

Efficacy of Combination Therapy With Pirfenidone and Low-Dose Cyclophosphamide for Refractory Interstitial Lung Disease Associated With Connective Tissue Disease: A Case-Series of Seven Patients

Lichong SHEN , Qingran YAN , Xiaoxiang CHEN 

Department of Rheumatology, Renji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai China

ABSTRACT

Objectives: This study reports a low dose combination therapy of cyclophosphamide (CYC) and pirfenidone (PFD) and the efficiency and safety of the therapy in refractory connective tissue disease associated interstitial lung disease (CTD-ILD) patients.

Patients and methods: The study included seven CTD-ILD patients (2 males, 5 females; mean age 48.8 years; range, 32 to 63 years) treated between January 2016 and December 2017 in our clinic. At enrolment, all patients had shown no improvement in their symptoms (dyspnea or cough) after at least one month of high dose steroids treatment. Patients who had received adjusted immunosuppressive agents other than steroids or anti-fibrotic medications within the three months before enrolment were excluded. We changed the treatment to a low dose combination of CYC 0.4 g/m² monthly and PFD 300 mg twice per day and quickly reduced the steroids. All the patients were followed-up for 12 months.

Results: Two patients had anti-synthetase syndrome, two had Sjögren syndrome, two had scleroderma and one had mixed connective tissue disease. The baseline forced vital capacity (FVC) was 39-81% and the six-minute walk distance (6MWD) was 202 m-324 m. Within 12 months follow-up, the median improvement in the FVC was 13.4% (range, 0-35.9%), the median improvement of carbon monoxide diffusing capacity was 6.3% (range, 1.7-16%) and the median improvement of 6MWD was 52.7% (range, 34.4-86.3%). All the patients were self-sufficient, and their dyspnea, chest high-resolution computed tomography scores, and quality of life improved simultaneously. Exceeding our expectations, no adverse events associated with CYC or PFD were observed during the follow-up period.

Conclusion: Our study provided preliminary while promising clinical evidence for combination therapy of CYC-PFD for CTD-ILD. A low dose combination of CYC and PFD was unexpectedly well tolerated, with satisfactory effects in refractory CTD-ILD patients. Well-designed controlled studies are needed to further establish the safety and efficacy of this approach.

Keywords: Cyclophosphamide, interstitial lung disease, pirfenidone.

Interstitial lung disease (ILD) is one of the most serious yet common conditions for patients with connective tissue diseases (CTDs), such as systemic sclerosis (SSc),¹ dermatomyositis (DM)² and Sjögren syndrome (SS).³ CTD-ILD begins as an autoreactive inflammation and can progress to fibrosis. Either of these morbidities can cause lung dysfunction, greatly shorten survival and bring heavy disease burden to the patients.

To treat inflammation in CTD-ILD patients, one immunosuppressant plus steroids is a common regimen. In recent clinical trials on CTD-ILD, cyclophosphamide (CYC) is one of the most evidence-supported immunosuppressants, particularly for SSc-ILD. CYC improved the pulmonary function of 49.2 to 61.5% of the subjects during the first one year of follow-up, and improved dyspnea, high-resolution computed

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Correspondence: Xiaoxiang Chen, MD. Renji Hospital, Shanghai Jiaotong University, School of Medicine, Rheumatology, 200025 Shanghai, China.
Tel: 021-53882254 e-mail: chenxiaoxiang@renji.com

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tomography (HRCT) appearance and the health-related quality of life.^{4,5}

To stop fibrosis, several small-molecule anti-fibrotic agents have been approved in recent decade. Pirfenidone (PFD) was the first of these agents. It is initially approved for idiopathic pulmonary fibrosis (IPF), the most treatment-resistant type of pulmonary fibrosis. In clinical trials on IPF, PFD improved patient forced vital capacity (FVC) and reduced the decline of the six-minute walk distance (6MWD).^{6,7} The application of PFD to CTD-ILD patients, including patients with SSc and DM, also showed acceptable efficacy,^{8,9} despite most trials still being in progress.

