# Online Supplement

## Supplemental Methods

### Severity Scoring of Immune-related Adverse Events

All immune-related adverse events (irAEs) were scored by severity using CTCAE guidelines. CTCAE scoring was refined using criteria developed in American Society of Clinical Oncology (ASCO) Clinical Practice Guidelines (25). A baseline score of 0 was assigned on the day prior to irAE diagnosis. Following irAE detection, a daily score was assigned. Unresolved irAEs were held at a constant value reflecting long-term severity, whereas resolved irAEs were scored as zero to indicate the return to baseline. For ICI-related myocarditis, daily scoring ranged from 0-5 based on CTCAE criteria (**Supplementary Table 1**):

**Online Table 1. Criteria for Myocarditis Severity Scoring**

| Myocarditis Severity Grade | Criteria |
|---|---|
| 0 | No clinical or laboratory signs of myocarditis |
| 1 | Troponin elevation above normal baseline, without clinical symptoms (e. g. angina, dyspnea, etc.) |
| 2 | Troponin elevation with clinical symptoms but not requiring patient hospitalization |
| 3 | Troponin elevation with clinical symptoms requiring hospitalization (NOT requiring intensive care unit level of care); abnormal cardiovascular diagnostic studies (echocardiography showing reduction in LV function or wall motion abnormalities; abnormal cardiac MRI) |
| 4 | Deterioration of Grade 3 clinical status or requirement for ICU level of care for cardiac symptoms with evidence of decreased cardiac output (cardiogenic shock) or arrhythmia |
| 5 | Death of the patient refractory to medical therapy |

### Data Abstraction

Data such as signs and symptoms, serial cardiac biomarkers, electrocardiographic parameters and parameters obtained from cardiac imaging were collected for all 23 patients identified with checkpoint associated cardiotoxicity. In addition, data on covariates such as demographic characteristics, cancer diagnosis and staging, ICI administrations, concurrent irAEs, past medical history, steroid use and dosages, and treatment outcomes were collected. Cardiac biomarkers on admission, such as admission troponin, were determined from the first measured value on the date of clinically suspected myocarditis or subclinical myocarditis. Outcomes such as

death, time until death, time until myocarditis resolution, were also collected. Myocarditis severity was scored on a scale of 0 to 5, as mentioned previously. Information was collected about the troponin monitoring protocol utilized by clinicians.

**Cardiac MRI Protocol**

Cardiac MRI's were obtained using a GE 1.5-T scanner with technical parameters recommended by the manufacturer. Scout images in coronal, sagittal and axial planes and fast spin-echo (FSE) axial slices were obtained. Steady-state free precession (SSFP) sequences were employed to obtain short-axis and two, three, and four-chamber images. Additionally, T2-weighted triple-inversion recovery images and T1-weighted FSE sequence before and after intravenous (IV) gadolinium injection (Omniscan 0.1 mmol/kg), and delayed enhanced images 7–10 minutes after gadolinium injection were obtained. Cardiac function was performed by using Segment version 3.1 R8123

**Immunological Methods**

One patient (#17), whose troponin rose to 0.46 ng/mL after the second dose of ICI, was consented and enrolled into a mechanistic study of immune response to ICI therapy. Peripheral blood mononuclear cell (PBMC) samples were obtained before treatment and every 4 weeks during therapy, resulting in a total of three samples for this patient who received two doses of anti-PD-1 therapy.

To gain mechanistic insight into cardiac toxicity and response to ICI therapy, we evaluated the changes in the frequency of circulating $CX3CR1^{+}$ $CD8^{+}$ T cells, which was recently found to correlate with response to ICI therapy in preclinical models and patients (1). PBMC samples were evaluated for expression of CX3CR1 in peripheral CD8+ T cells by flow cytometry. For phenotypic analysis of peripheral blood derived T cells, cryopreserved PBMC samples were stained with master mix of antibodies for surface stains including CD3 (UCHT1, BD Biosciences), CD4 (SK3, BD Biosciences), CD8 (RPA-T8, eBioscience), and, CX3CR1 (2A9-1, Biolegend). Samples were acquired using LSRFortessa (BD Biosciences) and data analyzed with FlowJo software v10.1.5 (TreeStar).

## Supplemental Results

### Immune System Biomarkers

We evaluated changes in the frequency of $CX3CR1^+$ $CD8^+$ T cells in longitudinally obtained blood samples from patient #17. In this patient, the frequency of PBMC $CX3CR1^+CD8^+$ T cells markedly decreased from the pre-treatment baseline (**Supplemental Figure 1A**), suggesting no response to ICI therapy. The patient developed extensive hyper-progressive disease 8 weeks following initiation of ICI therapy that was refractory to IV steroids, with a mild elevation of troponin presumptively due to cardiac strain (**Supplemental Figure 1C**).

In previous studies, we have observed a correlation between the frequency of PBMC derived $CD8^+$ T cells expressing CX3CR1, a marker of T-cell differentiation (2, 3) and response to ICI therapy in preclinical models and patients (1). Here, we used the CX3CR1 score in identifying a non-responder (patient #17) with a troponin rise unrelated to ICI myocarditis. This patient's sharp decrease of CX3CR1 score, extensive hepatopulmonary cancer progression, and steroid-refractory serologic end-organ damage together suggested their mild elevation of troponin and right bundle branch block was caused by cardiac strain rather than initially suspected ICI-related pneumonitis or myocarditis.

