastic anemia induced by antiprogrammed-cell-death-protein-1 immunotherapy, but our case suggests that danazol is an effective therapy.

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Introduction

The treatment of patients with metastatic non–small cell lung cancer (NSCLC) has undergone a significant transformation in the past 10-15 years due to the development of precision medicine based on molecular characterization. In patients with metastatic NSCLC, there are several distinct targetable driver mutations (eg, epidermal growth factor receptor, rearrangements of anaplastic lymphoma kinase, rearrangements of c-ros oncogene 1 receptor tyrosine kinase *ROS-1*, and v-raf murine sarcoma viral oncogene homolog B1 mutations). These mutations can be targeted therapeutically with mutation-specific tyrosine kinase inhibitors. In addition to these targetable genetic alterations, the expression of programmed death-ligand 1 (PD-L1) must also be considered when making therapeutic decisions. Pembrolizumab, a programmed cell death protein 1 (PD-1) immune checkpoint inhibitor, has been proven to be effective as first-line treatment for NSCLC, whether used alone or in combination with anticancer drugs.

However, the use of pembrolizumab comes at the cost of immune-related adverse events (irAEs) affecting various organ systems. Common irAEs include thyroid dysfunction, interstitial pneumonia, and colitis. The following hematological irAEs have also been reported, although rarely: autoimmune hemolytic anemia, pure red cell aplasia, immune thrombocytopenia (ITP), thrombotic thrombocytopenic purpura, aplastic anemia, and pancytopenia. As these events are rare, there remain many unknowns.

We herein report a patient who developed danazol-dependent aplastic anemia induced by pembrolizumab.

Cases and Methods

A 77-year-old female patient with an abnormal chest shadow was referred to our institution by her primary physician. She had a history of Sjögren's syndrome without treatment, and she had gastric cancer when she was 50 years old. Chest computed tomography revealed consolidation of approximately 10 cm in the right lower lobe, which had grown over the previous year.

We strongly suspected lung cancer and performed a biopsy with bronchoscopy for consolidation in the right lower lobe. Adenocarcinoma tissue was found on histological examination of the biopsy specimen (Fig 1). In addition, immunohistochemistry analysis of anaplastic lymphoma kinase was negative, and epidermal growth factor receptor mutation and *Ros-1* rearrangement were not found. The expression of PD-L1 was negative.

Positron emission tomography revealed multiple pulmonary metastases, including in the right middle lobe and left upper lobe, and supraclavicular lymph node metastasis. Thus, the patient

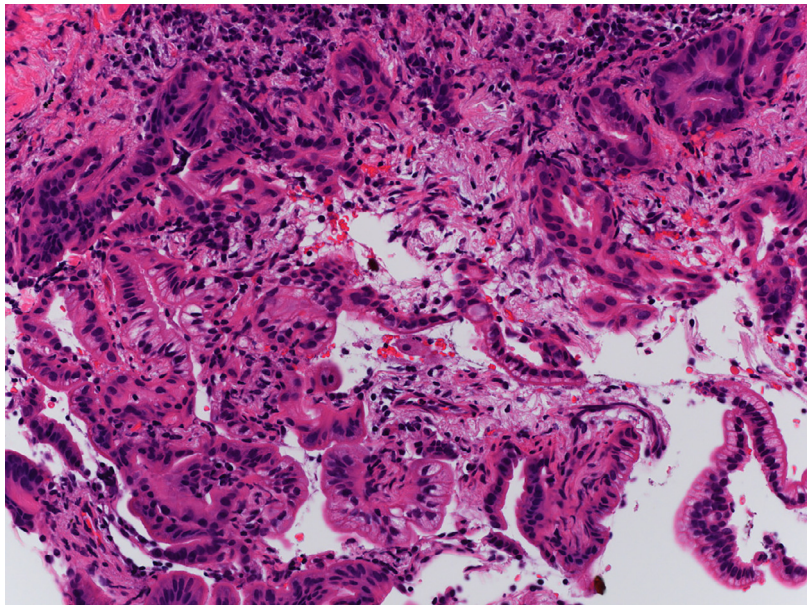

Fig. 1. Right lower lobe lung biopsy: atypical tubular gland duct hyperplasia is observed.

was diagnosed with cT4N3M1a stage IVA disease according to the 8th edition of the International Union Against Cancer staging system. Her Eastern Cooperative Oncology Group performance status was grade 1, so we started pembrolizumab with carboplatin and pemetrexed as first-line treatments.

