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INTERSTITIAL LUNG DISEASE

Endobronchial autologous bone marrow–mesenchymal stromal cells in idiopathic pulmonary fibrosis: a phase I trial

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ABSTRACT

Rationale: Idiopathic pulmonary fibrosis (IPF) has a dismal prognosis. Mesenchymal stromal cells (MSCs) have shown benefit in other inflammatory diseases.

Objectives: To evaluate the safety and feasibility of endobronchial administration of bone marrow autologous MSCs (BM-MSC) in patients with mild-to-moderate IPF.

Methods: A phase I multicentre clinical trial (ClinicalTrials.gov NCT01919827) with a single endobronchial administration of autologous adult BM-MSCs in patients diagnosed with mild-to-moderate IPF. In a first escalating-dose phase, three patients were included sequentially in three dose cohorts (10×10^6, 50×10^6 and 100×10^6 cells). In a second phase, nine patients received the highest tolerated dose. Follow-up with pulmonary function testing, 6-min walk test and St George's Respiratory Questionnaire was done at 1, 2, 3, 6 and 12 months, and with computed tomography at 3, 6 and 12 months.

Results: 21 bone marrow samples were obtained from 17 patients. Three patients were excluded from treatment due to chromosome aberrations detected in MSCs after culture, and one patient died before treatment. Finally, 13 patients received the BM-MSC infusion. No treatment-related severe adverse events were observed during follow-up. Compared to baseline, the mean forced vital capacity showed an initial decline of 8.1% at 3 months. The number of patients without functional progression was six (46%) at 3 months and three (23%) at 12 months.

Conclusions: The endobronchial infusion of BM-MSCs did not cause immediate serious adverse events in IPF patients, but a relevant proportion of patients suffered clinical and/or functional progression. Genomic instability of BM-MSCs during culture found in three patients may be troublesome for the use of autologous MSCs in IPF patients.

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Endobronchial autologous mesenchymal stromal cells (MSCs) did not cause direct serious adverse events in IPF patients. However, significant progression was seen in seven out of 13 patients. Genomic instability of autologous MSCs may limit use in IPF. https://bit.ly/39akv7z

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This study is registered at www.clinicaltrials.gov with identifier number NCT01919827. Individual participant data collected during the trial, after deidentification, as well as the study protocol, statistical analysis plan and code analysis, will be shared from the publication date onwards. Data will be shared to researchers who provide a methodologically sound proposal. Proposals should be directed to acampoe@unav.es. Requestors will need to sign a data access agreement. Data are available for 5 years upon request.

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Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic interstitial lung disease characterised by progressive fibrosis, loss of pulmonary function and high mortality with an average survival from the time of diagnosis of 2–5 years. It is the most common and severe form of idiopathic interstitial pneumonia. Aetiology involves genetic susceptibility [1–3], exposure factors and autoimmunity, leading to complex mechanisms such as cell senescence, impairment of repair mechanisms and host defence, and bronchoalveolar epithelial dysfunction [4].

Identification of new targets may allow the development of new drugs for this condition [5]. New commercialised drug treatments in the past few years, such as pirfenidone and nintedanib, may slow the progression of the disease [6–11], reduce exacerbations [12, 13] and improve survival [14]. However, prognosis remains poor [15–17], and treatments that can cure or definitively halt the disease are still lacking.

Cell therapies have been proposed as a possible therapy for IPF. Immune-modulatory effects of mesenchymal stromal cells (MSCs) [18, 19] have been proven to be beneficial and safe in laboratory conditions. Studies animal models of lung injury, or bleomycin-driven fibrosis, showed attenuation of fibrosis and reduced inflammation [19–24]. MSCs can be obtained from different sources (bone-marrow, adipose tissue, umbilical cord, placenta, dental pulp, menstrual blood or lung, among others) and expanded by culture. In the lung, MSCs engraft for a short period of time, although they may engraft for longer periods in damaged lungs [24, 25]. The therapeutic effect observed in different conditions is mediated mainly through paracrine mechanisms. Immunomodulation is mediated through interaction with other cells within the innate and adaptive immune systems, paracrine actions with release of soluble mediators, release of extracellular vesicles or by transfer of organelles such as mitochondria [26].

Three phase-1b clinical trials using adult MSCs have been conducted in IPF patients. The origin and type of cells and the route of administration differ, namely autologous adipose-derived MSCs by endobronchial infusion in 14 patients [27], intravenous placenta-derived MSCs in eight patients [28] and *i.v.* allogeneic MSCs in nine patients [29]. None of the three studies reported any serious adverse events at follow-up between 6 and 12 months. One study with a longer follow-up reported lung function decline during the subsequent 2 years [30].

The benefits, best source of the MSCs (autologous *versus* allogeneic), their origin, route of delivery (*i.v.*, endobronchial/endotracheal or even aerosolised) and dosing remain questioned for lung diseases, including IPF. MSCs originating from different sources might contain different secretome properties. Bone marrow (BM)-derived MSCs might have shown superior immunomodulatory effects compared to cells obtained from other sources [31]. Additionally, endobronchial infusion might confer an advantage regarding homing and retention of the cells in the lungs.

This study aimed to evaluate the safety and feasibility of the endobronchial administration of mesenchymal autologous stromal cells derived from bone marrow in patients with mild-to-moderate IPF.

