The First Allogeneic Transplantation of Human Umbilical Cord-derived Mesenchymal Stem/stromal Cells for Bronchopulmonary Dysplasia: Preliminary Outcomes in Four Vietnamese Infants

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Research

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Abstract

Background: Bronchopulmonary dysplasia (BPD) is a severe condition in premature infants that compromises theirlung function and necessitatesoxygen support. Despite major improvements in perinatal care minimizing the devastating effects, BPD remains the most frequent complication of extreme preterm birth. Our study reports the safety ofthe allogeneic administration of umbilical cord-derived mesenchymal stem/stromal cells (allo-UC-MSCs) and the preliminary efficacy of the treatment in four infants with established BPD.

Methods: UC tissue was collected from a healthy donor, followed by propagation at the Stem Cell Core Facility at Vinmec Research Institute of Stem Cell and Gene Technology. UC-MSC culture was conducted under xeno-free and serum-free conditions. Four patients with established BPD were enrolled in this study between May 25, 2018, and December 31, 2018. All four patients received two intravenous doses of allo-UC-MSCs (1 million cells/kg patient body weight (PBW) per dose) with an intervening interval of 7 days. Safety and efficacy were evaluated during hospitalization and at 7 days and 1, 6 and 12 postdischargemonths.

Results: No transplantation-associated severe adverse events or prespecified adverse events were observed in the four patients throughout the study period. At the time of this report, all patients had recovered from BPD and been weaned off of oxygen support. Chest X-rays and CT scans confirmed the dramatic reduction infibrosis.

Conclusions: Allo-UC-MSC transplantation is safe and might improve respiratory function anddecrease lung fibrosis in preterm infants with established BPD.

Trial registration: This preliminary study was approved by Vinmec International Hospital Ethics Board, approval number: 88/2019/QĐ-VMEC, registered 12 March 2019 - retrospectively registered.

Background

First discovered in 1967, bronchopulmonary dysplasia (BPD) has since emerged as the most prevalent chronic lung disorder in premature infants, resulting in reductions in alveolarization, vascular growth and overalllung function (1). The pathologic hallmarks of BPD are hyperoxia-induced pulmonary inflammation and tissue degeneration, resulting in significant cell death and culminating in impaired and immature alveolarization and dysregulated vascularization of the lung, finally leading to the formation of a fibrotic lung (2). These patients require prolonged mechanical ventilation and oxygen support (3). Infants with gestational ages younger than30 weeks are at particularly high risk of immature development of the respiratory system and suffer from detrimental long-term outcomes includinghigh morbidity and mortality rates. In the last 50 years, advances in neonatal medicine, including the discovery of neonatal steroid treatments (4, 5), surfactants (6-8), gentle ventilation treatments(9, 10), and effective noninvasive ventilation devices, have significantly improvedthe clinical outcomes inpremature newborns with BPD. However, the rates of complications and mortality are still high among infants with BPD (11).

Recently, stem cell transplantation was used to treatBPD in an animal model. Proof-of-concept experiments in neonatal BPD rodent models demonstrated that the injection of bone marrow mesenchymal stem cells (BM-MSCs) via either the intravenous or intratracheal route exertedlung-protective functions, including reductions inf lung inflammation and pulmonary hypertension and reformation of the alveolar structure, leading toa subsequent improvement in the survival rate (12-15). Furthermore, a single dose of human UC-MSCs administeredintratracheally prevented and rescued neonatal rats from hyperoxia-induced lung damage(16). In humans, Ahn and colleagues conducted the first phaseI clinical trial using umbilical cord blood-derived MSC (UCB-MSC) transplantation to prevent the manifestation of BPD in premature infants in 2014. Their results confirmed that UCB-MSC transplantation was safe and could reduce the risk of BPD in premature infants (17). In 2017, our group reported the first patient with established BPD treated successfully withautologous bone marrow mononuclear cells (18). However, obtaining bone marrow from established BPD newborns is a challenging task and carries a major risk of complications, especially infection. Therefore, utilizing the immune-privilege feature of UC-MSCs as allogeneic cell sources, from May 25, 2018, to December 31, 2018, we performed UC-MSC transplantations for four patients with established BPD in Vietnam. This study reports the safety and initial outcomesof UC-MSC transplantation inthose patients.

Methods

Ethics: This study was approved by the Scientific and Ethics Committee of Vinmec International Hospital (approved number: 88/2019/QĐ-VMEC). Written consent was obtained from both the donor of the umbilical cord and from the patients' parents.

Donor screening criteria for UC tissue

Healthy women with an uncomplicated, at term pregnancy underwent serological testing, including tests for HIV, cytomegalovirus (CMV), Epstein-Barr virus (EBV), hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis, and chlamydia, at 38 weeks of pregnancy. The umbilical cord tissues were collected at delivery and transferred to the laboratory for further processing.

Allo-UC-MSC preparation

The UC sample was processed at the Stem Cell Core Facility at the Vinmec Research Institute of Stem Cell and Gene Technology under ISO 14644-1 (certification number: CR61119-1). Culture reagents were purchased from Thermo Fisher Scientific (https://www.thermofisher.com/) and Pan Biotech (PowerStem MSC1 culture media, P04-77355K, hereinafter MSC culture media), unless stated otherwise. hUC-MSC cultures were conducted under xeno-free and serum-free conditions at 37°C in a humidified incubator containing 5% CO_2. The medium was changed every 3 days until the culture reached 80% confluence, followed by passaging using CTS^{TM}TrypLE^{TM} Select (A1285901). Aliquots of hUC-MSCs at P3 were thawed inCTS™ CELLstart™ substrate-coated flasks and cultured using TryPLE passaging in xeno-free and serum-free MSC culture media; under these conditions, hUC-MSCs were routinely passaged by

incubation with 1X CTS™TrypLE™ Select for 4 minutes at 37°C to liberate single cells or, preferably, small clumps of cells and subcultured for further expansion at a seeding density of 5000 cells/cm^2. Following the absence of the detection of bacteria, fungi, mycoplasma, and endotoxin, the cells were suspended in 10 ml of NaCl 0.9% (Braun, USA) at a final dose of $1x10^6$ cells/kg PBW.