### Case Reports: Severe Myocarditis (Grade 3-4)

Summaries of severe myocarditis cases are presented in **Supplementary Table 2**. Cardiac parameters for severe myocarditis patients are presented in **Supplementary Table 3.**

**Online Table 2. Patient Case Summary for ICI-related Severe Myocarditis Patients**

| Patient | Gender | PMH | Cancer | Checkpoint Inhibitor | Number of doses | Time from ICI Initiation | Time from previous dose | Symptoms and Signs | Other Side Effects | Time to initiation of steroids from symptom onset | Treatment | Treatment Outcome | Days to death | Cause of Death |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F | HTN, Valvular Disease, Prior PE | Cutaneous Squamous Cell Carcinoma, Stage IV | Nivolumab + cimiVAX | 1 | 7 days | 7 days | Dyspnea, Edema, Bradycardia, Reduced EF | | 6 days | oral prednisone then ATG | Myocarditis resolving when patient expired due to other causes | 22 | hemorrhagic stroke |
| 2 | F | HTN, CAD, CKD, Hypothyroidism | Melanoma, Stage IV | ipilimumab + nivolumab | 1 | 16 days | 16 days | Chills, Pruritus, Diastolic Heart Failure | Acute Kidney Injury, Rhabdomyolysis, | 8 days | IV methylprednisolone, oral prednisone | death | 15 | Myocarditis – |
| 3 | M | HTN, Hyperlipidemia, A flutter/A fib, Type 2 DM | Melanoma, Stage III-C | pembrolizumab | 2 | 31 days | 10 days | Chest pain, Dyspnea, Myalgia | | 21 days | oral prednisone then ATG | death | 30 | Myocarditis – |
| 4 | M | HTN | Melanoma, Stage IV | pembrolizumab | 1 | 21 days | 21 days | Myalgia, Dyspnea | Hepatitis, Myasthenia Gravis, Pancreatitis, Thyroiditis | 8 days | IV methylprednisolone, oral prednisone with mycophenolate for myasthenia gravis | Myocarditis resolved | | |
| 5 | M | HTN, HFpEF, CAD, prior MI, h/o triple bypass, PVD, A flutter, Hyperlipidemia | Melanoma, Stage IV | nivolumab | 3 | 42 days | 14 days | Bradycardia, Chest Pain, | Optic Neuritis | 6 days | IV methylprednisolone, infliximab, then oral prednisone | Myocarditis resolved | | |
| 6 | M | HTN | Non-small Cell Lung Cancer, Stage IIIA | pembrolizumab | 1 | 30 days | 30 days | Dyspnea, Fatigue, Edema limbs, Bradycardia | Myasthenia Gravis, | 20 days | oral prednisone | death | 37 | Myocarditis – |
| 7 | M | HTN, Hyperlipidemia, A. fib, HFrEF, prior MI, CAD, CABG, AICD implant, Basal cell carcinoma and Non-Hodgkin's lymphoma | Melanoma, Stage IV | Ipilmumab + nivolumab | 5 | 94 days | 17 days | Hypotension, Atrial fibrillation with RVR | Thyroiditis, Colitis, DVT | | IV methylprednisolone, the oral prednisone | Myocarditis resolved | | |
| 8 | M | HTN | Head & Neck Squamous Cell Carcinoma, Stage IV-C | pembrolizumab | 6 | 139 days | 20 days | Heart failure, NSTEMI | Neuropathy, | 23 days | IV methylprednisolone, the oral prednisone | Myocarditis resolved | 75 | ICI related axonal neuropathy and respiratory failure |
| 9 | F | HTN, CKD, smoker | Urothelial Cancer Stage IV | atezolizumab | 4 | 138 days | 48 days | Rash | Encephalitis, | 1 day | IV methylprednisolone, then oral prednisone | Myocarditis resolved | | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | M | HTN, A fib, COPD, Peripheral Vascular Disease, Obesity | Non-small Cell Lung Cancer, Stage IV | pembrolizumab | 1 | 13 days | 13 days | Dyspnea, Heart failure, NSTEMI, | | 3 days | IV methylprednisolone, then oral prednisone | death | 11 | Myocarditis – cardiac arrest |
| 11 | M | HTN, Hyperlipidemia, Type 2 DM, COPD | Melanoma IIIC | Nivolumab + ipilimumab | 6 | 147 days | 14 days | Chest pain, fatigue, fever | Thyroiditis | 9 days | IV methylprednisolone, then oral prednisone | Myocarditis resolved | | |

