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NARRATIVE REVIEW

# Mesenchymal stem cell therapy and its application to bronchopulmonary dysplasia of the preterm neonate. A review

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## Abstract

Bronchopulmonary dysplasia (BPD) remains a significant contributor to the morbidity and mortality of infants delivered at very low gestational ages. The therapeutic approach to BPD is essentially preventive and supportive. Inflammation is the key contributor to the disease development. Mesenchymal cells (MSCs) have been described over 40 years ago and have been shown to possess potent anti-inflammatory and proangiogenic properties and to contribute to tissue regeneration in a variety of adult and children´s clinical diseases, leading to enthusiasm for their use in BPD. Preclinical animal studies and clinical trials in human preterm neonates point to a positive preventive, as well as reparative role in BPD. Recent work revealed that MSCs release extracellular vesicles, called exomes, that contain the therapeutic activity of MSCs. Several clinical trials on MSCs in BPD are currently taking place worldwide. This review briefly discusses the different types of stem cells, their clinical applications in paediatric contexts, with particular involvement in their use in BPD, and provides information on the current use of MSCs in BPD.

***Keywords:*** Bronchopulmonary dysplasia. Mesenchymal stem cell. Preterm infant.

## *Terapia com células-tronco mesenquimais e sua aplicação na displasia broncopulmonar do recém-nascido prematuro. Uma revisão*

## Resumo

A displasia broncopulmonar (DBP) permanece uma causa de significativa morbilidade e mortalidade em crianças nascidas com idades gestacionais muito baixas. A abordagem terapêutica da DBP é essencialmente preventiva e de suporte. A inflamação é o principal contribuinte para o desenvolvimento da doença. As células mesenquimatosas (CMs) foram descritas há mais de 40 anos e demonstraram possuir potentes propriedades anti-inflamatórias e pró-angiogénicas, contribuindo para a regeneração tecidual numa variedade de doenças clínicas de adultos e crianças, levando ao entusiasmo pelo seu uso na DBP. Estudos pré-clínicos em animais e ensaios clínicos em recém-nascidos pré-termo humanos apontam para um papel preventivo, bem como reparador na DBP. Estudos recentes revelaram que as CMs libertam vesículas extracelulares, denominadas exomas, que contêm a atividade terapêutica das CMs. Vários ensaios clínicos com CMs na DBP estão em curso, atualmente, em todo o mundo. Esta revisão discute brevemente os diferentes tipos de células-tronco, suas aplicações clínicas em contextos pediátricos, com particular envolvimento na DBP, e fornece informações sobre o estado atual da utilização de CMs na DBP.

***Palavras chave:*** Célula mesenquimatosa. Displasia broncopulmonar. Recém-nascido pré-termo.

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## Keypoints

**What is known**

- The use of mesenchymal cells in pediatrics, more specifically in neonatology and bronchopulmonary dysplasia of the great preterm is a practice not yet available and therefore not used routinely.
- The few studies carried out to date point to a benefit of its use in bronchopulmonary dysplasia, as well as in other pathologies of the child. The treatments on bronchopulmonary dysplasia were well tolerated, without serious adverse effects or dose-limiting toxicity attributable to the transplantation. The severity of bronchopulmonary dysplasia was also shown to be lower in the transplant recipients.
- Clinical trials are taking place in several countries, already in phase 3, the results of which will be re- leased over the next decade.

**What is added**

- This review allows the reader to acquire knowledge on mesenchymal cells, their use in pediatrics and especially in bronchopulmonary dysplasia.
- This manuscript mentions all known clinical trials currently taking place around the world on mesenchymal stem cell therapy for bronchopulmonary dysplasia.
- The information contained in this review allows the reader to acquire knowledge in a new area of medicine.

## Introduction

Sequelae of pulmonary and neurological injury are common in preterm infants of extremely low gestational age (ELGA). Chronic respiratory problems caused by bronchopulmonary dysplasia (BPD) are the most frequent long-term complications of preterm birth[1,2]. BPD is the most common long-term pulmonary morbidity experienced by infants born prematurely[1,2]. Severe BPD is associated with neurodevelopmental impairment, pulmonary hypertension, cor pulmonale, and death[3].