Product release criteria

To generate and release the final product, hUC-MSCs at P5 were freshly harvested and subjected to a quality control process including (1) cell enumeration, (2) cell viability measurement (>85%), (3) hMSCmarker analysis by a Naviosflow cytometer system (Beckman Counter) using a human BD Mesenchymal Stem Kit (562245, BD Biosciences), (4) microbiological tests for sterility, (5) test for mycoplasma, (6) determination of the endotoxin level, (7) karyotyping, (8) CFU assay, and (9) trilineage differentiation using StemPro™ Adipogenesis (A1007001), StemPro™ Chondrogenesis (A1007101), and StemPro™ Osteogenesis Differentiation (A1007201) kitsaccording to the manufacturing protocols. Oil Red O, Alicante Blue, and Alizarin Red S stains were used to specifically stain adipocytes, chondrocytes, and osteocytes, respectively.

Patient enrollment

Four premature infants who required oxygen support for more than 28 days with findings of lung fibrosis on chest CT were enrolled in the study. Prior to intervention, all patients underwent a thorough clinical examination with blood gas analysis, total blood count and chest CT. BPD diagnosis was determined as previously described, including preterm birth (at less than 37 weeks), evidence of respiratory distress syndrome, and the need for oxygen support higher than 21% for up to 56 days in preterm infants (> 32 weeks) and more than 36 weeks of age in preterm infants less than 32 weeks of age (19).

Mode of cell administration

All four patients received two transplantations of allo-UC-MSCs at a dose of 1 million cells/kg patient's bodyweight (PBW) with a 7-dayintervening interval via the intravenous (IV) route. On the day of infusion, harvested cells at the targeted dose were prepared in 10 mL of 0.9% NaCl (Braun, USA) and delivered to the transplantation ward for IV infusion at a rate of 20 mL/hour.

Outcome measures

To assess safety, any major or minor adverse events during the stem cell infusion (72 h) and during the 7 days after transplantation were monitored. Body temperature, blood pressure, respiratory rate, heart rate, and SpO2 were recorded regularly. All four patients were requested to attend re-examination at the hospital at 7 days, 1 month, 6 months and 12 months after discharge. At each visit, a full clinical assessment was performed, including measuring the infant's height and body weight. All medication, home oxygen therapy, and rehospitalization since the last visit were documented. SpO2 and arterial blood

gas analysis (ABG) were examined at baseline and at each visit. Chest X-rays and CT scans were performed prior to transplantation, at the 6-month visit (CT scan), and at the 12-month visit (chest X-ray).

Statistical analysis

The data were analyzed using one-way ANOVA with Prism GraphPad software unless otherwise stated. ANOVA was performed to compare the means of the four patients as indicated in the test. Statistical significance was defined as $P<0.05$ unless otherwise indicated.

Results

hUC-MSC characterization

Our data showed that the UC-MSC line exhibited plastic adherent properties and a spindle- and fibroblast-like morphology (Figure 1A), with a population doubling time of 24 ± 0.6 hours (n=3, mean ± SEM). Propagation of UC-MSCs up to passage 6 did not introduce any karyotypical abnormality, and the cells maintained normal 46XY as indicated by the G-banding technique (Figure 1B). These cells were also able to form 519 ± 80 CFU/1000 cells (mean ± SEM, n=3) (Figure 1C). Further analysis of the differentiation potential confirmed that the UC-MSC line could undergo adipogenic, chondrogenic, and osteogenic differentiation processes, illustrated by positive staining with Oil O Red, Alcian Blue, and Alizarin Red, respectively (Figure 1D). Analysis of the expression patterns of positive markers, including CD73, CD90, and CD105, showed that more than 99% of the cells expressed all these markers and less than 2% expressed negative markers, including CD11b, CD19, CD34, CD45, and HLR-DR (Figure 1E). These results fulfilled the minimum criteria for mesenchymal stem cells proposed by the ISCT (Table 2).

Patient Outcomes as Case Report

Patient 1

An extremely premature girl (24 weeks and 5 days, first born of twins) was born by C-section due to premature rupture of the placental membrane with a bodyweight (BW) of 720 grams. Soon after birth, the patient developed signs of respiratory distress syndrome with retraction followed by apnea and cyanosis (SpO_2 ranged from 60% to 70%) and bradycardia with a heart rate below 100 bpm. She was immediately intubated and placed on mechanical ventilation, with a peak inspiratory pressure (PIP) of 18 cmH_2O and a positive end-expiratory pressure (PEEP) of 5 cmH_2O. Chest X-ray showed a stage 2 hyaline membrane requiring one dose of surfactant (Curosurf) at 200 mg/kg BW. Heart ultrasound detected patent ductus arteriosus, which closed after one course of paracetamol (15 mg/kg/6 h) for seven days, with no evidence of pulmonary artery hypertension (PAH) on echocardiogram after treatment. In addition, the patient suffered from septicemia caused by *Staphylococcus epidermidis*,resulting in necrosis at the distal phalanx of the left little and ring fingers and requiring antibiotic treatment. In the first 2 months, the patient was supported with synchronized intermittent mandatory ventilation (SIMV) and switched to continuous positive airway pressure therapy (CPAP) at7 cmH_2O and 50% FiO_2 for the following 1.5

months. At 3.5months postnatal age, the patient was diagnosed with BPD and continued to receiveoxygen support via anasal cannula at 0.5 – 1 L/min.Nebulized corticosteroids at 100 mcg/kg 4 times/day for a 1-month period wereadministered. A combination of diuretics (furosemide at 1 mg/kg/12 h), spironolactone (2 mg/kg/12 h) and bronchodilators (inhaled b2-agonists) together with nutrient enhancement (high-calorie nutrition and vitamins E, A, K supplementation) were initiated for 2 months. However, at 4.5 months postnatal age, the patient's BPD was not improved, with the SpO_2offoxygen support dropping to 90%. The chest CT scan and X-ray at 3.5months postnatal age confirmed the formation of diffuse fibrosis, atelectasis in the upper lobes of both lungs and significant air trapping in both lower lobes (Figure 2A and 3A).

