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Consecutive intrabronchial administration of Wharton's jelly-derived mesenchymal stromal cells in ECMO-supported pediatric patients with end-stage interstitial lung disease: a safety and feasibility study (CIBA method)

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Abstract

Background Patients ineligible for lung transplant with end-stage Interstitial Lung Disease (ILD) on Extra-Corporeal Membrane Oxygenation (ECMO) face an appalling prognosis with limited therapeutic options. Due to the beneficial effect of Mesenchymal Stromal Cells (MSC) on inflammatory, immunological and infectious diseases, cell therapy has been proposed as an option, but administration is hampered by the ECMO.

Methods Cryopreserved Wharton-jelly derived MSC (WJ-MSC) were conveniently diluted and directly applied consecutively on each lobule (5,1 ml = 10^7 cells) at a continuous slow rate infused over one hour via flexible bronchoscopy (Consecutive IntraBronchial Administration method, CIBA method).

Results Intrabronchial administration of MSC to a patient on ECMO was well tolerated by the patient even though it did not reverse the patient's ILD. This manuscript presents preliminary evidence from ongoing clinical trials

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program on Cell Therapy of Inflammatory, Immune and Infectious Diseases and, to our knowledge, is the first report of intrabronchial administration of MSC in a paediatric ECMO patient with ILD. Even more, MSC administered by this method do not reach the systemic circulation and do get blocked on ECMO membrane.

Conclusions Direct intrabronchial administration of MSC in a patient on ECMO is feasible and safe, and may be a new avenue to be assayed in ECMO patients with inflammatory, immunological and infectious diseases of the lung.

Keywords Cell therapy, Extracorporeal membrane oxygenation (ECMO), Mesenchymal stromal cells (MSC), Bronchoscopy, Interstitial lung disease, Bronchus associated lymphoid tissue (BALT)

Background

Intratracheal administration of Mesenchymal Stromal Cells (MSC) has been reported in preclinical studies [1–5], but there is limited data on their use in humans. Moreover, Extracorporeal Membrane Oxygenation (ECMO) support prevents intravenous administration since cells would be retained by the ECMO membrane [6]. Intravenous infusion of MSCs in ECMO patients poses significant challenges, including the risk of circuit obstruction due to cell size and aggregation, which could compromise ECMO function and patient safety [7]. Additionally, MSCs express tissue factor (TF/CD142), a potent activator of the coagulation cascade. Even in an anticoagulated ECMO setting, intravenous administration of MSCs may induce an undesirable coagulation response, potentially increasing the risk of thrombotic complications [8, 9]. While ECMO inherently prevents systemic MSC circulation by retaining cells in the membrane [10], this retention further exacerbates the challenges associated with IV administration. As an alternative, we explored an alternative method: direct and consecutive administration in each of the five lobes of the lungs, naming this new method slow consecutive intrabronchial lobe-by-lobe administration (**CIBA method**) (Fig. 1A).

The intrabronchial route offers the advantage of avoiding immediate systemic circulation, thereby minimizing the risks of ECMO obstruction and coagulation activation. Moreover, this method enables direct MSC delivery to the affected lung tissue, enhancing local therapeutic effects [11, 12]. Intratracheal administration of Mesenchymal Stromal Cells has been reported to treat bronchopulmonary dysplasia in neonates with a 2-year follow-up [13, 14] and in one adult aged 59 [15] in South Korea. Other clinical trials (see Table 1) did not report results nor provide a detailed description of the method. The article by Chang et al. [14] reports the results with a single patient, a 58 y old man who received a single infusion of 1×10^6 cells (kg and died at day + 3. To note that the PO_2/FiO ratio improved from 200 to more than 300. However he was not in ECMO. We could then say that this is the first intrabronchial administration in an ECMO pediatric patient. Additionally for the sake of the next trials we described our method in detail and give a list of biomarkers that may be used (Table 2). Table 1 summarizes the clinical trials included in the NIH Clinicaltrials.gov database.

To our knowledge this is the first report of intrabronchial administration of MSC in a paediatric ECMO patient with interstitial lung disease [5, 16]. So far there have been described five well-recognized routes for MSC delivery: intravenous, intramuscular, intralesional, topical and intra-arterial [17–19]. We introduce a new method allowing intrabronchial administration in ECMO patients.

MSC have become the most clinically studied experimental cell therapy platform worldwide, with more than 1500 clinical trials reported in the NIH Clinicaltrials.gov database. They have also become commercially available for graft-versus-host disease in South Korea and and more recently in the USA and for complex fistulae of Chron´s disease in Japan and Europe. Despite the numerous results published in Phase I-IIa and Phase II randomized clinical trials, only one cellular medicament based in MSC has been approved in Europe to treat complex fistulae of the Crohn´s disease [20, 21] and recently another in USA to treat pediatric graft vs. host disease. Although attempted in animal models and in humans (neonatal: less than 4 weeks old) for pulmonary bronchodysplasia via intratracheal administration, to our knowledge, this manuscript marks the first safe and feasible trial of selective intrabronchial infusion of mesenchymal cells in non-neonatal patients which opens an avenue for a new therapy on Inflammatory, Immunological and Infectious Diseases of the lungs.

Lungs, as the front line of contact with external agents, contain 7×10^{10} immune cells [22], most of them NK cells, mast cells, neutrophils, basophils, monocytes (including dendritic cells and macrophages), and B and T-cells. These immune cells play crucial roles in the lung's defense mechanisms and immune responses. Mesenchymal stromal cells (MSCs) interact with key immune cell populations such as alveolar macrophages (AM), M1 and M2 macrophages, and regulatory T cells (Treg). This interaction leads to the secretion of anti-inflammatory cytokines, including IL-10, which aids in mitigating pro-inflammatory cytokines like TNF-α and IL-6, thereby potentially preventing lung damage and promoting tissue repair (Fig. 1B).