To improve efficacy, a combination of an immunosuppressant and anti-fibrotic agent could be a reasonable approach. CYC is a traditional cytotoxic agent that kills activated lymphocytes to stop inflammation. On the other hand, PFD is an anti-fibrotic agent that inhibits collagen synthesis and fibroblast proliferation via pro-fibrotic growth factors.^{10,11} Based on their working mechanisms, PFD and CYC might work in synergy. In fact, PFD plus rapamycin have shown benefits in an alveolar cell line with pro-fibrotic stimulation.¹²

To our knowledge, thus far, the combination of PFD and CYC has never been reported, while other similar combinations have been assessed in two clinical trials of SSc-ILD: PFD with mycophenolate in Scleroderma Lung Study (SLS) III study (ClinicalTrials.gov: NCT03221257) and mycophenolate add on nintedanib in the Safety and Efficacy of Nintedanib in Systemic Sclerosis (SENSCIS) trial.¹³ The two studies are still in progress and both make use of full-dose combination.

A concern of full-dose combination is adverse effects. As monotherapy, pulse CYC at 1.0 g/m² caused nausea in 36.4% patients, hematuria in 13.6% patients and respiratory tract infections in 13.6% patients¹⁴ while daily CYC can cause even higher incidence of side effects.⁴ When taking daily PFD up to 2400 mg, almost all patients suffered drug-associated adverse events such as rash or gastrointestinal intolerance.¹⁵ According to a meta-analysis of IPF trials, drug intolerance caused greater withdrawal of patients treated with PFD, compared with controls.¹⁶ Overall, a full-dose combination of

CYC and PFD might be risky for patients and hide potential benefits.

Therefore, we hypothesized that a low-dose combination may improve tolerance. Thus, in this study, we reported a low dose combination therapy of CYC and PFD and the efficiency and safety of the therapy in refractory CTD-ILD patients.

PATIENTS AND METHODS

Seven CTD-ILD patients (2 males, 5 females; mean age 48.8 years; range, 32 to 63 years) who had not shown improvement of their symptoms (dyspnea or cough) after at least one-month of steroid treatment (prednisone ≥ 1 mg/kg daily or were equivalent) were enrolled sequentially between January 2016 and December 2017, from Renji Hospital, Shanghai Jiao Tong University School of Medicine. Patients who had adjusted their immunosuppressive agents (including biological agents) other than steroids or had received anti-fibrotic medications within the three months before enrolment were excluded. Patients with dominant infection or other complications outside of the lungs that required daily prednisone over 1 mg/kg were also ruled out. The study protocol was approved by the Renji Hospital Ethics Committee. A written informed consent was obtained from each patient or his/her legal representative. The study was conducted in accordance with the principles of the Declaration of Helsinki.

We used the 6MWD to assess the patient activity tolerance¹⁷ and St. George's Respiratory Questionnaire (SGRQ) to evaluate quality of life.¹⁸ Pulmonary function tests (PFTs) and HRCT were performed at our hospital at three-six-month intervals, according to the changes of symptoms. The HRCT scores of the patients were calculated based on the Ichikado criterion by the same two radiologists.¹⁹

We generally changed the patients' previous treatments into a standard protocol consisting of intravenous CYC 0.4 g/m² per month and oral PFD 300 mg twice daily. Additionally, we cut the oral steroids to prednisone 0.5 mg/kg daily (or equivalent) and tapered it to a stable dose (prednisone ≤ 10 mg per day) within six months.

Statistical analysis

All patients had HRCT scores, PFTs and 6MWD at baseline or follow-up to 12 months after changing their therapy. The differences in the median changes of diffusing capacity of lung for carbon monoxide (DLCO), FVC percentage, HRCT scores and 6MWD before and after treatment were assessed by Wilcoxon signed rank sum test. A p value <0.05 was considered statistically significant.

RESULTS

Detailed demographic information is shown in Table 1. Two patients had anti-synthetase syndrome, two had SS, two had SSc and one had mixed connective tissue disease (MCTD). These patients were diagnosed as ILD by chest computed tomography (CT) two to five years before. At enrolment, the median 6MWD was 275 m (range, 202 to 324 m), the median DLCO was 51% of the prediction (range, 47.7 to 63%) and the median FVC was 72.3% of the prediction (range, 39 to 81%). The median HRCT score was 150 (range, 136 to 203). All these patients had suffered severe dyspnea or cough, with the median baseline SGRQ score of 51 (range, 41 to 64).

After 12 months of CYC-PFD treatment, all patients showed significant improvement in their clinical status and HRCT. The PFTs were improved or stable. All patients who received

steroids were successfully maintained at 10 mg prednisone daily or less. The 6MWD showed a median increase of 52.7% (range, 34.4 to 86.3%, $p<0.05$). For quality of life assessment, the SGRQ total score showed a median improvement of 53.3% (range, 19.5 to 61.7%, $p<0.05$). The HRCT score showed a median decrease of 20.1% (range, 11.7 to 29.6%, $p<0.05$). Regarding lung functions, the DLCO percentage showed a median improvement of 6.3% (range, 1.7 to 16%, $p<0.05$) and the FVC percentage showed a median improvement of 13.4% (range, 0 to 35.9%, $p<0.05$) (Table 2, Figure 1a-f).

