BRIEF REPORT

Fulminant Myocarditis with Combination Immune Checkpoint Blockade

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SUMMARY

Immune checkpoint inhibitors have improved clinical outcomes associated with numerous cancers, but high-grade, immune-related adverse events can occur, particularly with combination immunotherapy. We report the cases of two patients with melanoma in whom fatal myocarditis developed after treatment with ipilimumab and nivolumab. In both patients, there was development of myositis with rhabdomyolysis, early progressive and refractory cardiac electrical instability, and myocarditis with a robust presence of T-cell and macrophage infiltrates. Selective clonal T-cell populations infiltrating the myocardium were identical to those present in tumors and skeletal muscle. Pharmacovigilance studies show that myocarditis occurred in 0.27% of patients treated with a combination of ipilimumab and nivolumab, which suggests that our patients were having a rare, potentially fatal, T-cell–driven drug reaction. (Funded by Vanderbilt–Ingram Cancer Center Ambassadors and others.)

Immune checkpoint inhibitors have transformed the treatment of several cancers by releasing restrained antitumor immune responses.[1] Ipilimumab, an anti–cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) antibody, and nivolumab, an anti–programmed death-1 (PD-1) antibody, have individually improved survival in patients with melanoma, and early results suggest that their combination further enhances antitumor activity and survival.[2-5] Other adverse events associated with these agents include dermatitis, endocrinopathies, colitis, hepatitis, and pneumonitis, which are all thought to arise from aberrant activation of autoreactive T cells.[6,7] These toxic effects are more frequent and severe when ipilimumab and nivolumab are used in combination.[4] Here, we report two cases of lethal myocarditis accompanied by myositis in patients treated with a combination of nivolumab and ipilimumab.

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CASE REPORTS

PATIENT 1

A 65-year-old woman with metastatic melanoma was admitted to the hospital with atypical chest pain, dyspnea, and fatigue 12 days after receiving her first doses of nivolumab (1 mg per kilogram of body weight) and ipilimumab (3 mg per kilogram). An initial workup revealed myocarditis and myositis with rhabdomyolysis (creatine phosphokinase level, 17,720 U per liter [normal range, 29 to 168]; creatine kinase–myocardial band [CK-MB] level, >600 ng per milliliter [normal level, <5.99]; and troponin I level, 4.7 ng per milliliter, increasing to 51.3 ng per milliliter [normal level, <0.03]). Electrocardiography (ECG) revealed prolongation of the PR interval with normal QRS complexes and no indication of ischemia. Within 24 hours, new intraventricular conduction delay developed and was later followed by complete heart block (Fig. 1A). Serial echocardiograms revealed preserved left ventricular systolic function, with an ejection fraction calculated as 73% (see Video 1, available with the full text of this article at NEJM.org). She was treated with high-dose glucocorticoids (intravenous methylprednisolone administered at 1 mg per kilogram per day) within 24 hours after admission, but progressive clinical deterioration followed, with multisystem organ failure and refractory ventricular tachycardia (Fig. 1B), from which she could not be resuscitated.

Videos showing echocardiograms are available at NEJM.org

PATIENT 2

A 63-year-old man with metastatic melanoma was admitted to the hospital with fatigue and myalgias 15 days after receiving his initial doses of nivolumab (1 mg per kilogram) and ipilimumab (3 mg per kilogram). A diagnostic workup revealed profound ST-segment depression, a new intraventricular conduction delay, myocarditis (troponin I level, 47 ng per milliliter [normal level, <0.03] and CK-MB level, 451 ng milliliter [normal level, <5.99]), and myositis (creatine phosphokinase level, 20,270 U per liter [normal range, 29 to 168]) (see Fig. S1 in the Supplementary Appendix, available at NEJM.org). Serial echocardiography revealed low-normal left ventricular systolic function, with an ejection fraction of 50% (see Video 2). He was treated with high-dose glucocorticoids (intravenous methylprednisolone administered at 1 g per kilogram daily for 4 days) and infliximab (5 mg per kilogram). Despite these measures, complete heart block developed and a temporary pacemaker was placed. Cardiac arrest occurred later. An initial return of spontaneous circulation was achieved, but the patient had a second cardiac arrest, after which supportive care was withdrawn.

