CASE REPORT

An Autopsy Case of Late-onset Fulminant Myocarditis Induced by Nivolumab in Gastric Cancer

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Abstract:
Nivolumab is an immune-checkpoint inhibitor (ICI) that can induce unique treatment-related toxicities, such as immune-related adverse events (irAEs). Myocarditis is a serious irAE with an incidence between 0.06% and 1.14%. Although the peak onset of irAE is generally within three months from the start of treatment, we experienced an autopsy case of late-onset fulminant myocarditis caused by nivolumab in Epstein Barr virus-associated gastric cancer. Pathological complete remission of the primary lesion was confirmed by the autopsy. We should consider possible complications of cardiac irAEs, especially fulminant myocarditis, even beyond three months after starting ICI therapy.

Key words: gastric cancer, nivolumab, myocarditis, immune-related adverse event

(Intern Med 61: 2867-2871, 2022)
(DOI: 10.2169/internalmedicine.9161-21)

Introduction

Nivolumab is an anti-programmed cell death-1 (PD-1) monoclonal antibody approved for treating patients with advanced gastric cancer based on the results of the ATTRACTION-2 trial (1, 2). However, immune checkpoint inhibitors (ICIs) can induce unique treatment-related toxicities, such as immune-related adverse events (irAEs). Myocarditis is a serious irAE, with an incidence of 0.06-1.14%, and its peak onset is generally observed within 3 months from the start of treatment (3-5). Late-onset fulminant myocarditis caused by ICIs is rare but has been reported in melanoma (6). We herein report an autopsy case of late-onset fulminant myocarditis induced by nivolumab in gastric cancer.

Case Report

A 74-year-old Japanese man was diagnosed with anemia by a screening test and referred to our hospital for a further evaluation. He had a history of transurethral resection of early-stage bladder cancer at 73 years old. On upper gastrointestinal endoscopy, a type 3 tumor measuring approximately 40 mm was observed at the posterior wall of the middle body of the stomach. Biopsy results indicated that the tumor was an human epidermal growth factor receptor (HER)-2-negative moderately differentiated tubular adenocarcinoma with lymphoid stroma. In addition, Epstein Barr virus (EBV)-encoded small RNA 1 \textit{in situ} hybridization was positive in the tumor cells (Fig. 1). Thoracoabdominal enhanced computed tomography revealed at least seven enlarged lymph node metastases along the gastric region, and multiple liver metastatic lesions were also observed. Based on these findings, the patient was finally diagnosed with HER-2-negative metastatic EBV-associated gastric cancer.

He received nine courses of capecitabine and oxaliplatin as first-line treatment, two courses of nab-paclitaxel and ramucirumab regimen as second-line treatment, and six courses of weekly paclitaxel regimen as third-line treatment. The best response of each treatment was partial response, stable disease and stable disease, respectively, according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria ver. 1.1. After a weekly paclitaxel regimen, he re-

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Received: December 6, 2021; Accepted: January 19, 2022; Advance Publication by J-STAGE: March 5, 2022
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Figure 1. Pathological findings of the biopsy from the primary gastric lesion. (a) Adenocarcinoma with lymphoid stroma; Hematoxylin and Eosin staining. (b) Epstein Barr virus (EBV)-encoded small RNA in situ hybridization was positive for tumor cells. (c) Programmed cell death-ligand 1 (PD-L1) expression was observed in most of the tumor cells and some of the immune cells.

received nivolumab monotherapy as fourth-line therapy, and the tumor response was shown to be stable disease. After 12 courses of nivolumab, organizing pneumonia (OP) developed and was highly suggestive of irAE. Nivolumab was therefore discontinued, and he received prednisone (PSL) (30 mg, 0.5 mg/kg) as immunosuppression therapy. After the start of PSL, the OP subsequently improved, and the PSL dose was gradually reduced to 5 mg.

