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Cyclophosphamide for Refractory Acute Cellular Rejection After Lung Transplantation

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Background. Acute cellular rejection (ACR) is a major risk factor for chronic lung allograft dysfunction after lung transplantation. Acute cellular rejection can persist or recur despite augmentation of immunosuppression by conventional methods. There are limited therapeutic options in treating these recurrent and refractory ACRs. We describe our experience with cyclophosphamide therapy for recurrent and refractory ACR in lung transplant recipients. **Methods.** Six consecutive patients who were treated with cyclophosphamide for recurrent or refractory ACR were included in the series. The primary outcome measures were improvement in ACR score and forced expiratory volume at 1 second. Secondary outcome measures included adverse drug events including bone marrow suppression, gastrointestinal side effects, and infections. **Results.** Five of the 6 patients treated demonstrated complete resolution of ACR on follow-up biopsies. Acute cellular rejection score improved after cyclophosphamide treatment ($P = 0.03$). None of the patients had high grade (≥A3) ACR in the 3 months after cyclophosphamide administration. Cyclophosphamide had no effect on forced expiratory volume at 1 second trend or bronchiolitis obliterans score. All patients tolerated cyclophosphamide with minor gastrointestinal side effects, mild bone marrow suppression, and nonfatal infections that were amenable to treatment. **Conclusions.** Cyclophosphamide therapy is an option in treating recurrent and refractory ACR in patients who have failed conventional treatments. Cyclophosphamide is tolerated well without serious adverse drug events (ADE).

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Lung transplantation represents the definitive life-saving treatment option that also confers quality of life benefits for patients affected with end-stage lung diseases.[1-3] Short-term outcomes after lung transplantation are comparable with other transplanted organs, with 89% and 80% of lung transplant recipients (LTRs) surviving 3 months and 1 year posttransplantation, respectively. However, long-term survival remains poor, with 65% of LTRs surviving 3, 54% surviving 5, and 32% of LTRs surviving 10 years posttransplantation.[4] Although the most common cause of death within the first 30 days after lung transplantation is attributed to non–CMV-related infections and primary graft dysfunction, the highest percentage of death at 3 and 5 years after lung transplantation is due to bronchiolitis obliterans syndrome (BOS) or obliterative bronchiolitis.[4] Bronchiolitis obliterans represents an obstructive phenotype of chronic lung allograft dysfunction (CLAD) characterized by persistent forced expiratory volume at 1 second (FEV_1) decline of 20% or greater below peak values in the absence of other clinical confounders such as infection.[5,6] Risk factors for the development of BOS in LTRs include refractory or high-grade acute cellular rejection (ACR), *Pseudomonas* infection, CMV disease, antibody-mediated rejection, gastroesophageal reflux disease, and medication nonadherence.[7-12]

The short-term outcomes after lung transplantation described above have improved over the past 3 decades.[13] The calcineurin inhibitors (CNIs), tacrolimus (TAC), and cyclosporine (CSA), serve as maintenance immunosuppression to which

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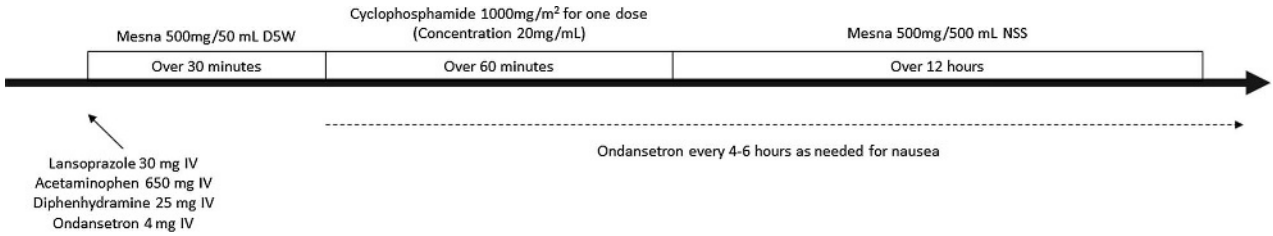

FIGURE 1. CP protocol for the treatment of ACR.

other agents such as mycophenolate mofetil (MMF) are added.[14] Calcineurin inhibitor–based immunosuppression results in over 50% of LTRs experiencing ACR within the 6 posttransplant months.[15,16] Some of these patients may also be refractory to standard corticosteroid pulse therapy and require alternative forms of treatment involving lymphodepletion.[17,18]

Cyclophosphamide (CP) is an alkylating agent that halts cell division by cross-linking DNA strands. It is a nonspecific cell cycle inhibitor and is a prodrug that must be metabolized into active metabolites via the liver.[19] Cyclophosphamide has some selectivity toward T and B lymphocytes in proliferating and differentiating stages of the cell cycle, but remains relatively nonspecific and affects most cell lines. Cyclophosphamide therapy in lung transplantation has been described in the context of rescue therapy for chronic rejection.[20,21] To our knowledge, this is the first case series describing CP use in LTRs experiencing recurrent or refractory rejection.