METHODS

We performed postmortem gross and microscopic evaluations of both patients. Immunofluorescence studies and next-generation sequencing (with the use of ImmunoSeq) were performed to identify the cell types in the infiltrates that were found in the myocardium, skeletal muscle, and tumors. Whole-transcriptome sequencing of afflicted tissues was used to further characterize the infiltrates. To investigate other possible causes of these reactions, we performed four-digit class I and II typing of HLA (with Illumina MiSeq) for the HLA antigens ABC, DR, DQ, and DP on DNA extracted from peripheral blood and formalin-fixed tissue.[8] Polymerase-chain-reaction (PCR) assays were performed to test for viral infections of the heart and serum. We also used a deep-sequencing, target-enrichment system designed to detect the genomes of 472 DNA and RNA viruses known to infect humans. Bristol-Myers Squibb corporate safety databases were interrogated to assess the frequency of myocarditis and myositis in a larger population treated with nivolumab, ipilimumab, or both, with a cutoff date of April 2016.

RESULTS

Both patients had hypertension but did not have other cardiac risk factors, and neither patient had a history of statin use, treatment with systemic drugs, radiation, or cardiac metastases. Each had received ipilimumab and nivolumab in clinical trials (NCT02320058 and NCT02224781). Histopathological analysis of the heart in Patient 1 revealed an intense, patchy lymphocytic infiltrate within the myocardium that also involved the cardiac sinus and atrioventricular nodes (Fig. 1C). No eosinophilic granulomas or giant cells were noted. Likewise, skeletal muscle showed lymphocytic destruction of isolated myocytes (Fig. S2 in the Supplementary Appendix). Infiltrating cells within the myocardium and skeletal muscle were positive for the T-cell marker CD3 (Fig. 1D) or the macrophage marker CD68. T-cell infiltrates