No abnormalities in blood cell counts were observed at the start of treatment. On day 13, the patient had oral bleeding, epistaxis, and systemic purpura. On day 16, pancytopenia (hemoglobin level 9.0 g/dL, white blood cell count 2.16×10^9 /L, platelet count 1×10^9 /L) was observed. Initially, we thought that myelosuppression by cytotoxic anticancer drugs was the main cause, rather than irAE. We discontinued her antitumor therapy and administered 20 units of platelet transfusion, and the next day, the patient's platelet count had improved to 50×10^9 /L. Non-platelet blood cell counts increased gradually, but the thrombocytopenia persisted, and her platelet count decreased to less than 20×10^9 /L after 2 days of platelet transfusion.

Without platelet transfusions for 3 days, the patient's platelet counts dropped again to 1×10^9 /L without obvious morphological abnormalities on peripheral blood smear. In addition, anti-*Helicobacter pylori* antibodies were negative. We consulted with a hematologist for the possibility of irAE, rather than thrombocytopenia due to myelosuppression of cytotoxic anticancer drugs.

Bone marrow aspiration and biopsy were not diagnostically significant because of peripheral blood contamination. Otherwise, platelet-associated IgG was markedly elevated at 922 ng/10^7 cells.

Based on these laboratory findings and the use of pembrolizumab, we diagnosed immune-related thrombocytopenia. Consequently, we started prednisolone at 40 mg (1mg/kg) on day 24 and thrombopoietin receptor agonists (TPO-RAs) and eltrombopag on day 34. These drugs were continued at the same doses for 4 weeks, but thrombocytopenia persisted, and the patient required frequent transfusions.

Based on concerns about side effects, we decided to gradually decrease the prednisolone dose. In addition, the expected effect of eltrombopag was not observed, so we discontinued its use and instead administered romiplostim. Meanwhile, we considered concomitant use of immunosuppressive agents, but ultimately did not administer them due to their potential to cause

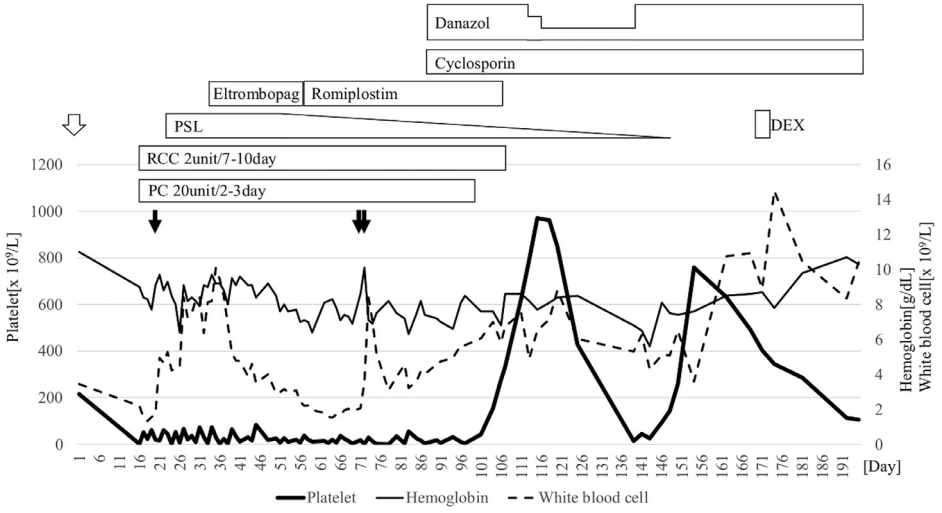

Fig. 2. Clinical course of blood cell count and therapy. ⇩: chemotherapy, (carboplatin+pemetrexed+pembrolizumab); DEX, dexamethasone; PC, platelet transfusion; PSL, prednisolone; RCC, red blood cell transfusion, ↓: granulocyte-colony stimulating factor.