Before transplantation, chronic hypercapnia was confirmed by ABG analysis with the following measured values: pH of 7.31, $PaCO_2$ of 68 mmHg, HCO_3- of 41.3 mmol/L, and PaO_2 of 73 mmHg. PAH was determined based ona maximum pulmonary artery pressure (PAP) of 40 mmHg and illustrated onechocardiogram, and the pro-BNP level was high (1942 ng/mL). Oral sidenafil (1.5 mg/kg/6 h) and bosentan (1 mg/kg/8 h) were administered when the patient was4 months old. The allo-UC-MSC transplantation was performed at 144 days postnatal age (47 weeks gestational age). No signs of serious adverse events were observed during the two transplantations. Three days after the second transplantation, the patient could breathe spontaneously withan SpO_2 of 96% without oxygen support. The patient was discharged at 161 days postnatal age (17 days posttransplantation).

At the first follow-up visit, the patient was alert, hada BW of 4 kg and was spontaneously breathing, with an SpO_2 of 96% without oxygen support. Blood gas analysis revealed a significant reduction in thesaturated CO_2 in the blood as follows: pH of 7.5, $PaCO_2$ of 33.6 mmHg, HCO_3- of 26.9 mmol/L, BE of 4 mmol/l and PaO_2 of 46 mmHg. These results suggested that the hypercapnic condition was ameliorated after stem cell transplantation. The pro-BNP level had dropped to 351.9 ng/ml, leading to the termination of PAH treatment at4 months posttransplantation. At the 1-month follow-up examination, the patient was cognizant and active, and her BW had increased to 4.3 kg with air fully entering both lungs. She was spontaneously breathing and had an SpO_2 of 97% onroom air without oxygen support. The laboratory tests revealed that herpH (7.37), $PaCO_2$ (46.3 mmHg), HCO_3- (27 mmol/L), and PaO_2 (42 mmHg) remained stable posttransplantation. Hematological analysis also confirmedthe absence of inflammation andsepsis, as indicated by the Hgb level (129 G/L), white blood cell count (WBC, 6.1 G/L), and neutrophillevel(6.4%). At the 6-month visit, the patient no longer required oxygen support, with her SpO_2 reaching100%, good air entry into the lungs, no sign of dyspnea and ABG results in the normal ranges (pH: 7.37, $PaCO_2$: 38 mmHg, HCO_3-: 21.9 mmol/L, PaO_2: 41 mmHg, and SpO_2: 100%); the pro-BNP level was 283.1 ng/ml. A significant reduction in lung fibrosis was observed on chest CT without PAH (Figure 2B). At 12months postdischarge, the patient's condition was improved. Her BW had increased to 7.5 kg. ABG results werein the normal ranges (pH: 7.34, PaCO2: 35.5 mmHg, PaO2: 87 mmHg, BE: -6; HCO3-: 19.3 mmol/l), and her pro-BNP level was 154.2 without PAH treatment. A significant reduction in lung fibrosis was observed on chest X-ray at the 12-month visit (Figures 3B). The detailed progression of the patient's condition is described in Supplement Table 1.

Patient 2

The second premature neonate was patient 1's twin, who was enrolled in this study with a BW of 650 grams. Similar to her twin sister, the patient suffered from respiratory distress syndrome, including gasping, followed byapnea, bradycardia, and cyanosis with an SpO_2 between 50% and 60%. The patient was positive pressure ventilated by bag-mask and then intubated and placed on aventilator inSIMV mode (with ventilator parameters similar to those in the first case). Chest X-ray revealed a stage 3 hyaline membrane, and a surfactant was given at a dose of 200 mg/kg BW on the 1^{st} and 3^{rd}days after birth. A large patent ductus arteriosus (PDA) was detected by cardiac echography, requiring one course of indomethacin (0.2 mg/kg/12 hours) within the firstpostnatalweek as previously described(20). After 2 months onSIMV, ventilation support was switched to CPAP with a PEEP of6 cmH_2O and 40% FiO_2. After treatment, the PDA size was reduced(1 mm), andthe shunt size was small; however, the size increased gradually and reached 3.6 mm at 3 months. The PDAwas maintained at a large size and required surgical closure at 3.5 months of age. Although the PDA was closed without complications, PAH wasobserved (38 mmHg), and the pro-BNP level was 2223 ng/ml, leading to treatment with 1 mg/kg/6 h sildenafil and 2 mg/kg/8 h bosentan. After the operation, the patient was on CPAP at 6 cmH_2O at a FiO_2 of 30% before switching to nasal cannula oxygen after 1 month at a rate of 1 L/min to maintain a stable SpO_2 between 93% and 97%. X-rays and chest CT scans at 4 months of age indicated diffuse fibrosis in the lung structures with atelectasis in the upper lobes of both lungs and significant air trapping in both lower lobes (Figure 2A and 3A). The patient was confirmed as havingBPD and treated with nebulized corticosteroids (100 mcg/kg 4 times/day), diuretics (furosemide 1 mg/kg/12 hours), spironolactone (2 mg/kg/12 hours), bronchodilators (inhaled β2-agonists) in combination with ipratropium bromide, and other supportive measures (high-calorie nutrition, vitamins E and A, etc.) for 6 weeks. However, the patient's condition didnot improve, and she remained dependent on oxygen support, leading to the indication forallo-UC-MSC transplantation at 151 days postnatal age. Prior to transplantation, ABG analysis revealed a pH of 7.6, $PaCO_2$ of 37.9 mmHg, PaO_2 of 35 mmHg, increased HCO_3- of 29.1 mmol/L and BE of 8 mmol/L. Three days after transplantation, the patient was discharged with oxygen support via nasal cannula at 1 L/minute, a respiration rate of 64-67 times/minute, and an SpO_2 of 83% (FiO_2: 21%).

At the first follow-up visit, the patient's body weight had increased to 4.3 kg, her heart rate was145 bpm, and she still required oxygen support at 0.5 l/min to maintain an SpO2 over 92% (83% without oxygen support). The ABG results showed a $PaCO_2$ of 67 mmHg and an HCO_3- of 32.3 mmol/L, a PaO_2 of 36 mmHg, and a BE of 6 mmol/l. The total hemoglobin level, WBC level, and neutrophil percentage were 129 G/L, 6.1 G/L, and 6.4%, respectively. The patient's condition had improved by her 1-month follow-up visit, with both reductions in both her$PaCO_2$ and HCO_3-, while her SpO_2 was maintained at 94-98% onoxygen via a cannula a 0.5 l/min. Two months after the first hUC-MSC transplantation, home oxygen monitoring results confirmed that the patient could breathe normally, and her SpO_2 had reached 95%.