Methods

Subjects and study design

This study was a phase I clinical trial of a single endobronchial administration of adult BM-MSCs in patients diagnosed with IPF, recruited between 2013 and 2016 and followed for 12 months. The centres for recruitment, MSC production and treatment were Clínica Universidad de Navarra (Pamplona, Spain) and IBSAL-Hospital Universitario de Salamanca (Salamanca, Spain). The estimated number of patients to be included was 18.

The protocol and all the procedures were approved by the Navarra Ethics Committee for Clinical Trials with Human Subjects (CEIC Navarra 01/2012) and by the Spanish Agency for Medicines and Health Products (AEMPS) (EudraCT 2011-006240-75, ClinicalTrials.gov NCT01919827). All patients provided written informed consent.

Criteria for eligibility of patients

Inclusion criteria were 1) males or females aged 30–80 years; 2) diagnosed with definite or probable IPF by high-resolution computed tomography (HRCT) and/or biopsy, according to American Thoracic Society

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(ATS) 2011 criteria [32] and after the exclusion of other known causes by history, examination, complementary blood tests and multidisciplinary discussion; 3) mild-to-moderate IPF defined by pulmonary function tests (PFTs) (functional vital capacity (FVC) ⩾50% predicted and diffusing capacity of the lung for carbon monoxide (D_{LCO}) ⩾35% pred); and 4) able to perform a 6-min walk test (6MWT) and fulfil all the requirements of the study protocol at the time of inclusion.

Exclusion criteria were current pregnancy or lactation, significant emphysema or any obstructive or restrictive respiratory conditions other than IPF, hospitalisation or active respiratory infection within 60 days before inclusion, frequent exacerbations of IPF (>2 episodes per year over the past 2 years), chronic severe heart or renal failure, previous neoplasm within 5 years, active treatment (pirfenidone, nintedanib, immunosuppressant or corticosteroids >10 mg prednisone), any medical or psychiatric condition that may limit life expectancy or interfere with the treatment of the study, and positive tests for HIV, hepatitis B surface antigen, hepatitis C virus antibody or syphilis screening assays.

Treatment groups

Patients enrolled were sequentially assigned to three groups with escalating doses: low (10×10^6 cells), intermediate (50×10^6 cells) and high (100×10^6 cells) dose. The study followed two phases: in phase I, three patients were included in each of the three dosing groups for a total of nine patients. Before escalating to the next dose, the three patients had to complete 3 months of follow-up without severe adverse events. In a second phase, nine patients received the highest tolerated dose.

Cell culture and infusion

BM-MSCs were generated under good manufacturing practice conditions with standard operating procedures, as described previously [33]. Bone marrow (100 mL) was harvested from the pelvic bone (iliac crest) under sterile conditions. The mononuclear cell fraction was isolated by Ficoll density gradient centrifugation (Ficoll-Paque; GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Cells, ranging between 20×10^6 and 60×10^6, were subsequently seeded in 175 cm^2 flasks with a growth medium, which consisted of αMEM without ribonucleosides (Gibco, Life Technologies, Carlsbad, CA, USA) supplemented with 5% platelet lysate, 2 units·mL^{-1} heparin, penicillin–streptomycin at 1% (Gibco) and 1 ng·mL^{-1} human fibroblast growth factor (Sigma-Aldrich, St. Louis, MO, USA). The flasks were maintained in culture at 37°C in a 5% carbon dioxide atmosphere. The growth medium was changed every 3–4 days. ∼10–15 days later, once colonies formed, the cells were split with TrypLE Select (Life Technologies) and seeded at 3000–5000 cells·cm^{-2}. Once 70–80% confluence was reached, cells were split again and cultured until they were available at the amounts required. Finally, cells were harvested with TrypLE Select, washed three times with PBS, and re-suspended in Ringer's lactate buffer (Grifols, Barcelona, Spain) containing 1% human albumin (Grifols), for administration within 24 h of harvesting of the cells. Cells were characterised according to International Society for Cellular Therapy criteria. Cells were then analysed by flow cytometry (FACSCalibur; BD Biosciences, San José, CA, USA) with the appropriate antibodies (BD Biosciences) to confirm the expression of surface markers CD90, CD73 and CD44, as well as the absence of CD34 and CD45. Genetic stability was assessed by array comparative genomic hybridisation (CGH) (NIM Genetics, Madrid, Spain). Serial microbiological controls were performed alongside MSC expansion and before the infusion.

Once appropriateness was confirmed, MSCs were diluted in Ringer's lactate solution with human albumin 1% and divided into four aliquots for administration. Under conscious sedation, a flexible bronchoscope was inserted and advanced to one of the lower lobes. MSCs were infused by a telescoping protected catheter (Combicath; Prodimed, Neuilly en Thelle, France) in four different segments of one of the lungs, followed by an infusion of 2 mL of saline. The total amount of fluid ranged from 20 mL to 50 mL for the different doses. Unilateral infusion was performed to avoid a large amount of fluid into the lungs, keep one of the lungs preserved and allow future comparison. As a general rule, the lung with less fibrotic changes was selected for the infusion.

Follow-up assessment and outcomes of interest

The study's primary objective was to evaluate the safety and feasibility of the endobronchial administration of BM-MSCs in patients with mild-to-moderate IPF. A secondary objective was to assess the disease's course as a possible effect of BM-MSC infusion in slowing or stopping the decline in pulmonary function.