**Online Table 3. Cardiac Parameters for ICI-treated Severe Myocarditis Patients**

| Patient | Troponin at Onset (ng/mL) | Max of Troponin (ng/mL) | Max of BNP (pg/mL) | Max of CK (IU/L) | Max of CK-MB (ng/ml) | Max of myoglobin (mg/dL) | ECG Findings | LVEF % | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.11 | 0.83 | 1047 | 85 | 7.6 | | 1st AV block, ST deviation and moderate T wave abnormality | 25 | Death due to hemorrhagic stroke |
| 2 | 2.85 | 65.02 | 612 | 8421 | 379 | 24000 | 1st AV block, progressing to 3rd AV block | | Death due to Myocarditis – |
| 3 | 5.78 | 5.78 | 151 | 998 | 104.4 | 10374 | h/o RBBB, 1st degree AV block at baseline → ST depression in anterolateral leads, → 2nd degree AV block → 3rd degree AV block | 70 | Death due to Myocarditis – |
| 4 | 1.37 | 1.37 | 82 | 1681 | 171.9 | 4.69 | RBBB | 55 | Survived |
| 5 | 0.66 | 1.86 | | 190 | | | 2nd AV block, T wave abnormalities | 31 | Survived |
| 6 | 3.82 | 8.3 | 367 | 6124 | 99.1 | 2971 | RBBB → LBBB, 3rd AV block → 2nd AV block→ normal | 62.9 | Death due to Myocarditis – |
| 7 | 0.12 | 1.05 | 2593 | 144 | 11.2 | | No significant findings | 31 | Survived |
| 8 | 7.61 | 7.61 | 108 | 99 | | | Non specific T wave changes | 35 | Death due to ICI related axonal neuropathy and respiratory failure |
| 9 | 1.52 | 1.52 | 3998 | 29 | 2.2 | | No significant findings | 22 | Survived |
| 10 | 12.12 | 20.54 | 9942 | 787 | 75 | 1651 | RBBB, Atrial Fibrillation, NSTEMI, left axis deviation | 35 | Death due to myocarditis – cardiac arrest |
| 11 | 0.31 | 0.44 | 750 | 26 | | 67 | 1st AV block at baseline, non specific ST/T wave abnormality | 60 | Survived |

Patient #1 was a female in his mid 60's with stage IV squamous cell carcinoma on an experimental treatment of nivolumab with cimiVAX. Seven days after this first dose of nivolumab, the patient began experiencing dyspnea and bradycardia. Two days later the patient presented and was found to have an elevated troponin of 0.11 ng/mL and a severely reduced EF from a baseline of 50% to 25%. The patient was

treated with a total of 8 doses of methylprednisolone 1 g IV. Three doses were administered to the patient on presentation. The patient was then converted to oral steroids, with troponin rising back to 0.19 ng/mL, and therefore methylprednisolone IV was restarted from days 14-18 for another 5 doses. The patient continued to exhibit signs of active myocarditis and was therefore trialed on two doses of ATG 125mg IV before succumbing unexpectedly to a hemorrhagic stroke that was probably caused by a metastatic lesion.

Patient #2 was a female in her early 80's with stage IV melanoma treated with combination ipilimumab and nivolumab. Sixteen days after receiving her first dose of combination immunotherapy, she began experiencing shortness of breath, pruritis, and intermittent low-grade fever. Seven days after symptom onset the patient presented and was found to have an elevated troponin of 2.85 ng/mL, complete heart block, rhabdomyolysis, acute on chronic renal failure, VTE and bilateral pulmonary embolism. She was given her first dose of 1 g methylprednisolone IV 8 days after symptom onset. Troponin continued to rise to a peak of 65 ng/mL even though she received daily doses of methylprednisolone 1 g IV. Tacrolimus was also added in a futile attempt to control the progression of myocarditis. The patient declined to be intubated and expired due to complications of myocarditis on day 15 after myocarditis onset.

Patient #3 was a male in his late 80's with stage IIIC melanoma who received two doses of pembrolizumab. Ten days after receiving his second dose, the patient began experiencing acute chest pain and myalgia. The patient was only discovered to have myocarditis 21 days after the onset of these symptoms, when a significant troponin elevation of 5.78 ng/mL and AV block were found. The patient then received methylprednisolone 1 g IV daily for 6 days, followed by oral steroids. When the patient failed to exhibit satisfactory response to treatment, four doses of ATG 125 mg IV, in addition to oral steroids, were administered. The patient developed staphylococcal sepsis, with multi-organ failure, and succumbed to complications of myocarditis and its treatment 10 days after their myocarditis was discovered, 31 days from onset.

Patient #4 was a male in his early 60's with stage IV melanoma. The patient began experiencing dyspnea and myalgia 21 days after receiving his first dose of pembrolizumab. The patient was discovered to have myocarditis 8 days after first exhibiting symptoms, with a significant troponin elevation of 1.37 ng/mL and right bundle branch block on ECG. The patient received 3 doses of methylprednisolone 1 g IV starting that day and continuing until day 10. The patient was then converted to a high dose oral prednisone taper, starting at prednisone 120 mg twice daily, with good response, as evidenced by a normalization of troponin down to 0.06 ng/mL by day 14. However, the patient continued to exhibit right bundle branch block for two months after onset and was continued on a prolonged oral steroid taper for 79 days. The patient exhibited multiple concurrent ICI-related toxicities including hepatitis, pancreatitis, thyroiditis, and myasthenia gravis, for which he was treated with five doses of immunoglobulin IV and a prolonged taper of oral pyridostigmine. The patient was also treated with oral mycophenolate mofetil for autoimmune hepatitis.