Although only seven patients were included in this study, it is worth mentioning that all patients tolerated the combination therapy unexpectedly well. During the 12-month follow-up, none of the patients had observable adverse events that could be attributed to PFD or CYC, such as gastrointestinal (nausea, dyspepsia, diarrhea, and anorexia), neurological (dizziness and fatigue), dermatological (photosensitivity, rash and pruritus), bone marrow suppression, hematuria, infection or menstrual disorders.

Patient 1: This patient is a 55-year-old male non-smoker, who was diagnosed with SS and ILD two years before. He was treated with oral methylprednisolone 8 mg per day and hydroxychloroquine 0.2 g twice a day for at least one year. He had slowly progressive dyspnea for six months, but did not exhibit satisfactory improvement after one-month of 50 mg/day

Table 1. Baseline characteristics of patients

Patient	Age (year)/Sex	ILD diagnosis (year)	Serum antibody	CTD diagnose*	Baseline medication other than steroids†
1	55/M	3	Anti-SSA	SS	Hydroxychloroquine
2	43/F	5	Anti-OJ	ASS	Cyclosporine
3	41/F	2	Anti-U1RNP, Anti-SSA	MCTD	Methotrexate
4	53/F	2.5	Anti-Scl-70	SSc	Hydroxychloroquine/colchicine
5	32/F	3	Anti-Scl-70	SSc	Methotrexate
6	55/M	2	Anti-SSA	SS	Hydroxychloroquine
7	63/F	2	Anti-Jo-1	ASS	Azathioprine

ILD: Interstitial lung disease; CTD: Connective tissue disease; SS: Sjögren syndrome; ASS: Anti-synthetase syndrome; MCTD: Mixed connective tissue disease; SSc: Systemic sclerosis; * Connective tissue disease diagnoses were established by consultant rheumatologists; † These medications did not change during large dose steroids treatment (prednisone ≥ 1 mg/kg daily) before enrolment.

Table 2. Parameters along with follow-up

Patients/parameter	Pre-treatment		12 months post-treatment		Change
	n	%	n	%	%
Patient 1					
DLCO		50.4		51.3	+1.7
FVC		76.1		88.4	+16.2
6MWD	253		488		+92.8
SGRQ total score†	45		21		-53.3
HRCT score§	146		116		-20.5
Patient 2					
DLCO		50		58	+16
FVC		39		53	+35.9
6MWD	202		377		+86.3
SGRQ total score	64		36		-43.8
HRCT score	203		143		-29.6
Patient 3					
DLCO		47.7		53.2	+11.5
FVC		72.3		79.3	+9.7
6MWD	279		375		+34.4
SGRQ total score	53		31		-41.5
HRCT score	143		121		-15.4
Patient 4					
DLCO		57.2		60.3	+5.4
FVC		62.6		74.5	+19
6MWD	275		420		+52.7
SGRQ total score	62		25		-59.6
HRCT score	150		128		-14.6
Patient 5					
DLCO		55		57	+3.6
FVC		81		81	0
6MWD	324		442.5		+36.5
SGRQ total score	41		33		-19.5
HRCT score	136		120		-11.7
Patient 6					
DLCO		63		67	+6.3
FVC		67		76	+13.4
6MWD	267		405		+46.7
SGRQ total score	47		18		-61.7
HRCT score	169		135		-20.1
Patient 7					
DLCO		51		58	+13.7
FVC		76		84	+10.5
6MWD	274		433		+58.0
SGRQ total score	51		23		-54.9
HRCT score	176		140		-20.5

DLCO: Diffusing capacity of lung for carbon monoxide; FVC: Forced vital capacity; 6MWD: Six-minute walk distance; SGRQ: St. George's Respiratory Questionnaire; HRCT: High-resolution computed tomography; † St. George's Respiratory Questionnaire is a tool to assess quality of life based on pulmonary dysfunction; § High-resolution computed tomography scores were calculated by an invited radiologist according to criteria by Ichikado et al.¹⁹

prednisone. At enrolment, he suffered an obvious aggravation in dyspnea and could only climb one flight of stairs. He had infiltration and fibrous cords as shown by HRCT. After two months of combined treatment, he was able to enjoy an overseas tour and could climb a hill over 200 meters high. His steroid treatment was changed to methylprednisolone 8 mg per day at the seventh month of follow-up.