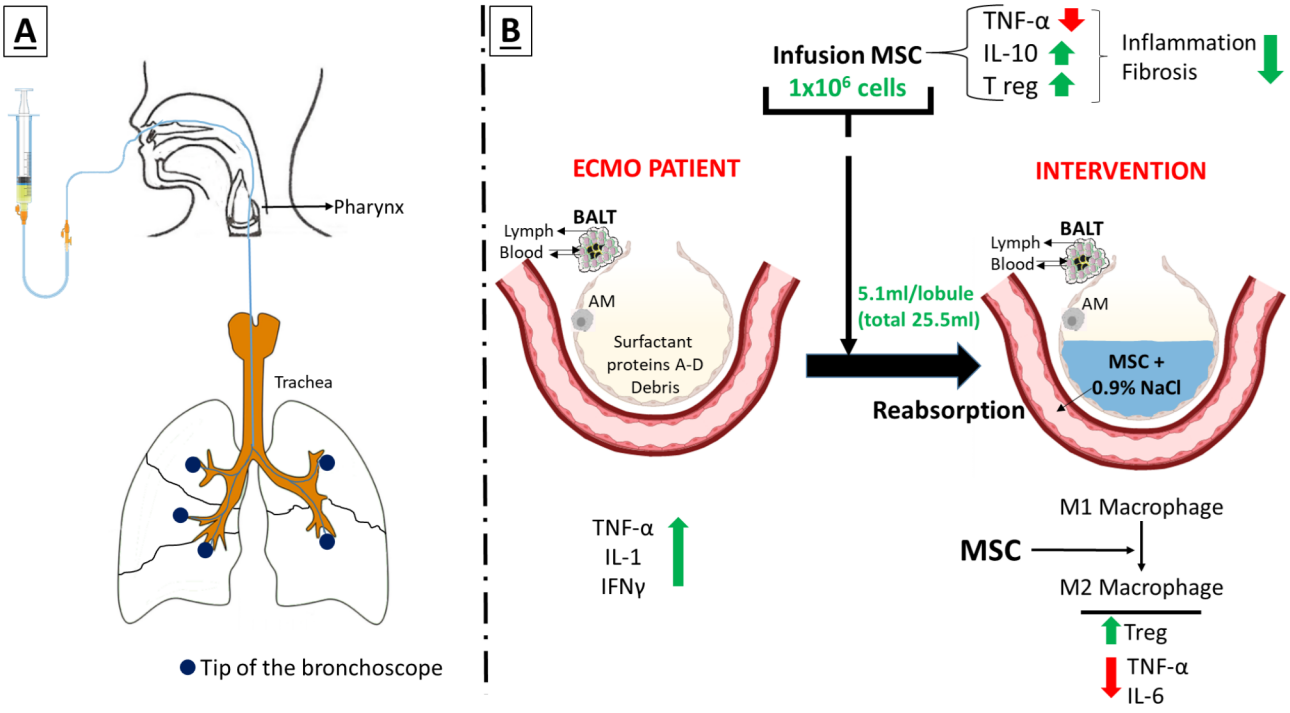

Fig. 1 CIBA Method and the Rationale for MSC administration. MSCs administered intrabronchyally using a bronchoscope (CIBA method) migrate through the lungs (**A**), where they interact with immune cells, including alveolar macrophages (AM), M1 and M2 macrophages, and regulatory T cells. This interaction leads to the secretion of anti-inflammatory cytokines (IL-10), which reduces pro-inflammatory cytokines (TNF-α and IL-6) (**B**). BALT: Bronchus Associated Lymphoid Tissue, ECMO: Extracorporeal Membrane Oxygenation, MSC: Mesenchymal Stromal Cells, TNF-α: Tumor Necrosis Factor α, IL-10: Interleukin 10, IL-6: Interleukin 6, Treg: Regulatory T cells, AM: Alveolar Macrophage, M1 and M2 macrophages

Table 1 Clinical trials reported in clinicaltrials.gov. ATMP: advanced therapy medicinal product. UC-MSC: umbilical cord mesenchymal stromal cells. NCT: National clinical trials number. BPD: bronchopulmonary dysplasia. ARDS: acute respiratory distress syndrome

Disease	ATMP	Phase	NCT number	Dose (cells/ kg)	Location	Results
Bronchopulmonary Dysplasia (BPD)	UC-MSC	I ($n=100$)	03683953	25×10^6	Guanddong, China	No Results reported
Bronchopulmonary Dysplasia (BPD)	UC-MSC	I ($n=180$)	0364555	20×10^6	Fudam, China	No Results reported
Bronchopulmonary Dysplasia (BPD)	UC-MSC	I (vs. saline) ($n=10$)	01207869	3×10^6	Taiwan, China	No Results reported
Bronchopulmonary Dysplasia (BPD)	Pneumostem UC-MSC	II Follow-up 6–24 months	04003857	10×10^6	Seoul, South Korea	No Results reported
Bronchopulmonary Dysplasia (BPD)	Pneumostem UC-MSC	I ($n=69$) Open label single center	01297205	A: 10×10^6 B: 20×10^6	Seoul, South Korea	[14]
Bronchopulmonary Dysplasia (BPD)	Pneumostem UC-MSC	I ($n=9$) Follow-up 2 years	01632475	Low: 1×10^7 High: 2×10^7	Seoul, South Korea	[13] Follow-up 2 years
Bronchopulmonary Dysplasia (BPD) Extremely Low Weight	UC-MSC	I ($n=12$)	02381366	Low: 1×10^7 High: 2×10^7	Chicago, USA	[16]
Bronchopulmonary Dysplasia (BPD) Extremely Low Weight	UC-MSC	II ($n=70$)	01828957	1×10^7	Seoul, South Korea	[17]
Adult ARDS patient	UC-MSC			Case Report Female 59 y old	Seoul, South Corea	[14]

In steady-state, PPARγ, GM-CSF, and TGFβ1 maintain a balance between pro- and anti-inflammatory signals, ensuring proper immune function in the lungs. PPARγ is involved in the differentiation and function of AMs, which plays a crucial role in maintaining lung homeostasis (Fig. 2). GM-CSF promotes the survival and function of AMs, while TGFβ1 regulates the development and function of regulatory T cells (Tregs) and type 2 innate lymphoid cells (ILC2s). During inflammation, the balance between these factors is disrupted, leading to an altered immune response. PPARγ, GM-CSF, and TGFβ1 work together to coordinate the appropriate immune response by regulating the function of various immune cells, such as AMs, dendritic cells (DCs), Tregs, and ILC2s. The rationale behind this treatment is the tolerogenic and immunomodulatory profile of MSC, which