Patients were followed-up 1, 2, 3, 6, 9 and 12 months after treatment. All the follow-up visits included PFTs, 6MWT, dyspnoea and cough questionnaires, St George's Respiratory Questionnaire (SGRQ) and blood analysis. PFTs included spirometry, lung volumes and diffusing capacity (Vmax 22; Sensormedics Corp, Yorba Linda, CA, USA), following ATS recommendations. Values are presented as percentage of predicted reference values. HRCT was done at 3, 6 and 12 months.

Primary safety end-points were the incidence and severity of adverse events during follow-up for up to 12 months. Adverse events are named using the Medical Dictionary for Regulatory Activities version 22.0. The severity of adverse events was graded as mild, moderate, severe and life-threatening, according to clinical intensity, impact on usual activities and need for medical care.

A serious adverse event was defined by any medical occurrence that resulted in death, was life-threatening, required inpatient hospitalisation or prolongation of existing hospitalisation, resulted in persistent or significant disability/incapacity or was a congenital anomaly/birth defect.

Efficacy end-points were 1) change in FVC as a continuous variable and 2) progression of the disease defined by death, need for transplantation or deterioration in pulmonary function defined by fall in FVC $\geqslant$10% or D_{LCO} $\geqslant$15%.

Statistics

Quantitative variables were summarised using mean±SD or median (interquartile range (IQR)), and categorical data by frequencies and percentages. The Shapiro–Wilk test checked the assumption of normality. Values at the follow-up visits were compared with baseline levels using paired t-tests or Wilcoxon matched-pairs signed-rank tests. Two-tailed p-values <0.05 were considered statistically significant. All analyses were performed with Stata 14 (Stata Statistical Software: release 14; StataCorp, College Station, TX, USA).

Results

Demographics of patients

18 patients were assessed for eligibility from April 2013 to December 2016. One patient was determined to be a screening failure, since results of the first PFTs did not meet inclusion criteria.

The diagram in figure 1 shows the patients initially screened, those excluded and the patients who received treatment. In summary, 21 bone marrow samples were obtained from 17 patients (15 males, two females). Seven expanded cultures from four patients resulted in nonappropriate cells (three had mosaic trisomy in chromosome 5 and one did not have viable cells), and were discarded for treatment. A second attempt conducted in these four patients obtained trisomy in chromosome 5 in three (75%) cases. For those individuals with trisomies in the BM-derived stromal cells after culture, BM cells prior to culture were studied using CGH array and confirmed to be normal. One patient died unexpectedly before treatment. Finally, 13 patients received MSCs at the corresponding dose.

The baseline characteristics of the 13 patients who received treatment are shown in table 1. Patients were predominantly male (12 out of 13), with a median age of 68 years (range 54.4–79.5 years), and a history of smoking in 10 (76.9%). The median time since the onset of symptoms was 3.3 years (range 0.5–7.8 years) and the median time since diagnosis was 1.2 years (range 0.2–8.1 years).

Diagnosis of IPF was based on HRCT features in eight patients and on HRCT and lung biopsy in five patients. Underlying known causes or other diagnoses were discarded by history, examination and complementary tests and multidisciplinary discussion. PFTs at baseline showed a mean FVC 75.6±20.3% pred, total lung capacity 61.5±13.2% pred and D_{LCO} 46.9±12.9% pred. 6-min walk distance (6MWD) was 488±69.2 m.

Safety

The median follow-up for the 13 patients was 10.1 months (IQR 6.8–12.9 months; range 2.2–13.6 months). Three patients died during the 12-month follow-up period because of progression of the underlying pulmonary fibrosis, *Legionella* pneumonia and general progressive deterioration of unknown cause, respectively. One patient received lung transplantation at 6 months due to the progression of the disease. Finally, 10patients completed follow-up at 6 months and six patients at 12 months.

30 adverse events were reported in 13 patients (table 2). The severity of adverse events was considered mild in 16 (53.3%), moderate in 10 (33.3%), severe in three (13.7%) and life-threatening in one case (3.3%). The more frequent adverse events besides IPF progression were bronchitis (eight patients), upper tract infection [2] and flu-like symptoms [2]. Fever and increase in dyspnoea within the first 24 h after infusion not requiring hospitalisation occurred in three patients who had received the highest dose of MSCs (100×10^6).

Adverse events were serious in four patients, leading to four hospitalisations in three patients and death in three. Besides IPF progression, two adverse events were fatal (*Legionella* infection and general deterioration). This last patient had weight loss, abdominal pain and a debilitating process; work-up was conducted with abdominal and thorax computed tomography, gastrointestinal tract endoscopy and blood