Patient 2: This patient is a 43-year-old female non-smoker with anti-synthetase antibody-associated ILD. She experienced quickly worsening dyspnea and deteriorating PFTs over six weeks after being treated for four years with cyclosporine (75 mg twice a day) and prednisone (15 mg daily). She did not improve after increasing her steroid regimens (methylprednisolone 80 mg for one week and prednisone 50 mg for three weeks). She had resting dyspnea at enrolment. Her HRCT demonstrated the presence of infiltration and consolidation along the peripheral lung fields. After three months of combined treatment, she had no resting dyspnea. Twelve months later, her condition showed strikingly improved PFT with a 39.5% improvement in FVC, with her prednisone dose maintained at 10 mg per day.

Patient 3: This patient is a 41-year-old female non-smoker with MCTD-ILD. She was referred with rapidly progressive dyspnea within one month after being treated with methotrexate (MTX, 15 mg per week) and prednisone (10 mg per day) for one year. After relapsing, she received 40 mg/day prednisone but the progression of her symptoms did not stop. At enrolment, she had dyspnea when taking a shower or washing clothes. Her HRCT showed reticular fibrosis at the base of the lung. After six months of combined treatment, she had no difficulties with everyday housework or taking care of herself. Twelve months later, she could walk at least 1000 m on the ground without any rest or dyspnea, her prednisone dose maintained at 10 mg for four months and 5 mg for two months.

Patient 4: This patient is a 53-year-old female non-smoker diagnosed with SSc-ILD one-and-a-half years before. She had received hydroxychloroquine (0.2 g per day), colchicine (1 mg per day) and prednisone (10 mg per day) for over one year. She experienced progressive

dyspnea for three months, although she had received 40 mg/day prednisone in the last two months. At the time of enrolment, she could not even talk freely because of dyspnea. She had large scale of consolidation at the base of the lung. After 12 months of combined treatment, she could talk normally and walk on the ground for at least 500 m without dyspnea, with prednisone dose maintained at 10 mg per day for six months.

Patient 5: This 32-year-old female non-smoker was diagnosed with SSc-ILD and treated with MTX (10 mg per week) and prednisone (15 mg per day). For six months before enrolment, she had dry cough accompanied with chest pain when breathing. Her HRCT demonstrated diffused gross-glass opacity along peripheral lung fields. Because we first thought that MTX might be the reason, MTX was stopped and changed to prednisone 40 mg/day for one month. Unfortunately, her symptoms were still aggravated and they bothered the patient in daily speaking and activity before enrolment. Her HRCT showed almost no change compared to one month earlier. Three months after the combination treatment, her cough and chest pain disappeared. After another 10-month follow-up, her PFTs remained stable, with an improved 6MWD and quality of life.

Patient 6: This patient is a 55-year-old male with more than 20-year smoking history (20 cigarettes a day on average). He was diagnosed as SS-ILD two years before, at which time he quit smoking. He was treated with prednisone (15 mg per day) and hydroxychloroquine (0.4 g per day) for over eight months. At enrolment, because of severe dyspnea, he had difficulty in talking for more than 10 minutes or climbing two flights of stairs because of dyspnea. Additionally, he had mild cough and felt severe fatigue all day. His chest HRCT showed diffused infiltration and reticular fibrosis, mostly along the peripheral area of the lungs (Figure 1f). After three months of combined treatment, he had no talking difficulties; 12 months later, he could climb five flights of stairs without dyspnea. His prednisone dose was maintained at 7.5 mg per day since the ninth month of follow-up.

Patient 7: This patient is a 63-year-old female with a 15-year smoking history (approximately seven cigarettes per day) until