Patient #5 was a male in his late 60's with stage IV melanoma and a past medical history of chronic heart failure, significant coronary artery disease with triple bypass, and dilated cardiomyopathy. The patient began experiencing chest pain 14 days after receiving his third dose of nivolumab. Five days after the onset of chest pain, the patient was discovered to have myocarditis by cardiac MRI, when he exhibited a mildly elevated troponin of 0.66 ng/mL and new onset 2$^{nd}$ degree AV block. The patient also suffered an MI during his presentation with myocarditis, with negative catheterization and had new onset atrial flutter. Notably, this patient did not receive high dose IV steroids early in their treatment, instead receiving a high dose oral steroid taper starting at prednisone 160mg twice daily on the day his troponin elevation was discovered. This patient exhibited poor response to oral steroids and had a prolonged course of troponin elevation, which peaked 29 days after symptom onset at 1.86 ng/mL and continued for a total of 91 days after symptom onset. The patient eventually received two doses of methylprednisolone 1g IV at days 29 and 81. Patient 5 was received a trial of infliximab IV on day 86 and continued to receive steroids for concomitant ICI-related optic neuritis as well. Ejection fraction recovered to 56% 71 days after onset of myocarditis, but troponin remained persistently elevated for 263 days following symptom onset, with concurrent exacerbations of chronic heart failure.

Patient #6 was a male in his early 70's with stage IIIA non-small cell lung cancer treated with pembrolizumab. The patient developed fatigue, dyspnea, edema and muscle weakness 30 days after receiving his first dose of pembrolizumab. The patient was discovered to have myocarditis and myasthenia gravis 3 days after onset of symptoms, when he presented with an elevated troponin of 3.82 ng/mL and new onset 2$^{nd}$ degree AV block which progressed to complete heart block and alternating left and right bundle branch blocks. This case occurred in early 2016 when clinical experience with ICI-related myocarditis was limited. The patient received 1 dose of oral prednisone 80 mg on the day he presented, and two subsequent doses of prednisone 60 mg for concurrent ICI-related myasthenia gravis on days 19 and 20. The patient was treated with a pacemaker with improvement in symptoms. The patient received plasmapheresis three times in conjunction with immunoglobulin IV therapy over 6 days. The patient died 27 days after symptom onset from complications of myocarditis and acute myasthenic crisis.

Patient #7 was a male in his late 70's with stage IV melanoma who received combination ipilimumab and nivolumab. The patient began experiencing hypotension and atrial fibrillation with RVR 17 days after receiving his fifth dose of combination immunotherapy. The patient was found to have a troponin elevation of 0.12 ng/mL when he presented to the hospital with acute coronary syndrome with concurrent atrial fibrillation. Elevation in troponin was attributed to acute on chronic ischemia heart disease as this patient had a significant history of heart failure with reduced EF (33%), two prior MI's, 6 vessel coronary artery bypass, and atrial fibrillation. Of note, this patient received 10 doses of methylprednisolone 40 mg IV every 12 hours for 5 days for severe ICI-related colitis, starting 1 day prior to his presentation with ACS. The patient was subsequently converted to oral prednisone taper starting at 70 mg twice daily. The patient also received amiodarone for tachyarrhythmia and a watchman implant for atrial fibrillation. This patient

experienced a prolonged elevation in troponin lasting for 107 days after his presentation with ACS, peaking at 1.05 ng/mL at day 33. Importantly, this patient's troponin was normal at baseline with multiple readings of 0.05 ng/mL before initiating and while receiving ICI therapy. Immunotherapy was permanently discontinued due to risk of further decompensation of the patient's condition. Patient also experienced other irAEs, including ICI-related thyroiditis requiring levothyroxine, and severe colitis.

Patient #8 was a male in his early 60's with stage IVC squamous cell carcinoma of the head and neck. The patient experienced an NSTEMI 20 days after his 6$^{th}$ dose of pembrolizumab and was subsequently determined to have probable ICI-related myocarditis 9 days after this event, with persistent troponin elevation of 0.18 ng/mL and decreased LVEF of 35% on echo. The patient was then given 5 doses of methylprednisolone 1 g IV over the course of days 10-14 after symptom onset, before conversion to oral prednisone 60 mg daily, which he received from days 15-22. Unfortunately, the full course of this patient's treatment is unknown because he was transferred to hospice care, but the patient did expire 75 days after symptom onset due to respiratory failure, possibly secondary to ICI-related axonal sensorimotor polyneuropathy.

Patient #9 was a female in her early 70's with stage IV urothelial cancer, who was treated with 4 doses of atezolizumab. The patient presented 48 days after receiving her last dose with elevated troponin of 1.52 ng/mL, reduced LVEF of 22% from their baseline of 55% and acute MI on ECG with no previous history of cardiovascular disease. At the time of presentation, there were no apparent symptoms of myocarditis. The patient exhibited concomitant ICI-related encephalitis for which they received methylprednisolone 40 mg IV every 6 hours from days 1-9 after diagnosis of suspected myocarditis. This patient exhibited rapid normalization of LVEF, rising back to 65% 11 days after diagnosis but was continued on a prolonged oral prednisone taper for 42 days after symptom onset.