From the 6-month follow-up onwards, the patient's health had stabilized under normal conditions, with her BW reaching 8 kg at the 12-month visit. All ABG tests were within normal parameters at the 6-month

visit, further confirming the recovery of the patient from BPD. Her SpO_2 was maintained at 95% at the 6-month visit and reached 100% at the 12-month follow-up. Blood gas analysis at 12 months showed all parameters were within the normal limits without oxygen support. The chest CT scan at the 6-month visit revealed a significant reduction in lung fibrosis (Figure 2B). A normal chest X-ray was observed at the 12-month follow-up (Figure 3B). It is important to note that the maximal PA recorded at the 6-month visit was 46 mmHg,with a pro-BNP level of 511 ng/ml, leading to the administration of sildenafil (1 mg/kg/12 h). At the 12-month visit, the maximum PA was 37 mmHg, and her pro-BNP levelwas reduced to 202 ng/mL; therefore, a lower dose of sildenafil (0.5 mg/kg/12 h) was given.

Patient 3

A 34-week-old male infant was prematurely born due to premature rupture of the placental membrane and had a BW of 2.4 kg at birth. The patient was diagnosed with hyaline membrane disease and required ventilator support. After 3 consecutive treatments with a surfactant, he was successfully weaned off of mechanical ventilation at 3months postnatal age. However, he still depended on oxygen support at a rate of 1 L/min via a sponge cannula. The diagnosis of BPD with vocal cord cirrhosis and laryngomalacia combined with periventricular leukomalacia was confirmed using nasopharyngoscopy, CT and MRI.

Upon admission toVinmec International Hospital, the patient was supported with oxygen at a rate of 1 L/min via nasal cannula to maintain the target SpO_2 above 92%. The SpO_2 dropped dramatically to 60% without oxygen support or crying. The patient suffered from severe chronic hypercapnia with pH, BE, $PaCO_2$ and HCO_3- levels maintained at 7.35, 12 mmol/L, 63.6 mmHg and 67.2 mmol/L, respectively, whereas his SpO_2 and PaO_2 were relatively low (60% at a FiO_2 of 21%, and 44 mmHg, respectively). No cardiovascular malfunction or PAH was detected on echocardiogram, with a pro-BNP level of 176.5 ng/ml. The patient was diagnosed with CMV infection, with a viral load of 1.44 $x10^5$ copies/ml in the endotracheal fluid. When CMV treatment with 3 weeks with valganciclovir was complete, the chest CT scan and radiograph revealed lung fibrosis with significant air trapping in both lungs and lung inflammation (Figure 2A and 3A), and the patient could not be weaned off ofoxygen. He was dependent on oxygen at a rate of 1 L/min via nasal cannula to maintain an SpO_2 between 94-96%.

Before transplantation, the patient still suffered from chronic hypercapnia with the following parameters: pH 7.51, $PaCO_2$ 59 mmHg, HCO_3- 47.2 mmol/L, and PaO2 57 mmHg. He required oxygen support via nasal cannula at 1 L/min to maintain an SpO_2between 92%-97%; without oxygen support, his $SpO_2$was as lowas 70% (FiO_2: 21%). The PCR results and hematological analysis (WBC: 23.9 G/L, neutrophils: 20.9%, and Hgb: 95 G/L) confirmed that the patient no longer carried CMV; he did not suffer from inflammation, nor did he have sepsis. Allo-UC-MSC transplantation was performed at 173 days postnatal age with no signs of severe adverse events. The patient was discharged 13 days after the first transplantation with oxygen support via nasal cannula at 0.5 L/min with an SpO_2ranging between 93% and 98%.

At the first visit, the patient's general condition was fair, and he was cognizant, with his BW slightly increased to 5.3 kg. He was still receiving oxygen at 0.5 l/min via cannula to maintainan SpO_2at92-98%. The patient's hypercapnic condition was reduced, with the following ABG test results: pH of 7.46, PaO_2 of 45 mmHg, $PaCO_2$of 52.6 mmHg and HCO_3- of 38 mmol/L. His SpO_2 without oxygen support had increased to 85% on room air. The total blood count results remained in the normal ranges. The blood CRP level was 0.2 mg/L, confirming that the patient hadnot developed aninflammatory response. At the 1-month follow-up, the patient was still dependent on oxygen support at a rate of 0.5 L/min to maintainan SpO_2 level between 95% and 98. Two months posttransplantation, the patient was independent of active oxygen support, with an SpO_2 of 96-98%.

The clinical team observed improvements at the 6-month visit. The patient was cognizant and was able to crawl, laugh, and actively respond to his parent's voice. Due to the complication of periventricular leukomalacia, an additional Denver II test was conducted at the 6-month examination, and the results confirmed that the patient's gross motor function was similar to that expected at 3 months, his language ability was equivalent to that expected at 5-6 months, his fine motor adaptive skills were equivalent to those expected at 3 months, and his personal-social skills were equivalent to those expected at 5 months. Moreover, improved respiratory function was also documented, with better airflow in both lungs, no crackles or rales, and no signs of retraction ornasal flaring atthe 6-month visit. All ABG results remained stable at the 12-month visit, with no sign of respiratory distress syndrome, an improved saturated oxygen level (SpO_2: 100%) and a normal CO_2 level in the blood (pH of 7.4, PaO_2 of 72 mmHg, $PaCO_2$ of 34.8 mmHg, HCO_3- of 21.5 mmol/L; BE of -3 mmol/l) (Supplemental Table 4). Investigation of the patient's lungswithCT at the 6-month visit indicated areduction infibrosis and the recovery offunction. Chest X-rays at the 12-month visit further confirmed the significant improvement (Figure 3B).