Table 2 CIBA method follow-up. Biomarkers, role in disease and therapy, detection methods. This table presents key biomarkers of therapy response. BAL: Bronchoalveolar lavage. IL: interleukin. TNF: tumor necrosis factor. TGF: transforming growth factor. VGEF: vascular endothelial growth factor. MMP: matrix metalloproteinase. CXCL8: chemokine (C-X-C motif) ligand 8. SP-D: surfactant protein.D. CRP: C-reactive protein

Biomarker	Role in Disease and Therapy	Detection Method	References
IL-6	Pro-inflammatory cytokine, elevated in lung injury	BAL, Blood	[35]
IL-10	Anti-inflammatory cytokine, linked to MSC-mediated repair	BAL, Blood	[35]
TNF-α	Pro-inflammatory cytokine, indicates immune activation	BAL, Blood	[35]
TGF-β	Fibrosis marker, involved in tissue remodeling	BAL, Blood, Biopsy	[39]
VEGF	Angiogenesis factor, may indicate vascular repair	BAL, Blood	[37]
MMP-9	Matrix metalloproteinase, involved in ECM degradation	BAL, Blood	[37, 39]
CXCL8 (IL-8)	Neutrophil chemotactic factor, reflects inflammation	BAL, Blood	[39]
SP-D	Lung epithelial integrity marker	BAL, Blood	[37, 39]
CD4+/CD8+T-cell ratio	Immune balance indicator	Blood	[34]
Alveolar Macrophage Phenotype (M1/M2 ratio)	Inflammatory vs. anti-inflammatory response	BAL, Biopsy	[38]
C-reactive protein (CRP)	Systemic inflammation marker	Blood	[36]
Fibronectin	ECM turnover and fibrosis marker	BAL, Blood, Biopsy	[39]

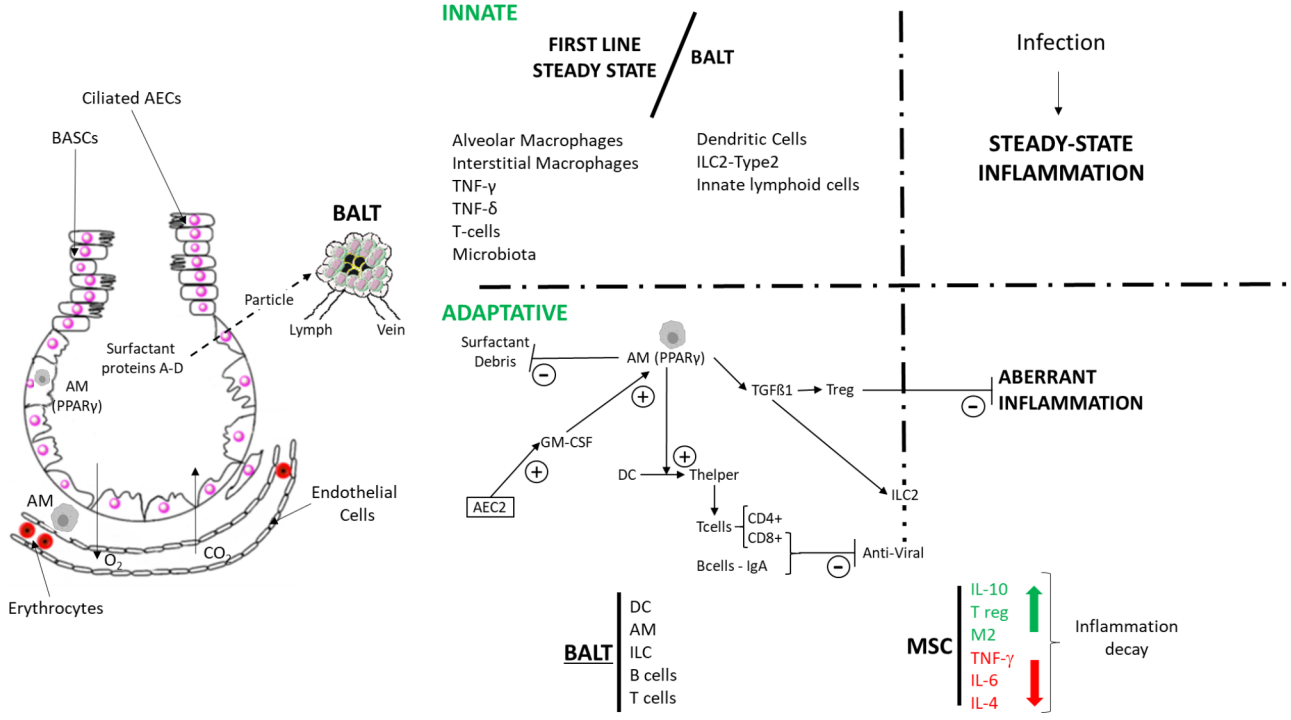

Fig. 2 Key host factors controlling tissue-specific lung immunity in the steady-state and in inflammation are shown including transcription factor PPARγ, GM-CSF, and TGFβ1. In the steady-state, PPARγ, GM-CSF, and TGFβ1 maintain lung homeostasis by regulating AMs, promoting their survival and function, and regulating the development of regulatory T cells and innate lymphoid cells. During inflammation, the disruption of this balance can lead to tissue damage and disease. TGFβ1: Transforming Growth Factor beta1; AEC2: type 2 Alveolar Epithelial Cell; BALT: Bronchus-Associated Lymphoid Tissue; BASC: Bronchoalveolar Stem Cell; TNF-γ: Tumour Necrosis Factor γ; TNF-δ: Tumour Necrosis Factor δ; AM: Alveolar Macrophage; DC: Dendritic Cell; GM-CSF: Granulocyte-Macrophage Colony-Stimulating Factor; PPARγ: Peroxisome-Proliferator Activated Receptor; TGFβ: Transforming Growth Factor β; Th: T helper; Treg: Regulatory T cell; M2: Macrophage M2; IL: Interleukin; ILC: Innate Lymphoid Cell

are able to elicit tolerogenicity in the lung by migrating through the injured alveoli, reaching the interstitial space and the Bronchus Associated Lymphoid Tissue (BALT). BALT is a lymphoid organ next to the alveoli that contains different immune cells, such as NK cells, dendritic cells, neutrophils and alveolar macrophages. These cells collectively orchestrate the appropriate immune response in the lungs, with the innate response provided by cells like neutrophils and natural killer cells, and the adaptive response mediated by Tregs and DCs (Fig. 2).