BPD is the result of a complex process in which multiple prenatal and/or postnatal factors interfere with the development of the lower airways, leading to a chronic dependency on supplemental oxygen in the neonatal period and to life-long respiratory illness[4]. It is currently clear that BPD is associated with persistent pulmonary disability later in life, and has a significant impact on healthcare services once subjects with BPD have, in most cases, frequent respiratory episodes and a reduction in their quality of life[5].

The treatment of BPD is challenging and is based more on its prevention. Prevention of prematurity, systematic use of non-aggressive ventilatory measures, avoidance of supra-physiological oxygen exposure, and administration of surfactant, caffeine and vitamin A may significantly reduce the risk of BPD development[5].

Cell therapy is now emerging as the most exciting new treatment for pulmonary lesions due to BPD and several clinical trials of mesenchymal stem/stromal cel(MSC) therapy for BPD are underway[6].

Drugs with proven efficacy in meta-analyses and systematic reviews comprise exogenous surfactant application, vitamin A, caffeine, postnatal corticosteroids and azithromycin (low-quality evidence suggests azithromycin therapy reduces BPD or death in preterm infants with positive Ureaplasma, but not in all preterm infants)[7].

## Stem cells

Stem cells are cells that remain undifferentiated, that is, they have not yet gone through the process of cell differentiation; they are unspecialized cells of the human body. The stem cell, once divided, produces a cell which retains this undifferentiated character and a second daughter cell capable of responding to its environment, differentiating itself into any cell of an organism with a functional role[8].

Stem cells are also classified based on the range of their differentiation potentials into totipotent, pluripotent, multipotent, oligopotent and unipotent, Table 1[8-12]. The development power is reduced at each potential stage, which means that a unipotent stem cell is not able to differentiate in as many cell types as a pluripotent[9].

Over the last several years, stem cell therapy has become a very promising and cutting-edge scientific research topic. There are three possibilities of stem cell extraction. They may be embryonic, adults, or mesenchymal (MSCs), Table 2[9,13-15]. MSCs have been used to repair or regenerate damaged tissues since can differentiate according to the place where they are placed. In addition, due to its powerful immunosuppressive activity, they can be used in immune mediated diseases, such as autoimmune diseases and also in transplant rejections.

## MSC therapy for paediatric disorders

Named for the first time in the 1980s by Arnold Caplan, MSC appeared to be an extremely promising

**Table 1.** Classification of stem cells based on the range of their differentiation potentials[8-12]

| | |
|---|---|
| TSC | TSC have the highest differentiation potential and are able to divide and differentiate into cells of the whole organism. One example of a totipotent cell is a zygote, which is formed after a sperm fertilizes an egg and has the capacity to form both embryo and extra-embryonic structures. |
| PSC | PSC form cells of all germ layers but not extra-embryonic structures, such as the placenta. Embryonic stem cells (ESCs) are an example. |
| MSC | MSC have a narrower differentiation spectrum than PSC, but they may specialize in discrete cells of specific cell lines. An example is a hematopoietic stem cell, which may develop into several kinds of blood cells. Once differentiated, a haematopoietic stem cell turns into an oligopotent cell, and its differentiation abilities are then restricted to cells of its lineage. Some multipotent cells are capable of conversion into unrelated cell types, which suggests naming them pluripotent cells. |
| OSC | OSC can differentiate into several cell types. A myeloid stem cell is an example that can divide into white blood cells but not red blood cells.[8] |
| USC | USC are characterized by the narrowest differentiation capacities and a peculiar property to divide over and over again. These cells are only able to form one cell type, e.g., dermatocytes. |

MSC: multipotent stem cells; OSC: oligopotent stem cells; PSC: pluripotent stem cells; TSC: totipotent stem cells; USC: unipotent stem cells.