PATIENTS AND METHODS

This is an institutional review board approved, single-center retrospective chart review of 6 consecutive patients who were treated with pulse CP between May 2013 and November 2016 for recurrent or refractory ACR. Recurrent ACR was defined as at least 2 treated episodes of ≥ grade A2 acute rejection with return to A0 or A1 between episodes. Refractory rejection was defined as 3 consecutive treated episodes of ≥ A2 acute rejection without return to A0 or A1 between episodes. All patients were maintained on triple drug immunosuppression with a CNI, MMF/sodium or azathioprine and prednisone. Patients underwent surveillance bronchoscopies on posttransplant day (PTD) 14 followed by every 2 to 3 months for the first 2 years posttransplant. Additional bronchoscopies were also performed if there was a suspicion for ACR based on clinical, radiological, or spirometric findings. Beyond 2 years, protocol transbronchial biopsies were performed for suspected causes. All transbronchial biopsies were graded according to the 2006 criteria of International Society of Heart and Lung Transplantation.[22] ACR score was calculated by summing the A-grade in all the biopsies in the 3 months before and after CP and dividing this number by total number of biopsies as described previously.[17]

Cyclophosphamide was administered at 1000 mg/m^2 of actual body surface area as a single IV infusion over 60 minutes along with pre/postmesna, intravenous fluids and premedication as shown in Figure 1. Maintenance immunosuppression regimen was altered at the discretion of the attending pulmonologist as part of routine clinical care. After CP, all patients received infection prophylaxis with valganciclovir, triazole antifungals, and were continued on sulfamethoxazole-trimethoprim prophylaxis.

Primary outcome measures were improvement in ACR score and FEV_1. Secondary outcomes included adverse reactions to CP including bone marrow suppression (serial measurements of WBC counts, hemoglobin, and platelet counts), gastrointestinal side effects, infections, and other known ADEs. All positive bacterial, viral, and fungal cultures up to 180 days posttreatment were recorded.

RESULTS

Six consecutive patients treated with CP for recurrent or refractory ACR were included in this series. The baseline demographics are shown in Table 1. All patients had failed multiple pulse doses of methylprednisolone and lymphocyte depletion treatments before CP (Table 2). Cyclophosphamide was administered on PTD 79 to 1332 (mean = 782). Five of the 6 patients were biopsied post-CP and all of them showed complete resolution of ACR in follow-up biopsies. Median ACR score in the 3 months before CP was 2.15 (1.6-3.3) and improved to 1 (0.3-1) in the 3 months after CP. There were 2.5 biopsies per patient in the 3 months pre-CP and 1.83 biopsies per patient in the 3 months post-CP treatment. Among the 5 patients who had follow-up biopsies after CP, none had high grade ACR (A3 or A4) in the 3 months after CP treatment. FEV_1 declined post-CP in 2 patients, remained stable in 1 and improved in 3 patients. However, the rate of decline in mean FEV_1 appeared to slow post-CP (Figure 2) defined by change in slope. The BOS score 3 months before and after CP remained stable among all patients (data not shown) except patient 2 who had sustained deterioration in FEV_1 and progression to BOS stage 3. Three of 6 patients developed donor-specific antibodies de novo.

Patient 1

A 36-year-old male underwent bilateral sequential lung transplant (BSLT) for mixed connective tissue disease/polymyositis related interstitial lung disease (ILD). Baseline immunosuppression included alemtuzumab induction, TAC, MMF, and prednisone. Routine posttransplant surveillance biopsies revealed moderate ACR on PTD 16 (A3B1R) and severe ACR on PTD 42 (A4B1R) which were treated with pulse dose

TABLE 1.

Demographic data

Recipient no.	Age, y	Sex	Transplant indication	BOS grade
1	36	Male	MCTD	0
2	51	Female	COPD	2
3	31	Male	Idiopathic pulmonary fibrosis	0p
4	62	Female	Sarcoidosis	0
5	34	Male	Systemic sclerosis	1
6	22	Male	Cystic fibrosis	0

MCTD, mixed connective tissue disease; COPD, chronic obstructive pulmonary disease.