Patient 4

A premature female infant was born at another hospital at 28 weeks gestation with a birth weight of 1400 grams due to premature rupture of the placental membranes. She rapidly developed respiratory distress syndrome and required mechanical ventilation. A single dose of surfactant was given (100 – 200 mg/kg) on the first day. After that, the patient was placed on CPAP for a month, followed by oxygen support at 0.5 – 1 L/min until she reached 36 weeks old. Dexamethasone treatment using the DART protocol was advised for one week to further improve the patient's condition. The patient was successfully weaned from oxygen supportand discharged at 37 weeks with an SpO_2 ranging between 93% and 95%. However, 2 days postdischarge, the patient developed dyspnea with acute respiratory distress and returned to the hospital, where she stayed for the next 2 months.

The patient was referred to VinmecHospital at 4 months old with malnutrition (BW of 3 kg). Although the oxygen support was maintained at 1 L/min via nasal cannula, herSpO_2 was relatively low (80%). Auscultation showed poor air entry into the lungs with crackles andrales. Her heart rate was high (200 – 220 bpm), with evident cyanosis and an SpO_2 of 80% on 24%oxygen. The patient was intubated

immediately and placed ona ventilator inSIMV mode (PIP at 23 cm H_2O, PEEP at 5.5 cm H_2O, and FiO_2 at 50%). Five days after the treatment, ventilation support was switched to sponge cannula with oxygen flowing at 1 L/min. The ABG examinations revealed the following: pH of 7.49, $PaCO_2$of 38.6 mmHg, HCO_3- of 29.5 mmol/l, and PaO_2 of 60 mmHg with FiO2: 40%. Furthermore, a complete blood count showed a low platelet count (53 G/L), while the WBC, neutrophil, and Hgb results were 5.8 G/L, 1.3 G/L and 112 G/L, respectively. Anechocardiogram was performed when the patient was stable and showed a pressure gradient through the tricuspid valve at 28 mmHg. The pro-BNP level was 8065 pg/ml. Hence, the patient was treated with 0.5 mg/kg/8 h sildenafil. The viral tests confirmed a CMVinfection (460 copies/ml), which was treated with valganciclovir for 21 days. The results of a chest X-ray and CT scan indicated severe lung fibrosis and substantialairtrapping in both lungs (Figure 2A and 3A).

Two UC-MSC transplantations were carried out without adverse events when the patient was160 days old. Four days after the first transplantation, the patient could breathe spontaneously at 55 – 62 breaths/minute. On the day of discharge (a week after the second transplantation), the patient breathed spontaneously with an SpO2 of 95% without oxygen support.

At the 7-day examination, the patient still suffered from dyspnea, with a respirationrate of 53 breaths/minutes. An increase in the SpO_2 level to 95% was also recorded. The pro-BNP level was reduced significantly to 136.7 ng/ml. The hematological analysis confirmed that no sepsis or inflammatory reaction had occurred after stem cell transplantation, with a WBC count of 9.8 G/L, neutrophil percentage of 12.1%, Hgb level of 112 G/L and platelet count of 61 G/L. Onemonth postdischarge, the patient was cognizant and active, with a BW of 4kg. There was an increase in the SpO_2 to 98% without oxygen support, suggesting that the patient's respiratory function had recovered.

At the 6-month visit, respiratory distress was assessed as mild. The SpO_2had stabilized at 97%. The 12-month follow-up corroborated the conclusion that the patient had recovered from BPD, witha normal SpO2 of 97%, pH of 7.34, $PaCO_2$ of 39.8 mmHg, HCO_3- of 20.8 mmol/l, BE of -4 mmol/l, and PaO2 of 73 mmHg. At 12 months after transplantation, it is worth mentioning that the patient hadrecovered well with regard to both her SpO_2 and PaO_2 levels, which were 100% and 72 mmHg, respectively. Evaluation of the lung structure onCT scans demonstrated that the fibrotic area was reduced (Figure 2B), while alveolation and maturation of the lung had becomeobvious. A further assessment of the lung structure using chest X-rays at the 12-month follow-up showed no signs of atelectasis or hyperexpansion in either lung (Figure 3B).

Discussion

All four patients tolerated the allo-UC-MSC infusion well, and no prespecified infusion-related adverse events were recorded after either the first or second transplantation. Specifically, no significant changes in heart rate, mean arterial pressure, oxygen saturation or body temperature were observed in any of the four infants (Supplementary Figure 1). These results, together with the detailed hematological analysis reported in each case, confirmed that allo-UC-MSC transplantation does not trigger an inflammatory

response during or 72 h after infusion. Previously, a study reported the safety outcomes of the allogeneic transplantation of human umbilical cord blood-derived MSCs, in which nine preterm infants received either a single dose of $1x10^7$ cells/kg or $2x10^7$ cells/kg (21). In another single-center, open-label phase 1 trial, Lim's group administered $1x10^6$ human amnion epithelial cells tosix preterm infants with established BPD and reported the safety profile 2 years posttransplantation. In these trials, as in ours, no infusion toxicity or allogeneic UC-MSC transplantation-associated adverse events were recorded. These results were also supported by a preclinical study evaluating the long-term safety and efficacy of the allogeneic transplantation of MSCs in a rodent model of BPD, which reported no adverse lung effects posttransplantation together with persistent improvement in respiratory function and lung condition (22).

Additionally, our preliminary data support the efficacy of allo-UC-MSC transplantation in neonates with established BPD. This is, to our knowledge, the first report of the use ofallo-UC-MSC transplantation to treat premature infants with established BPD. During the follow-up period, all patients exhibited significant improvements in lung function. All patients stopped being dependent on mechanical oxygen support as early as 4 days after the first transplantation and as late as 2 months posttransplantation. These results make our study different from previous studies, as our study focused on the treatment of established BPD in premature infants. Ahn's study suggested that the injection of allogeneic MSCs could reduce the risk of BPD (11, 23, 24). It was suggested that the earliest time at whichBPD can be predicted is day 4 of life, when the peak inspiratory ventilator pressure is recorded and assisted ventilation is required (25). To confirm the diagnosis of BPD, subsequent studies found that BPD patternstypical oflung diseasesemerge during the first 14 days of life. Hence, during this period, several risk factors could affect the disease, including late surfactant deficiency, sepsis, inflammation, and PDA(26, 27). Furthermore, it was reported that 50% of infants with pulmonary deterioration and nearly 70% of infants with early persistent pulmonary deterioration develop BPD (28). Therefore, intervention withstem cell therapy during the first 14 postnatal days shouldbe considered as a preventive treatment ininfants at risk of BPD.