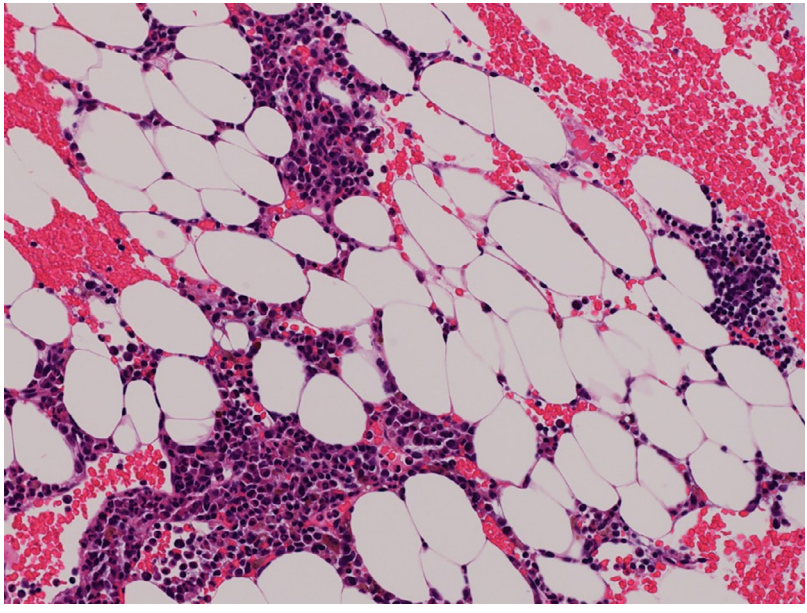

Fig. 3. Bone marrow biopsy (day 57): marrow cellularity is low to 20% with a few erythroblasts and bone marrow megakaryocytes.

progression of lung cancer. We also considered splenectomy, although it was unclear whether it would be effective for immune-related thrombocytopenic purpura.

One month later, the patient's laboratory data began to show pancytopenia (Fig 2). Folic acid and vitamin B12 levels were sustained within normal ranges. Initially, we had suspected immune-related thrombocytopenic purpura; however, at this point, we suspected that she may

have aplastic anemia, and thus we repeated bone marrow aspiration and biopsy. The nucleated cell count was 8×10^9 /L, and no dysplasia was found. Bone marrow biopsy specimens showed that marrow cellularity was low to 20% with a few erythroblasts and bone marrow megakaryocytes (Fig 3). We administered granulocyte-colony stimulating factor (G-CSF) to her on days 71 and 72 and initiated cyclosporine treatment but feared the exacerbation of lung cancer. We also started danazol 300 mg (7.5 mg/kg). After 18 days of administration of these 3 drugs, the patient's blood cell counts increased. As her platelet count increased to 970×10^9 /L, we continued cyclosporine at the same dose, stopped romiplostim, and then reduced danazol. However, 24 days after the reduction of danazol, her platelet count dropped to 14×10^9 /L. Increasing danazol improved the platelet count to 758×10^9 /L in a few days. Because the platelet count was danazol-dependent, prednisolone was discontinued and the cyclosporine dose was reduced, and the platelet count was subsequently maintained.

The patient's performance status declined, and no further chemotherapy was administered until her death. We provided the best supportive care possible, including dexamethasone, and the patient died 3 months later. For 3 months after discharge, the platelet count never dropped to a level requiring further platelet transfusions.

Discussion and Conclusion

This was a case of aplastic anemia caused by pembrolizumab, an immune checkpoint inhibitor as well as a treatment option for lung cancer.

The reported frequency of hematological irAEs induced by anti-PD-1 or anti-PD-L1 immunotherapy is 4.1% for all grades and 0.7% for grades III–IV.[1] The breakdown of hematological irAEs is as follows: ITP, 26%; autoimmune hemolytic anemia, 26%; neutropenia, 26%; pancytopenia or aplastic anemia, 14%; bicytopenia, 6%; and pure red cell aplasia, 3%.[2] Several cases of pancytopenia or aplastic anemia induced by anti-PD-1 immunotherapy have been reported (Table 1).[3-8] The primary treatments are corticosteroids and supportive therapy, such as transfusions and G-CSF. However, only 20%-25% of patients with pancytopenia or aplastic anemia induced by anti-PD-1 immunotherapy experience resolution, and recovery takes approximately 6-8 weeks.[1,2] The associated mortality rate is high, and no effective treatment has been established.

We considered 2 major important points in our case. First, thrombocytopenia was initially noticeable, but pancytopenia appeared after 1 month. Second, danazol was highly effective against thrombocytopenia, but corticosteroids, immunosuppressants, and TPO-RAs were not.

With respect to the first point, we initially suspected ITP. According to the Japanese guidelines for ITP,[9] the recommended treatment is corticosteroids. If this treatment is ineffective or not tolerated, TPO-RAs, rituximab, and splenectomy are considered as second-line treatments. We chose TPO-RAs, as they are less invasive and less likely to have negative effects on lung cancer. Neither steroids nor TPO-RAs were effective; therefore, we considered splenectomy. However, the patient eventually developed pancytopenia and was diagnosed with aplastic anemia. Pancytopenia took longer to develop due to differences in the speed of decline of each blood cell. Because the treatments for ITP and aplastic anemia are quite different, we considered it especially important to monitor over time to determine whether this decline was limited to only platelets or if other blood cells would also decline. Treatment based on an incorrect diagnosis is inappropriate and may cause unnecessary invasion for the patient.