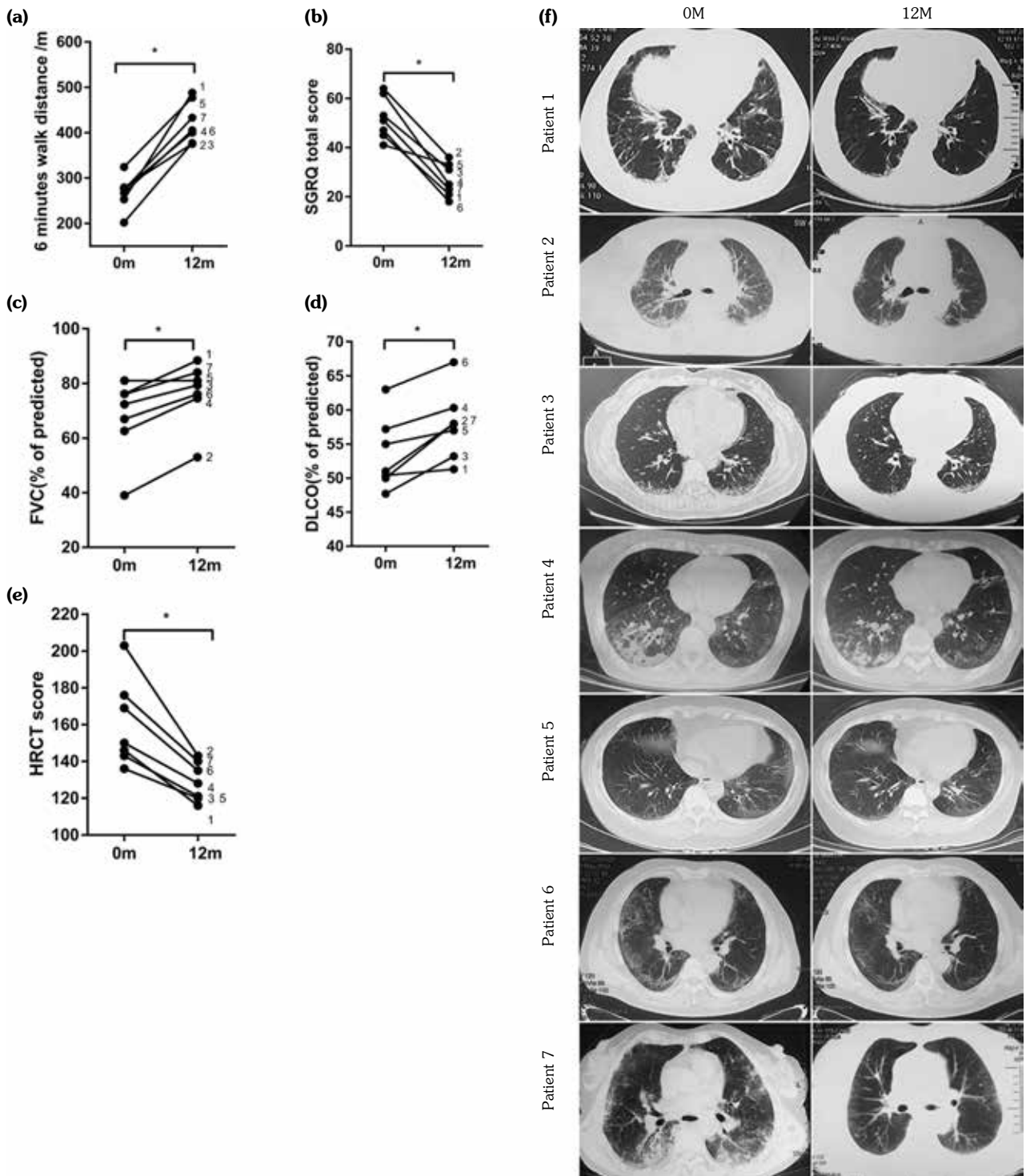


Figure 1. Parameters along with follow-up. **(a)** Patient activity tolerance was assessed by six-minute walk distance. **(b)** Patient dyspnea quality of life was assessed by St. George's Respiratory Questionnaire total score. **(c and d)** Pulmonary function tests, including forced vital capacity and diffusion capacity of lung for carbon monoxide. **(e and f)** High-resolution computed tomography scores and images. Various radiologic patterns, including infiltration, gross-glass opacity and fibrosis were found in seven patients. Numbers besides each point from picture A to E represent patient 1 to 7.

* $P < 0.05$, Wilcoxon signed rank sum test.

SGRQ: St. George's Respiratory Questionnaire; FVC: Forced vital capacity; DLCO: Diffusion capacity of lung for carbon monoxide; HRCT: High-resolution computed tomography.

she was diagnosed as anti-synthetase antibody-associated ILD two years before. She was treated with azathioprine (50 mg per day) for one year. She developed dry cough three months prior to enrolment. She was first treated with 15 mg/day prednisone for two months, but her symptoms still progressed. Her dose of prednisone was increased to 60 mg per day for one month without additional improvement. At enrolment, she suffered dyspnea and dry cough, with difficulties in her daily housework. She had consolidation and ground glass opacity over almost 50% of her lung field (Figure 1f). After two months of combined treatment, she was not bothered by dyspnea or cough when doing housework or climbing three flights of stairs. After 12-month follow-up, her status remained stable with a prednisone dose at 7.5 mg per day for six months.