Although it was suggested that the direct delivery of stem cells to a patient's lung via intratracheal administration could allow the stem cells to reach their designated location, a systematic analysis of preclinical studies suggested that the intravenous administration of MSCs resulted insuperior effects than the intratracheal administration of MSCs(29). Moreover, it was previously reported that the intravenous route is more effective than local infusion via intratracheal delivery(30). Our results support the argument that using a less invasive administration method, such as the intravenous infusion of stem cells or drugs, in established BPD infants could further enhance the effectiveness of the therapy andreduce the risks of both respiratory and systemic inflammation (31, 32). Moreover, the delivery of a high concentration of stem cells in a relatively small volume (<5 ml) might increase the risk of pulmonary embolism regardless of whether intravenous administration orintratracheal infusion was used, as was demonstrated previously in mouse models (33).

BPD is a clinical syndrome characterized by the disruption of the alveolarization process and the microvascular development of the lung. Inflammation is the common pathway that leads to the phenotype of the disease and further compromises the structure and function of the patient's respiratory

system (24). However, pro-inflammatory cascades are complex and redundant, involving numerous different cell types and molecular pathways triggered by either cell-to-cell interactions or paracrine signals from damaged tissues (34). Therefore, MSC transplantation emerges as a promising therapy investigated in a recently reported phase I clinical trial (21). Two potential mechanisms supporting the action of MSCs in BPD patients are the immunological regulatory functions and the secretion of a wide range of growth factors, cytokines, and exosomes that are involved in the initiation of tissue regeneration (35, 36). Despite the rapid evolution of MSC therapy forthe treatment of BPD, it is important to emphasize that cell-based therapies for lung regeneration are still in their infancy, and major knowledge gaps regardinghow these MSCs may be involved in the repair of the damaged lung remain to be addressed. Hence, a better understanding of the biological activity of MSCs and technological advances in the manufacturing of these cells couldsupport the development of such therapies in the near future (36).

Conclusions

Our current study provides the first evidence of the safety of the allogeneic transplantation of hMSCsin established BPD patients.The results also showed that the allogeneic transplantation of hMSCsmight decrease lung fibrosis and improve lung function. However, our major limitation is the small cohort size (only 4 preliminary cases) and the lack of a control group. A study with a larger sample size and a control group is needed to draw more accurate conclusions.

List Of Abbreviations

- **allo-UC-MSCs:** allogeneic transplantation of umbilical cord-derived mesenchymal stem/stromal cells
- **ABG:** arterial blood gas analysis
- **BE:** base excess
- **BPD:** bronchopulmonary dysplasia
- **BW:**body weight
- **HCO_3-:** bicarbonate
- **ITP:**idiopathic thrombocytopenia purpura
- **$PaCO_2$:**partial pressure of carbon dioxide
- **PAH:** pulmonary artery hypertension
- **PaO_2:**partial pressure of oxygen
- **PAP:** pulmonary artery pressure
- **PBW:** patient body weight
- **PEEP:** positive end-expiratory pressure
- **PIP:** peak inspiratory pressure
- **SpO_2:** saturated oxygen level in blood
- **UC-MSCs:** umbilical cord-derived mesenchymal stem/stromal cells

Declarations

Ethics approval and consent to participate

All clinical procedures, stem cell administration and patient follow-up processes used inthis study were approved by the Scientific and Ethics Committee of Vinmec International Hospital (approved number: 88/2019/QĐ-VMEC). Written consent was obtained from both the donor who donated the umbilical cord and from the patients' parents.

Consent for publication

Not applicable

Availability of data and materials

The authors declare that [the/all other] data supporting the findings of this study are available within the article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Liem Nguyen Thanh, Thai Trieu Thi Hong, Hue T. H. Bui and Duc M. Hoang contributed equally to this work.

- Liem Nguyen Thanh: Conception and design, administrative support, provision of study material or patients, data analysis and interpretation, manuscript writing, and final approval of manuscript.
- Thai Trieu Thi Hong: provision of study material or patients, collection and/or assembly of data, data analysis and interpretation, manuscript writing, and approval of clinical assessment.
- Hue T.H. Bui: Stem cell processing and characterization, preparation of transplantation product, and data analysis and interpretation.
- Van T. Hoang: quality control of stem cell product, flowcytometry analysis of stem cell markers, data analysis and interpretation.
- Anh T.T. Nguyen: Stem cell processing and characterization.
- Nhung T.H. Trinh: Characterization and differentiation of UC-MSCs.
- Kien T. Nguyen: Statistical analysis and data analysis.

- Duc M. Hoang: administrative support, provision of study material or patients, data analysis and interpretation, manuscript writing, and final approval of manuscript.

We confirmed that the manuscript has been read and approved for publication by all of the abovementioned authors.

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Tables

Table 1: General characterization of patients enrolled in the study.

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4
DOB	25/05/2018	25/05/2018	01/08/2018	07/07/2018
Gestational age at birth, weeks	24 (+5 days)	24 (+5 days)	34	28
Birth weight (grams)	720	650	2400	1400
Sex	Female	Female	Male	Female
Prenatal steroids used	Yes	Yes	No	No
Pulmonary hypertension	Yes	Yes	No	Yes
Mechanical ventilation duration before transplantation	3.5 months	4.5 months	1 month	3 months
$PaCO_2$ level (mmHg) before transplantation	37.9	68	59	38.6
HCO_3- (mmHg) before transplantation	29.1	41.3	47.2	29.5
Oxygen support before transplantation	Nasal cannula 0.5 l/min	Nasal cannula 1.0 l/min		Nasal cannula 0.5 l/min
$PaCO_2$ at 6 months	38	32.8	28.6	39.8
SpO_2 (%) FiO_2 (21%) before transplantation	75	91	70	91
SpO_2 at 6 months	100	95	99	97
SpO_2 at 12 months	100	100	100	97
Postnatal age at UC-MSC administration, days	144	151	173	160
Weight at UC-MSC administration (grams)	3600	4000	5400	3800
Duration from birth to discharge (days)	161	161	183	173
Duration from transplantation to independence from oxygen support	3 days after the 2nd transplantation	2 months	2 months	4 days after the first transplantation

