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Successful use of equine anti-thymocyte globulin (ATGAM) for fulminant myocarditis secondary to nivolumab therapy

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Background: Immune-mediated myocarditis is an uncommon adverse effect of immune checkpoint inhibition and is associated with a high rate of mortality.

Methods: In this reported case, a 64-year-old woman with right temporo-parietal glioblastoma IDH-WT was treated with nivolumab, temozolomide and radiation therapy on a clinical trial. She developed malignant arrhythmias secondary to histologically confirmed severe immune-mediated myocarditis. She was treated with equine anti-thymocyte globulin (ATGAM) due to development of malignant arrhythmias refractory to high-dose corticosteroids.

Results: This report describes the only case of immune-mediated myocarditis treated with ATGAM resulting in a favourable outcome.

Conclusions: Use of ATGAM should be considered in cases of steroid-refractory immune-mediated myocarditis and administered in close consultation with a cardiac transplant team experienced in the use of this agent.

Immune checkpoint inhibitors have revolutionised the treatment of malignancy by facilitating enhanced anti-tumour immune response. Nivolumab, an anti-programmed death-1 (PD-1) antibody has been established as a standard of care in melanoma (Larkin *et al*, 2015; Robert *et al*, 2015; Weber *et al*, 2015), lung (Borghaei *et al*, 2015), renal cell (Motzer *et al*, 2015), bladder (Sharma *et al*, 2017) and head and neck (Ferris *et al*, 2016) cancers and is being tested in clinical trials for many other tumour types.

Nivolumab, like other PD-1 directed antibodies, releases restraint on the innate immune system. This unique mechanism of action gives rise to a side-effect profile distinct to that of traditional cytotoxic chemotherapy agents. Toxicities are autoimmune in nature and most commonly include rash, endocrinopathies, colitis, hepatitis and pneumonitis. Less frequently reported side-effects are myasthenic syndrome, nephritis, myopathies and myocarditis (Naidoo *et al*, 2015).

Steroids are currently the mainstay of treatment for the serious toxicities from immunotherapy. In steroid-refractory cases, other immunosuppressants such as infliximab, mycophenolate and intravenous immunoglobulin have been trialled with variable success (Postow, 2015).

Recent case reports of immune-mediated myocarditis reveal the extremely high mortality of this particular toxicity (Johnson *et al*, 2016). Death due to refractory arrhythmias is the outcome in most reported cases (Heinzerling *et al*, 2016; Johnson *et al*, 2016). Here, we describe the only case of immune-mediated myocarditis treated with intravenous anti-thymocyte globulin (ATGAM) resulting in a favourable outcome.

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CASE REPORT

A 64-year-old woman with no prior history of autoimmune or cardiac disease was diagnosed with right temporo-parietal glioblastoma IDH-WT. She was consented and enroled onto the CheckMate CA209–548 clinical trial (A Randomised Phase 2 Single Blind Study of Temozolomide plus Radiation Therapy combined with Nivolumab or Placebo in Newly Diagnosed Adult Subjects with MGMT-Methylated (tumour O6-methylguanine DNA methyltransferase) Glioblastoma). Eight days after her second trial drug infusion, she presented with diplopia, diffuse myalgias and proximal weakness. No chest pain, dypsnoea, palpitations or fever was reported.

On admission, creatine kinase was 3538 U per litre (normal range 60–285 U per litre) and troponin I was 8375 ng l^{-1} (normal range <26). Electrocardiograph (ECG) revealed ventricular bigeminy with frequent ventricular ectopics. Echocardiogram demonstrated normal left ventricular size with moderate systolic dysfunction and a reduced left ventricular ejection fraction of 37%. Electromyography excluded neuromuscular junction pathology. Viral serology for herpes simplex virus type 1 and 2, varicella zoster virus, cytomegalovirus, enterovirus and adenovirus were all negative.

A clinical diagnosis of autoimmune myositis and myocarditis was made. She commenced intravenous methylprednisolone 500 mg daily for 3 days before tapering to oral prednisolone 100 mg daily. A single dose of intravenous infliximab 5 mg kg^{-1} was administered on Day 2 of admission given its use in previous case reports. Over the subsequent days, persistent ventricular bigeminy and episodic non-sustained ventricular tachycardias was treated with amiodarone and low-dose beta blocker therapy. Following this treatment, she remained haemodynamically stable with cardiac monitoring, confirming intermittent ventricular bigeminy with no further malignant arrhythmias.

On day 8 of admission, her clinical condition acutely deteriorated with sustained conscious ventricular tachycardia and haemodynamic compromise. She required direct current cardioversion that resulted in complete heart block with a junctional escape rhythm. Subequently, an emergency cardiac biopsy, coronary angiogram and placement of a temporary pacing wire was undertaken. Cardiac biopsy (taken following administration of corticosteroids and infliximab) revealed multifocal lymphocytic (CD3+, CD8+ predominant with mixed CD3+ CD4+ subtypes) and histiocytic infiltrates associated with focal areas of myocyte necrosis (Figures 1 and 2; panel A) consistent with an immune-mediated myocarditis. All immunohistochemistry was performed using DAKO-Omnis apparatus with ready-to-use DAKO antibodies to CD3, CD4, CD8, CD20 and CD68. Coronary angiogram was unremarkable.