**Table 2.** Classification according to the possibility of extraction[9,13-15]

| | |
|---|---|
| ESC | ESC are found in the human embryo in the blastocyst state and are classified as pluripotent. |
| ASC | ASC are found in various tissues, such as bone marrow, blood, liver, umbilical cord, placenta, and other organs. |
| MSCs | MSC, a multipotent stroma cell population, have the ability to differentiate in various tissues. They have a great plasticity, and because of this plasticity, these cells have been used to repair or regenerate damaged tissues. MSCs can differentiate according to the place where they are placed, that is, according to the elasticity of the surface of these sites their differentiation occurs. For example, a hard matrix causes MSCs to differentiate into bone cells; on the contrary, in a moderately elastic matrix, it will cause them to become muscle cells. These stem cells are associated with normal conditions of growth and repair of the human body. In addition, these cells have a powerful immunosuppressive activity, which opens the possibility of their clinical application in immune mediated diseases, such as autoimmune diseases and also in transplant rejections. |

ASC: adult stem cells; ESC: embryonic stem cells; MSCs: mesenchymal stem cells.

therapy[16]. MSCs are a potentially revolutionary therapy for a wide variety of paediatric diseases, but the optimal cellular therapy for such diversity has yet to be determined[15].

The published clinical trials for pediatric pulmonary, cardiac, orthopedic, endocrine, neurologic, hematologic diseases provide evidence that MSCs are efficacious, but the significant heterogeneity in therapeutic approaches between studies raises new questions[15].

The published literature includes numerous case reports and clinical trials on the use of MSC therapy for paediatric conditions, as BPD, cardiomyopathy, hypophosphatasia and osteogenesis imperfecta, cerebral palsy and spinal muscular atrophy, autism spectrum disorders, and inborn errors of metabolism[17-27].

Some of the sources of MSCs studied in paediatric diseases include bone marrow-derived MSCs (BM-MSCs), Wharton's jelly or umbilical cord tissue-derived MSCs (UC-MSCs), umbilical cord blood-derived MSCs (UCB-MSCs), placenta-derived MSCs (P-MSCs), amniotic fluid-derived MSCs (AF-MSCs), and adipose tissue-derived MSCs (ADSCs)[28-30]. Allogeneic MSCs of related and unrelated donors with various levels of HLA and autologous MSCs have been investigated.

## Bonemarrow derived MSCs

BM-MSCs are the MSC prototype and have been the most extensively studied. Pittenger et al. reported the chondrogenic, osteogenic and adipogenic potential of BM-MSC in humans in 1999[31]. Although they are relatively easy to obtain from the human iliac ridge, tibia and femur, and vertebrae, other types of MSCs (e.g., umbilical cord or placenta) are easier to obtain[15]. In addition, BM-MSCs are typically isolated from adults, and increased donor age is associated with reduced proliferation capacity, turning the use of human adult BM-MSCs in the paediatric population less desirable[15].

BM-MSCs have been used in paediatric osteogenesis imperfecta[32], in hypophosphatasia[33,34], for graft versus-host disease (GvHD)[35,36], in treating patients with

lysosomal storage disorders, such as metachromatic leukodystrophy, Hurler syndrome (muco-polysaccharidosis type I), and Hunter syndrome (mucopolysaccharidosis type II)[37,38], and in type I spinal muscle atrophy[39,40].

## UC-MSCs and UCB-MSCs

Both UC- and UBC-MSCs are available in relatively large quantities from morally acceptable sources, since collection does not involve any painful or invasive techniques. Also, they may be richer sources of MSCs, depending on the fibroblast effectiveness of colony-forming units, and may generate MSCs with higher immunomodulatory potential than BM-MSC[41].

MSCs in general are immune-evasive, not exhibiting major histocompatibility complex (MHC) class II, but UC- and UCB-MSCs may be even more immune-evasive than BM-MSCs since they have the ability to inhibit T-cell alloreactivity and B-cell proliferation and to interfere with the function of antigen-presenting cells[42]. Therefore, umbilical cord products can be used in scenarios where myeloablative treatment is contraindicated (e.g., in neonates)[43].