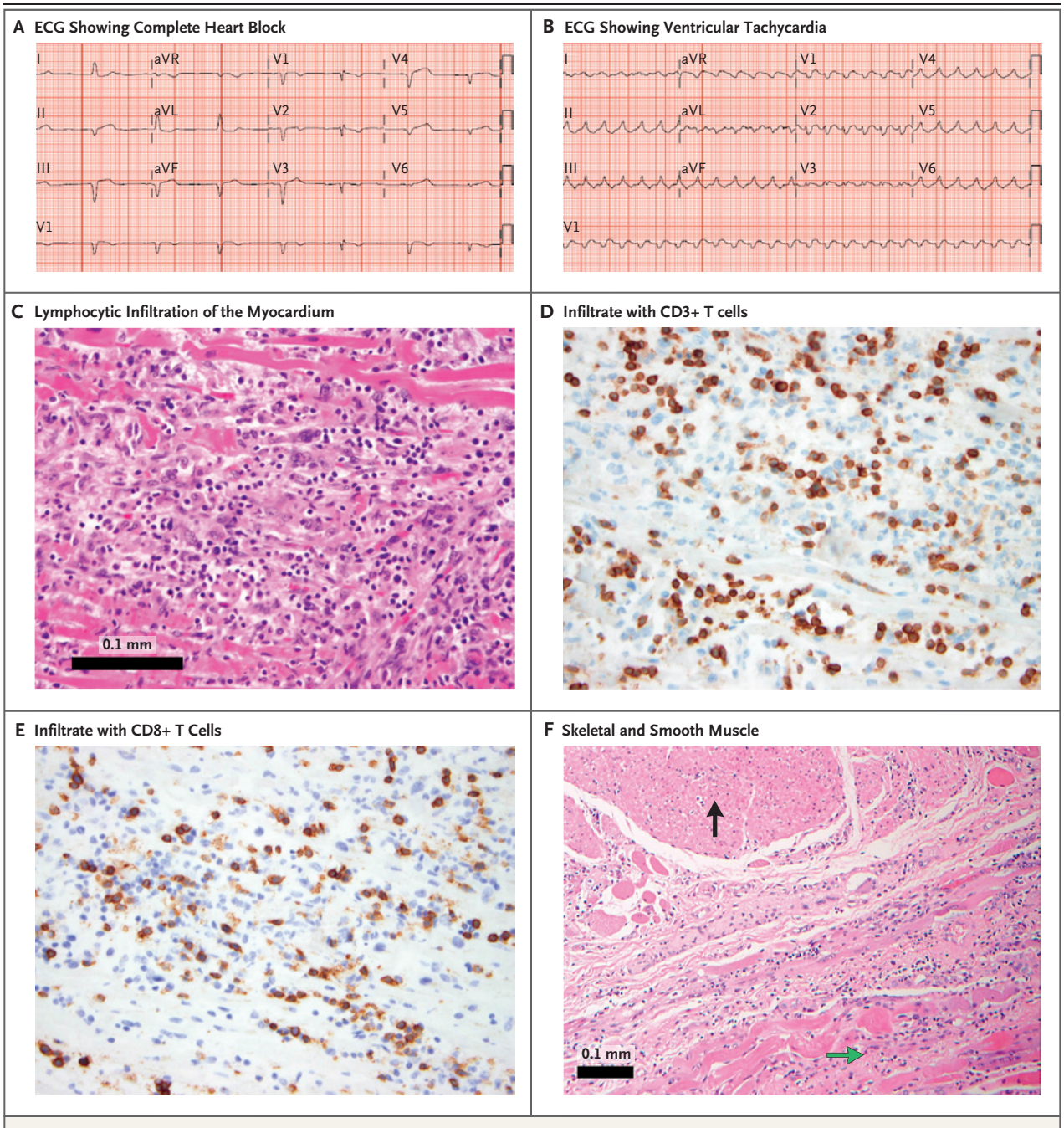

Figure 1. Results on ECG and Immune Effects in Cardiac Muscle after Treatment with Ipilimumab and Nivolumab in Patient 1.

Patient 1 had rapid progression to complete heart block (as shown on electrocardiography [ECG] in Panel A), followed by ventricular tachycardia (Panel B). Autopsy revealed lymphocytic infiltration of the myocardium (shown in the intraventricular septum in Panel C; staining with hematoxylin and eosin). The inflammatory infiltrate included CD3-positive T lymphocytes (Panel D), many of which were positive for CD8 (Panel E). Only cardiac and skeletal muscle was affected; smooth muscle and other tissues were spared (Panel F, hematoxylin and eosin). The black arrow points to esophageal smooth muscle without immune infiltration, and the green arrow points to esophageal skeletal muscle, which is heavily infiltrated by immune cells.

contained an abundance of CD4+ and CD8+ T cells (CD8+ T cells are shown in Fig. 1E). The infiltrating cells were negative for CD20 and contained no antibody deposits. Postmortem histopathological analysis of the heart in Patient 2 showed similar T-cell and macrophage infiltrates

in the myocardium, the cardiac conduction system, and skeletal muscle that were indicative of lymphocytic myocarditis and myositis (Fig. S1 and S2 in the Supplementary Appendix). It is noteworthy that in both patients, immune infiltration was restricted to cardiac and skeletal muscle; no other tissues were affected, including adjacent smooth muscle (Fig. 1F). Biopsy specimens of tumors that were obtained from both patients before treatment showed modest amounts of immune infiltrates or appeared to contain none. In contrast, postmortem evaluations revealed substantial amounts of intense lymphocytic infiltrates in metastases from both patients, particularly Patient 1 (Fig. S2C through S2F in the Supplementary Appendix).

To further characterize the infiltrating lymphocytes in the myocardium, skeletal muscle, and tumors, we performed next-generation sequencing of T-cell receptors (specifically, the CDR3 region and the antigen-binding portion of the T-cell–receptor beta chain).[9,10] The distribution, clonality, and diversity of T-cell receptors were analyzed. Both patients had common high-frequency T-cell–receptor sequences in infiltrates from cardiac muscle, skeletal muscle, and tumors (Fig. 2). Specific shared clones were expanded in tumors after checkpoint inhibitor therapy. The most abundant T-cell receptor in the myocardium and skeletal muscle in Patient 2 also greatly expanded in the tumor after treatment. (Fig. S3 and S4 in the Supplementary Appendix). In contrast, no one specific clonotype predominated in Patient 1. These findings raise the possibility that antigens (epitopes) that are present in the myocardium, skeletal muscle, and perhaps tumors were recognized by the same T-cell clones. Whole-transcriptome sequencing of the afflicted tissues revealed high expression of inflammatory T-cell cytokines and considerable expression of muscle-specific transcripts in tumor specimens (Fig. S5 in the Supplementary Appendix), possibly providing support for the hypothesis that there is a common (shared) epitope between the tumor and striated muscle.

