

A comparative study of treatment with azathioprine or interferon-g for patients with idiopathic pulmonary fibrosis

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SUMMARY. **Introduction:** Idiopathic pulmonary fibrosis (IPF) is characterized by progressive deterioration of lung function, leading ultimately to death. No pharmacological treatment has been found to stabilize the evolution of the disease, but interferon-g and azathioprine have been used as therapeutic options. **Aim:** To compare the effectiveness of treatment with interferon-g plus low dose prednisone or azathioprine plus low dose prednisone in patients with IPF. **Materials and methods:** Patients newly diagnosed with IPF were recruited, 22 in total, of whom 10 received azathioprine plus prednisone and 12 patients received interferon-g plus prednisone for six months. Clinical evaluation, lung function tests, HRCT, bronchoscopy and bronchoalveolar lavage (BAL) were performed at baseline and after six months of treatment. **Results:** All patients were alive after six months of treatment. No statistically significant difference between the two groups was detected regarding clinical deterioration, inflammatory biomarkers such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and BAL cell subpopulations. There was a trend, not statistically significant, towards a greater reduction in forced vital capacity and diffusing capacity for carbon monoxide in the interferon-g group. **Conclusion:** Interferon-g does not offer any therapeutic advantage over azathioprine as regards the clinical course, lung function tests and BAL cell counts of patients with IPF. *Pneumon 2009, 22(3):247-253.*

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is the most common interstitial lung disease. It is characterized by progressive deterioration of lung function, leading ultimately to death. No effective treatment has been found to date that can stabilize or reverse the evolution of the disease¹.

Azathioprine is a derivative of thioguanine, a purine antimetabolite, which is administered for prevention of graft rejection, for the treatment

of rheumatoid arthritis, and as maintenance treatment for systemic vasculitis². Azathioprine has also been shown to retard deterioration of lung function and it may offer a slight increase in survival in patients with IPF, compared to corticosteroid treatment^{3,4}. The combination of azathioprine with small doses of corticosteroids has been proposed as a treatment regimen in IPF, according to the American Thoracic Society and the European Respiratory Society (ATS/ERS) consensus statement⁵.

Interferon-g is a powerful antifibrotic agent which promotes cellular death and decreases fibroblast proliferation. It interacts with the TH1/TH2 cytokine balance, diminishing the secretion of profibrotic cytokines. The rationale for treatment with interferon-g in IPF is based on the hypothesis that decreased levels of this cytokine have been detected in pulmonary fibrosis⁶.

Bronchoalveolar lavage (BAL) is a useful research tool for the study of the pathogenesis of interstitial lung disease⁷. An increase in neutrophils and eosinophils in the BAL of patients with IPF has been correlated with worse prognosis⁸⁻¹⁰, while BAL lymphocytosis suggests a more benign course of the disease¹¹.

In this study the efficacy of interferon-g as an antifibrotic therapeutic option in patients with IPF was investigated in comparison to the therapeutic regime proposed by ATS/ERS of azathioprine plus low doses of corticosteroids.

The primary aim of this study was to compare two therapeutic regimes, i.e., azathioprine and interferon-g, according to the evolution of the disease and clinical deterioration, lung function parameters, BAL components and biochemical markers, in patients with IPF, before and after six months of treatment.

MATERIALS AND METHODS

All 22 patients in the study had newly diagnosed IPF. The diagnosis of IPF was made from:

- 1) the typical histological pattern of interstitial pneumonia on biopsy obtained by video-assisted thoracoscopy or open-lung biopsy in patients with interstitial lung disease (11 patients).
- 2) the application of the ATS/ERS criteria⁵: presence of all major and of at least of 3 of the 4 minor criteria (11 patients):

Major criteria:

- Non-identification of a secondary cause which causes

interstitial lung disease (drug toxicity, environmental exposure, collagen vascular disease).

- Lung function tests showing a restrictive pattern and blood gas abnormalities (increased alveolo-arterial gradient for PaO₂ at rest or after exercise)
- Typical high resolution computerized tomography (HRCT) findings.
- BAL or transbronchial biopsy findings that do not support an alternative diagnosis.

Minor criteria:

- Age above 50 years.
- Progressive dyspnoea on exercise.
- Disease duration of more than 3 months.
- Bilateral fine crackles on lung auscultation ("Velcro").