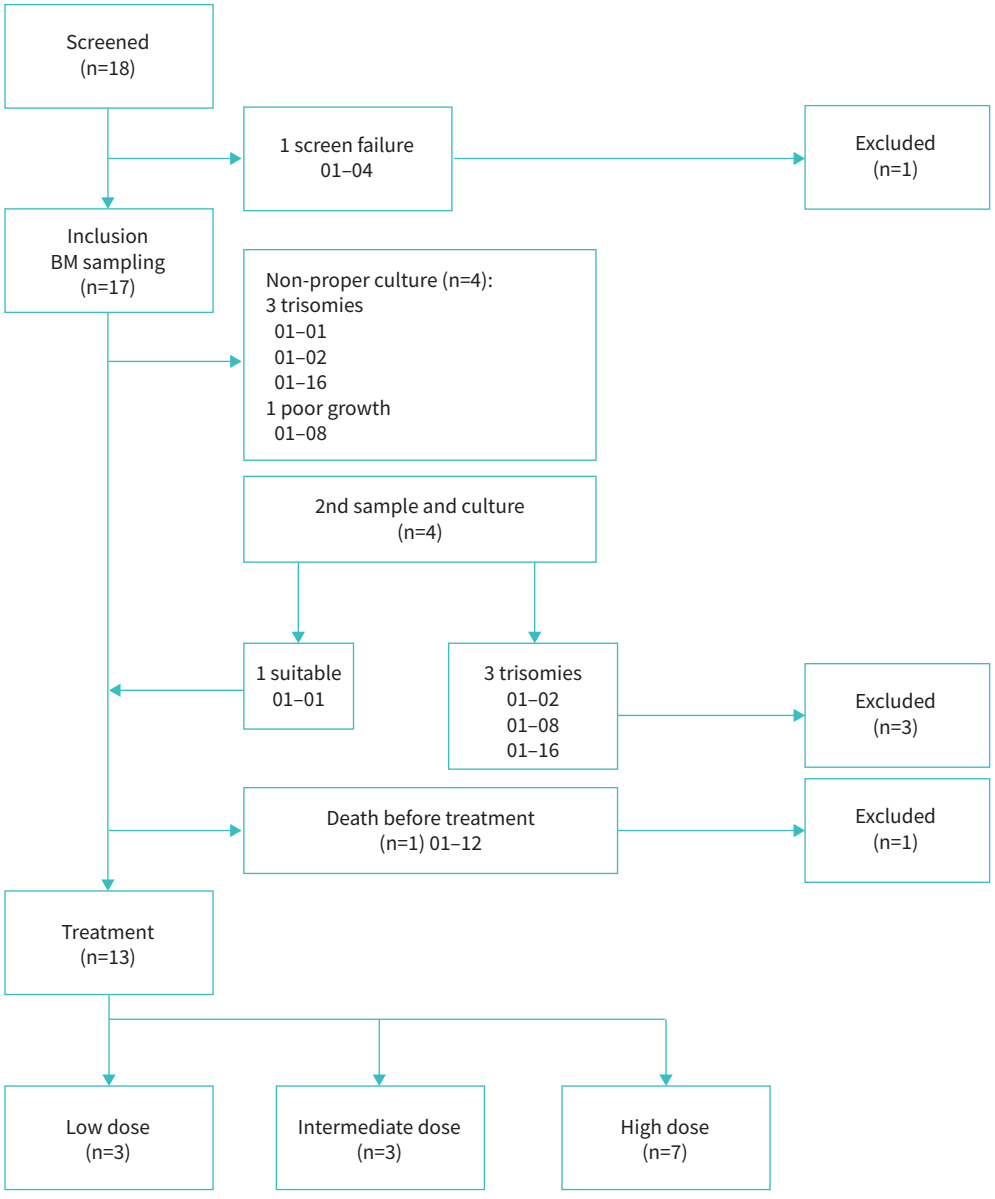

FIGURE 1 Study flow diagram for treatment. Patients identified by 01-XX. BM: bone marrow.

tests without evidence of neoplastic or infectious disorder. He continued clinical follow-up locally in another centre, and the final cause of death could not be determined.

One patient received bilateral lung transplantation at 6 months due to IPF progression, manifested mainly by exercise-induced hypoxia. The gross and microscopic examination of the explanted lungs reported diffuse and chronic interstitial pneumonia with honeycomb change in both lungs, without evidence of malignancy or other remarkable findings; final diagnosis was usual interstitial pneumonia. Evidence of engraftment or regeneration by administered cells was not found.

Two patients developed cancer during the follow-up period. One patient, aged 79 years, with previous bladder cancer 6 years before entry, was diagnosed with prostate cancer 6 months after MSC infusion. Another patient, aged 61 years, a former smoker with a baseline 23×20 mm atypical consolidation in the left lower lobe (LLL) not considered suspicious of malignancy, received MSCs in this LLL and developed a contralateral nodule in the right lower lobe (11×9 mm) at 3 months, with subsequent growth at 6 months (17×14 mm), without significant changes in the LLL. A 2-fluoro-2-deoxy-D-glucose positron emission tomography study revealed abnormal metabolic activity in both lesions. A subsequent transthoracic biopsy of the new nodule demonstrated small cell lung cancer. The clinical interpretation was that he had a previous primary neoplasm in LLL and which metastasised to the contralateral lung. Chemotherapy and