Four-digit class I and II typing of HLA antigens revealed that the highly prevalent major histocompatibility complex class II allele HLA-DQB1*03:01 was the only HLA allele shared by the patients. PCR assays that were performed to test for viral infections of the heart and serum were negative for adenovirus, cytomegalovirus, parvovirus, respiratory syncytial virus, influenza A, enterovirus, hepatitis C, and human herpesvirus 6. To look more broadly for viruses, we used a deep-sequencing target-enrichment system designed to detect the genomes of 472 DNA and RNA viruses known to infect humans. In Patient 1, we detected herpes simplex virus 1 sequences in heart tissue (HSV-1, 38,583 nucleotides, which constituted 25.34% of the viral genome) but not in skeletal-muscle tissue. We detected Epstein–Barr virus (EBV) (5456 nucleotides, which constituted 3.18% of the viral genome) in heart tissue from Patient 2 only. HSV-1 and EBV have rarely been associated with myocarditis.[12-14] Given that detection of viral genomes does not necessarily reflect active infection, it is uncertain whether our patients' myocarditis was of viral origin.

We also assessed samples of tumor tissue and inflamed cardiac- and skeletal-muscle tissue for the expression of programmed death ligand 1 (PD-L1). PD-L1 (CD274) was expressed on the membranous surface of injured myocytes (Fig. S6 in the Supplementary Appendix) and on infiltrating CD8+ T cells and histiocytes from the inflamed myocardium. In contrast, specimens of skeletal muscle and tumor were negative for PD-L1 expression (threshold for detection, 1%). Similarly, messenger RNA transcriptional data from Patient 2 showed expression of PD-L1 in affected cardiac tissue that was more than 10 times as high as that in nondiseased smooth muscle and 5 times as high as that in affected skeletal muscle (Fig. S7 in the Supplementary Appendix).

To assess the frequency of myocarditis and myositis in a larger population, Bristol-Myers Squibb corporate safety databases were interrogated with a cutoff date of April 2016 to identify the occurrence of these events in patients being treated with nivolumab, ipilimumab, or both. Among 20,594 patients, 18 drug-related severe adverse events of myocarditis were reported (0.09%). Patients who received combination therapy with both drugs appeared to have more frequent and severe myocarditis than those who received nivolumab alone (0.27% vs. 0.06%; $P<0.001$; five fatal events vs. one event) (Table 1). Among patients receiving combined therapy with ipilimumab and nivolumab for many different types of cancer, myocarditis was diagnosed at a median of 17 days after the first treatment (range, 13 to 64 days) (Table S1 in the Supplementary Appendix). Severe myositis (grade 3 to 4) also appeared more frequently when the combination of drugs was used than when nivolumab