Patient #10 was a male in his mid-70's with stage IV non-small cell lung cancer and a past medical history of atrial fibrillation, lymphedema, and COPD. He began experiencing dyspnea 13 days after his first dose of pembrolizumab. The patient was hospitalized one day later when he was found to have an NSTEMI and an elevated troponin of 12.12 ng/mL with a LVEF of 35% on echocardiography. A coronary angiogram was negative for ischemia. The patient was started on that day with methylprednisolone 1 g IV. The patient was then briefly converted to methylprednisolone 60 mg every 8 hours, however, his troponin remained elevated at 12.3 ng/mL. The patient was given another dose of methylprednisolone 1 g IV, 6 days after myocarditis onset, and then discharged on oral prednisone taper starting at 80 mg daily. The patient was readmitted the next day for worsening symptoms and was found to have new heart failure with LVEF of 35%. The patient received two more doses of methylprednisolone 1 g IV. During his second hospitalization, he began to develop runs of ventricular tachycardia, which became sustained. He expired in hospital two days after admission due to cardiac complications of myocarditis.

Patient #11 was a male in his early 80's with stage IIIC melanoma, treated with 6 doses of combination immunotherapy with ipilimumab and nivolumab. The patient began experiencing chest pain, two weeks after his 6th dose of combination immunotherapy. The patient presented 9 days later and was found to have an elevated troponin of 0.31 ng/mL and a peak troponin elevation of 0.44, as well as 1st degree AV block on ECG. He was given methylprednisolone 1 g IV daily for 3 days, with a fall in troponin to 0.19 ng/mL. The patient was subsequently discharged 12 days after myocarditis onset on oral prednisone 80 mg daily. He returned to this outpatient cancer clinic on day 16 and was found to have normalization of troponin back down to 0.02 ng/mL.

**Case Reports: Mild Subclinical Myocarditis (Grade 0-2)**

Summaries of individual subclinical myocarditis cases are presented in **Supplementary Table 4**. Cardiac parameters for subclinical myocarditis patients are presented in **Supplementary Table 5**.

**Online Table 4. Patient Case Summary for ICI-related Subclinical Myocarditis Patients**

| Patient | Gender | PMH | Cancer | Checkpoint inhibitor | Number of doses | Time from ICI Initiation | Time from previous dose | Symptoms and Signs | Effects | Time to initiation of steroids from symptom onset | Treatment | Immunotherapy re-initiated? |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12 | M | CAD, Prior MI, Prior PE, HTN, A fib, pacemaker | Melanoma, Stage IV | pembrolizumab | 2 | 60 days | 39 days | Dyspnea | Colitis | | IV methylprednisolone, oral prednisone | No |
| 13 | M | prior MI ,CAD, HFrEF, PVD, HTN, A fib, Hyperlipidemia, COPD | Melanoma | pembrolizumab | 1 | 22 days | 22 days | Asymptomatic | Colitis | | Immunotherapy discontinued, no steroids | No |
| 14 | F | None | Non-small Cell Lung Cancer | nivolumab + cabiralizumab | 3 | 43 days | 15 days | Dyspnea, Edema, | | | IV methylprednisolone, oral prednisone | Re-initiated 1 and a half years later |
| 15 | M | HTN, Hyperlipidemia, hypothyroidism, Gout, BPH | Melanoma, Stage IV | nivolumab + NKTR-214 (IL-2 prodrug) | 9 | 172 days | 4 days | Dyspnea, | Pneumonitis, Stroke | | Immunotherapy discontinued, no steroids | No |

**Online Table 5. Cardiac Parameters for ICI-treated Subclinical Myocarditis Patients**

| Patient | Troponin at Onset (ng/mL) | Max of Troponin (ng/mL) | Max of BNP (pg/mL) | Max of CK (IU/L) | Max of CK-MB (ng/ml) | ECG Findings | LVEF % | Outcome |
|---|---|---|---|---|---|---|---|---|
| 12 | 0.09 | 0.15 | 129 | 32 | 1.1 | T wave abnormalities, Baseline 1st degree AV block and left anterior hemiblock | | Survived |
| 13 | 0.11 | 0.15 | 1189 | 37 | 2.4 | Borderline repolarization abnormality and ST depression at baseline | 70 | Survived |
| 14 | 0.37 | 0.37 | 57 | 699 | 1.3 | No significant findings | 55 | Survived |
| 15 | 0.19 | 0.77 | 20 | 71 | 5.1 | No significant findings | 65 | Survived |

Patient #12 was a male in his late 70's with stage IV melanoma with a past medical history of coronary artery disease, atrial fibrillation with pacemaker implantation, and prior MI and PE. Thirty-nine days after receiving his second dose of pembrolizumab, the patient began experiencing dyspnea. The next day the patient presented for routine weekly troponin monitoring and was suspected of having myocarditis with elevated troponin of 0.14 ng/mL. Notably this patient was already taking oral prednisone 50 mg daily for colitis when cardiotoxicity manifested. The patient was treated with 2 doses of methylprednisolone 100 mg twice daily IV for one day and then converted to a higher oral prednisone dose for suspected myocarditis, up to prednisone 60 mg twice daily which was tapered over a month-long period. The patient's troponin normalized 9 days after the onset back down to 0.06 ng/mL.