Use of MSC to treat lung diseases has been proposed for sepsis, acute respiratory distress syndrome (ARDS), lung injury and eronchiolitis [23–25]. We also used adipose tissue-derived MSC to treat lung

hyperinflammatory response in intubated and mechanically ventilated patients in COVID-19, no ECMO [26]. During the first wave of COVID-19 (March-May 2020) in Spain, the corresponding author of this report designed and coordinated a study using adipose tissue-derived MSC on intubated and mechanically ventilated patients in the Intensive Care Unit [26]. We used intravenous administration with a Swan-Ganz catheter in order to reach the pulmonary artery and deliver the MSC directly in the lung territory. As reported before we knew that MSC from type 2 diabetic patients could promote thrombosis [27] or an inflammatory response in animal models [28]. Even more, Moll et al., [9] pointed out the patient coagulopathy concerns since the intravenous infusion of MSC, which express variable levels of Tissue Factor (TF/CD142) could exacerbate coagulation issues, potentially triggering blood clotting. In the design of **CIBA method** we followed the guidelines and recommendations previously published [7–9, 11, 12]. Even more, we previously described that MSC could prevent neurological complications of radiotherapy [29]. With these preliminary data and the amount of data on the anti-inflammatory and regenerative properties of MSC we estimated that MSC could have a beneficial effect of our patient. Figures 1 and 2 depict how MSC migrate and, given this method of administration (CIBA method) do not end with pulmonary congestion or even with lung edema.

Given that all prior immunosuppressive therapies had been unsuccessful in this patient and MSC have demonstrated their tolerogenic and immunosuppressive effects when no alternative therapy is available, we decided to explore the compassionate usage of MSC. Previous research has shown that administering MSC can induce a reconfiguration of the immune system, including the regulation of M2 macrophage polarization, the production of immunosuppressive factors, and the inhibition of the production of pro-inflammatory cytokines such as IL-2, IL-6, IL-10, PGE2, TGF-β, HGF, CCL-2, and IFN-α [30–33]. In addition, umbilical cord-derived mesenchymal stromal cells (UC-MSC) exhibit trophic and regenerative properties, showcasing benefits in other diseases, such as models of type 1 diabetes [32]. This study aims to comprehensively explore the potential tolerogenic and immunosuppressive effects of MSC in refractory cases, focusing on immune system modulation through M2 macrophage polarization regulation, immunosuppressive factor enhancement, and pro-inflammatory cytokine inhibition. Future evaluation studies should include biomarkers of immune profile. Table 2 summarizes the most relevant and the detection methods [34–39].

Methods

Patient and study design

We report the case of a 2-year-old patient with past history of pineoblastoma in remission after chemotherapy, three autologous stem cell transplantation and proton beam therapy who developed Interstitial Lung Disease (ILD) requiring ECMO. We anticipated that the infusion of MSC will induce a shift in macrophage polarization, transforming M1 macrophages (pro-inflammatory) into M2 macrophages (anti-inflammatory). Simultaneously, this process was expected to decrease the levels of TNF-α while increasing IL-10 and regulatory T cells (Treg). The ultimate outcome of these cellular responses anticipated to be a reduction in inflammation and fibrosis. Our hypothesis was based on previous observations with COVID-19 critical patients with pneumonia and lung hyper-inflammation. Infusing adipose-derived mesenchymal stromal cells directly into the lung territory using a Swan-Granz catheter resulted in a significant improvement in clinical conditions, leading to reduced mortality, inflammatory markers, and partial resolution of lymphopenia [26].

Ethics and regulatory approvals

Following advice from the European Medicines Agency (EMA) on Advanced Therapy Medicinal Products (ATMP), we obtained informed consent from the patient's family, and Compassionate Use permission from the Spanish Agency of Medicines and Medical Devices (AEMPS) (Permission number SLC59890178876, August 16, 2023).

Cell Preparation and characterization

Wharton-Jelly-derived Mesenchymal stromal cells (WJ-MSC) were harvested from specifically donated umbilical cord tissue following previously described GMP-compliant procedures [40], in the clean room of the Banc de Sang I Teixits de Catalunya (BSTC). Briefly, clinical-grade WJ-MSC were obtained using a two-tiered cell banking system comprising an initial Master Cell Bank (MCB), and a second cell bank (working cell bank or WCB). Immediately after cell expansion, WJ-MSC were cryopreserved and stored in cryovials containing $2.5 \times 10^7 \pm 20\%$ cells in a solution containing Plasmalyte 148 (Baxter, Deerfield, IL, USA) supplemented with 4% w/v human serum albumin (Grifols, Barcelona, Spain) and 10% v/v dimethyl sulfoxide (DMSO) (Cryoserve, Bioniche Pharma, Lake Forest, IL, USA) according the AEMPS-PEI number 16/017.

For administration, cells from one cryovial of a released WCB batch were rapidly thawed in a 37 °C water bath, then slowly diluted 1:10 using pre-cooled thawing solution consisting of 10% (w/v) albumin in Plasmalyte 148. DMSO was washed out by centrifugation and the

solution re-suspended in the desired volume and transported at 4 °C to the clinical center for infusion in the following 24 h. The Pharmacy Unit of the University Hospital *12 de Octubre* (Madrid) received one bag of 25.4 mL containing a cellular suspension of 1.2 million of WJ-MSC per kg of the patient. The viability of the product was 93.28%. Phenotype specifications of flow cytometry and endotoxin were compliant. Microbiological and particle environmental control in the production area were compliant. The entire product was distributed in two 20 mL syringes under sterile conditions in a laminar flow hood up to 25.4 mL.