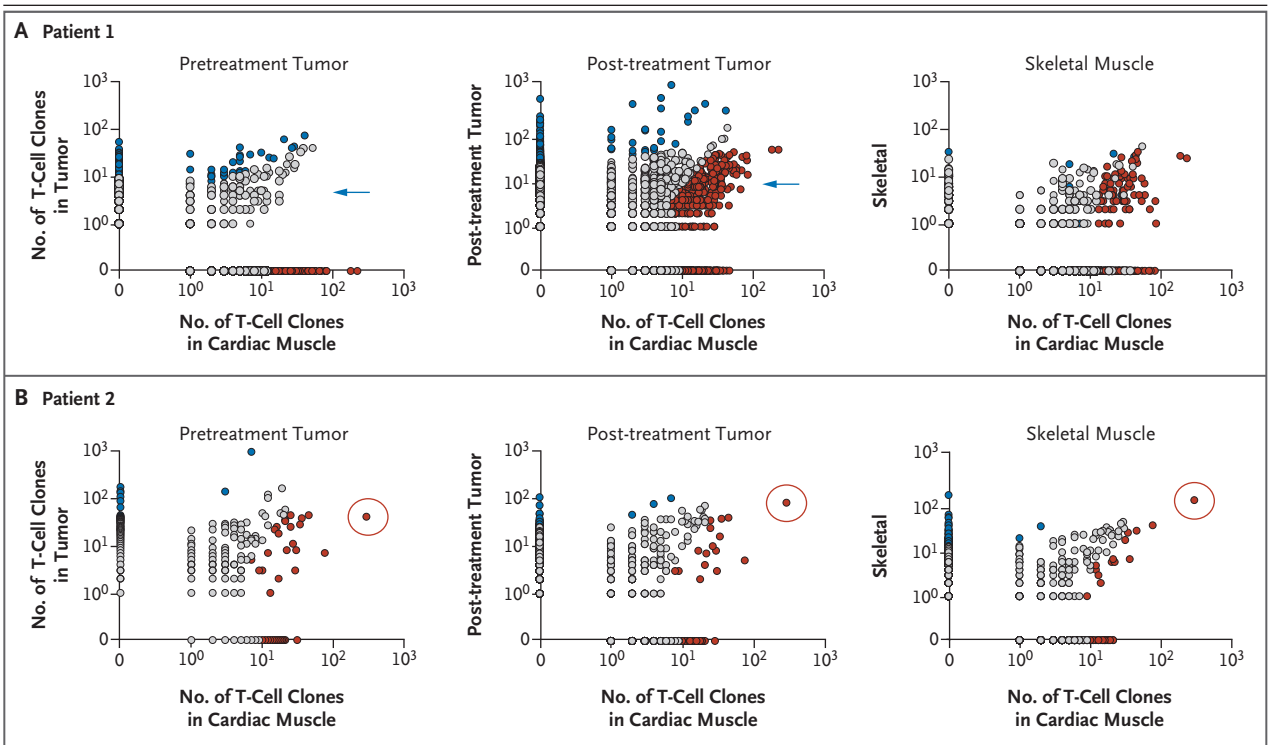

Figure 2. T-Cell–Receptor Clones in Striated Muscle and Tumor in Patients 1 and 2.

Next-generation sequencing of the CDR3 region and the antigen-binding portion of the T-cell–receptor beta chain was performed (as previously described[9,10]) with specimens of cardiac and skeletal muscle and tumors from Patients 1 and 2 before and after treatment. The prevalence of T-cell clones according to count are displayed in various tissue types, with each x-axis showing the counts in cardiac muscle, and the y-axes showing the counts in other tissues. Numerous T-cell clones were present in all affected tissues in Patient 1 (Panel A) and Patient 2 (Panel B). The color red denotes T-cell clones that are more prevalent in cardiac muscle than in other tissue, blue denotes clones that are more prevalent in other tissues (pretreatment or post-treatment tumor or skeletal muscle), and gray denotes clones present in approximately equal numbers. P values for each clone in any two samples being compared were calculated by means of Fisher's exact test and adjusted for a positive false discovery rate with the use of the Storey method, as described by DeWitt et al.[11] In Panel A, the arrows highlight the relative prevalence of shared T-cell clones in the pretreatment tumor, where there were relatively few shared clones, and the post-treatment tumor, where the number of shared clones was greatly increased. In Panel B, the circled dot indicates that the T-cell clone that was found with highest frequency in the heart was also highly prevalent in skeletal muscle and that the frequency was higher in the post-treatment tumor than in the pretreatment tumor.

was the only agent used (0.24% vs. 0.15%) (Table 1). No obvious cardiac-specific or cancer-specific clinical features predisposed patients to these severe adverse events (Table S1 in the Supplementary Appendix). These events were recorded by investigators in reports to Bristol-Myers Squibb. In clinical trials involving nivolumab, ipilimumab, or both, there was no routine testing for myocarditis by means of either biochemical analysis or cardiac imaging.

DISCUSSION

Combined immune checkpoint inhibition with ipilimumab and nivolumab has produced frequent and durable antitumor responses in patients with advanced melanoma, along with promising activity in the treatment of other cancers.[4] However, immune-related adverse events frequently complicate therapy and require discontinuation in nearly 40% of patients.[4,5] These events are generally manageable with the administration of high-dose glucocorticoids, although clinically severe, prolonged, and even fatal events have occurred in rare instances.[4,5] Characterizing these severe toxic effects, even if uncommon, is a major priority.

Myocarditis was rarely reported in early clinical trials with anti–CTLA-4 and anti–PD-1 agents (nivolumab and pembrolizumab, respectively), which resulted in one death in a patient receiving adjuvant treatment with ipilimumab at a dose of

Table 1. Incidence of Myocarditis and Myositis in Patients Receiving Nivolumab or Ipilimumab plus Nivolumab.