The 22 patients were divided into two groups: 10 patients received azathioprine combined with a low dose of corticosteroids (azathioprine 2-3mg/kg/d, with a maximum dose of 150mg/d, with prednisone 0.5mg/kg/d x 4wk - 0.25/kg/d x 8wk - 0.125/kg/d), and 12 patients were put on treatment with interferon-g and low-dose corticosteroids (interferon-g 200 µg x 3 /wk sc and prednisone 0.5mg/kg/d x 4wk - 0.25/kg/d x 8wk - 0.125/kg/d).

The patients were evaluated before, and at 3 and 6 months after starting treatment. Lung function testing, HRCT, bronchoscopy and BAL were performed before treatment and at 6 months.

Clinical stabilization or deterioration were defined according to the ATS/ERS consensus statement criteria^[5].

Bronchoscopy and BAL were performed as described previously^[12, 13]. BAL was performed in the middle lobe or the lingula, according to the HRCT findings. Aliquots of 60 ml of sterile normal saline were instilled through the bronchoscope and the fluid was retrieved by mechanical suction. The standard introductory volume was 180 ml.

Cells were separated from the BAL fluid by low-speed centrifugation at 300 g for five minutes at 4 °C, and were washed three times with cold minimal essential medium (MEM) containing 25mM Hepes buffer. BAL cell counts were completed as previously reported^[12,13]. Total cell counts were determined using an improved Neubauer counting chamber and expressed as the total number of cells per ml of aspirated fluid. Slide preparations for differential percentage counting of the cells were made with a Shandon cytocentrifuge (Cytospin II, Shandon Ltd, Runcorn, Cheshire, U.K.) using 100-µl aliquots of the lavage cell suspensions, adjusted to 1.25x10⁶ cells per millimeter in MEM. The differential count was determined

on a stained preparation stained by May-Grunwald Giemsa staining and Papanicolaou staining, counting more than 1000 cells.

The study was approved by the Bioethics Committee of Sismanoglio Hospital and all participants gave written informed consent.

The two groups were compared according to clinical deterioration, lung function parameters, biochemical markers and BAL cell populations.

Statistical analysis was performed with t-test and mixed model analysis for repeated measurements.

RESULTS

Clinical characteristics, lung function tests, BAL and biochemical parameters prior to treatment

Newly diagnosed patients with IPF were recruited for the study, 10 male and 12 female, with a mean age of 68.91 ± 6.2 years. The mean duration of their symptoms was 13.08 months. All patients reported cough and dyspnoea and chest auscultation revealed Velcro type crackles.

The mean forced vital capacity (FVC) was $70.45 \pm 19.14\%$ of predicted and the mean diffusing capacity for carbon monoxide (DLCO) $54.18 \pm 27\%$ of predicted. The mean partial oxygen pressure (PaO₂) was 72.61 ± 11.54 mm Hg and the alveolo-arterial gradient for oxygen partial pressure (PA-PaO₂) was 29.30 ± 11.33 mm Hg.

BAL analysis showed an increase in the neutrophil and eosinophil sub-populations (table 1).

No significant differences were found between the two groups of patients in clinical and laboratory baseline parameters, with the exception of the PA-PaO₂, which was higher in the azathioprine group (table 2).

Clinical characteristics, lung function tests, BAL and biochemical parameters after six months of treatment

Clinical characteristics

All patients were alive after six months of treatment. Five patients in the interferon-g and four patients in the azathioprine group had deteriorated according to the ATS criteria during treatment. The difference in the deterioration rate was not statistically significant ($p=0.39$). Three patients in the interferon-g and three in the azathioprine group showed worsening of their dyspnoea, from class II to class III of NYHA classification. This difference between groups was non-significant ($p=0.53$).