CIBA method

MSC were slowly administered by Consecutive Intrabronchial lobe-by-lobe Administration (**CIBA method**-see Fig. 1). Before intrabronchial administration of MSC, the patient was spontaneously breathing on nasal cannulas with low flow oxygen therapy with peripheral Veno-Venous ECMO (VV-ECMO) support. He was electively intubated, sedated and muscle relaxed to prevent any movement during infusion and for the following 48 h. In view of the cleanness of the airway and to minimize procedural risks, no additional bronchoalveolar lavage prior to the infusion was performed. Cells were administered through a videobronchoscope Olympus BF-XP190 3.1 mm, with a final diameter of approximately 1.2 mm, consecutively into the 5 lobes (5.1 mL/lobe, total volume 25.5 ml). Intrabronchial administration of MSC dissolved in physiological solution and with 1% DMSO was successfully performed. The total volume of the product was infused very slowly during 1 h and 5 s (0.42 mL/min, approximately 12 min by lobe) consecutively lobe-by-lobe (Fig. 1). Intrabronchial administration of WJ-MSC (1.2×10^6 cells/kg; body weight: 11 kg) was performed 87 days after ECMO implantation. Patient remained sedated and muscle relaxed, avoiding any airway suctioning, for 48 h following the procedure.

Lung computerized tomography

Prior to MSC administration, the patient underwent comprehensive computed tomography to assess the extent of disease and guide treatment planning. Transverse scans were performed (Fig. 3). No signs of fibrosis were observed, in contrast, the patient show bronchiectasis and interstitial lung disease.

X-ray imaging

The patient underwent serial chest X-rays to monitor disease progression and response to treatment. Standard postero-anterior views were acquired with the patient in an upright position. Exposure parameters were adjusted based on patient size to optimize image quality while minimizing radiation dose. Images were obtained at baseline prior to MSC administration, and at regular intervals during the patient's hospital course.

Results

Patient history and treatment protocol

A 2-year-old boy weighing 11 kg on VV-ECMO for support with refractory ILD with localized pineoblastoma diagnosed at 7 months of age was diagnosed, treated and followed by the Pediatric Oncology Unit of the University Hospital *12 de Octubre* (Madrid). Pineoblastome was partially resected, cerebrospinal Fluid (CSF) cytology showed no malignant cells and he received treatment according to protocol CCG-99703 [41]. After induction treatment, the subsequent evaluation revealed complete remission. Afterwards, three consolidation cycles of high-dose chemotherapy (carboplatin and thiotepa) with stem cell rescue were performed. The only relevant complication observed during treatment was a persistent lower respiratory infection caused by metapneumovirus. Craniospinal Magnetic Resonance Imaging (MRI) showed persistent complete remission at the end of treatment.

Proton beam therapy and Immunomodulatory treatment

Although the patient responded exceptionally well to the initial treatment, the prognosis remained poor. Consequently, we opted for a more targeted approach, administering local proton beam therapy to improve the patient's outcome and scheduled metronomic and antiangiogenic treatment, including oral thalidomide, celecoxib, fenofibrate, and intravenous bevacizumab (combined with alternating cycles of oral VP-16 and cyclophosphamide), to begin 6 months after the last dose of high-dose Concurrent Chemotherapy (CT) [42]. Five weeks after starting metronomic chemotherapy, he developed a lower respiratory infection caused by rhinovirus with mild respiratory distress and hypoxemia (Sat O_2, 89%). This was resolved after treatment with inhaled salbutamol and oral prednisone.

One week later, the patient experienced a recurrence of moderate respiratory distress and hypoxemia again. Viral screening was negative, and despite restarting salbutamol and prednisone, polypnea and hypoxemia worsened. Lung computerized tomography revealed diffuse pulmonary parenchymal involvement, consisting of extensive ground-glass opacities of panlobular distribution and a few poorly defined centrilobular nodules scattered in both lungs, with discrete pneumomediastinum, indicative of interstitial pneumonitis (Fig. 3).

Infection was ruled out, and despite receiving several pulses of steroids, there was no improvement. Following a diagnostic bronchoalveolar lavage, the patient experienced worsening respiratory distress, necessitating orotracheal intubation. However, connecting him to