Patient #13 was a male in his late 80's with melanoma and a significant past medical history of heart failure with reduced ejection fraction, atrial fibrillation, coronary artery disease, prior MI, atrial fibrillation and COPD. The patient was asymptomatic but found to have a slightly elevated troponin of 0.11 ng/mL, 22 days after his first dose of pembrolizumab, which persisted for 12 days, but there were no concurrent ECG or echocardiographic changes. Pembrolizumab was held until troponin normalized, however the administration of the second dose resulted in another elevated troponin reading of 0.15 ng/mL, after which immunotherapy was permanently discontinued. The patient's myocarditis subsequently resolved.

Patient #14 was a female in her late 40's with non-small cell lung cancer treated with nivolumab + cabiralizumab and no other significant past medical history. The patient began experiencing dyspnea and edema 15 days after receiving her third dose of this combination. The patient was diagnosed with suspected myocarditis 7 days after the onset of symptoms when she was found to have an elevated troponin of 0.37 ng/mL. This troponin elevation persisted for 14 days after onset, with no accompanying ECG or echocardiographic changes. Immunotherapy was withheld and the patient received 6 doses of methylprednisolone 100 mg IV twice daily for 3 days and was subsequently converted to a high dose oral prednisone taper starting at 100mg twice daily for approximately two months, with resolution of troponin elevation and accompanying symptoms. Because the patient showed clinical progression on CT, immunotherapy was withdrawn. The patient was successfully stabilized on oral cabozantinib. Approximately 1.5 years after this incident, the patient was successfully restarted on combination immunotherapy with ipilimumab and nivolumab without cardiotoxicity. However, after 6 doses the patient expired due to progressive disease.

Patient #15 was a male in his late 60's with stage IV melanoma, who was being treated with an experimental protocol of nivolumab with NKTR-214 (an IL-2 prodrug). The patient began experiencing dyspnea 4 days after his 9$^{th}$ dose of immunotherapy. The patient presented the next day and was found to have a troponin elevation of 0.19 ng/mL which subsequently increased to a max of 0.77 ng/mL and persisted over the course of 15 days. The patient was also diagnosed with suspected pneumonitis with hypoxemia and findings consistent with pneumonitis on CT of chest. There were no accompanying changes on ECG or echocardiogram. The cardiologist did not favor steroids for low grade troponin elevation in this case, so immunotherapy was permanently discontinued, after which the patient's pneumonitis and myocarditis resolved.

**Case Reports: Other Cardiotoxicities**

Case summaries and cardiac parameters for other cardiotoxicities are provided in **Supplementary Tables 6 and 7**.

## Online Table 6. Patient Case Summary for Other Cardiotoxicity Patients

| Patient | Gender | PMH | Cancer | Checkpoint Inhibitor | Cardiotoxicity | Number of doses | Time from ICI Initiation | Time from previous dose | Symptoms and Signs | Other Side Effects | Treatment | Treatment Outcome | Days to death | Cause of Death |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 | F | HTN – severe, Hyperlipidemia, | Ovarian Cancer, Stage III-C | pembrolizumab + bevacizumab | Heart failure exacerbation | 1 | 8 days | 8 days | Dyspnea, Edema, Acute Decompensated HF | | Immunotherapy temporarily halted, nifedipine and diuresis | Cardiotoxicity resolved, immunotherapy reinitiated 3 months later | | |
| 17 | M | HTN, COPD, T2DM, Hyperlipidemia, | Melanoma, Stage IV | nivolumab | Hyper-progression, pneumonitis | 2 | 35 days | 8 days | Dyspnea, | Pneumonitis, | IV methylprednisolone, patient expired before any further treatment | Patient expired from complications of metastatic disease | 23 | Respiratory failure due to metastatic cancer |
| 18 | M | Type 2 Diabetes, Hypothyroidism | Melanoma, Stage IV | Nivolumab + NKTR214 | Acute Coronary Syndrome | 1 | 6 days | 6 days | Chest pain, fatigue, fever | | 2 stents placed, supportive care, immunotherapy temporarily held and later resumed | Cardiotoxicity resolved | | |
| 19 | M | HTN, Hyperlipidemia, Hypothyroidism, Pancreatitis, Gout, Neuropathy | Urothelial Cancer of bladder | atezolizumab | Acute Coronary Syndrome | 3 | 82 days | 40 days | Chest pain, fatigue, | Adrenal insufficiency | Supportive care, immunotherapy stopped | Cardiotoxicity resolved | | |
| 20 | F | Neuropathy | Adenocarcinoma of colon | pembrolizumab | Demand ischemia | 1 | 17 days | 17 days | Dyspnea, chills, nausea | Pneumonitis | Supportive care, immunotherapy stopped, | Not followed due to poor prognosis, patient discharged to palliative care | 38 days | Respiratory failure due to extensive metastatic disease |
| 21 | F | CAD w h/o 3x CABG, h/o pacemaker implant, Type 2 Diabetes | Melanoma, Stage IV | pembrolizumab | Heart failure exacerbation | 11 | 216 days | 0 days | Dyspnea, peripheral edema, | | Immunotherapy stopped | Cardiotoxicity self-resolved | | |
| 22 | F | HTN, Hyperlipidemia, A. fib., CAD, Hypothyroidism | Melanoma, Stage IIIC | pembrolizumab + interferon alfa-2b | Coronary artery spasm | 1 | 14 days | 14 days | Dyspnea, chest pain, fatigue, flu-like symptoms, | | IV methylprednisolone with short steroid taper, immunotherapy temporarily halted | Cardiotoxicity resolved | | |
| 23 | F | HTN, CAD, Hypothyroidism, Thyroid Cancer | Squamous cell carcinoma, head and neck | nivolumab | Chronic Ischemic Heart Disease | 3 | 57 days | 15 days | Myalgia | | Supportive care, Immunotherapy stopped | Not followed due to concurrent morbidity, patient expired due to other cases | 74 | pneumonia |

