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Longitudinal outcomes of patients enrolled in a phase Ib clinical trial of the adipose-derived stromal cells-stromal vascular fraction in idiopathic pulmonary fibrosis

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Abstract

Background and objective: Cell-based therapies have been used for the management of several diseases, holding promising results. Few studies have evaluated their use in chronic lung diseases. Idiopathic pulmonary fibrosis (IPF) remains a lethal disease although new therapies have emerged the recent years. We have recently published a phase I study of 14 patients receiving endobronchially adipose-derived stem cells (ADSCs). The aim of this report is to assess the outcome for our patients' population.

Patients and methods: Patients who originally participated in this phase I study were followed up until the time of death. Pulmonary function tests as well as disease progression and survival time points were recorded.

Results: After first administration, a significant functional decline was observed as assessed by the changes (delta—Δ) of diffusion capacity for carbon monoxide (DLco) (mean ΔDLco = 6.2%, $P = .04$) and forced vital capacity (FVC) (mean ΔFVC = 6%, $P = .029$) at 18 and at 24 months, respectively. Median overall progression-free survival was 26 months and median overall survival was 32 months. All patients were alive for at least 2 years (survival rate, 100%) after first administration. Twelve patients (85.7%) died owing to disease progression. None of the patients experienced tumor development.

Conclusions: Significant functional decline occurred at 24 months after first administration. The median survival and time to progression are in line with the published epidemiologic data. Further clinical trials complemented by mechanistic studies are sorely needed to delineate the role of ADSCs in IPF pathogenesis and treatment.

KEYWORDS
clinical trial, follow-up, idiopathic, IPF, pulmonary fibrosis, stem cells, survival

1 | INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a devastating parenchymal lung disease that affects 13–20 individuals per 100 000.[1] It is characterized by progressive distortion of normal lung architecture that leads to gas exchange abnormalities and subsequently death mainly owing to respiratory failure. Despite major advances during the last decade, the pathogenesis of IPF remains poorly understood. Current disease pathogenesis assumes an aberrant wound-healing process within the alveolar epithelium in response to repetitive

Eleni Manoloudi and Argyris Tzouvelekis contributed equally to this study.

injurious stimuli in genetically predisposed and aged individuals.[2] On the other hand, the contribution of immune deregulation in disease pathogenesis has been recently reinforced.[3]

Regenerative medicine and particularly the potential use of cell therapies to treat acute lung injury or chronic lung diseases has been recently emerged as an alternative option or even salvage treatment for end-stage lung diseases.[4,5] Mesenchymal stem cells (MSCs) represent a cell therapy option with many advantages. They can be easily harvested from many tissues (bone marrow, stromal vascular fraction [SVF] of the adipose tissue, umbilical cord, and peripheral blood) and expanded in vitro with slight modifications.[6] Numerous experimental studies support the concept of anti-inflammatory, immunomodulatory, and potential antifibrotic properties of MSC.[7–9] Importantly, MSCs are considered to be "immune privileged," lacking expression of class II major histocompatibility complex, and thus making them ideal candidates for allogeneic transplantation.[6] Despite the above encouraging mechanistic potential concerns regarding the functionality, fate, and role of MSCs within a profibrotic microenvironment remain to be addressed and hamper their widespread clinical applicability.

The recent FDA approval of 2 novel antifibrotic agents, pirfenidone and nintedanib, finally shifted the therapeutic dial of IPF.[10,11] Nevertheless, both drugs present with major adverse events and only slow down disease progression; thus, at the best case leaving patients with considerable functional disability. To this end, the need for alternative therapeutic options is amenable. Before pirfenidone and nintedanib became commercially available, our study group launched a phase Ib study,[4] aiming to explore the safety profile of the endobronchial infusion of autologous adipose-derived stem cells—stromal vascular fraction (ADSCs-SVF) in a small cohort of patients with IPF ($n = 14$) of mild-to-moderate disease severity as assessed by forced vital capacity (FVC) of >50% and diffusion capacity for carbon monoxide (DLco) of >35%.[3] Our secondary endpoint was to assess efficacy based on the pulmonary function tests (PFTs), exercise capacity, and indices of quality of life.[4] The results of this study demonstrated an acceptable safety and tolerability profile of endobronchial infusion of ADSCs-SVF during the entire study period (12 months) as indicated by the absence of acute or chronic adverse events. Safety profile of MSC treatment in patients with IPF was further validated by 2 small nonrandomized phase 1b studies.[5,12]