DISCUSSION

The hypothesis that PFD and CYC might work in synergy is based on the different working mechanisms of these two agents. CYC is a classic alkylating agent that can induce cell death in resting and dividing lymphocytes and is highly potent in controlling a range of autoimmune diseases.^{20,21} PFD, a small molecular agent, can suppress the expression of growth factors, such as transforming growth factor-beta, fibroblast growth factor and platelet-derived growth factor, suppress proliferation of fibroblasts and inhibit collagen synthesis. Furthermore, it also inhibits monocyte secretion of inflammatory cytokines, such as tumor necrosis factor-alpha, interleukin (IL)-1 beta, and IL-6, and suppresses the production of reactive oxygen species *in vivo* and *in vitro*.^{10,11} Therefore, the combination of the two medicines may exhibit synergy in inhibition of the activation of inflammation and fibrosis as well as the progression of CTD-ILD.

Although the combination therapy is always an attractive idea, the likelihood of adverse effects is always a concern. In fact, there have been attempts to reduce the dose to improve the tolerance of either for CYC or PFD as monotherapy. Martin-Suarez et al.²² first suggested reducing the CYC dose to 0.5 g per injection (first, 0.5 g weekly for three weeks and then 0.5 g monthly) to achieve

less adverse effects. This regimen was verified in SSc-ILD patients by Paone et al.²³ and Ando et al.,²⁴ who further reduced the dose to 0.5 g monthly, which is comparable to the dose of CYC selected in our combination treatment.

Compared with Caucasian patients, the adverse effect of PFD is a more prominent issue in East Asian patients. Because of its adverse effects, most clinical trials in East Asia chose 1800 mg/day PFD, rather than the dose of 2400 mg/day for American patients. Even so, the incidence of PFD related adverse events (such as photosensitivity, rash, gastrointestinal reaction, dizziness and fatigue) was over 50%.^{6,25} In a phase I trial for Chinese individuals,²⁶ the incidence of adverse events was 20% at 600 mg/day PFD and rose up to 83% at 1200 mg/day. Therefore, we chose 600 mg/day PFD for the combination treatment to improve the tolerance.

In this cohort, all the patients unexpectedly tolerated the low dose combination therapy unexpectedly well. This approach might be feasible to improve the tolerance when treating CTD-ILD.

For low dose combination, insufficient efficacy is a potential concern. Although our study included a limited number of patients, our data show no apparent inferiority of the low dose combination therapy to CYC monotherapy. In this cohort, the median FVC and DLCO at baseline were 72% and 51% of the prediction, respectively, which appear to be similar to patients from the SLS I and II studies.^{4,5} In these clinical trials, approximately 20-30% patients from the CYC group had decreased FVC/DLCO at the end of the follow-up, while in our study, three/seven patients had elevated predicted FVC $\geq 15\%$ or predicted DLCO $\geq 10\%$, six/seven patients had improved FVC, seven/seven patients had improved DLCO and no patient exhibited deterioration in PFTs after one-year of treatment. Our data for the first time suggest that the low dose combination of PFD and CYC warrants further investigation.

One confounder of this observation is the use of steroids before the enrolment. We could not rule out the effects of steroids. However, at enrolment, we had to step-up the treatment without washing-out as clinical practitioners. According to reports on ILD associated with DM or polymyositis,^{27,28} once a patient began treatment with high dose steroids, if the respiratory symptoms were not

improved within four weeks, the survival would be poor (less than 40% in 10 weeks after the treatment) if the patient did not promptly receive immunosuppressants. Until now, no strong evidence has demonstrated that ILD associated with most CTDs has a substantially different mode of progression. The only known exception is the anti-melanoma differentiation-associate gene (anti-MDA5) antibody associated rapidly progressing ILD,²⁹ which is not included in this study. Given the condition of our patients, although not all the patients had myositis, we thought waiting or washing-out would be relatively unsafe. And the concomitant steroids thus became an inevitable major confounder. Full-dose controlled clinical trials with longer observation are needed with restriction on the use of steroids to better assess the low dose combination therapy.

In conclusion, our study provided preliminary but promising clinical evidence for combination therapy of CYC and PFD for CTD-ILD. A low dose combination of CYC and PFD was well tolerated, with satisfactory effects in refractory CTD-ILD patients. Well-designed controlled studies are needed to further establish the safety and efficacy of this approach.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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