Three months after the discontinuation of nivolumab due to the irAE mentioned and 8 months from the start of nivolumab, the patient visited our hospital emergently due to fatigue and nausea. Echocardiography showed a preserved left ventricular ejection fraction (LVEF) of 73% without visible pericardial effusion. However, elevation of cardiogenic biomarkers (creatinine kinase, 402 U/L; troponin-I, 17,971 pg/mL) and abnormal electrocardiogram findings (ST segment elevation in leads II, III, and aVF, and subsequent complete atrioventricular block) (Fig. 2) prompted us to urgently admit the patient to our hospital, where we performed temporary pacemaker insertion and cardiac catheterization. Cardiac catheterization revealed no significant stenosis in the coronary arteries (Fig. 2) with a normal left ventricular wall motion. At the same time, a myocardial biopsy from the right ventricle was performed. The pathological findings showed marked inflammatory cell infiltration, mainly composed of cluster of differentiation (CD) 8-positive T cells focally surrounding myocardial cells. Myocardial necrosis was not found. In addition, programmed cell death-ligand 1 (PD-L1) expression in a few myocardial cells was observed by immunohistochemistry using an SP263 assay (Fig. 3). The patient was diagnosed with nivolumab-related myocarditis, for which he required PSL (60 mg, 1.0 mg/kg).

On the 3rd day post-admission, the EF decreased from 73% to 10%, and we diagnosed the patient with fulminant myocarditis based on the clinical course. Methylprednisolone (mPSL) (1 g daily for 3 days) was started on the same day. On the 4th day post-admission, intravenous gamma globulin (1 g/kg daily for 2 days) was started, and a percutaneous ventricular assist device (Impella®) and percutaneous cardiopulmonary support were initiated for cardiogenic shock. On the 5th day post-admission, plasma exchange was started daily for 3 days. From the 6th day post-admission, the corticosteroid dose (PSL) was reduced to 60 mg (1 mg/kg) daily. From the 8th day post-admission, plasma exchange was performed every other day for 4 days. Following these treatments, his EF improved to 59% at the 13th day post-admission. However, the patient developed sepsis caused by Klebsiella pneumoniae and Enterobacter cloacae and died on the 19th day post-admission.

An autopsy was performed. In addition to myocardial necrosis, inflammatory cell infiltration, mainly composed of CD8-positive T cells and CD68-positive histiocytes, was broadly observed in the left ventricle (Fig. 3). Immunohistochemistry revealed PD-L1 expression in a few myocardial cells. EBV-encoded small RNA in situ hybridization was negative for myocardial cells. Alveolar hemorrhaging and fibrin deposition were observed in the lungs bilaterally. Many Gram-negative bacilli with bacterial phagocytosis by histiocytes were observed in multiple organs. There was no residual cancer in the stomach, and we regarded it as a pathological complete response to nivolumab. However, metastatic gastric cancer remained in the liver and para-aortic lymph nodes. Given these findings, the definite diagnosis was late-onset fulminant myocarditis induced by nivolumab, and the causes of death were alveolar hemorrhaging and sepsis caused by Gram-negative bacilli.
We presented the case of a patient with HER-2 negative EBV-associated metastatic gastric cancer who developed late-onset fulminating myocarditis caused by nivolumab. To our knowledge, this is the first case report of late-onset fulminating myocarditis in gastric cancer. Samples from a myocardial biopsy and autopsy were pathologically analyzed, confirming the infiltration of inflammatory cells - mainly CD8-positive T cells - into the myocardium tissues.

Nivolumab is a human immunoglobulin G4 monoclonal immune checkpoint antibody against programmed death protein and is useful for the treatment of patients with previously treated advanced gastric cancer based on the results of the ATTRACTION-2 trial (1, 2). In this trial, there were no reports of cardiac irAEs (1, 2). In other cancers, Johnson et al. reported that the incidence of myocarditis in patients receiving nivolumab monotherapy was 0.06%, based on a large database (4). According to another study using WHO's global database, the incidence of myocarditis in patients using immunotherapy was 0.39%, and the odds ratio of the development of myocarditis was 11.21 (95% confidence interval: 9.36-13.43) (5). In a report on gastric cancer, Ohta et al. reported that 1 out of 15 patients who received nivolumab monotherapy developed myocarditis (7). Most cases of myocarditis as irAEs have been reported to occur within three months of receiving initial ICI therapy (3-5). Johnson et al. reported the median time from the initial treatment to the diagnosis of myocarditis was 17 days (range, 13-64 days) (4). Similarly, Salem et al. reported 30 days (range, 18-60 days) (5). However, in our case, myocarditis occurred eight months after the initiation of nivolumab and three months after its cessation. There has been a report of late-onset fulminating myocarditis in melanoma after receiving 13 cycles of nivolumab (6). Dolladille et al. reported that late-onset cardiac irAEs have a high mortality rate (27-44%), similar to cardiac irAEs occurring within 3 months (8). The symptoms of cardiac irAEs vary among patients. Thus, regular monitoring of serum troponin levels and ECGs is useful for the early detection of cardiac irAEs (9). The initial symptoms in our case were fatigue and nausea. These symptoms were nonspecific, but we suspected cardiac disease relatively earlier due to findings suggestive of myocarditis, such as elevated myocardial enzymes and abnormal ECG findings. As mentioned above, late-onset cardiac irAEs are very rare but become critical if they occur. Hence, we must always consider the possibility of cardiac irAEs, even beyond three months after starting ICI therapy.