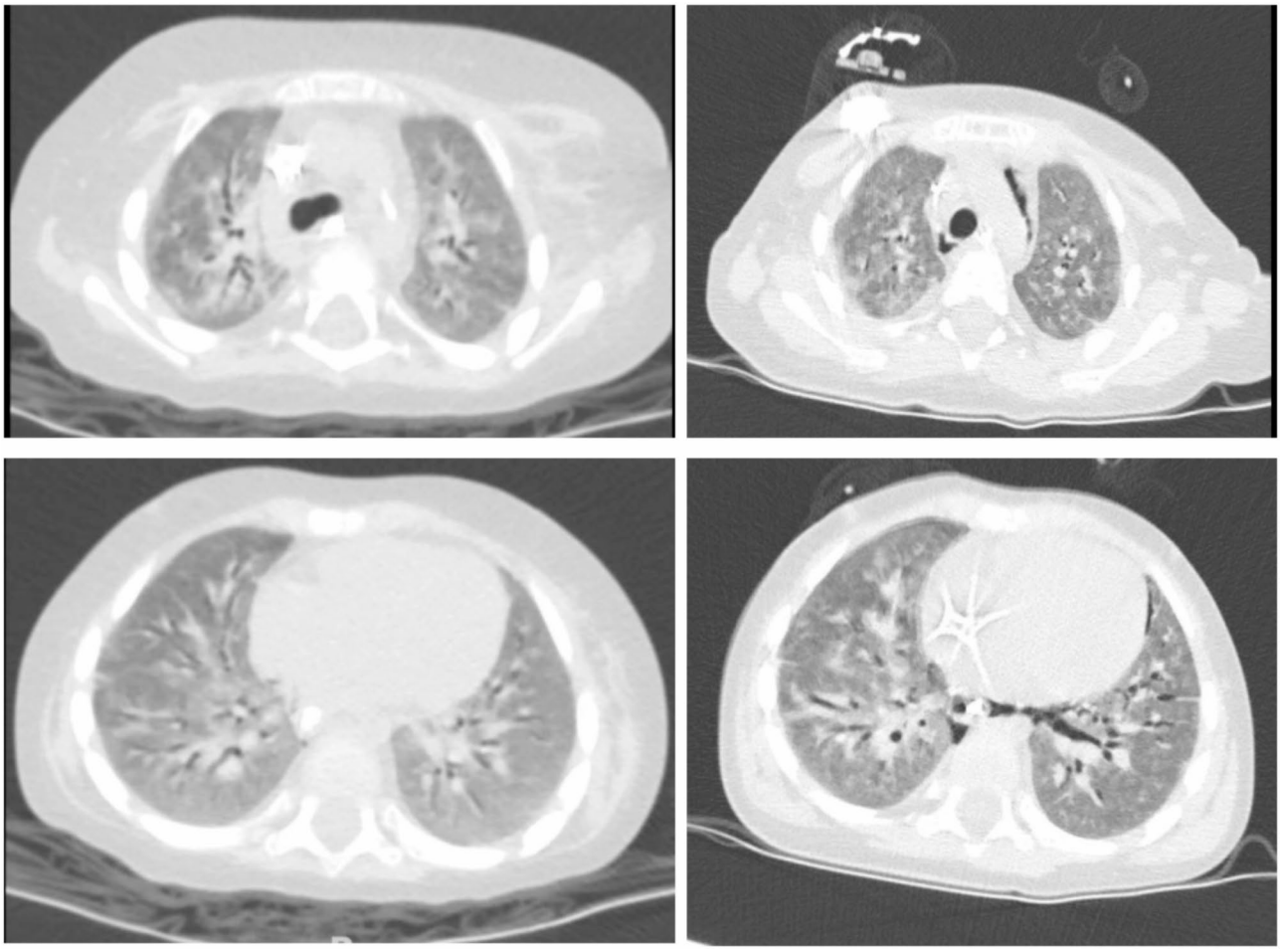

Fig. 3 Extensive panlobar ground-glass opacities and some ill-defined centrilobular nodules. Slight pneumomediastinum, minimal anterior pneumothorax. Reduced lung volumes; pulmonary involvement with an extensive ground-glass pattern, diffuse bilateral distribution with perihilar involvement, and also in the subpleural peripheral region. No signs of fibrosis. Dilation of the tracheobronchial tree, with a rosary-like morphology of the bronchi related to bronchiectasis/bronchiolectasis

Invasive Mechanical Ventilation (IMV) proved impossible due to his restrictive pathology, requiring very high peak pressures which led to the appearance of pneumomediastinum.

Emergent Veno-Arterial ECMO support

Given the frailty of his respiratory condition, the indication for ECMO prior to BAL (bronchoalveolar lavage) was discussed by the mutidisciplinary team. It was decided in favour of the indication either as a bridge to a possible cure in a barely treated and un-diagnosed lung injury, or as a bridge to decision until irreversibility and absence of therapeutic possibilities were established in a neurologically intact patient with an exceptional response to oncologic treatment of the underlying tumor. In any case, the need for continuous re-evaluation to avoid futility was clear from the onset.

In response to the critical situation, with oxygen saturation ranging from 75 to 80% and hypercapnia reaching 100 mmHg, the decision was made to initiate emergent Veno-Arterial (V-A) ECMO support while manually ventilating the patient. The patient was maintained on minimal ventilator settings (PEEP 4 cm H_2O, IP 4 cm H_2O, FiO_2 0.5), with resolution of the air leak. Four days later, the patient was transitioned to Femoro-Jugular Veno-Venous (V-V) ECMO. Simultaneously, intensive immunosuppressive treatment was commenced, beginning with high-dose steroids followed by rituximab and azathioprine. Subsequently, ruxolitinib was introduced, but no improvement was observed.

After an initial period of sedation and muscle relaxation, various ventilator strategies were attempted in an effort to wean the patient from ECMO, including Neurally-Adjusted Ventilatory Assist (NAVA). However, it was not possible to withdraw ECMO due to increased respiratory distress and hypercapnia. Two months later, the patient was extubated remaining on spontaneous

ventilation with low flow oxygen therapy and ongoing V-V ECMO.

Lung biopsy and genetic testing

After two months under ECMO support, due to the absence of respiratory improvement, a lung biopsy (pulmonary wedge) was performed, revealing the expansion of all interalveolar septa due to an inflammatory cellular response, primarily consisting of polymorphonuclear lymphocytes and occasionally eosinophils. Gomori's trichrome stain did not identify fibrotic expansion, but focal smooth muscularization of septa was observed, notably highlighted with smooth muscle actin. Some alveolar spaces were enlarged and rounded. The alveolar maturation state was generally appropriate, although in more peripheral areas, alveoli with reduced air space and hyperplasia of type 2 pneumocytes were evident. Thickening of pre-acinar arterial muscular walls was observed within bronchiole-vascular axes, without involvement of intra-acinar arteries. The final diagnosis was mixed pneumonitis, as there were neither granuloma nor clear signs of vasculitis.

Laboratory tests, including immunoglobulin quantification and assessment of lymphocyte subsets (T, B, and NK cells), did not raise suspicion of a hidden inborn error of immunity. To comprehensively investigate this aspect, as well as other monogenic interstitial lung diseases and cancer predisposition syndromes, whole exome sequencing revealed no germline pathogenic variants in RB1, DICER1, ADA2, STAT1, STAT2, STAT3 and those related to lung fibrosis or interstitial lung disease (100 genes).