Despite the above encouraging observations, these data derived from short-term, underpowered, nonrandomized studies with the absence of placebo arm and therefore definite conclusions cannot be drawn. Considering the major lack of knowledge on the longitudinal effects of stem cell therapies in patients with chronic lung diseases, we aimed to extend our observations and determine the longitudinal outcomes of endobronchial administration of ADSCs-SVF in the same small cohort of patients with IPF,[4,13] focusing on mortality, progression-free survival, lung function, and exercise capacity.

2 | PATIENTS AND METHODS

2.1 | Patients

The study was conducted at the Department of Pneumonology, Medical School, Democritus University of Thrace, and University Hospital of Alexandroupolis and was based on the previously published protocol.[4,13] The study was approved by the Local Ethics Committee and the Institutional Review Board of the University Hospital of Alexandroupolis (EHD33/1SC/16-02-2010). All patients agreed and signed an informed consent. All 14 patients who participated in the original study were followed up at our outpatient clinic on a prospective basis. After pirfenidone approval in 2012, 12 out of 14 patients fulfilling the criteria (80%>FVC > 55% and DLco > 35%) were treated with pirfenidone and therefore the analysis was performed on an intend-to-treat basis. Median time between stem cells infusion and initiation of pirfenidone treatment was 22 months. Median duration of pirfenidone treatment was 18 months. Follow-up was available for all patients.

2.2 | Methods

Overall survival and cause of death after first administration of ADSCs were noted. Pulmonary function tests, including FVC % of predicted, DLco % of predicted, and 6-minute walk test (6MWT), were prospectively collected. Pulmonary function tests were analyzed against the baseline values (before the initiation of treatment). Additionally, time to disease progression as defined by a relative decline from initial values (delta—Δ) in FVC of more than 10% and/or DLco of more than 15%, or death was recorded for each patient.[14–18]

2.3 | Statistical analysis

The analysis of PFT measurements was performed with Friedman test as data were not normally distributed. Progression-free survival and overall survival were calculated with the Kaplan–Meier method using log-rank product limit estimation. A P value of <.05 is considered statistically significant. Statistical analysis was performed with Statview 4.5 statistical software (Abacus Concepts Inc., Berkeley, California).

3 | RESULTS

During the follow-up period, a significant decline of FVC (ΔFVC = 6.2%pred, $P = .029$) and 6MWT (Δdistance = 180 m, $P = .0007$) were observed 24 months after the first

TABLE 1 Comparison of lung functional tests and 6MWT over time (values are expressed as mean ± standard deviation)[a]

TIME	Baseline data	6 Months after ADSCs infusion (*P*)	12 Months after ADSCs infusion (*P*)	18 Months after ADSCs infusion (*P*)	24 Months after ADSCs infusion (*P*)	30 Months after ADSCs infusion (*P*)	36 Months after ADSCs infusion (*P*)	42 Months after ADSCs infusion (*P*)	48 Months after ADSCs infusion (*P*)	54 Months after ADSCs infusion (*P*)
FVC %predicted	71.2 ± 15.2	73.4 ± 18 (.20)	74.4 ± 17.5 (.14)	73.9 ± 16 (.66)	65.2 ± 15.6 (.029)	60.4 ± 13.8 (.006)	61.9 ± 15.5 (.0612)	66.1 ± 17.7 (.11)	58.9 ± 16.9 (.2)	49.83 ± 11.7 (.039)
DLco %predicted	48.4 ± 11.1	48.9 ± 12.8 (.81)	47.3 ± 12.9 (.61)	42.2 ± 16.19 (.04)	33.9 ± 18.4 (.002)	36.8 ± 17.2 (.0016)	38.8 ± 25.4 (.048)	47.9 ± 17.6 (.01)	38.33 ± 8.2 (.12)	23.7 ± 4.3 (.007)
6MWT (m)	472.1 ± 55.2	477.4 ± 50.3 (.09)	476.4 ± 51.9 (.14)	440 ± 53.7 (.06)	292.1 ± 150.2 (.0007)	288.7 ± 135.1 (.0077)	292 ± 167.7 (.09)	293.33 ± 167.7 (.164)	213.3 ± 133.2 (.0622)	200 ± 28.3 (.0145)
Patients alive	14 (100%)	14 (100%)	14 (100%)	14 (100%)	8 (57%)	6 (43%)	6 (43%)	5 (36%)	4 (28%)	2 (14%)

Abbreviations: ADSC, adipocyte-derived stem cell; DLco, diffusion capacity for carbon monoxide; FVC, forced vital capacity.