The second point concerns treatment. In cases of aplastic anemia, allogeneic hematopoietic stem cell transplantation and immunosuppressive therapy with cyclosporine are standard therapies, and danazol is considered if these therapies are ineffective or a patient is ineligible to receive them.[10] Danazol, a synthetic anabolic steroid, suppresses apoptosis of CD34-positive cells (including hematopoietic stem cells) by inhibiting both interleukin-1 and tumor necrosis factor-α (TNF-α)[11,12] and 46% of patients with aplastic anemia are responsive to it.[13] Increased secretion of TNF-α is 1 of the mechanisms of aplastic anemia,[12] and anti-PD-1 immunotherapy has been reported to increase TNF-α secretion.[14] We hypothesized that such a pathway might be strongly induced in irAEs. Danazol has antitumor effects by some pathway[15] without any

Table 1
Cases of pancytopenia or aplastic anemia induced by anti-PD-1 immunotherapy.

Reference	Age/gender	Disease	ICI	Cycles of ICI	Type of Haem-irAE	Treatment	Outcome
Michot 2017[3]	73/F	NSCLC	Nivolumab	12	Pancytopenia/aplastic anemia	IVIG	No response to IVIG; death at 1 month from febrile neutropenia
Michot 2017[3]	70/M	NSCLC	Nivolumab	10	Pancytopenia/aplastic anemia	Prednisone 1mg/kg, norethandrolone	Persistent pancytopenia still ongoing at 4 months
Michot 2017[3]	78/M	NSCLC	Nivolumab	1	Pancytopenia/aplastic anemia	Prednisolone 1mg/kg, IVIG	No response to steroids and IVIG; death at 3 months from acute coronary syndrome
Tokumo 2018[4]	56/M	NSCLC	Nivolumab	3	Pancytopenia	Steroid-pulse therapy, prednisolone 50mg/day, IVIG	No response to steroids and IVIG; death at 4 months from progression of lung cancer
Comito 2019[5]	57/M	Glioblastoma multi-forme	Nivolumab	2	Pancytopenia/aplastic anemia	Eltrombopag	No response to eltrombopag; death at 73 days from febrile neutropenia
Rouvinov 2019[6]	74/F	Metastatic melanoma	Nivolumab	4	Pancytopenia/aplastic anemia	Prednisolone 1.5mg/kg, eltrombopag	No response to steroids and eltrombopag; death from aplastic anemia
Atwal 2017[7]	52/F	Metastatic melanoma	Pembrolizumab	18	Pancytopenia	Prednisone 1mg/kg, IVIG	Recovered from anemia 6 weeks later; doing well
Ni 2019[8]	67/M	Metastatic melanoma	Pembrolizumab	8	AIHA Pancytopenia	Prednisone 1mg/kg	2 months later, gradually recovered

Haem-irAE, hematological irAEs; IVIG, intravenous immunoglobulin; NSCLC, non–small cell lung cancer.

evidence of progression of lung cancer; therefore, it may be considered before using immunosuppressive drugs.

The white blood cell count seemed to have improved prior to initiation of danazol, and the hemoglobin did not appear to respond to danazol as robustly as the platelets. We administered G-CSF on days 71 and 72. In addition, she had norovirus enteritis on day 71. We believe that these factors increased the white blood cell count before the start of danazol. As shown in Figure 2, the platelet count increased prior to discharge from the hospital; we believe this was caused by dexamethasone, which was used for palliative care. Although the hemoglobin level did not rise to the same extent as the platelets, some effect was observed, as transfusion dependence disappeared 20 days after the start of danazol.

Although infrequent, aplastic anemia induced by pembrolizumab can be disruptive to cancer treatment. In our case, it was possible to control the situation using danazol. The reporting and sharing of information regarding these cases is crucial. The mechanism of disease onset and an effective treatment regimen must be investigated, and it is important to accumulate as many case reports as possible.

Ethical Approval and Consent to Participate

The patient in this report provided consent for participation in this study. The Institutional Review Board of the Japanese Red Cross Kyoto Daiichi Hospital approved this study.

Consent to Publish

The patient in this report provided consent for the anonymous publication of her experiences. All authors of this report agree with and are greatly obliged to the Editorial Board for the publication of this report.

Author Contributions

Shiho Goda: Conceptualization, Writing - Original Draft, Taisuke Tsuji: Writing - Review & Editing; Yosuke Matsumoto: Writing - Review & Editing; Shinsuke Shiotsu: Writing - Review & Editing, Shunya Tanaka: Writing - Review & Editing; Yoshifumi Suga: Writing - Review & Editing; Hiroyuki Fujii: Writing - Review & Editing; Aosa Matsuyama: Writing - Review & Editing; Ayaka Omura: Writing - Review & Editing; Tatsuya Yuba: Writing - Review & Editing; Chieko Takumi: Writing - Review & Editing; Noriya Hiraoka: Supervision.

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