TABLE 1 Baseline characteristics of patients

	Low dose (10×10^6 MSCs)	Intermediate dose (50×10^6 MSCs)	High dose (100×10^6 MSCs)	Total
Subjects	3	3	7	13
Age years	60.5 (54.4–68.0)	65.6 (64.3–78.0)	70.2 (66.1–78.0)	68.0 (64.3–77.2)
Male/female	3/0	3/0	6/1	12/1
Time since IPF symptoms years	4.1±1.7	3.5±3.7	3.0±1.6	3.3±2.1
Time since IPF diagnosis years	1.9±2.1	3.5±4.0	1.6±1.7	2.1±2.4
Current or former smoker	3 (100)	2 (66.67)	5 (71.43)	10 (76.9)
Smoking pack-years	19.7±4.5	20.0±0	22.5±22.1	21.0±13.8
BMI kg·m^{-2}	27.7±1.9	29.4±1.0	26.3±2.7	27.4±2.5
Baseline S_{pO_2}	96.0±2.6	94.7±0.6	95.3±1.6	95.3±1.7
MRC dyspnoea scale 0/1/2/3	1/1/0/1	1/2/0/0	0/6/1/0	2/9/1/1
Cough intensity 0/1/2/3	0/0/2/1	0/1/2/0	1/3/1/2	1/4/5/3
Cough frequency	5 (5–7)	3 (2–4)	3 (2–4)	3 (2–5)
SGRQ symptoms	39.4 (23.8–50.7)	21.2 (0–39.0)	30.5 (23.8–36.9)	30.5 (23.8–39.0)
SGRQ activity	67.2 (0–73.0)	67.1 (35.5–73.8)	48.3 (41.5–67.1)	48.5 (41.5–67.2)
SGRQ impact	49.7 (0–50.1)	24.1 (11.6–36.0)	15.3 (10.3–30.6)	16.0 (11.6–36.0)
SGRQ total	53.3 (4.0–57.1)	37.8 (20.5–49.0)	24.2 (23.2–44.1)	28.9 (23.2–49.0)
Baseline P_{aO_2} mmHg	76.7±14.2	72.7±4.7	68.0±4.1	71.3±7.9
FVC % pred	83.7±36.5	69.4±15.6	74.9±15.8	75.6±20.3
FEV_1 % pred	82.3±33.3	75.2±12.0	77.1±16.7	77.9±18.9
FEV_1/FVC %	78.7±2.1	84.0±7.5	80.1±7.3	80.7±6.4
TLC % pred	66.0±25.2	58.5±9.0	60.9±9.8	61.5±13.2
D_{LCO} adj % pred	45.7±18.5	57.0±18.7	43.1±5.7	46.9±12.9
6MWD m	476.0±114.5	491.0±56.7	491.9±63.6	488±69.2
6MWT S_{pO_2} nadir	87.0±8.9	88.0±1.7	77.9±6.8	82.3±7.9
HRCT findings				
Traction bronchiectasis	3	3	6	12
Reticular pattern	3	3	7	13
Honeycombing	1	2	7	10
Pulmonary biopsy	2 (66.67)	2 (100)	1 (14.29)	5 (38.5)

Data are presented as n, median (interquartile range), mean±SD or n (%). MSCs: mesenchymal stromal cells; IPF: idiopathic pulmonary fibrosis; BMI: body mass index; S_{pO_2}: peripheral oxygen saturation; MRC: Medical Research Council; SGRQ: St George's Respiratory Questionnaire; P_{aO_2}: arterial oxygen tension; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s; TLC: total lung capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide; 6MWD: 6-min walk distance; 6MWT: 6-min walk test; HRCT: high-resolution computed tomography.

nintedanib were initiated after diagnosis. Even though the original lesion was present before MSC treatment, any deleterious effect of the MSCs cannot be determined or discarded. None of these serious adverse events were considered directly related to the treatment.

Assessment of pulmonary function

As shown in table 3, PFTs, 6MWT and SGRQ were performed at every follow-up visit. Individual baseline FVC and D_{LCO} values and changes during follow-up are shown in table 4 and depicted in figure 2.

All 13 patients were revisited at 3 months and performed PFTs with a change in mean FVC of −212±275 mL ($p<0.05$) and a change in FVC of −8.1% pred (p-value nonsignificant); there were no significant changes in D_{LCO} and 6MWD. Six patients remained functionally stable, with falls in FVC of <10% and in D_{LCO} of <15%.

The number of patients followed-up at 6, 9 and 12 months was 10, seven and six patients, respectively. Table 3 shows mean FVC and D_{LCO} values and the number of patients with clinically significant declines. At the end of follow-up (12 months), three patients were stable in terms of pulmonary function considering both FVC and D_{LCO}. The overall outcome at the last visit or follow-up contact was stability for three patients, functional decline for six patients, lung transplantation for one patient, death due to progression in one patient and death from other causes in two patients, as shown in table 4.

Discussion

The main finding of this study is that infusion of MSCs into the lungs *via* bronchoscopy in patients with IPF results in a low incidence of directly attributable side-effects, yet significant progression of the disease