TABLE 1. Baseline characteristics of the patients with idiopathic pulmonary fibrosis (n=22)

	Minimum	Maximum	Mean	Std. Deviation
Ηλικία	54,00	80,00	68,9130	6,20054
Διάρκεια συμπτωμάτων	2,00	48,00	13,0870	11,65377
FVC	,77	4,00	2,0500	,78144
FVC%	37,00	99,00	70,4545	19,14786
DLCO%	6,00	122,00	54,1875	27,93616
DLCO	1,96	23,30	12,4438	6,18683
restpao ₂	53,00	95,00	72,6143	11,54376
pAao ₂	10,00	52,50	29,3048	11,33391
Μακροφάγα BAL	44,00	68,00	55,5238	6,95427
Λεμφοκύτταρα	,00	14,00	5,8095	3,73656
Ουδετερόφιλα	18,00	40,00	24,7619	4,58154
Ηωσινόφιλα	,00	16,00	10,8571	3,66450
Μαστοκύτταρα	,00	6,00	2,7000	2,07998
Περιφερικό αίμα	3,96	12,40	8,5776	2,08203
Λευκά αιμοσφαίρια				
Ουδετερόφιλα	46,00	80,50	64,1810	9,50214
Αιμοσφαιρίνη	11,20	15,40	13,9524	1,20483
TKE	1,00	103,00	28,4476	25,30707
CRP	,03	33,00	2,7760	8,38688
LDH	305,00	688,00	457,1905	105,09834

BAL= bronchoalveolar lavage, CRP= C-reactive protein, LDH= lactic dehydrogenase, PA-PaO₂= alveolo-arterial partial oxygen pressure gradient, PaO₂= partial oxygen pressure, FVC%= forced vital capacity percent predicted, DLCO%= diffusing capacity for carbon monoxide percent predicted

Lung function tests

There was a decrease in FVC in the interferon g group but not of significant difference compared to the azathioprine group ($p=0.32$). A similar trend to a greater decrease of the DLCO was observed in the interferon-g group but the difference was not statistically significant ($p=0.13$). The PaO₂ showed no difference before and after treatment while the increased PA-PaO₂ in the azathioprine group persisted (table 3, diagram 1).

Biochemical parameters

There was no statistically significant difference be-

TABLE 2. Comparison of the baseline parameters of the two groups of patients with idiopathic pulmonary fibrosis before treatment (AZA= azathioprine treatment group, IFN= interferon-g treatment group)

Variable	THERAPY	N	Statistics						
			Lower CL Mean	Mean	Upper CL Mean	Lower CL Std Dev	Upper CL Std Dev	Std Dev	Std Err
AGE	AZA	10	65.15	70.1	75.05	4.7594	6.9194	12.632	2.1881
AGE	IFN	12	64.514	68.25	71.986	4.165	5.8795	9.9826	1.6973
DURATION_ SYMPTOMS	AZA	10	5.3699	16.1	26.83	10.317	15	27.383	4.7433
DURATION_ SYMPTOMS	IFN	12	5.7094	11.083	16.457	5.9916	8.458	14.361	2.4416
restPO2	AZA	10	59.169	68.1	77.031	8.5877	12.485	22.793	3.9481
restPO2	IFN	11	70.44	76.718	82.996	6.5292	9.3446	16.399	2.8175
FVC100	AZA	10	52.953	68.4	83.847	14.853	21.593	39.421	6.8284
FVC100	IFN	12	60.954	72.167	83.379	12.501	17.647	29.963	5.0943
DLCO100	AZA	8	46.388	59.5	72.612	10.37	15.684	31.922	5.5453
DLCO100	IFN	8	18.023	48.875	79.727	24.399	36.903	75.108	13.047
BAL_macr	AZA	10	51.96	57.4	62.84	5.2304	7.6041	13.882	2.4046
BAL_macr	IFN	11	49.679	53.818	57.958	4.3051	6.1615	10.813	1.8578
lym	AZA	10	2.5989	5.6	8.6011	2.8856	4.1952	7.6589	1.3266
lym	IFN	11	3.6728	6	8.3272	2.4204	3.4641	6.0793	1.0445
neu	AZA	10	21.741	23.4	25.059	1.5951	2.319	4.2336	0.7333
neu	IFN	11	22.106	26	29.894	4.0501	5.7966	10.173	1.7477
eos	AZA	10	8.9224	10.4	11.878	1.4208	2.0656	3.771	0.6532
eos	IFN	11	8.0777	11.273	14.468	3.323	4.7559	8.3462	1.4339
mast	AZA	8	0.0179	1.5	2.9821	1.1721	1.7728	3.6082	0.6268
mast	IFN	8	1.506	2.75	3.994	0.9839	1.488	3.0286	0.5261
ESR	AZA	10	6.2714	28.6	50.929	21.47	31.213	56.983	9.8705
ESR	IFN	11	14.806	28.309	41.812	14.044	20.1	35.274	6.0604
CRP	AZA	10	-3.472	3.8632	11.198	7.0526	10.253	18.719	3.2424
CRP	IFN	5	-0.495	0.6016	1.6978	0.529	0.8829	2.5369	0.3948
LDH	AZA	10	387.13	467.8	548.47	77.562	112.76	205.86	35.659
LDH	IFN	11	378.93	447.55	516.16	71.367	102.14	179.25	30.797
PA_PaO2	AZA	10	27.833	36.4	44.967	8.237	11.975	21.862	3.7869
PA_PaO2	IFN	11	19.07	22.855	26.64	3.9366	5.634	9.8873	1.6987