## MSC therapy for BPD

The interesting results of the first clinical trial of UC-MSCs in infants for BPD of the preterm neonate were reported in 2014[44]. In this trial, Chang YS et al assessed the safety and feasibility of Pneumostem®, an allogeneic human umbilical cord blood (hUCB)-derived mesenchymal stem cell (MSC) transplantation in preterm infants. In a phase I dose-escalation trial, the authors assessed the safety and feasibility of a single, intratracheal transplantation of hUCB-MSCs in preterm infants at high risk for BPD. The first three patients were given a low dose ($1 \times 10^7$ cells/kg) of cells, and the next six patients were given a high dose ($2 \times 10^7$ cells/kg); the mean gestational age was 25.3 ± 0.9 weeks and the mean birth weight was 793 ± 127g, at a mean of 10.4 ± 2.6 days after birth. The treatments were well tolerated, without serious adverse effects or dose-limiting toxicity attributable to the transplantation. Levels of interleukin-6, interleukin-8, matrix metalloproteinase-9, tumour necrosis factor $\alpha$, and transforming growth factor $\beta$1 in tracheal aspirates at day seven were significantly reduced compared with those at baseline or at day three post-transplantation. BPD severity was lower in the transplant recipients, and rates of other adverse outcomes did not differ between the comparison group and transplant recipients.

Since then, several clinical trials of MSCs use for BPD are ongoing or are planned in some countries to investigate the efficacy of MSCs in the prevention or treatment of BPD in premature infants[45].

The MSC-BPD trial (ID: NCT03601416) is an ongoing randomized trial, single-center, open-label, dose-escalation, phase-II trial designed to investigate the safety and efficacy of hUC-MSCs in children with severe BPD. In this trial, 72 patients will be enrolled and randomly divided into two intervention groups and one control group. Patients in the intervention groups will receive a low dose of hUC-MSCs (n = 24; 2.5 million cells/kg) or a high dose of hUC-MSCs (n = 24; 5 million cells/kg) in combination with traditional supportive treatments for BPD. The patients in the control group (n = 24) will be treated with traditional supportive treatments alone without hUC-MSCs. The primary outcome measures will be cumulative duration of oxygen therapy. Follow-up assessments will be performed until 24 months post intervention, and the key outcome during follow-up will be changes on chest radiography. This will be the first randomized controlled trial to evaluate the safety and efficacy of intravenously administered hUC-MSCs in children with severe BPD. Its results will provide a new evidence-based therapy for severe BPD[46]. An open-label dose-escalation trial of intratracheal UC-MSCs administration for preterm infants is currently enrolling in the U.S. (clinicaltrials.gov; NCT02381366)[47].

Trials on MSCs for BPD are listed in Table 3 (information from *ClinicalTrials.gov Search Results*, assessed November 05/2022).

The addition of MSCs is unlikely to represent a significant burden above the current standard of care (ventilation, surfactant, etc.) in extremely preterm infants. However, the sequence of therapeutic inter-vention should be considered in the trial development: using an "off-the-shelf" allogeneic product may be more timely than using an individualized autologous product.

## Mechanism of MSC treatment

Two mechanisms of action of MSCs have been hypothesized: (1) MSCs exert their immunomodulatory/anti-inflammatory/angiogenic/antibacterial effects by different humoral factors; and (2) MSCs exert their regenerative effects through their potential for multiple lines in damaged tissue[48].

### *The paracrine effect*

The paracrine effect is an action of substances secreted from MSCs that enables the cells to exert a