**Online Table 7. Cardiac Parameters for ICI-treated Other Cardiotoxicity Patients**

| Patient | Troponin at Onset (ng/mL) | Max of Troponin (ng/mL) | Max of BNP (pg/mL) | Max of CK (IU/L) | Max of CK-MB (ng/ml) | ECG Findings | LVEF % | Outcome |
|---|---|---|---|---|---|---|---|---|
| 16 | 0.07 | 0.07 | 1083 | 49 | 1.9 | Left axis deviation, left atrial abnormality | 50 | Resolved |
| 17 | 0.39 | 0.39 | 153 | 41 | 4.9 | Partial RBBB, ventricular bigeminy | | Patient expired from other causes |
| 18 | 1.74 | 1.74 | | 48 | | Partial RBBB, anterior T wave abnormality | | Resolved |
| 19 | 0.98 | 1.08 | 448 | 25 | | anterior and inferior T wave abnormality | 60 | Resolved |
| 20 | 0.14 | 0.14 | 77 | 23 | 1 | No significant findings | 69 | Patient expired from other causes |
| 21 | 0.03 | 0.06 | 299 | 51 | 1.8 | No significant findings | 60 | Resolved |
| 22 | 6.18 | 6.18 | 318 | 360 | 11 | nonspecific ST-T changes | 56 | Resolved |
| 23 | 0.52 | 0.68 | | 38 | 0.9 | No significant findings | | Patient expired from other causes |

Patient #16 was a female in her mid 70's with stage IIIC ovarian cancer and a past medical history of severe hypertension. The patient developed dyspnea and peripheral edema 8 days after receiving their first dose of pembrolizumab and bevacizumab. The patient presented 19 days after the onset of symptoms, when they were found to have an elevated troponin of 0.07 ng/mL, elevated BNP of 1083 pg/mL and reduction in LVEF to 50% from a baseline of 65%. Patient's condition was attributed to an exacerbation of undiagnosed chronic heart failure with concurrent renal failure. Patient was treated with supportive care and immunotherapy was temporarily withheld due to suspicion of cardiotoxicity. The patient was subsequently successfully restarted on pembrolizumab approximately three months later with no return of cardiotoxicity.

Patient #17 was a male in his early 70's with stage IV melanoma, treated with nivolumab. The patient began experiencing dyspnea 8 days after his second dose of nivolumab. The patient presented 22 days after the onset of dyspnea with an elevated troponin of 0.39 ng/mL and transient right bundle branch block. The patient's symptoms and elevated biomarkers were initially attributed to possible myocarditis or pneumonitis. The patient was started on oral prednisone 50 mg twice daily on day 14 after dyspnea onset for what was determined at the time to be a COPD exacerbation. When myocarditis was suspected on day 22, the patient received 2 doses of methylprednisolone 1 g IV on that day and the following day with no response. CT revealed extensive metastatic progression of melanoma resulting in multiple pulmonary

nodules, innumerable masses in the liver, multiple metastatic lesions in the spleen, left adrenal gland, and lymph nodes, and massive left inguinal femoral adenopathy. On day 23, the patient succumbed to respiratory failure likely due to hyper-progressive disease. Troponin elevations and right bundle branch block were ascribed to right heart strain resulting from underlying metastatic pulmonary disease.

Patient #18 was a male in his late 60's with stage IV melanoma. The patient presented with a significant troponin elevation of 1.74 ng/mL and partial right bundle brand block 6 days after his first dose of nivolumab and an experimental agent NKTR-214 (an IL-2 prodrug). The patient was hospitalized for suspected myocarditis, but cardiac MRI was negative for myocarditis and the patient was determined to have non-Q wave myocardial infarction. Cardiac catherization revealed multiple vessel coronary artery disease and patient had two stents placed in left anterior descending coronary artery. Patient had no known heart or cardiovascular disease at baseline. Troponin remained elevated for 26 days after myocardial infarction, after which it returned to a baseline value of 0.01 ng/mL and nivolumab therapy was deemed safe to resume. Patient experienced no further potential cardiotoxicities while receiving an additional 7 doses of immunotherapy.

Patient #19 was a male in his late 60's with urothelial cancer of bladder treated with atezolizumab. The patient began to experience chest pain and fatigue and presented with an initial troponin elevation of 0.08 ng/mL occurring 40 days after the patient's third dose of atezolizumab. The patient was determined to have CAD with NSTEMI when hospitalized, and his troponin peaked at a max of 1.08 ng/mL day 6 after his initial presentation. Notably, this patient was receiving oral prednisone 100 mg daily for ICI-related adrenal insufficiency at the time of this event. This patient had no known history of CAD but did have hypertension and hyperlipidemia. Immunotherapy was never again pursued in this patient.