Chest X-ray before transplantation	diffuse fibrosis, atelectasis, diffuse haziness	diffuse fibrosis, atelectasis, diffuse haziness	air trapping, diffuse fibrosis, atelectasis, diffuse haziness	air trapping, diffuse fibrosis, atelectasis, diffuse haziness
Chest X-ray **12 months after transplantation**	reduction in fibrosis	reduction in fibrosis	normal	normal

Table 2: **Release criteria of allo-UC-MSC administration.**

	Patient 1		**Patient 2**		**Patient 3**		**Patient 4**	
Transplantation	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Cell doses (x10^6 cells/kg)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Cell viability	97%	97%	96%	98%	98%	98%	97%	89%
CD73	97.9%	98.5%	98.5%	98.3%	98.4%	99.63%	99.5%	99.6%
CD90	100%	100%	100%	100%	100%	99.7%	100%	99.9%
CD105	100%	100%	100%	100%	100%	100%	100%	100%
Negative markers*	0.9%	0.3%	0.3%	0.7%	0.1%	0.1%	0.0%	0.0%
Microorganism and fungal tests	Negative							
Mycoplasma	Negative							
Endotoxin	< 0.1 EU/ml	< 0.05 EU/ml	< 0.1 EU/ml	< 0.1 EU/ml	0.073 EU/ml	< 0.05 EU/ml	< 0.05 EU/ml	< 0.05 EU/ml
Karyotyping	46, XY, 16qh+							
CFU assay (CFU per 1000 cells)	519 ± 80							
Adipogenesis	Pass							
Chondrogenesis	Pass							
Osteogenesis	Pass							

* CD11b, CD19, CD34, CD45, and HLR-DR

Figures

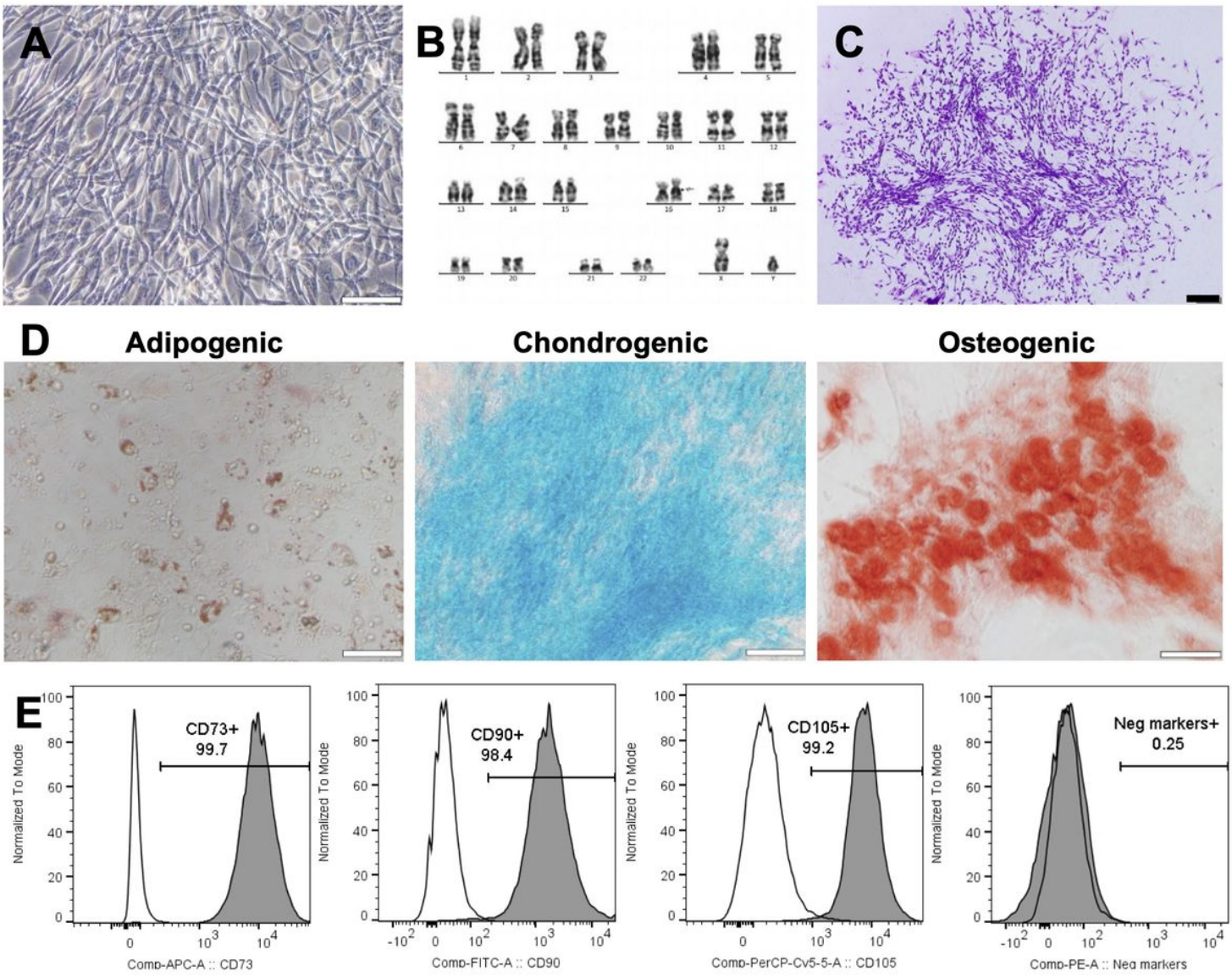

Figure 1

Characterization of hUC-MSC sources for allogeneic transplantation of severe BPD patients. (A)hUC-MSCs were obtained from a healthy donor after written informed consent was given. The morphology of hUC-MSCs (P3) expanded in xeno-free and serum-free culture medium wasspindle-shaped with adherence; they formed a monolayer in 2D culture. (B) The cells maintained a normal karyotype after 6 passages in culturein vitro with a population doubling time of 28 ± 1.3 hours. (C) The hUC-MSCs exhibit colony-forming features (140 ± 15 CFU/1000 cells, mean ± SEM, n=3) and (D) are able to differentiate into three lineages. (E) Assessment of MSC markers using flow cytometry confirmed the expression of MSC-positive markers (CD73, CD90, and CD105 >98%) and less than 2% negative markers. Scale bar: 100 μm.