**Table 3.** Trials on MSCs for BPD. ClinicalTrials.gov Search Results, assessed 11/05/2022

| Identifier | Status | Title | Results | Condition | Interventions | Locations |
|---|---|---|---|---|---|---|
| NCT03558334 | Unknown | Human MSC for BPD | No results available | BPD | Drug: transplantation of mesenchymal stem cell<br>Drug: no transplantation of mesenchymal stem cell | Children's Hospital of Chongqing Medical University, Chongqing, China |
| NCT03601416 | Unknown | Human MSCs for moderate and severe BPD | No results available | BPD | Drug: transplantation of mesenchymal stem cell<br>Drug: no transplantation of mesenchymal stem cell | Children's Hospital of Chongqing Medical University, Chongqing, China |
| NCT03645525 | Recruiting | Intratracheal Umbilical Cord- derived MSC for the Treatment of BPD | No results available | BPD | Drug: human umbilical cord-derived mesenchymal stem cell<br>Drug: placebo | Obstetrics and Gynecology Hospital of Fudan University; Children's Hospital of Fudan University; Shanghai First Maternity and Infant Hospital, Shanghai, China |
| NCT03873506 | Unknown | Follow-Up Study of MSCs for BPD | No results available | BPD | Drug: transplantation of hUC-MSCs | Children's Hospital of Chongqing Medical University, Chongqing, China |
| NCT04062136 | Unknown | Umbilical Cord MSC Transplantation in the Treatment of BPD | No results available | BPD | Combination product: stem cell transplantation | Vinmec Research Institute of Stem Cell and Gene Technology, Hanoi, Vietnam |
| NCT02443961 | Active, not recruiting | MSC Therapy for BPD in Preterm Babies | No results available | BPD | Biological: mesenchymal Stem Cell (MSC) therapy | Hospital Universitario A Coruña, A Coruña, Spain; Hospital Clínico San Carlos, Madrid, Spain; Hospital Universitario La Paz, Madrid, Spain; Hospital Universitario y Politécnico La Fe, Valencia, Spain |
| NCT03378063 | Recruiting | Stem Cells for BPD | No results available | BPD | Drug: transplantation of mesenchymal stem cell<br>Drug: no transplantation of mesenchymal stem cell | Department of Pediatrics, Daping Hospital, Research Institute of Surgery, Third Military Medical University, Chongqing, China |
| NCT03774537 | Unknown | Human MSC for Infants At High Risk For BPD | No results available | BPD | Drug: transplantation of hUC-MSCs<br>Drug: no transplantation of hUC-MSCs | Children's Hospital of Chongqing Medical University, Chongqing, China |

*(Continues)*

**Table 3.** Trials on MSCs for BPD. ClinicalTrials.gov Search Results, assessed 11/05/2022 *(continued)*

| Identifier | Status | Title | Results | Condition | Interventions | Locations |
|---|---|---|---|---|---|---|
| NCT03683953 | Unknown | The Treatment of BPD by Intratracheal Instillation of MSCs | No results available | Safety issues; effect of drugs | Other: mesenchymal stem cells therapy Drug: 0.9% sodium chloride | Guangdong Women and Children Hospital, China |
| NCT03631420 | Active, not recruiting | MSCs for Prevention of BPD in Infants | No results available | BPD | Biological: human umbilical cord derived MSCs | National Chen-Kung University Hospital, Tainan City, Taiwan |
| NCT01207869 | Unknown | Intratracheal Umbilical Cord- derived MSCs for Severe BPD | No results available | BPD Extremely premature infants Severe BPD that conventional therapies has failed No severe congenital anomalies No severe IVH neither cystic PVL | Biological: UC-MSCs Other: normal saline | China Medical University Hospital, Taichung, Taiwan |
| NCT02381366 | Completed | Safety and Efficacy of Pneumostem® in Premature Infants at High Risk for BPD - a US Study | No results available | BPD | Biological: human umbilical cord blood Derived-MSCs | Rush University Medical Center, Chicago, Illinois, United States |
| NCT01297205 | Completed | Safety and Efficacy Evaluation of Pneumostem® Treatment in Premature Infants With BPD | No results available | BPD | Biological: human umbilical cord blood Derived-MSCs | Samsung Medical Center, Seoul, Republic of Korea |
| NCT03857841 | Terminated | A Safety Study of IV Stem Cell-derived Extracellular Vesicles (UNEX-42) in Preterm Neonates at High Risk for BPD | Has results | BPD | Biological: UNEX-42 Biological: phosphate-buffered saline | University of Colorado Hospital, Aurora, Colorado, United States; Boston Children's Hospital, Boston, Massachusetts, United States; Brigham and Women's Hospital, Boston, Massachusetts, United States; Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States; University of Mississippi Medical Center, Jackson, Mississippi, United States; Children's Mercy Hospital, Kansas City, Missouri, United States |