Variable	P-value
AGE	0.5127
DURATION_SYMPTOMS	0.3634
restPO2	0.0939
FVC100	0.6638
DLCO100	0.4718
BAL_macr	0.2544
lym	0.8155
neu	0.1927
eos	0.5885
mast	0.1496
ESR	0.9803
CRP	0.3434
LDH	0.6723
PA_PaO2	0.0064

BAL= bronchoalveolar lavage, ESR= erythrocyte sedimentation rate, CRP= C-reactive protein, LDH= lactic dehydrogenase, PaO2= partial oxygen pressure, PA-PaO2= alveolo-arterial partial oxygen pressure gradient, FVC%: forced vital capacity percent predicted, DLCO%: diffusing capacity for carbon monoxide percent predicted, macr= macrophages, mast= mast cells, lym= lymphocytes, neu = neutrophils, eos= eosinophils

TABLE 3. Lung function tests and biochemical parameters in patients with idiopathic pulmonary fibrosis before and after six months of treatment (AZA= azathioprine treatment group, n=10, IFN= interferon-g treatment group, n=12)

TREATMENT	VISIT	Mean	S.E.	P-value
REST PaO2				
AZA	AFTER	64.9000	3.4443	0.1450
AZA	BEFORE	68.1000	3.4443	
IFN	AFTER	72.9551	3.6043	0.3172
IFN	BEFORE	76.7182	3.2840	
FVC%				
AZA	AFTER	68.5000	6.3863	0.3172
AZA	BEFORE	68.4000	6.3863	
IFN	AFTER	63.4119	6.1598	0.1329
IFN	BEFORE	72.1667	5.8299	
DLCO%				
AZA	AFTER	62.9771	10.1018	0.1329
AZA	BEFORE	59.5000	8.9661	
IFN	AFTER	34.0829	8.8073	8.8073
IFN	BEFORE	49.3920	8.8073	
PA-PaO2				
AZA	AFTER	42.9750	4.7531	0.0273
AZA	BEFORE	36.4000	4.7531	
IFN	AFTER	27.8237	4.9614	0.6873
IFN	BEFORE	22.8545	4.5730	
ESR				
AZA	AFTER	33.1292	8.2297	0.6873
AZA	BEFORE	28.6000	7.9208	
IFN	AFTER	26.8903	7.8146	2.8899
IFN	BEFORE	28.3091	7.5522	
CRP				
AZA	AFTER	0.7921	2.6479	0.6567
AZA	BEFORE	3.8632	1.9973	
IFN	AFTER	1.1125	2.4150	69.0156
IFN	BEFORE	0.6010	2.8899	
LDH				
AZA	AFTER	567.90	72.3842	0.7001
AZA	BEFORE	467.80	72.3842	
IFN	AFTER	484.40	76.7743	69.0156
IFN	BEFORE	447.55	69.0156	

ESR= erythrocyte sedimentation rate, CRP= C-reactive protein, LDH= lactic dehydrogenase, PaO2= partial oxygen pressure, PA-PaO2= alveolo-arterial partial oxygen pressure gradient, FVC%: forced vital capacity percent predicted, DLCO%: diffusing capacity for carbon monoxide percent predicted

tween the two groups regarding the erythrocyte sedimentation rate (ESR) or blood levels of C-reactive protein (CRP) and lactic dehydrogenase (LDH) as inflammatory biomarkers (table 3).