Lung transplant eligibility was ruled out following discussion with reference centers due to recent malignancy history. Given the patient's unresponsiveness to prior immunosuppression protocols and the ongoing requirement for V-V ECMO due to poor oxygenation resulting from pulmonary interstitial disease with an inflammatory component, and considering the continued complete remission of the underlying disease, we proposed administering intrabronchial mesenchymal stromal cells, with a single dose of one million cells per kilogram of the patient's body weight.

CIBA method administration

Three months after ECMO initiation, the patient was reintubated under sedoanalgesia and muscle relaxant for the procedure. Most commonly used intravenous administration for MSC was disregarded to prevent both potentially fatal clotting of the ECMO circuit as well as loss of therapeutic effect from the preferential binding of MCS to artificial membranes, and MSC were administered as described in Methodology Sect. (2.4 CIBA method).

The procedure was well-tolerated, aligning with the expectations based on data from mouse models [1–4, 6] and previous clinical data [13, 14, 43, 44]. The patient remained stable throughout, with no adverse reactions observed. Sedation and muscle relaxants were subsequently reduced with excellent tolerance. After 72 h, the patient was successfully extubated. For a full comparison among chest X-ray images see Fig. 4.

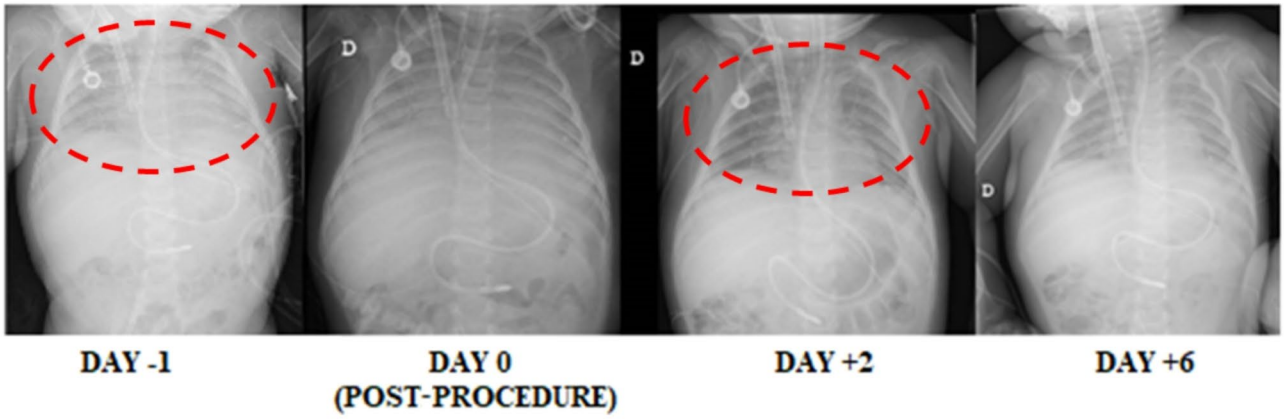

Fig. 4 Day − 9: Decreased lung volumes with bilateral opacities. Poor pulmonary aeration in the context of alveolo-interstitial involvement and ECMO. Day 0: Radiological deterioration signs consist of a greater extension of bilateral pulmonary opacities with air bronchograms, likely secondary to endotracheal infusion. Endotracheal tube placement is projected over the lower vertebral body of D3. The rest of the devices (ECMO cannula, nasogastric tube, weighted tube, and right jugular Port-a-Cath catheter) show no significant changes. Day + 2: ETT (EndoTracheal Tube) less advanced than in the previous X-ray, with the tip at the level of T1-T2. The rest of the devices (ECMO cannula, nasogastric tube, weighted tube, and right jugular Port-a-Cath catheter) show no significant changes. Lung volumes remain decreased. Compared to the previous examination, there is an improvement in aeration, with some less extensive opacities persisting and a reduction in areas of air bronchograms Day + 6: Limited lung volumes. No new pulmonary opacities. Surgical chain at the left pulmonary base. Abdomen shows no pathological findings. Other external devices: Veno-Venous ECMO cannulas, nasogastric tube in the gastric body, and weighted tube in a transpyloric position. The red dashed line highlights the differences between day − 1 and day + 2, suggesting transient amelioration following treatment

Four weeks after intrabronchial infusion of MSC ECMO gas flow was gradually reduced until closure, with poor tolerance, resulting in increased respiratory drive, progressive hypercapnia, and increased respiratory distress. Following this attempt, the patient's radiological condition worsened, with poor pulmonary aeration and sustained tachypnea, which was unresponsive to increased ECMO assistance.

Under these circumstances and without further treatment options and ineligibility for lung transplant, redirection of care was agreed following a multidisciplinary meeting with the family. After 127 days of ECMO support, the patient was disconnected from ECMO and passed away surrounded by his family.

Discussion

Stem cells sourced from bone marrow, umbilical cord, adipose tissue, or dental pulp among others, are able to migrate towards the injured tissues, reducing inflammation and promoting tissue restoration. Additionally, they can enhance the natural function of progenitor cells within the lung [45]. Over the last decade, intratracheal infusion has been used in the setting of bronchopulmonary bronchodysplasia in neonates [15]. The initial trial involved preterm neonates born at 25 weeks of gestational age. MSC were administered 10 days after birth. Patients were divided into two groups (10×10^6 cells/kg in three of them and 20×10^6 cells/kg in 6 patients). No significant adverse events were observed with a decrease in the Severity Respiratory Score along with a reduction of inflammatory markers (IL-6, IL-8, matrix metalloproteinase-9, TNF-α, TGD-1B) in the tracheal aspirates. The administration was performed using a 22G needle via the endotracheal tube [14]. Subsequently, the same group demonstrated safety, and no difference in growth and neurodevelopment compared to control groups at 2 years of follow-up [13].

In adults, intratracheal administration has been reported only once in a 59 year old female with acute respiratory distress syndrome (ARDS) and pulmonary fibrosis [15]. She achieved immediate improvement in terms of PaO2/FiO2 ratio, chest imaging, lung compliance and mental status within the first three days. Unfortunately, the patient died due to unrelated causes [15]. In a single paediatric case report with ARDS due to adenoviral pneumonia, the patient improved after intratracheal infusion of 6.25×10^6 cells/kg; with a total dose of 5×10^7 cells [46].