*(Continues)*

**Table 3.** Trials on MSCs for BPD. ClinicalTrials.gov Search Results, assessed 11/05/2022 *(continued)*

| Identifier | Status | Title | Results | Condition | Interventions | Locations |
|---|---|---|---|---|---|---|
| NCT01828957 | Completed | Efficacy and Safety Evaluation of Pneumostem® Versus a Control Group for Treatment of BPD in Premature Infants | No results available | BPD | Biological: Pneumostem® Other: normal saline | Asan Medical Center, Seoul, Republic of Korea; Samsung Medical Center, Seoul, Republic of Korea |
| NCT04003857 | Recruiting | Follow-up Study of Safety and Efficacy in Subjects Who Completed Pneumostem® Phase II (MP-CR-012) Clinical Trial | No results available | BPD | Biological: Pneumostem® Biological: normal saline | Asan Medical Center, Seoul, Republic of Korea; Samsung Medical Center, Seoul, Republic of Korea |
| NCT01897987 | Completed | Follow-up Safety and Efficacy Evaluation on Subjects Who Completed Pneumostem® Phase-II Clinical Trial | No results available | BPD | Biological: Pneumostem® Biological: normal saline | Asan Medical Center, Seoul, Republic of Korea; Samsung Medical Center, Seoul, Republic of Korea |
| NCT01632475 | Active, not recruiting | Follow-Up Study of Safety and Efficacy of Pneumostem® in Premature Infants with BPD | No results available | BPD | Biological: Pneumostem® | Samsung Medical Center, Seoul, Republic of Korea; Samsung Medical Center, Seoul, Republic of Korea |
| NCT03392467 | Unknown | Pneumostem® for the Prevention and Treatment of Severe BPD in Premature Infants | No results available | Severe BPD | Biological: Pneumostem® Other: Placebo | Asan medical Center, Seoul, Republic of Korea; Samsung Medical Center, Seoul, Republic of Korea |
| NCT02023788 | Completed | Long-term Safety and Efficacy Follow-up Study of Pneumostem® in Patients Who Completed Pneumostem® Phase-I Study | No results available | BPD; Respiratory Tract Infections; Premature Birth of Newborn | Biological: Pneumostem® | Samsung Medical Center, Seoul, Republic of Korea |
| NCT04255147 | Not yet recruiting | Cellular Therapy for Extreme Preterm Infants at Risk of Developing BPD | No results available | BPD | Biological: allogeneic umbilical cord tissue- derived MSCs | The Ottawa Hospital - General Campus, Ottawa, Ontario, Canada |

BPD: bronchopulmonary dysplasia; IVH: intraventricular hemorrhage; MSC: mesenchymal stem cell; Pneumostem®: human umbilical cord blood derived-mesenchymal stem cells; PVL: periventricular leukomalacia; US: United States.

local function via tissue fluid instead of via the bloodstream. Paracrine effects of MSC have been investigated using a conditioned medium, and have shown that the MSC-derived conditioned medium protected pulmonary epithelial and microvascular endothelial cells from oxidative stress, prevented alveolar developmental impairment induced by hyperoxia, and repaired the lung by stimulating bronchioalveolar stem cells[49-51]. This supports that the effects of MSCs on BPD animal models are primarily due to their paracrine effects instead of their capacity for regeneration.

MSCs exert their paracrine effects via secretion of various substances, including signaling peptides (interleukin [IL]-6, IL-8, and vascular endothelial growth factor), extracellular matrix proteins (collagen and elastin), and exosomes[52-54].

Exosomes are membrane-bound vesicles containing biological products as proteins and microRNAs, which

are phagocytosed by adjacent cells where they affect the gene expression. Target cells of MSC-derived- exosomes include fibroblasts, MSCs, epithelial and immune cells. MSCs have been shown to exert their anti-inflammatory properties in vitro through exosomes[55,56]. The effects of intra-tracheal (IT) treatment was compared between MSCs and MSC-derived exosomes, and exosomes have shown increased pulmonary compliance and survival rates in the DBP rat model compared to MSCs[57,58].

Mitochondrial transfer from MSCs to macrophage is reported to be responsible for the anti-inflammatory effects of MSCs on macrophages in inflammatory environments[59,60].

MSCs by the action of complex mechanisms are able to influence the functions of several immune cells, including activated T cells, B cells, natural killer cells, dendritic cells, monocyte/macrophages, neutrophils, and mast cells[61].