[a]Deaths were treated as censored.

infusion of ADSCs, compared to baseline values. DLco was significantly (ΔDLco = 6.2%, $P = .04$) reduced 18 months after the first ADSC administration compared to baseline values. Serial fluctuations of PFTs during the entire follow-up period are summarized and shown in Table 1 and Figure 1.

Median overall PFS was 26 months (range, 18–54 months) (Figure 2A). Disease progression was recorded at 18 months for 2 patients (14%), at 24 months for 5 patients (35%), at 28 months for 1 patient (7%), 30 months for 3 patients (21%), 36 months for 2 patients (14%), 1 underwent lung transplantation, and 54 months for 1 patient (7%).

Median overall survival (Figure 2B) was 32 months (range, 24–60 months). All patients were alive at 2 years (survival rate, 100%) after the endobronchial instillation of ADSCs. Three-year survival rate was 43%, 4-year was 35%, and 5-year was 14% (Table 1). Twelve patients (85.7%) died with IPF-related disease: 5 (35.7%) died from acute respiratory failure, 4 (6%) from pulmonary infection, and 3 (21.4%) from myocardial infarction owing to acute respiratory failure. The remaining 2 patients (14.3%) who are still alive; 1 had lung transplantation at 36 months, he is alive 60 months after first administration, and the second is alive 66 months after first administration.

None of the patients experienced any tumor development during the follow-up period.

4 | DISCUSSION

Overall, our patients experienced significant functional and exercise capacity decline at 2 years after first endobronchial infusion with a median overall survival of 32 months and progression-free survival of 26 months. Disease progression and survival rates were similar to those reported for natural clinical course of disease as well as the placebo arms of large phase III-randomized controlled trials of pirfenidone and nintedanib.[1,10,11,19,20]

Our study exhibited a number of significant attributes that should be highlighted. This is the first study in the literature that reports longitudinal outcomes (5 years) of cell therapies in patients with IPF. This is of vital importance, considering that it addresses residual questions and concerns on long-term safety issues of stem cell therapy.[21] In agreement with the parent phase Ib study,[4] as well as the 2 follow-up studies,[5,12] on stem cell therapies in patients with IPF, none of the patients experienced any longitudinal treatment-related adverse events including cancer development. Our patients experienced statistically significant reductions in both DLco and FVC at 18 and 24 months, respectively, after first endobronchial infusion; yet, declines in both indices were equal to 6% and thus did not exceed the clinically meaningful threshold of 10% and 15%, respectively, that would indicate the deterioration of disease.[16,17,22]