TABLE 2.

Acute cellular rejections

Recipient no.	PTD	ACR grade	Immunosuppression regimen[a]	Treatment
1	16	A3	TAC, MMF	Methylprednisolone
	42	A4	TAC, MMF	Methylprednisolone, RATG
	79	A3	TAC, MMF	CP
	114	A0	TAC, MMF, EVR	
2	837	A3	TAC, AZA	Methylprednisolone
	872	A2	TAC, AZA, EVR	RATG
	916	A2	TAC, EVR	CP
	979	A2	TAC, EVR	CP
3	1268	A4	None	Methylprednisolone, RATG
	1322	A4	TAC, MMF	CP
	1366	A2	TAC, MMF, EVR	CP
	1407	A0	TAC, MMF, EVR	
4	442	A2	TAC, MPA	RATG
	504	A3	TAC, MPA	Alemtuzumab
	545	A2	TAC, MPA, EVR	Methylprednisolone
	644	A2	TAC, MPA, EVR	CP
	656	A3	TAC, MPA, EVR	
	714	A0	TAC, MPA, EVR	
5	120	A3	CSA, MMF	Methylprednisolone
	148	A3	CSA, MMF	Methylprednisolone
	288	A3	CSA, MMF	RATG
	337	A3	CSA, MMF	Alemtuzumab, Methylprednisolone,
	449	A1	CSA, MMF	Rituximab
	603	A1	CSA, MMF	CP ×2 (14 d apart)
	650	A0	CSA, MMF	
6	758	A2	CSA	Methylprednisolone
	785	A2	CSA	Methylprednisolone
	863	A3	CSA, MMF	Methylprednisolone
	954	A2	CSA, MMF	RATG, Methylprednisolone
	1129	A2	TAC, MMF, EVR	CP
	1171	A0	TAC, MMF, EVR	

[a] All patients were on prednisone.

MPA, mycophenolate sodium.

methylprednisolone. After the second biopsy, the patient was treated with a 7-day course of rabbit antithymocyte globulin (RATG) and the dose of MMF was increased to 750 mg twice daily. Follow-up biopsy on PTD 79 revealed refractory, moderate ACR (A3B1R). He received another course of pulse methylprednisolone along with a pulse dose of CP and

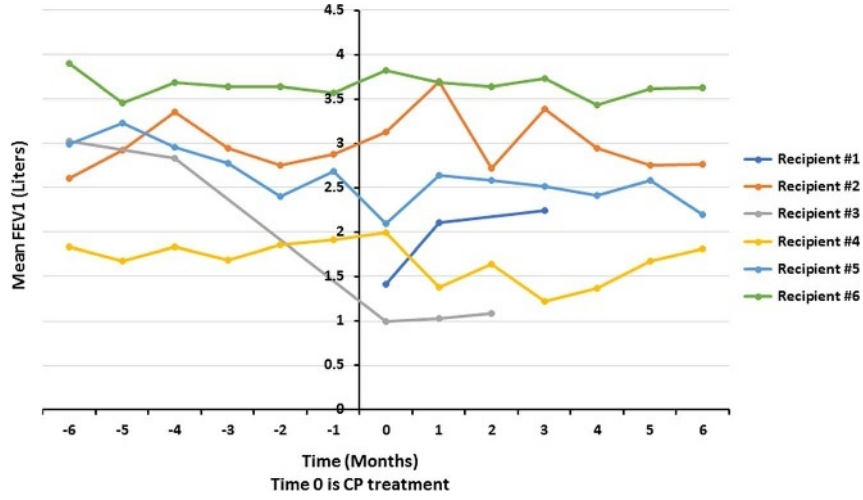

FIGURE 2. FEV_1 trend pre- and post-CP.

everolimus (EVR) was initiated. Follow-up biopsy on PTD 114 showed no definite evidence of ACR (A0B0R). His FEV_1 remained stable with no evidence of CLAD.