After consultation with the heart failure/cardiac transplantation unit, the decision was made to commence equine ATGAM therapy with a view to depletion of T-lymphocytic infiltrate as used in cellular rejection of cardiac allografts.

ATGAM administration was per local protocol for acute cellular rejection consisting of ATGAM 500 mg on day 1, titrating the dose by 250 mg increments to daily CD2/3 levels (aiming for levels of 50–100/μl) for a total of 5 days. During this time, the patient remained in a ventricular paced rhythm. Repeat echocardiogram showed a mildly improved left ventricular ejection fraction of 43%.

Clinical and biochemical improvement occurred within three days of ATGAM therapy with resolution of ventricular arrhythmias and near normalisation of cardiac enzymes occurring by day 5 of ATGAM initiation. ECG confirmed return to sinus rhythm with an incomplete right bundle branch block and the temporary pacing wire was removed. Mycophenolate 1 gm twice daily was commenced on day 5 of ATGAM therapy. Prednisolone 100 mg daily was continued throughout ATGAM dosing.

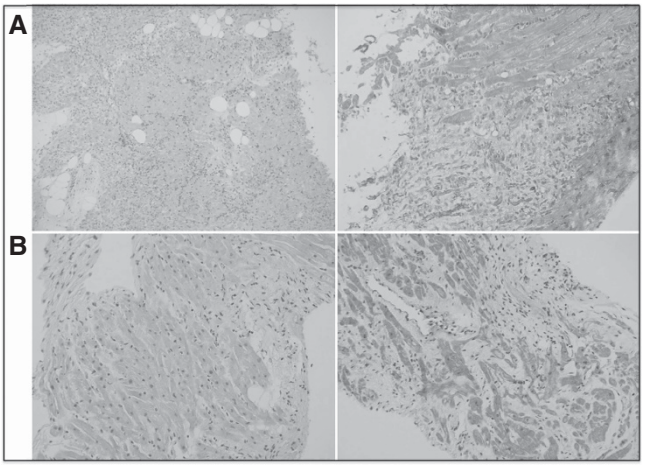

Figure 1. Comparison of endomyocardial biopsy histology. Comparison haemoatoxylin and eosin (left) and Masson-trichrome stain (right) of original endomyocardial biopsy (**A**) and 6-week biopsy (**B**) showing a marked reduction in lymphocytes and patchy early fibrosis by 6 weeks. At $\times 200$ magnification.

A second biopsy performed 10 days later demonstrates pathologic improvement with patchy lymphohistiocytic myocarditis with significantly less-myocyte necrosis. A third biopsy at 6 weeks after presentation revealed patchy foci of fibrosis, scant inflammatory cells and intervening viable myocytes in keeping with evidence of early repair (Figures 1 and 2; panel B).

Following removal of temporary pacing wires, the patient was able to complete a full course of radiotherapy for her glioblastoma. She was taken off study, unblinded and nivolumab permanently discontinued. Temozolamide concurrent with radiotherapy was also discontinued given the potential for prolonged cytopenias in the setting of ATGAM therapy.

Mycophenolate 1 gm twice daily was continued for 4 weeks then slowly weaned and ceased over a 12 week period. Prednisolone 100 mg was weaned by 5 mg every week. The diplopia and proximal muscle weakness that characterised her myopathy at presentation markedly improved with the institution of intravenous steroids and infliximab.

It is now 6 months since her presentation. Performance status has improved and she is now independent with all activities of daily living. There is no evidence of disease progression on her most recent cerebral magnetic resonance imaging. She is currently in her second cycle of standard temozolomide on a monthly schedule.

DISCUSSION

Immune-mediated cardiotoxicity is an uncommon adverse effect of immune checkpoint inhibition resulting in a spectrum of events that affect the myocardium including myocarditis, cardiomyopathy and myocardial fibrosis. (Heinzerling *et al*, 2016).

Interrogation of safety databases report the incidence of immune-mediated myocarditis to be 0.06% ($n=10/17620$) for single-agent nivolumab and 0.27% ($n=8/2974$) for combination nivolumab/ipilimumab. Combination checkpoint blockade is associated with both a higher incidence and severity of immune-mediated myocarditis (Johnson *et al*, 2016). The true incidence of immune-mediated myocarditis may be grossly underestimated with serial cardiac monitoring with ECGs or troponin levels not routinely undertaken in clinical practice.