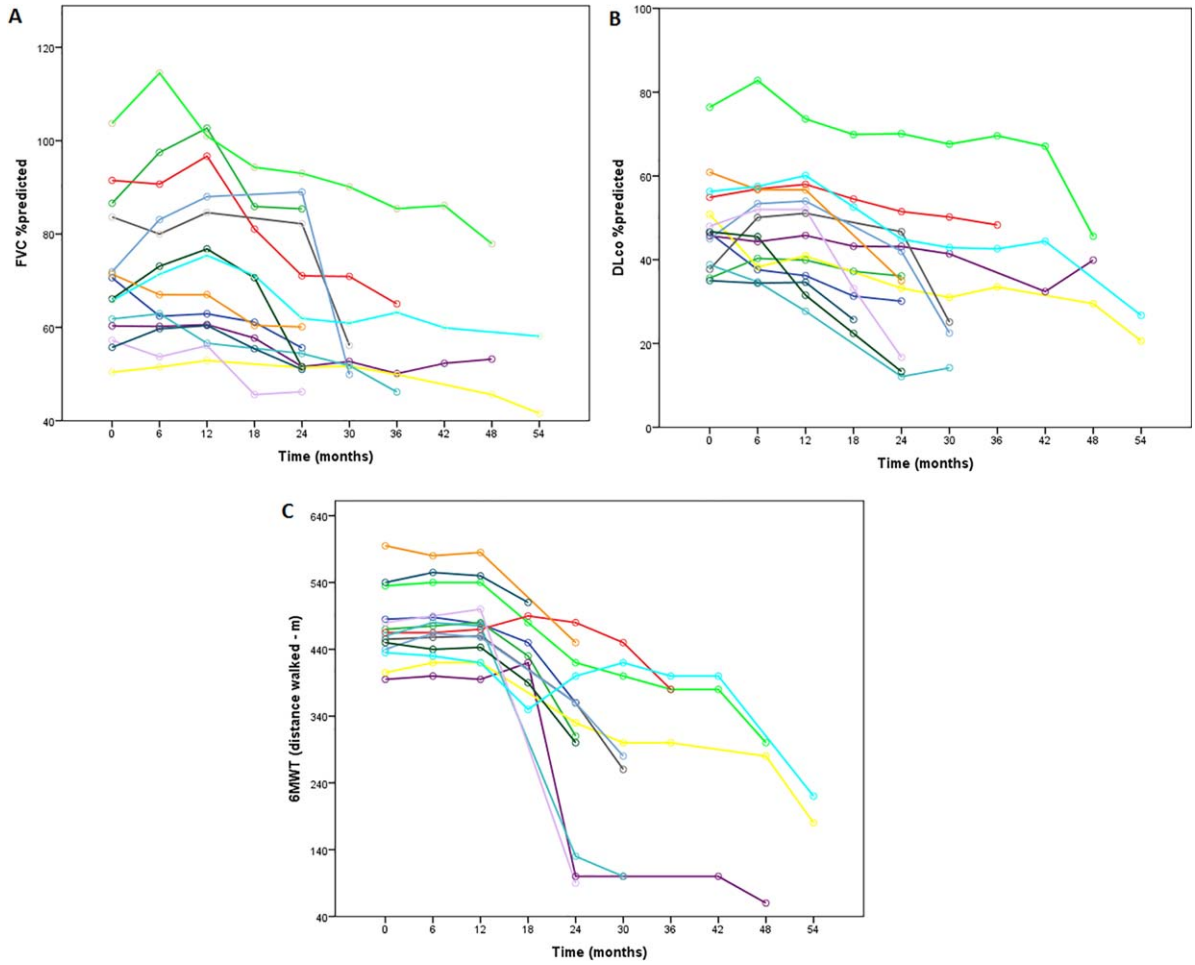

FIGURE 1 The evolution of PFTs for all patients during the follow-up period. Each line represents the measurements made in a single subject. Time point at 0 months indicates first study intervention. A: Forced vital capacity (as % of predicted) over time. B: Lung diffusion capacity for carbon monoxide (as % of predicted) over time. C: Distance walked during 6-minute walk test over time

Disease progression occurred 26 months after first administration of stem cells and median survival was 32 months. Importantly, all our patients were alive 24 months and 2 patients are still alive 5 years after the initiation of study. Significant reductions in DLco at earlier time points than FVC can also be attributed to the presence of emphysema or pulmonary hypertension in almost a third (4/14 and 5/14, respectively) of patients enrolled in the study. However, FVC seems to be a stronger predictor of mortality compared with DLco, and therefore it has been used as a primary-endpoint of efficacy in all randomized controlled trials, so far.[16,17,22,23] Finally our patients exhibited substantial reductions in measures of exercise capacity, including 6-MWT, 24 months following the initiation of study. On the other hand, clinically meaningful decline above the threshold of 24 m, as assessed by Du Bois and colleagues[24,25] was