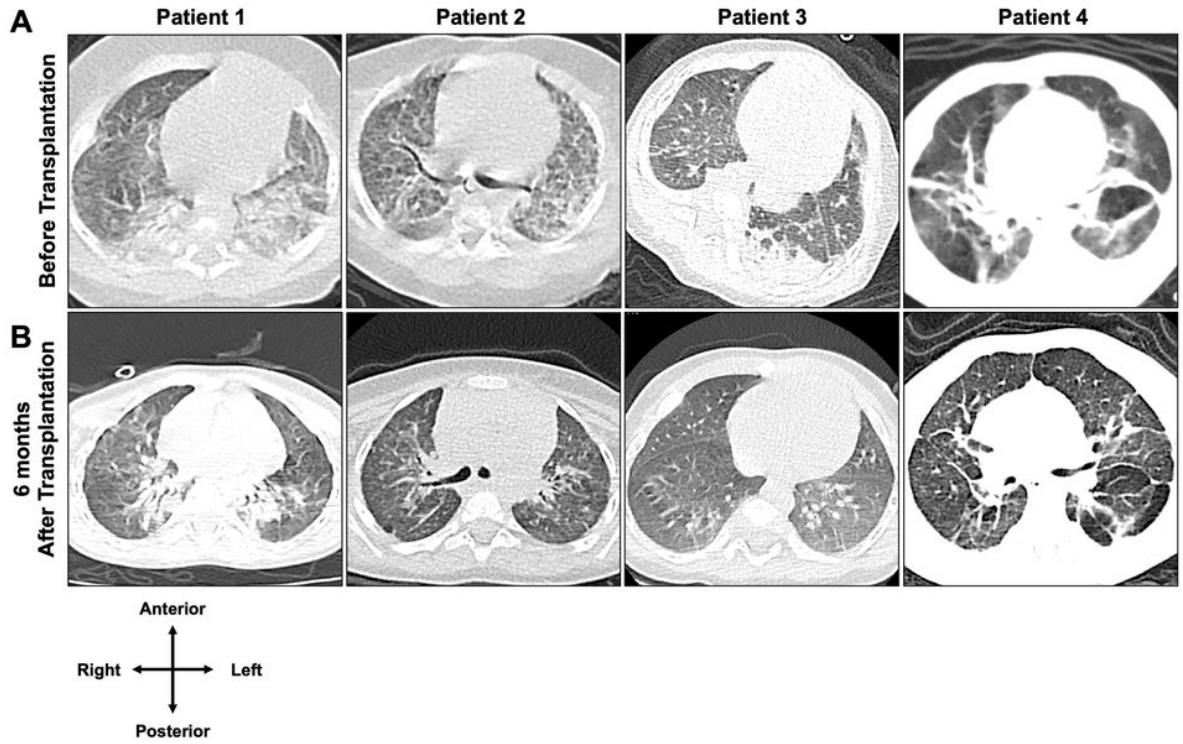

Figure 2

Chest CT scan indicating the improvement in lung structure before (A) and after (B) UC-MSC transplantation in all four BPD infants.

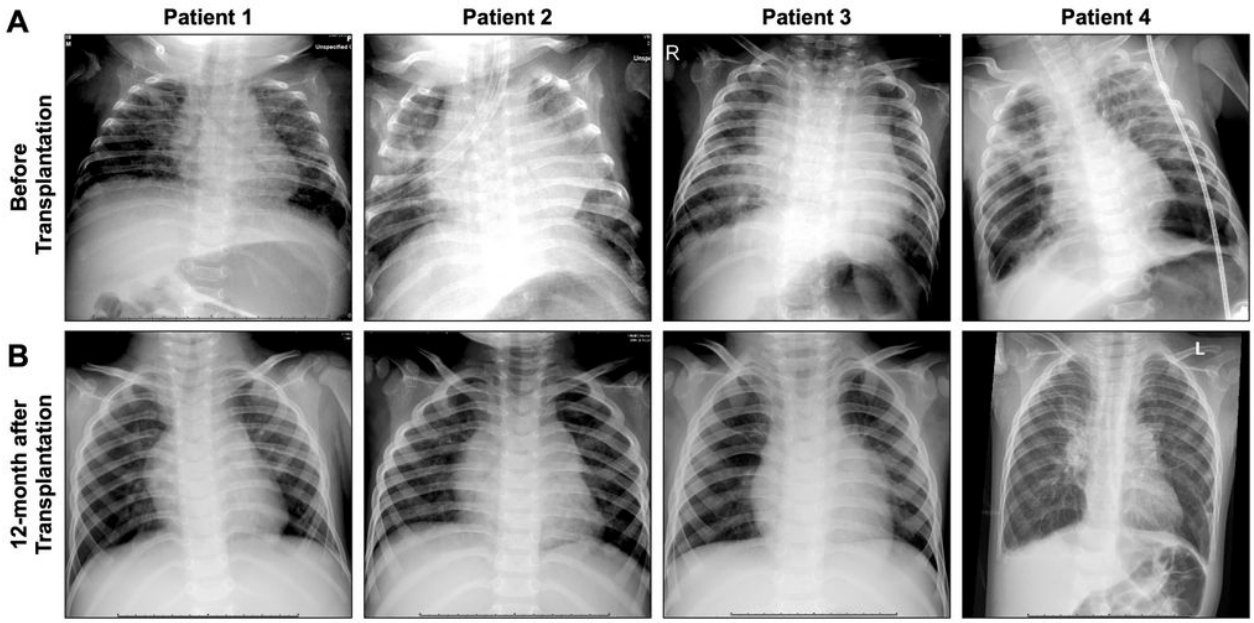

Figure 3

Chest radiographs of the four patients enrolled in the study showing the changes in cystic fibrosis before and 12 months after transplantation. The results indicate the progressive improvement of the lung with more air entering both lungs and a reduction infibrosis after allo-UCtransplantation.

Supplementary Files

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