Patient 2

A 51-year-old woman underwent BSLT for chronic obstructive pulmonary disease. Baseline immunosuppression included basiliximab induction, TAC, MMF, and prednisone. She was diagnosed with ACR on surveillance biopsies on PTD 11, 212, and 239 that were successfully treated with pulse dose methylprednisolone. Over time, her TAC target goal was reduced and MMF was discontinued due to gastrointestinal side-effects and replaced with azathioprine. On PTD 837, she underwent biopsy for an unexplained decline in FEV_1. The biopsy revealed moderate ACR (A3B1R) which was treated with 3-day course of pulsed dose methylprednisolone and EVR was initiated. Her FEV_1 continued to decline and she was diagnosed with new onset BOS (FEV_1 63% of posttransplant baseline, FEV_1/FVC 65). Follow-up biopsy on PTD 872 revealed persistent mild ACR (A2B0R) which was treated with a 5-day course of RATG. Her FEV_1 continued to decline and she was admitted with progressive dyspnea and underwent repeat biopsy on posttransplant day 916 that revealed refractory, moderate ACR (A2B1C1) for which she was treated with pulse dose IV CP. She improved clinically but postdischarge she had sustained deterioration in FEV_1 and progression to BOS stage 3 and further lung biopsies were not pursued.

Patient 3

A 31-year-old man underwent BSLT for ILD with UIP pattern. Baseline immunosuppression regimen included alemtuzumab induction, TAC MMF, and prednisone. Surveillance bronchoscopies revealed no ACR allograft function remained stable. On PTD 1268, he presented with acute hypoxemic respiratory failure and reported nonadherence to antirejection medications for several days. Chest CT revealed diffuse, bilateral infiltrates raising concern for ACR. Transbronchial biopsy revealed severe ACR (A4B1R) that was treated with 2 courses of pulse methylprednisolone along with increased TAC trough goal to 12 to 15 ng/mL and increased dose of MMF to 1000 mg twice daily. His clinical condition continued to deteriorate requiring mechanical ventilatory and tracheostomy. He was treated with 5-day course of RATG but follow-up biopsy on PTD 1322 revealed persistent severe ACR (A4B1R) for which he was treated with a single pulse dose of CP. After CP, he improved gradually and was weaned off the ventilator to aerosolized tracheostomy mask. Follow-up biopsy on PTD 1366 revealed mild ACR (A2B1R) that was treated with another pulse dose of CP. Over the next month, he continued to improve and his tracheostomy was successfully decannulated and he was discharged home on 2 L of oxygen at rest and 4 L with exertion. Follow-up biopsy on PTD 1407 revealed complete resolution of ACR. He continues to 2 L of oxygen at rest and 4 L with exertion due to advanced CLAD.

Patient 4

A 62-year-old woman underwent lobar BSLT for sarcoidosis. Baseline immunosuppression included alemtuzumab induction, TAC, MMF, and prednisone. Posttransplant course was complicated with prolonged respiratory failure and a need for tracheostomy. Routine surveillance biopsies revealed moderate ACR (A3B1R) on PTD 48 that resolved with a course of pulse dose methylprednisolone. She was eventually decannulated and discharged home on 2 L of oxygen. Follow-up biopsies on PTD 140, 294, 322, 350, 413, and 442 revealed recurrent mild to moderate ACRs despite multiple courses of methylprednisolone and increased TAC trough goal to 12 to 15 ng/mL. After her latest ACR, she was treated with a 7-day course of RATG. Follow-up biopsies on PTD 504 revealed persistent moderate ACR (A3B1R) for which she was treated with 1 dose of alemtuzumab and was started on EVR. Despite alemtuzumab, follow-up biopsies revealed persistent mild ACR (A2BxR) on PTD 545 and minimal ACR (A1B0R) on PTDs 581 and 644. At this time, she was pulse dose CP for persistent ACR and follow-up biopsy a short time thereafter revealed moderate ACR (A3Bx) on PTD 666. She was treated with a course of pulse dose methylprednisolone and subsequent biopsies on PTDs 714 and 777 revealed no ACR. Her FEV_1 remained stable with no evidence of CLAD.

Patient 5

A 34-year-old man underwent BSLT for systemic sclerosis related ILD and pulmonary hypertension. His baseline immunosuppression regimen included alemtuzumab induction, CSA, MMF, and prednisone. Surveillance biopsies revealed moderate ACR (A3B2R) on PTDs 120 and 148 treated with pulse dose methylprednisolone with resolution. The dose of MMF was increased to 1250 mg twice daily. Moderate ACR recurred on biopsies on PTD 288 and he was treated with 5-day course of RATG. Follow-up biopsy on PTD 337 revealed persistent moderate ACR (A3B1) for which he was treated with a dose of alemtuzumab and pulse methylprednisolone. Follow-up biopsies revealed no evidence of ACR but inactive bronchiolitis was observed despite preserved FEV_1. On PTD 603, lung biopsies revealed minimal ACR (A1B1R) with inactive bronchiolitis obliterans. Patient now had a decline in FEV_1 consistent with BOS stage 1 (FEV_1 68% of posttransplant baseline) and 2 pulse doses of CP was administered. After the dose of CP, multiple biopsies over the next 6 months showed no evidence of ACR. FEV_1 improved to 83% of posttransplant baseline after CP.