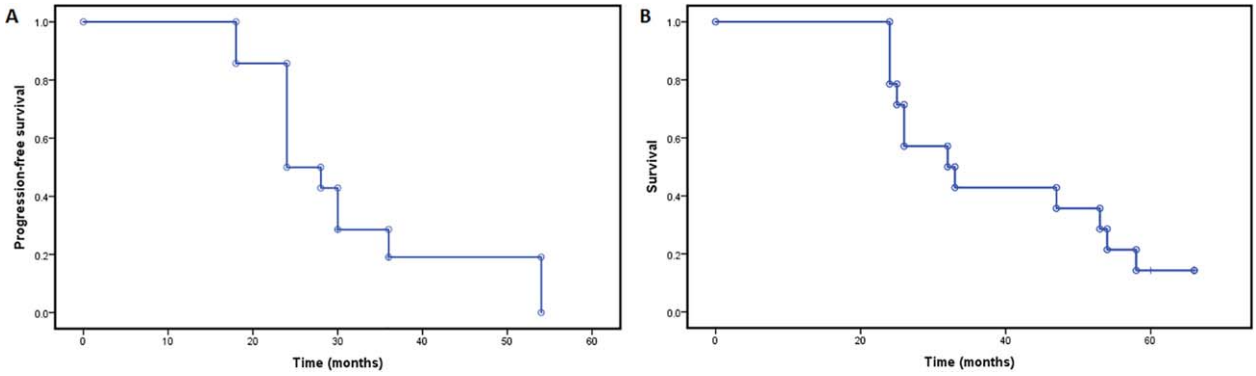

FIGURE 2 Kaplan–Meier curves for (A) progression-free survival and (B) overall survival of all patients ($n = 14$)

reported 24 months after first infusion. Nevertheless, 6MWT, as disease prognosticator, presents with major weaknesses, given that it is being affected by pulmonary hypertension and a variety of nonpulmonary comorbidities including peripheral arterial disease, musculoskeletal problems, and nutritional status, as well as factors such as age, sex, weight, and height.[23–25]

Despite the above preliminary observations on longitudinal safety of stem cell treatment in patients with IPF, our study exhibited some limitations. Although we report disease progression, as assessed by functional and physiological indices, as well as 43% of mortality at 3 years following first administration, we cannot demonstrate a causal-effect relationship between stem cell therapy and disease progression because our study was not designed to neither assess efficacy or survival. A limited number of patients underwent a short-term (3 months) treatment course and then followed up (1–5 years) to address, mostly, safety concerns. With regard to safety issues, none of our patients exhibited acute or long-term treatment-related side effects and all deaths reported were disease related. On the other hand, one cannot rule out the possibility that pirfenidone treatment may have positively interfered with longitudinal outcomes of stem cells infusion and this should kept into consideration.

Also, IPF is a disease paradigm with clinical course and response to available treatments largely heterogeneous and unpredictable. Our results indicate similar survival and annual functional decline rates to those reported in large epidemiological studies on disease natural course, as well as in the placebo arms of large randomized controlled trials.[3,10,11,20,26] In this context, our results do not signify a potential failure of cell-based therapies in IPF. It is possible that stem cell therapy could be beneficial for a subgroup of patients with IPF even in the context of add-on treatment to the current standard-of-care. Animal data showing attenuation of experimental lung injury upon stem cells administration[27,28] complemented with reassuring safety profiles of MSCs treatment in 4 different cohorts of patients with chronic lung diseases[4,5,12,29] provide encouragement to design-randomized controlled trials.

5 | CONCLUSIONS

To conclude, our study reports an acceptable longitudinal safety profile of endobronchial infusion of ADSCs in patients with IPF, on the basis of similar survival rates and time to disease progression with published epidemiologic data for untreated population. Stem cell therapy failed to demonstrate any beneficial functional effect during the entire study period. Larger randomized placebo-controlled clinical trials are sorely needed to assess safety and efficacy of stem cell treatment in patients with IPF.

CONFLICT OF INTEREST

Dr Tzouvelekis is a recipient of a Marie Sklodowska-Curie ERS-EU/RESPIRE 2 fellowship 8860-2015. All other authors have no conflict of interests related to this manuscript.

AUTHOR CONTRIBUTIONS

Study conception and design: Ntolios, Froudarakis
Data acquisition: Manoloudi, Steiropoulos, E. Bouros, Anevlavis
Data acquisition and interpretation: Ntolios, Tzouveleki
Statistical analysis and data interpretation: Froudarakis
Article draft, revision, and final approval: Ntolios, Manoloudi, Tzouveleki, Froudarakis
Article revision, and final approval: Steiropoulos, E. Bouros, Anevlavis
Revision for important intellectual content and final approval: D. Bouros, Froudarakis

ETHICS

The study was approved by the Local Ethics Committee and the Institutional Review Board of the University Hospital of Alexandroupolis (EHD33/1SC/16-02-2010). All patients agreed and signed an informed consent.

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