TABLE 2 Adverse events and outcomes

	MedDRA code	Patients	Severity (mild/moderate/severe/life-threatening)	Seriousness (nonserious/serious)	First 3 months after infusion	Outcome at 12 months
Bronchitis	10006451	8 (61.5)	8/0/0/0	8/0	5	Resolved
IPF clinical progression or worsening without hospitalisation	10067761	5 (38.5)	0/3/2/0	5/0	1	4 IPF progression 1 lung transplant
IPF worsening with hospitalisation (2 patients)	10067761 10054112	3 (23.1)[#]	0/2/1/0	0/3	1	1 death
Fever and/or dyspnoea after infusion	10013968 10037660	3 (23.1)	1/2/0/0	3/0	3	Resolved
Upper respiratory tract infection/sinusitis	10046309	2 (15.4)	2/0/0/0	2/0	2	Resolved
Flu-like syndrome	10022004	2 (15.4)	2/0/0/0	2/0	6	Resolved
Acute gastroenteritis	10066762	1 (7.7)	1/0/0/0	1/0	1	Resolved
Skin lesion	10040882	1 (7.7)	1/0/0/0	1/0	1	Resolved
***Legionella* pneumonia with hospitalisation**	10035718	1 (7.7)	0/0/0/1	0/1	0	1 death
Abdominal pain, weight decreased, and general deterioration	10000081 10000159 10049438	1 (7.7)	0/1/0/0	1/0	1	1 death
Prostate adenocarcinoma	10060862	1 (7.7)	0/1/0/0	0/1	0	On treatment
Small cell lung cancer	10041067	1 (7.7)	1/0/0/0	0/1	0	On treatment
Radiotherapy side-effects with hospitalisation (prostate cancer treatment)	10037759	1 (7.7)	0/1/0/0	1/0	0	Resolved

Data are presented as n (%) or n. MedDRA: Medical Dictionary for Regulatory Activities; IPF: idiopathic pulmonary fibrosis. [#]: one patient was hospitalised twice during a 2-month period.

was observed. Three patients had early adverse events attributable to the infusion, namely fever and/or dyspnoea, not leading to hospitalisation or subsequent IPF exacerbation. All three were in the higher dose group and thus received a greater volume of infused fluid during the procedure. Other adverse events were unlikely to be related to the treatment, as they were not temporally correlated, although this cannot be

TABLE 3 Changes in pulmonary function, 6-min walk test and quality of life

	Time after infusion					
	1 month	2 months	3 months	6 months	9 months	12 months
Subjects	13	11	13	10	7	6
ΔFVC mL	−93±240	−184±272[#]	−212±275[#]	−200±240[#]	−214±292	−212±189[#]
ΔFVC % change	−3.7±10.8	−6.8±12.7	−8.1±11.7	−7.7±10.5	−7.0±10.5	−7.6±6.4
ΔTLC mL	27±267	−79±187	−151±315	−169±370	−260±338	−345±374
ΔTLC % change	0.5±4.1	−1.3±2.9	−2.3±4.9	−2.7±5.9	−4.3±5.0	−5.5±5.5
ΔD_{LCO} mL·mmHg^{-1}·min^{-1}	−0.2±1.3	−0.2±1.7	−1.1±1.7[#]	−0.9±1.9	−1.2±2.3 n=6	−1.5±2.6
ΔD_{LCO} %	−1.7±11.1	−2.6±11.6	−9.5±14.2	−8.3±14.3	−12.1±16.8 n=6	−11.5±19.5
Δ6MWD m	5.3±32.4	9.0±34.6	1.8±71.4	−9.8±37.3	−40.0±83.1	−16.7±41.3
Patients with FVC decline <10%	11	7	7	6	4	4
Patients with D_{LCO} decline <15%	12	10	8	7	2	4
ΔSGRQ symptoms	6.0±14.3	4.9±16.6	5.6±19.9	3.1±16.4	10.8±30.8	3.6±27.6
ΔSGRQ activity	3.7±16.7	−1.4±20.2	−0.8±23.2	−3.8±27.1	7.0±27.9	3.8±28.6
ΔSGRQ impact	−0.5±11.3	−0.7±10.8	0±16.0	1.6±13.0	6.0±20.8	−2.9±15.3
ΔSGRQ total	0.7±11.2	−0.8±12.6	−0.5±16.2	−0.5±15.7	5.2±19.8	−2.0±17.6

Data are presented as n or mean±SD. FVC: forced vital capacity; TLC: total lung capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide; 6MWD: 6-min walk distance; SGRQ: St George's Respiratory Questionnaire. [#]: significant change compared to baseline values, p<0.05.

TABLE 4 Individual baseline and functional outcomes[#] at the end of follow-up

Patient identifier	Dose n cells	Baseline FVC %	Baseline D_{LCO} %	Functional outcome at 3 months	Last visit	Overall outcome at last visit/ or follow-up contact	Observations: death/ lung transplant[¶]
01_01	10×10^6	53	35	Progression	6 months	Progression	
01_03	10×10^6	74	35	Stable	3 months	Other	Lung transplant at 6 months
01_05	10×10^6	124	67	Stable	12 months	Stable	
01_06	50×10^6	57	42	Stable	9 months	Progression	
01_07	50×10^6	87	78	Progression	12 months	Stable	
02_01	50×10^6	64	51	Progression	12 months	Progression	
01_09	100×10^6	73	42	Progression	3 months	Other	Death at 5 months
01_10	100×10^6	61	35	Stable	9 months	Progression	
01_11	100×10^6	96	53	Stable	12 months	Stable	
01_13	100×10^6	90	47	Progression	12 months	Progression	
01_14	100×10^6	52	39	Stable	6 months	Other	Death at 8 months
01_15	100×10^6	69	43	Progression	3 months	Other	Death at 9 months
01_17	100×10^6	83	43	Progression	12 months	Progression	

FVC: forced vital capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide. [#]: progression defined by fall in FVC ⩾10% and/or fall in D_{LCO} ⩾15%, otherwise considered stable; [¶]: causes of death: 01_09 *Legionella* pneumonia, 01_14 IPF progression, 01_15 not determined.

ruled out entirely. Most adverse events were common syndromes like bronchitis, flu-like syndrome, upper respiratory tract infections and acute gastroenteritis, accounting for 45% of adverse events.