Patient 6

A 22-year-old man underwent BSLT for cystic fibrosis. His baseline immunosuppression regimen included alemtuzumab induction, CSA, MMF, and prednisone. His surveillance lung biopsies on PTD 15 revealed mild ACR (A2B1R) that resolved with a course of pulse dose methylprednisolone. He had recurrent ACR on PTDs 758, 785, 954 that were treated with pulse dose methylprednisolone and a 5-day course of RATG. Subsequently, he remained rejection free on lung biopsies until PTD 1094 when his biopsies revealed mild ACR (A2B1R) for which he was treated with another course of pulse dose methylprednisolone but repeat biopsy on PTD 1129 showed persistent mild ACR (A2B0). He remained asymptomatic with stable spirometry tests but given the persistent ACR, he was treated with a pulse dose of CP along with another course of pulse dose methylprednisolone. A follow-up biopsy on PTD 1171 showed no evidence of ACR. His FEV_1 has remained stable with no evidence of CLAD.

Adverse Drug Events

Most people tolerated CP infusion well. One patient experienced cutaneous flushing during infusion which resolved with intravenous diphenhydramine. All patients

developed leukopenia which nadired between 7 and 14 days (Figure 3). Two patients developed neutropenia requiring 1 to 2 doses of subcutaneous filgrastim. All patients experienced mild drop in their hemoglobin which nadired between 3 and 14 days and none required red cell transfusion. Three patients had worsening thrombocytopenia which nadired between 3 and 7 days but none had bleeding complications nor did they require platelet transfusion.

Three patients developed gastrointestinal side effects including nausea, vomiting and diarrhea within 24 hours that resolved spontaneously without interruption of nutrition. None positive cultures were noted in 4 patients ranging from 9 to 59 days after CP. They included BAL cultures positive for *serratia marsescans, pseudomonas aeruginosa, klebsiella pneumoniae, aspergillus fumigatus* and *parainfluenza*. Urine culture positive for *escherichia coli* and stool cultures positive for *rotavirus* and *norovirus*. None of the infections were life-threatening and were easily treated with antibiotics with complete recovery. There were no instances of hemorrhagic cystitis or cardiotoxicity reported. No critical illness or mortality was attributed to CP therapy. All patients are alive at the time of this report with post-CP survival ranging from 170 to 1437 days (mean = 633).

DISCUSSION

Conventional treatment of ACR involves immunosuppression augmentation with the use of pulse dose steroids. However, in a small percentage of patients, ACR remains steroid resistant. In these patients, treatment options are limited and include antithymocyte globulin, alemtuzumab, methotrexate (MTX), and photopheresis. Antithymocyte globulins recognize most of the molecules involved in the T-cell activation cascade, such as CD2, CD3, CD4, CD8, CD11a, CD18, CD25, HLA DR, and HLA class I.[23] Rabbit antithymocyte globulin promotes expansion of regulatory T (Treg) cells responsible for preventing immune system activation and therefore eliminating self-reactivity.[24] Rabbit antithymocyte globulin induction in addition to conventional immunosuppression has been shown to decrease the frequency of biopsy-proven ACR in lung transplant recipients.[25] Although RATG is routinely used in the treatment of refractory ACR, all patients in this series failed to respond to RATG necessitating further immune augmentation with CP. Alemtuzumab is a humanized anti-CD52 monoclonal antibody targeting mature lymphocytes and causes prolonged suppression of both CD4+ and CD8+ for over a year. Alemtuzumab has been shown to improve the mean ACR score in patients with refractory ACR.[17,18] In a study by Ensor et al ACR resolved in follow-up biopsies of all the patients treated with alemtuzumab and fewer than 40% of the patients had ACR on biopsies during the months after alemtuzumab treatment.[18] However, in the 2 patients who received alemtuzumab, ACR recurred or persisted but resolved after CP treatment. Extracorporeal photopheresis (ECP) is an immunomodulatory treatment in which a patient's leukocytes are exposed to ultraviolet-A light after pretreatment with 8-methoxypsoralen. The exact mechanisms remain unknown, but ECP results in leukocyte apoptosis, changes in cytokines profile, and upregulation of Treg cells. The use of ECP has been described in LTRs with advanced BOS in whom it has been shown to stabilize the decline in FEV_1 and resolution of ACR.[26,27] Andreu and colleagues[28] describe successful treatment of ACR in patient with concurrent infection.[29] However, ECP is resource intensive requiring multiple treatments over a prolonged period of time with some patients requiring an indwelling catheter throughout the course of treatment.[27] Methotrexate has also been used to treat refractory ACR. It is a potent anti-inflammatory medication and has antiproliferative and proapoptotic effects on activated T lymphocytes.[30] In a report by Cahill et al,[29] Steroid-resistant ACR resolved completely in all the patients treated with at least 4 weeks of MTX administered weekly. Ten of the 12 patients had no ACR in the 12 months after MTX treatment. However, these patients did not receive any prior attempts at immune augmentation.