The gold standard for the diagnosis of myocarditis is histopathologic evidence of CD8-positive T cells and macrophages infiltrating myocardial tissue on an endomyocardial
Figure 3. Pathological findings of each myocardial specimen obtained via the right ventricular biopsy (a-c) and autopsy (d-f). (a) Lymphocytic infiltration into the myocardium: Hematoxylin and Eosin (H&E) staining. (b) CD8-positive T cells surrounded myocardial cells, indicating cytotoxicity. (c) Programmed cell death-1 (PD-L1) expression in a few myocardial cells. (d) Myocardial necrosis (left) and fibrous replacement (right) with marked inflammatory cell infiltration: H&E staining. (e) CD8-positive T cell infiltration in the non-necrotic myocardium. (f) PD-L1 expression in a few myocardial cells.

biopsy or autopsy (9, 10). In addition to the presence or absence of pathological findings, cardiac biomarkers, cardiac imaging, and symptoms may be combined to make a diagnosis (10). In the present case, immunohistochemical analyses of the myocardial specimens obtained via the right ventricular biopsy and the autopsy revealed CD8-positive T cell infiltration into the myocardium and PD-L1 expression in a few non-necrotic myocardial cells. A previous study showed the initiation of T cells infiltration with the same T cell receptor in the tumor and myocardium (4). This suggests that the antigens present in the myocardium and tumors were recognized by the same T cell clone. Interferon γ (IFN-γ)-induced PD-L1 in the myocardium is an important factor in protecting the heart from immune-inflammatory injury (11). The administration of high-dose (500-1,000 mg/day) mPSL within 24 hours of admission is effective as an initial therapy for cardiac irAE and improves the survival (12). The present patient received combination therapy including mPSL pulse therapy, resulting in a recovered EF; however, he still ultimately died due to sepsis.

EBV-associated gastric cancer accounts for approximately 10% of gastric cancers and has pathological lymphoepithelial-like features (13). Most infiltrating lymphocytes are CD8+ T cells (14). The Cancer Genome Atlas classified gas-
tric cancer into four molecular subtypes: microsatellite unstable tumors, genomically stable tumors, tumors with chromosomal instability, and EBV-associated gastric cancer which show the activation of immune cell signaling and high expression of PD-L1/PD-L2 (15). Kim et al. indicated that EBV-associated gastric cancer is a highly effective factor for ICIs (16). Several studies have shown that irAEs are associated with the efficacy of ICIs in melanoma, lung cancer, and gastric cancer (17-19). Interestingly, our case had both mentioned predictive factors and showed a good response to an ICI based on the clinical course and autopsy findings (the primary site had pathological complete remission). Further studies will be required to elucidate the prognostic factors and predictors of ICI therapy.

In conclusion, we reported a patient with EBV-associated gastric cancer who developed late-onset fulminant myocarditis after treatment with nivolumab. We must therefore consider possible complication with cardiac irAEs even beyond three months after starting ICI therapy.

All procedures were performed in accordance with the ethics laid down in the 1964 Declaration of Helsinki and its subsequent amendments.

Informed consent was not obtained because the patient died; permission was instead obtained from the patient’s family for this case report.

The authors state that they have no Conflict of Interest (COI).

Acknowledgements
We express our deep thanks to Dr. Hatsue Ishibashi-Ueda for her help in the pathological diagnosis of acute myocarditis.

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