In the patient reported here, due to the uncertain diagnosis, the patient underwent a lung biopsy. This procedure, deemed feasible even under ECMO assistance, aimed to establish a potential diagnosis and provide a rationale for potential therapies [47, 48]. The biopsy results indicated a mixed pulmonary pathology with toxic and inflammatory characteristics, which justified the initiation of immunosuppressive therapy initially, and, as a last resort, regenerative and immunomodulatory therapy with MSC. Considering the risk associated with intravenous MSC infusion in patients under ECMO support, as previously reported [6] with a rapid decline in oxygenator performance, we explored the alternative intrabronchial route (**CIBA method**). This approach was chosen to ensure effective cell delivery while minimizing these risks. By directly targeting the injured lung tissue, the intrabronchial route aligns with previous studies on intra-tracheal MSC administration for pulmonary diseases [11, 12].

Despite limited knowledge regarding the technicalities of this route, we optimized the procedure for safety and intended efficacy. Another concern was the direct effect of DMSO on the lung. While DMSO has been found to have respiratory toxicity, it has also been found to have potential therapeutic effects in certain conditions, such as acute lung injury [49, 50]. We deliberately reduced the DMSO content through centrifugation and, fortunately, did not observe any attributable toxic effect.

Due to lack of authorization by the Spanish Agency of Medicines and Medical Devices (AEMPS), the administration of alternative products like aerosolized allogeneic secretome or extracellular vesicles was not possible. Consequently, we sought to deliver the cellular product in proximity to the alveoli [51, 52] through intrabronchial administration. It is known that MSC are typically cleared from the lungs within a few days after administration [53, 54]. Nevertheless, this clearance may not impede site-specific effects, as evidenced in other administration routes such as intraperitoneal, where therapeutic effects are initiated despite MSC clearance [32]. The patient tolerated the infusion well for a period of four weeks, as expected based on the data from the humanized mouse model [55] and previous human data. In rats, the model of acute lung injury induced by bleomycin demonstrated promising results [56].

Conclusions

This case demonstrates that administering intrabronchial MSC in a patient on ECMO support is both feasible and safe, despite the procedure not reversing the patient's interstitial lung disease. To the best of our knowledge, this is the first report of intrabronchial MSC administration in a pediatric patient on ECMO. It is relevant to note that ECMO filter capacity was not affected by administration of MSC directly into the lungs (**CIBA method**), thus MSC did not reach the circulation. Other important questions, such as the potential for multiple doses, increased dose to 1×10^7 cells/kg or early administration to prevent the development of established and irreversible interstitial lung disease, warrant further investigation

Table 3 Clinical trials on advanced therapies medicinal products in adult patients with inflammatory, immunological and infectious diseases using intravenous administration of ATMPs. COPD; chronic obstructive pulmonary disease. ILD; interstitial lung disease. RSV; respiratory syncytial virus. ARDS; acute respiratory distress syndrome. Taken from clinicaltrials.gov, last accessed Feb. 18th, 2024

Lung Diseases		
Inflammatory	**Immunologic**	**Infectious**
COVID-19 (NCT04753476)	Systemic Sclerosis (NCT06058091)	COVID-19 (NCT02924818, NCT04392778)
Cystic Fibrosis (NCT02924818)	Respiratory Bronchiolitis ILD (NCT06058091)	Influenza-A (NCT04896853)
COPD (NCT02924818)	Sjögren Syndrome (NCT06058091)	Metapneumovirus (NCT04896853)
ILD (NCT02924818)	COVID-19 (NCT04720612, NCT06166030)	RSV Infection (NCT04896853)
	Primary Immunodeficiency (NCT06092528)	ARDS (NCT04366830)

in future research. It is tempting to speculate whether earlier and/or repeated doses may result in better clinical outcomes in the Case reported.

Thus, this technique allows targeted distribution to the alveoli, maximizing therapeutic effects and reducing systemic hazards, especially for individuals suffering from acute lung damage, ARDS, or bronchopulmonary dysplasia. Although MSC clearance from the lungs occurs within days, sustained site-specific effects suggest promise for broader application. Although more study and regulatory approval are required, these findings show that intra-bronchial injection has the potential to be a novel and successful approach for regenerative and immunomodulatory therapy in pulmonary diseases.

This observation, limited by the clinical conditions of our patient, open the door to the use of CIBA to administer Advanced Therapies Medicinal Products in adult patients with Inflammatory, Immunological and Infectious Diseases (ILD, ARDS and other pathologies) (see Table 1) and ECMO [57]. For example, in inflammatory airway diseases. Table 3 summarizes registered clinical trials in which ATMPs were administered intravenously.

This manuscript presents prospective proof of data which undergoes the design of a Clinical Trial still under way by four institutions: Bioengineering Institute of University Miguel Hernández (Elche, Spain), ISABIAL- Institute of Biomedical Research of the Dr Balmis University Hospital of Alicante (Alicante, Spain), University Hospital *12 de Octubre* (Madrid, Spain) and *Banc de Sang I Teixits de Catalunya* (Barcelona, Spain).

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Author contributions
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Data availability
All data is included in the manuscript. Availability of data and materials are all included in the manuscript.

Declarations

Ethics approval
University Hospital *12 de Octubre* (Madrid, Spain) Ethical Committee and AEMPS Permission number SLC59890178876, to the project entitled "Consecutive IntraBronchial Administration of Wharton's Jelly-Derived Mesenchymal Stromal Cells in ECMO-Supported Pediatric Patients with End-Stage Interstitial Lung Disease: A Safety and Feasibility Study (CIBA Method)".

Informed consent
Informed consent was obtained from all subjects involved in the study.

Conflict of interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Institutional review board statement
The study was conducted in accordance with the Declaration of Helsinki, and following advice from the European Medicines Agency (EMA) on Advanced Therapy Medicinal Products (ATMP), we obtained informed consent from the patient's family, and Compassionate Use permission from the Spanish Agency of Medicines and Medical Devices (AEMPS) (Permission number SLC59890178876, August 16, 2023).

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