Two patients had a diagnosis of cancer during the follow-up period. Although carcinogenesis has been a concern in any treatment with stromal cells, the nature and development of the tumours seen, and previous knowledge from other clinical trials in which no adverse events related to malignancy were reported make it unlikely that the cancers observed during this study were due to treatment with MSCs [25].

Clinical IPF progression was observed as an adverse event in seven patients during the follow-up, causing three hospitalisations in two patients, lung transplantation in one patient and death in one patient. Compared to baseline, median FVC declined significantly at 3 months. During follow-up, pulmonary function was stable in six patients at 3 months and only in three patients at 12 months. As shown in table 4, patients with better outcomes had higher baseline pulmonary parameters, meaning that patients with mild disease are more stable and less prone to deterioration. Although IPF progression has hardly been reported in other clinical trials [27, 28], the AETHER (Allogeneic Human Mesenchymal Stem Cells in Patients With Idiopathic Pulmonary Fibrosis via Intravenous Delivery) study [29] showed that two out of nine patients died due to IPF progression, a similar proportion as in our study. Longitudinal outcomes obtained from a previous phase I study with autologous adipose tissue driven MSCs [27] showed a median survival of 32 months, with 12 out of 14 patients dying due to disease progression [30].

The study was designed before the final approval of antifibrotic drugs in the European Union and Spain. During the trial, the availability and authorisation of these drugs for the individual patients were diverse. Recommendations for therapy with antifibrotic drugs (pirfenidone or nintedanib) were made in all pre-screened patients where and when available. The recommendations were reiterated for recruited patients during the clinical follow-up, and especially if there was any deterioration. All this caused a delay in patient recruitment and probably a worse clinical course in the patients selected. The need for the initiation of treatment in the early stages of the disease should be emphasised.

Patients with IPF seemed to have good tolerance to treatment with BM-MSC *via* bronchoscopIc infusion. However, even though small volumes of fluid were administered, the procedure may cause symptoms in some patients and might eventually cause clinical deterioration in individuals with more severe IPF. When the study was designed, the treatment choice was the endobronchial infusion of autologous BM-MSC. One of the limitations of the systemic administration of MSCs cells is the inefficient homing of the cells, although it can be enhanced in the acutely injured lung [24, 25]. The endobronchial infusion may overcome this issue. However, some of the patients in our study experienced symptoms after the infusion, especially those who received higher volumes of fluid.

As a general rule, the chosen site of infusion was the lower lobe of the less fibrotic lung, considering that presumably the immunomodulatory and/or regenerative effect of the MSCs could be null in an intensely fibrotic parenchyma. Conversely, infusing cells into the less fibrotic lung might compromise more