Characteristic	Nivolumab (N=17,620)	Nivolumab plus Ipilimumab (N=2974)
	no. (%)	
Myocarditis		
Any*	10 (0.06)	8 (0.27)
Fatal events	1 (<0.01)	5 (0.17)
Myositis		
Any	27 (0.15)	7 (0.24)
Fatal events	2 (0.01)	1 (0.03)

* The number of patients with myocarditis includes six patients with concurrent myocarditis and myositis.

10 mg per kilogram.[15-18] Our review of a large safety database suggests that myocarditis is more frequent and severe with the combination of ipilimumab and nivolumab than with nivolumab monotherapy, but the condition remains rare with both regimens, occurring in less than 1% of patients. Since cardiac monitoring (e.g., with ECG or assessment of troponin levels) is not routinely performed in most immunotherapy trials, the true incidence is unknown.

Clinicians should be vigilant for immune-mediated myocarditis, particularly because of its early onset, nonspecific symptomatology, and fulminant progression. There are no known data regarding what monitoring strategy may be of value; in our practice, we are performing baseline ECG and weekly testing of troponin levels during weeks 1 to 3 for patients receiving combination immunotherapy. In our experience with the cases reported here, both patients had strikingly elevated troponin levels and refractory conduction-system abnormalities with preserved cardiac function. The findings on pathological examination were reminiscent of those observed in patients with acute allograft rejection after cardiac transplantation. In this regard, high-dose glucocorticoids appeared to blunt ongoing inflammation and had the effect of decreasing creatine phosphokinase and troponin levels, but the data are not directive.

We sought to characterize these aberrant immune responses mechanistically. Notably, striated muscle (cardiac and skeletal) and tumor were the only affected tissues. Robust T-cell infiltration, activation, and clonal expansion were observed across tissue types, with indications of shared high-frequency T-cell receptors. There are several possible mechanisms for the observed toxic effects. T cells could be targeting an antigen shared by the tumor, skeletal muscle, and the heart, or the same T-cell receptor may be targeting a tumor antigen and a different but homologous muscle antigen. Alternatively, it is possible that clonal, high-frequency, T-cell–receptor sequences across tumor and muscle samples are misleading and that distinct T-cell receptors are targeting dissimilar antigens. These include the possibilities that T cells are targeting an antigen shared by the tumor, skeletal muscle, and the heart, that the same T-cell receptor is targeting a tumor antigen and a different but homologous muscle antigen, or that clonal, high-frequency, T-cell receptor sequences across tumor and muscle samples are misleading and that distinct T-cell receptors are targeting dissimilar antigens. In keeping with the first possibility, we observed high levels of muscle-specific antigens (desmin and troponin) in tumors from both patients. It is also conceivable that subclinical viral infection could generate T-cell targets, although extensive viral profiling did not reveal a clear cause. Although the single HLA class II allele HLA-DQB1*03:01 was shared by the two patients, up to 30% of white people carry this allele. No specific class I or II HLA haplotype was shared. Ultimately, defining which epitopes are being recognized by these T-cell receptors within the universe of potential antigens is a difficult task. Moreover, only early mechanistic insights have been proposed for any toxic effects derived from immune checkpoint inhibitors.[19,20] Further studies are needed to elucidate the causative antigens and molecular mechanisms involved in these events.

The development of myocarditis from immune checkpoint inhibition does have biologic plausibility. In studies in mice, PD-1 plays a role in myocardial immune responses and protects against inflammation and myocyte damage in models of T-cell–mediated myocarditis.[21] Genetic deletion of PD-1 in mice leads to cardiomyopathy that is caused by autoantibodies against cardiac troponin I.[22,23] We did not observe IgG-autoantibody deposition in the affected tissue, which argues against a directly analogous mechanism. Thus, the underlying cause of the T-cell

reactivity to myocardial and other striated muscle tissue is not clear, and it is certainly not universal across patients. It is interesting that we found increased expression of PD-L1 in the injured myocardium in our patients, which is consistent with the up-regulation of myocardial PD-L1 in studies of T-cell–mediated myocarditis in mice.[24] PD-L1 up-regulation in the myocardium is probably a cytokine-induced cardioprotective mechanism that is abrogated by immune checkpoint blockade. A better understanding of the mechanism of this drug-induced toxicity may provide us with valuable insight into the occurrence of idiopathic myocarditis in patients who do not have cancer and into the general interaction of the immune system with the myocardium.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

APPENDIX

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