Immunotherapy-induced myocarditis has a highly variable clinical presentation. Symptoms range from subclinical disease to

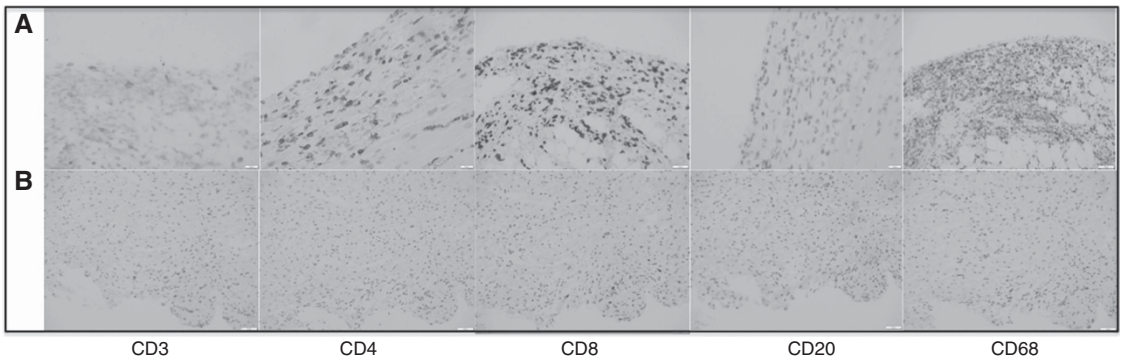

Figure 2. Comparison of endomyocardial biopsy immunoperoxidase stains. Comparison immunoperoxidase stains for CD3, CD4, CD8, CD20 and CD68 of original endomyocardial biopsy (**A**) and 6-week biopsy (**B**) showing a marked reduction over 6 weeks in the initial T-cell-rich CD8 > CD4 and histiocytic infiltrate (CD68), with no B cells (CD20). At × 200 magnification.

chest pain, cardiac failure, cardiogenic shock, arrhythmias and sudden death (Läubli *et al*, 2015; Heinzerling *et al*, 2016; Johnson *et al*, 2016). The onset of symptoms tend to occur within the first few weeks of initial anti PD-1 and/or anti-CTLA4 therapy with the median time to onset of symptoms being 17 days from the first infusion (Johnson *et al*, 2016). Although an uncommon event, the importance of identifying immune-mediated myocarditis is underscored by its potentially fatal sequelae.

Histopathological examination of the endomyocardium typically demonstrates a patchy CD3-positive lymphocytic infiltrate, involving the myocardium and conduction system. Drug-induced immune-mediated myocarditis can share some morphologic similarity but usually contains eosinophils with other causes of a multifocal lymphocytic myocarditis, including severe cellular allograft rejection post cardiac transplantation, severe viral myocarditis and connective tissue disease associated lymphocytic myocarditis.

Corticosteroids are used to manage immune-related adverse events through suppression of lymphocyte activity and inhibition of cytokine synthesis. High-dose corticosteroids have been initiated in the majority of reported cases of immune-mediated myocarditis. Induction doses range from 1–2 mg kg^{-1} methylprenisolone, followed by a taper to oral corticosteroids (Heinzerling *et al*, 2016; Johnson *et al*, 2016). Benefit from corticosteroid therapy should occur within days and can be monitored with serial troponin and creatinine kinase levels.

High-dose corticosteroid therapy alone may not be sufficient to resolve immune-related cardiac toxicity with multiple published reports, (Heinzerling *et al*, 2016; Johnson *et al*, 2016) describing immune-mediated myocarditis treated with corticosteroids alone resulting in death owing to the development of malignant arrhythmias or cardiac failure.

Infliximab is a chimeric IgG 1 monoclonal antibody that blocks tumour necrosis factor-alpha, a proinflammatory cytokine implicated in the pathogenesis of immune-mediated colitis. Infliximab is indicated for use in steroid refractory immune-related colitis secondary to checkpoint inhibition (Pagès *et al*, 2013). Early introduction of infliximab for severe cases of immune-related colitis is also supported by the literature (Merrill *et al*, 2014). No published cases of immune-mediated myocarditis have been successfully treated with infliximab, suggesting tumour necrosis factor-alpha is not a primary driver of T-cell hyperactivation within the myocardium.

ATGAM is a polyclonal antibody derived by immunisation of horses with lymphoid cells derived from the thymus or cultured B-cell lines. Labelled use includes treatment of allograft rejection and aplastic anaemia (Lexicomp, Inc, 2017). ATGAM reverses acute allograft rejection by inducing T lymphocyte depletion via complement-dependent cell lysis. ATGAM has been successfully used to treat at least two cases of severe ipilimumab-induced hepatotoxicity (Chmiel *et al*, 2011; Ahmed *et al*, 2015). No other data are published regarding the use of ATGAM for immune-related adverse events, including cases of immune-mediated cardiotoxicity.

ATGAM is administered via a central line with dosing titrated to CD2 and CD3 levels. We advise that ATGAM is administered under the guidance of a cardiac transplant unit experienced in treating acute allograft rejection.

In this case, we attribute the successful termination of malignant arrhythmias and improvement in cardiac function to the administration of ATGAM given the temporal relationship between the time of infusion and clinical improvement. We hypothesise that ATGAM resulted in the rapid reduction of T-cell hyperactivation as demonstrated by reduction of lymphocytic infiltration seen on serial cardiac biopsies and the near normalisation of troponin levels.

CONCLUSION

Immune-mediated myocarditis is a rare, often fatal immune-related adverse effect of checkpoint inhibitor therapy. This case demonstrates that the use of ATGAM should be considered in cases of steroid-refractory immune-mediated myocarditis. ATGAM should be administered in close consultation with a cardiac transplant team experienced in the use of this agent.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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