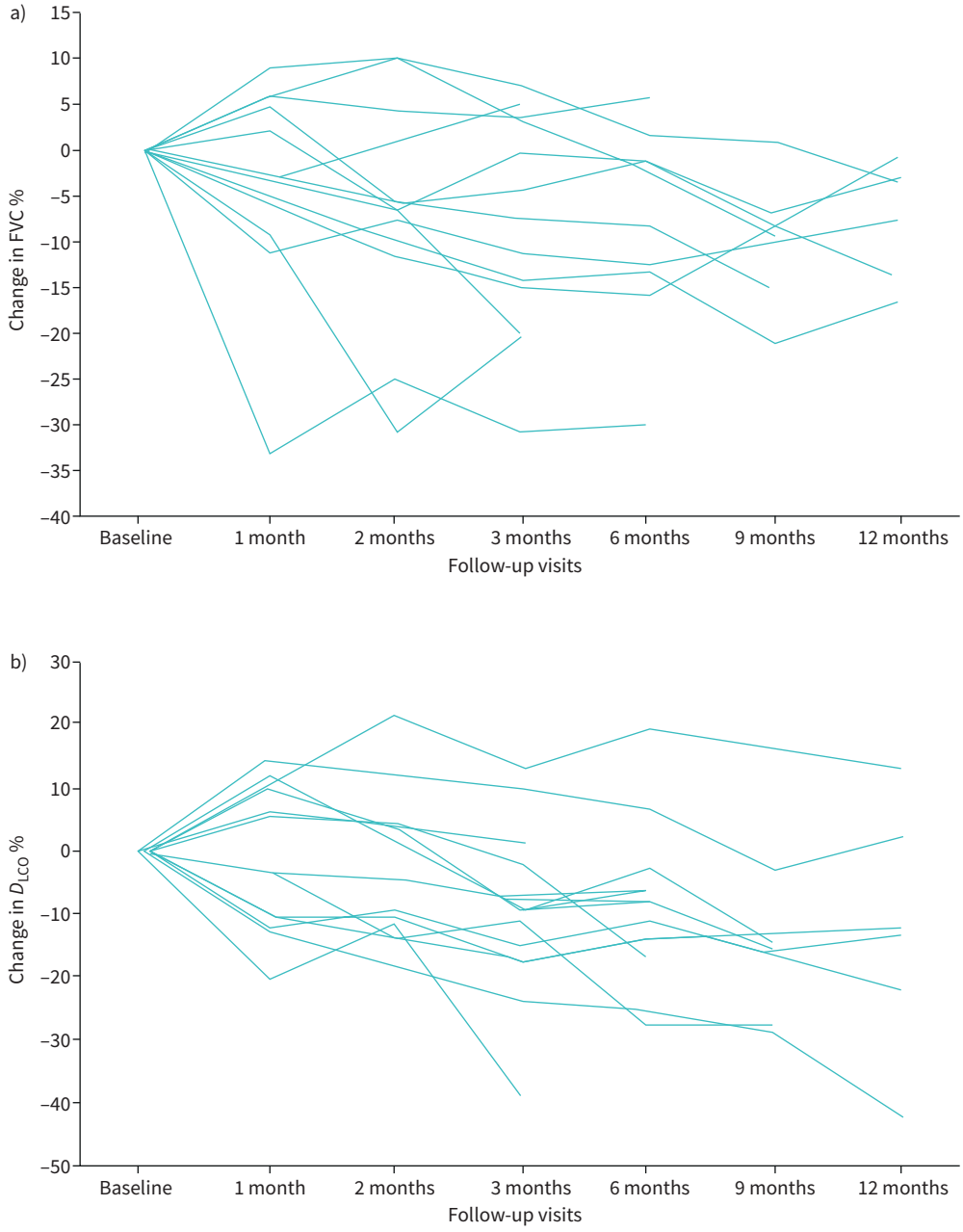

FIGURE 2 Individual changes in a) forced vital capacity (FVC) and b) diffusing capacity of the lung for carbon monoxide (D_{LCO}) during follow-up.

functional parenchymal areas if any local adverse effects were derived, such as inflammation, infection or (although hardly expected) carcinogenesis. Thus, although the patients had diffuse and quite symmetrical damage in both lower lobes, for safety reasons future studies should be aware of patients with predominantly right or left fibrosis.

A phase I clinical trial used autologous adipose-driven MSCs by three endobronchial infusions in 14 patients and provides some comparative data [27]. Adipose-derived cells were neither expanded nor cultured. After the infusion, half of the patients experienced fever, and 14% experienced some minor effects such as mild cough, dyspnoea or desaturation, but no clinically severe effects in follow-up to 1 year.

Two other clinical trials administered MSC intravenously. One administered placenta-derived MSCs in eight patients [28]. During the MSC infusion there was a mild fall in arterial oxygen saturation after 15 min (1%, range 0–2%), but without changes in haemodynamics, which may be attributed to transient vascular obstruction due to the size of MSCs. The AETHER study administered *i.v.* allogeneic MSCs in

nine patients [29] and did not report any relevant adverse events during the infusion, but some patients deteriorated during long-term follow-up.

The potential advantage of using autologous cells is avoiding immune reactions, especially if the cells differentiate over time. However, allogeneic cells are easier to develop and stock, resulting in higher availability, although they tend to show less anti-inflammatory response [34]. In older patients or those with specific conditions, autologous cells have limited expansion due to increased cellular senescence.

In this regard, one unexpected finding in the study was that six cultures in four patients yielded MSCs with chromosome aberrations and which were not appropriate for treatment. In our experience, these alterations are uncommon when BM-MSCs are obtained from patients with other diseases and raise the question whether bone marrow stromal cells in IPF patients have more genomic instability. An extensive analysis of chromosome aberrations in adult stromal cells reported a frequency of ~4% for MSCs [35], markedly lower than found in our cohort. As reported by others, the recurrence of trisomy 5 in repeated samples from three patients suggests this is a donor-dependent phenomenon [36, 37]. Features of senescence in BM-MSCs obtained in IPF patients have been reported previously [38] and may be involved in IPF pathogenesis [39]. Further studies are warranted to understand the mechanisms beyond IPF, including pulmonary and bone marrow stromal cell abnormalities. Meanwhile, we conclude that autologous bone marrow might not be a good source of MSCs in IPF.

The high incidence of chromosomal alterations seen after culture in IPF patients in the present study seems to limit the use of autologous cells and favour allogeneic cells for further studies.

Other important issues not considered in this discussion are the cells' different properties depending on their processing. Finally, and due to the main paracrine effect, the use of MSC-derived products has been advocated for exploration of potential immunomodulatory therapy in well-designed studies.

Limitations

Conclusions from this study cannot be easily extrapolated to current patients with IPF, since most patients will be receiving new antifibrotic therapies. In future studies, there might be a validation control group to prove the efficacy and comparative safety of the treatment. Newer studies should be conducted in patients on currently approved pharmacological treatments. Furthermore, half of the patients in this study did not complete the 12-month follow-up due to death (three patients), lung transplantation (one patient) and IPF progression precluding follow-up visits (two patients). However, all recruited patients were followed and checked for adverse events for $\geqslant$3 months.

This small phase I study was designed to evaluate safety and is not powered to detect significant lung function changes.

Conclusions

In a group of patients with IPF, the endobronchial infusion of BM-MSCs did not directly cause serious adverse events. Acute effects of endobronchial infusion were infrequent, transient and not serious, although this therapy might be challenging in severe patients, particularly with higher doses. Two patients developed cancer within the follow-up period, although it was not likely to be related to the treatment. At the end of the follow-up period, clinical progression of the disease or significant functional decline was high, as observed in eight out of 13 patients.

Finally, a relatively frequent genomic instability of MSCs is found during culture, which may preclude the use of autologous MSCs in IPF patients.

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