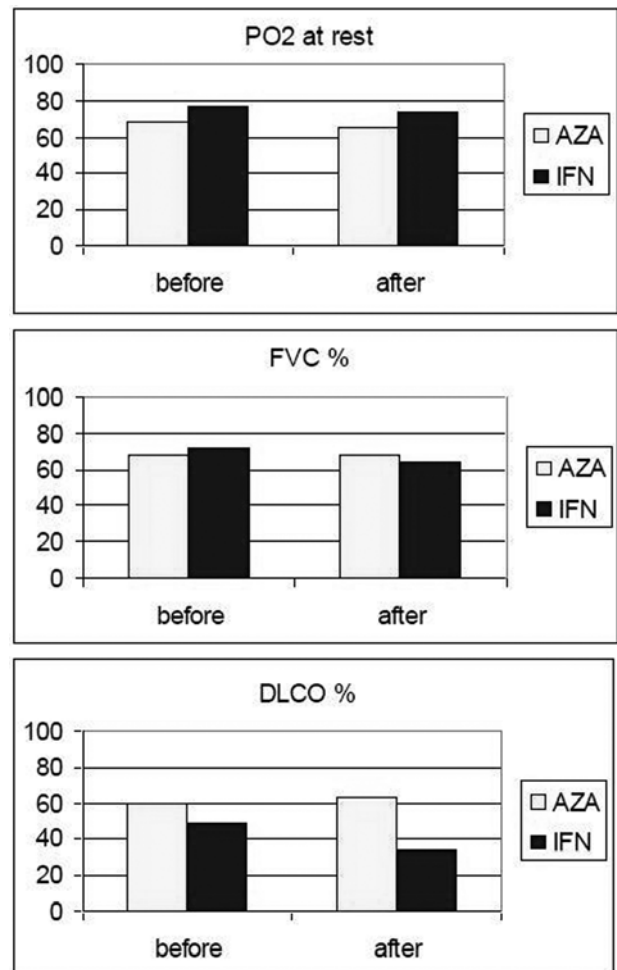


DIAGRAM 1. Change of partial oxygen pressure (PaO2), forced vital capacity (FVC) and diffusing capacity for carbon monoxide (DLCO) before and after six months of treatment with azathioprine (AZA, n=10) or interferon-g (IFN, n=12) plus prednisone.

Bronchoalveolar lavage

No statistically significant differences were found in the total BAL cell count or the cell percentages. A small but not significant increase in the lymphocyte sub-population of the azathioprine group was observed (table 4, diagram 2).

DISCUSSION

In this study the effects of two treatment regimens for IPF were compared, and specifically the clinical course, lung function tests, BAL and biochemical markers of the disease. The ATS suggested azathioprine plus low dose

TABLE 4. Bronchoalveolar lavage (BAL) cell percentages in patients with idiopathic pulmonary fibrosis before and after six months of treatment (AZA= azathioprine treatment group, n=10, IFN= interferon-g treatment group, n=12).

TREATMENT	VISIT	Mean value	S.E.	P-value
TOTAL BAL CELLS				
AZA	AFTER	22.4945	1.5770	0.1465
AZA	BEFORE	25.8000	1.3975	
IFN	AFTER	26.6080	1.4034	
IFN	BEFORE	26.7273	1.3325	
BAL macr				
AZA	AFTER	59.1090	2.9209	0.4579
AZA	BEFORE	57.4000	2.6072	
IFN	AFTER	55.0414	2.6096	
IFN	BEFORE	53.8182	2.4858	
BAL lymph				
AZA	AFTER	9.5132	1.5810	0.5572
AZA	BEFORE	5.6000	1.3997	
IFN	AFTER	7.1825	1.4063	
IFN	BEFORE	6.0000	1.3346	
BAL neu				
AZA	AFTER	21.1219	2.0918	0.4352
AZA	BEFORE	23.4000	1.8610	
IFN	AFTER	24.0427	1.8655	
IFN	BEFORE	26.0000	1.7744	
BAL Eos				
AZA	AFTER	8.7468	1.5009	0.5410
AZA	BEFORE	10.4000	1.3284	
IFN	AFTER	10.7984	1.3348	
IFN	BEFORE	11.2727	1.2666	
BAL Mast				
AZA	AFTER	3.0000	0.6299	0.5099
AZA	BEFORE	1.6716	0.7058	
IFN	AFTER	2.6449	0.6012	
IFN	BEFORE	2.6393	0.7071	

mast= mast cells, lym= lymphocytes, neu = neutrophils, eos= eosinophils, macr= macrophages

prednisone regime was compared with the promising, according to various reports, combination of interferon-g with low dose prednisone. To the best of the authors' knowledge, such a study has not been reported previously.