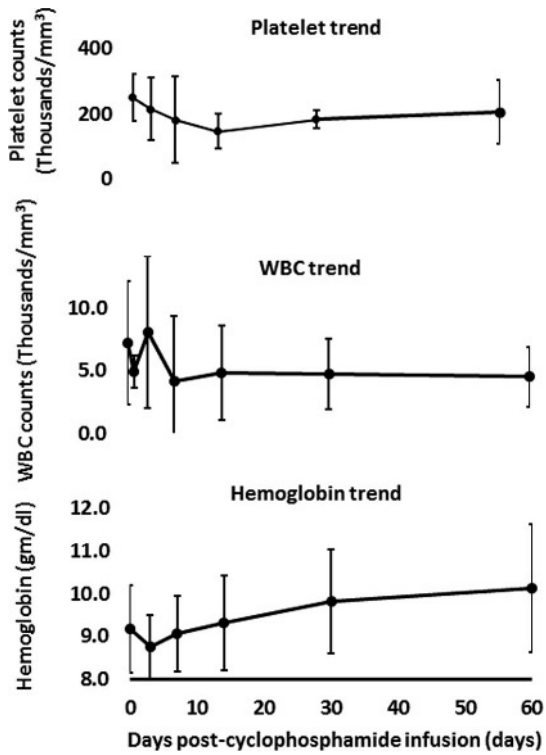

FIGURE 3. CBC trend post-CP infusion.

Cyclophosphamide is a potent cytotoxic and lymphoablative agent.[31] It is an alkylating agent that halts cell division by cross-linking DNA strands and thus, is a nonspecific cell cycle inhibitor. Animal studies have revealed that newly generated alloreactive T cells are particularly sensitive to high-dose CP.[32] In humans, immune reconstitution after CP is well studied in bone marrow transplant recipients. In the first 2 months after CP, there are reductions in naive alloreactive CD4+ T cells, persistence of activated Treg cells and phenotypically stem cell–like memory CD4+ T cells.[33] Posttransplantation CP effectively prevents graft-versus-host disease in these patients.[34,35] The acute reduction in circulating naïve alloreactive T-cells likely explains the resolution of ACR in our patients with recurrent and refractory ACR. Our results complement the previously reported effects of CP on stabilization of FEV_1 in chronic rejection. In a case series of 7 LTRs with BOS, FEV_1 decline was shown to be effectively stabilized after CP treatment.[20] CLAD leads to scarring and fibrosis in the alveolar and is regarded as a fibroproliferative process. Inflammatory processes that lead to CLAD and ACR are mediated by cytokines such as transforming growth

factor (TGF)-β1. CPA has been shown to inhibit inflammatory cytokines like TGF-β1 in idiopathic pulmonary fibrosis and may explain the mechanistic pathway for its efficacy in ACR and CLAD.[21,36,37] In our patients, the rate of decline in FEV_1 pre-CP slowed down post-CP but this change was not statistically significant. This could reflect the small sample size and limited follow-up in our study population.

Our study has important limitations. First, it is a small study involving 6 patients. The retrospective nature of the study cannot ensure freedom from potential biases and also, there was no control population for comparison to account for confounding factors that may influence the outcomes.

CONCLUSIONS

Cyclophosphamide offers a potential treatment option in patients with recurrent or refractory ACR who have previously failed conventional treatments. Cyclophosphamide is associated with improvement in ACR grade and is tolerated without serious ADEs. Future studies are warranted to examine the impact of CP on other forms of CLAD and also antibody-mediated rejection.

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