Patient #20 was a female in her mid-60's with adenocarcinoma of the colon treated with pembrolizumab. The patient presented with dyspnea, chills, and nausea, with a troponin elevation of 0.14 ng/mL 17 days after her first dose of pembrolizumab. The patient was diagnosed with grade 4 pneumonitis and respiratory failure. ECG and echocardiogram were reported normal. Full cardiac workup and continued monitoring were not pursued in light of severe pneumonitis and poor cancer prognosis. Patient received methylprednisolone IV for pneumonitis as well as palliative radiation but ultimately succumbed to pneumonitis 22 days after presenting with elevated troponin.

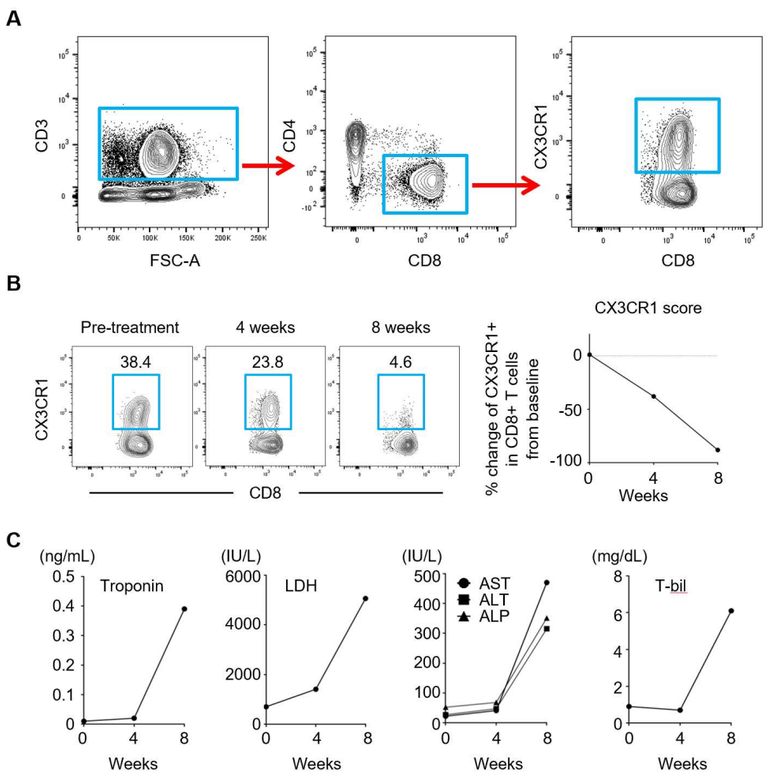
Patient #21 was a female in her mid-80's with stage IV melanoma, with a past medical history significant for coronary artery disease with triple bypass and pacemaker implantation. The patient presented with peripheral edema, persistent BNP elevation (277 pg/mL) and marginal (0.06 ng/mL) troponin elevation from baseline of 0.03 ng/mL after receiving her 11$^{th}$ dose of pembrolizumab. Elevated cardiac biomarkers

were attributed to exacerbation of undiagnosed heart failure although her last echocardiogram was essentially negative. Immunotherapy was halted but no specific treatment was pursued and patient was referred to outpatient cardiology for management of presumed heart failure.

Patient #22 was a female in her mid-70's with stage IIIC melanoma, enrolled in a trial of pembrolizumab and interferon alfa-2. The patient presented with transient chest pain and dyspnea and an elevated troponin of 6.18 ng/mL with accompanying elevations in BNP, CK and CK-MB, 14 days after her first dose of pembrolizumab. Patient received four doses of methylprednisolone IV followed by oral steroid taper starting with prednisone 100 mg daily for suspected myocarditis. However, the patient's cardiac MRI was negative and coronary artery spasm was deemed the most likely explanation in this case. Troponin rapidly normalized within 15 days back to 0.01 ng/mL as did CK and CK-MB. Patient was quickly tapered off steroids and immunotherapy was subsequently reinitiated with no return of cardiotoxicity.

Patient #23 was female in her early 70's with squamous cell carcinoma of the head and neck. The patient was found to have a troponin elevation of 0.52 ng/mL incidentally during hospitalization for pneumonia, 15 days after her 3$^{rd}$ dose of nivolumab. Patient had history of mild CAD but due to pneumonia, cardiac work up was not pursued. Due to the mild nature of the troponin elevation and concurrent morbidity, myocarditis was not deemed likely but nivolumab was discontinued and the patient succumbed to pneumonia two weeks later.

## Online Figures




**Online Figure 1. Analysis of circulating CX3CR1+ CD8+ T cells and acute myocardial infarction panel.**

**(A)** Gating strategy for identifying $CX3CR1^+$ $CD8^+$ T cells in peripheral mononuclear blood cells. Cells were first gated for lymphocytes (SSC-A vs. FSC-A) and for singlets (FSC-H vs. FSC-A).
**(B)** Left panel shows CX3CR1 expression in peripheral blood (PB) $CD8^+$ T cells before and during treatment in a patient who developed fulminant myocarditis to anti-PD-1 therapy. Numbers denote percent $CX3CR1^+$ cells. Right panel shows % change of $CX3CR1^+$ subset in PB $CD8^+$ T cells (CX3CR1 score).
**(C)** Changes in plasma values of troponin, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin (T-bil) before and during treatment in the patient as described in **Fig. 6B**.

## Supplemental References

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