Interferon-g has been shown in three earlier studies to offer a survival benefit in patients with mild to moderate disease[14-16]. However, these results have not been confirmed: the large, multicentre, randomized double-blind study INSPIRE failed to show any survival benefit in patients with IPF treated with interferon-g compared

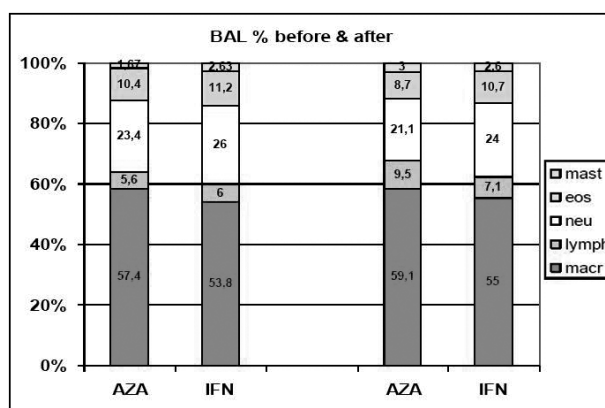


DIAGRAM 2. Differences in bronchoalveolar lavage (BAL) cell sub-populations before and after six months of treatment with azathioprine (AZA, n=10), or interferon-g (IFN, n=12) plus prednisone. mast= mast cells, lym= lymphocytes, neu = neutrophils, eos= eosinophils, macr= macrophages.

to placebo and it was prematurely stopped¹⁷.

Azathioprine has been shown to offer a small survival benefit compared to monotherapy with corticosteroids alone[4] and an improvement in the functional parameters of patients with IPF: in the IFIGENIA trial, treatment with combination therapy of azathioprine, prednisone and N-acetylcysteine resulted in an increase in the distance walked in the six minutes walk test[18].

The main conclusion of the present study is that neither of the two therapeutic options tried was superior to symptomatic treatment or lung function stabilization of patients with IPF. The number of patients who presented clinical deterioration was similar in the two groups. Despite the more rapid decrease of vital capacity and the DLCO in the interferon group, this difference was not statistically significant.

Although IPF has long been considered not to be an inflammatory process¹⁹, here it was examined whether treatment could affect inflammatory biomarkers such as CRP and ESR. Indeed the values of these parameters were in the normal range before and after treatment.

Increased lactic dehydrogenase (LDH) levels are commonly found in patients with IPF without depicting disease activity²⁰. In this study, LDH levels did not change after treatment.

The effect of treatment on BAL sub-populations was investigated. Previous studies have reported that an increase in neutrophils is commonly observed, while an increase in eosinophils is associated with worse prognosis⁸⁻¹¹. On

the contrary, an increase of lymphocytes is associated with better prognosis and a lymphocyte percentage of more than 20% suggests an alternative diagnosis, such as non-specific interstitial pneumonia, hypersensitivity alveolitis or sarcoidosis^{7,21}. In this series of patients, BAL neutrophils and eosinophils were increased and treatment did not cause a difference in the percentage of cell counts after six months of treatment. There was a trend towards an increase of lymphocytes in the azathioprine group, which was not statistically significant.

There were significant limitations to this study, and in particular the small number of patients included and the relatively short duration of the observation period. Despite these limitations, a slower, although not statistically significant, deterioration of lung function and an increase in BAL lymphocytes were observed in the azathioprine group, raising the suspicion of increased "effectiveness" compared to interferon-g.

In conclusion, treatment with low doses of prednisone and interferon-g for six months was not superior to treatment with azathioprine and prednisone regarding clinical deterioration and lung function deterioration in patients with IPF. For the first time it was observed that treatment had no effect on the BAL lavage cell populations or the inflammatory biomarkers.

Since pharmacological therapies have not been shown to offer a survival benefit in patients with IPF, early enrolment in a lung transplantation programme could be the most effective therapeutic option for these patients at present. Enrollment in clinical trials is essential in the pursuit of treatment for this debilitating disease²².

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