## *The regenerative effect*

MSC utilization in BPD animal models has been reported to increase the number of bronchioalveolar and distal stem cells and epithelial progenitor cells in the lung compared with that observed in the non- treated controls[62]. Results from animal studies point to the fact that this greater stem cells number, or progenitor cells, do not result from replacing with the exogenous MSCs, but instead, results from a process of endogenous repair improved by paracrine signalling[48].

## *Treatment methods (route, dose, and timing)*

In BPD animal models, MSCs are given by intravenous (IV), intraperitoneal, intranasal, and intratracheal (IT) routes. In a systematic review and meta-analysis of treatment potential of MSCs in experimental BPD, the treatment effect favoured MSC therapy over con- trols in all subgroups except in those that received MSCs by intranasal route[63].

In a preclinical study of IV versus IT differences MCS on hyperoxic-induced lung lesions in newborns rats, IT therapy attenuated pulmonary lesions in quadruple doses inferior to those used for IV treatment, indicating that the IT route is preferred for BPD[64].

For the success of treatment, the determination of cell dose is an important issue. Doses of approximately 105 MSCs have been used by IT route in the rat model, on postnatal days 3–10[47,49,62-64]. One study compared the different IT doses of MSCs of 5 x $10^3$, 5 x $10^4$, and 5 x $10^5$ cells, and found the dose of 5 x $10^5$ cells to be the most effective for the maintenance of lung architecture, survival rates, inflammation reduction, and collagen production[65]. The dose of $10^7$ cells/kg was selected as the used dose on further clinical trials. A phase I dose-escalation trial, used the doses 1 x $10^7$ and 2 x $10^7$ MSCs/kg given by IT route to human BPD patients, and both doses were used safely and were not associated to any short or long-term adverse event[44,66].

The optimal time of MSC treatment is another important issue to prevent or to treat the lung injury associated with BPD development. Early (postnatal day 3) IT administration of MSCs versus late (postnatal day 10) was shown to be more effective for the prevention of experimental BPD in a hyperoxia-induced lung injury model in neonatal rat[67]. Another study reported that MSC exerted a therapeutic benefit in both, experimental hyperoxia-induced BPD in rats as preventive (on postnatal day 4) and as regenerative (on postnatal day 14), highlighting the potential for their use in developing as well as in established BPD[49].

In the first clinical trial of MSC for BPD in human preterm neonates performed by Chang, et al[44]. in Korea, MSCs decreased the rate of moderate or severe BPD from 72% (13/18) to 33% (3/9), when compared with 18 patients in the historical cohort. The rate of home oxygen therapy at discharge was 0% in the MSC group, compared with 22% in the control group. At 18–24 months of corrected age, the mean bodyweight was significantly higher in the MSC group than in the control group; no differences were observed between groups in terms of height, head circumference, incidence of cerebral palsy, blindness, and neurodevelopmental outcomes suggesting that IT MSC treatment in preterm infants can be used safely.

In the USA, a phase I/II, open-label dose escalation trial using hUCB-MSC (Pneumostem®; Medipost) enrolled 12 infants, with a mean gestational age of 24.9 weeks and mean birthweight of 676g. In all, 1 x $10^7$ and 2 x $10^7$ MSC/kg were administered by IT route, at a mean of 10.6 ± 2.9 days of life. MSCs were used safely without any adverse events directly related to MSC therapy[47].

The current clinical practice is to avoid or remove endotracheal tubes early in preterm infants, so this may limit the use of autologous MSCs because of the weeks required to generate MSCs from umbilical cord tissue or blood. In this case IV administration may be an acceptable alternative to IT delivery. Questions regarding the use of MSCs as either prophylactic or therapeutic should be answered to determine the optimal route of administration.

## Difficulties in using MSC in a clinical setting

Important challenges will exist in translating MSC therapy into clinical setting, even if clinical trials of MSC for BPD are successful. The first is the significant het- erogeneity of MSC populations[68], on the dependence of the donor and tissue type from which they are harvested, and therefore, their different levels of effectiveness. MSC derived from gestational tissues, such as umbilical cord blood, Wharton's jelly, and placenta, are known to present less antigenicity, higher proliferative capacity, and stronger paracrine potency compared with MSC derived from bone marrow[69]. An interesting finding is that MSC from female subjects can secrete higher levels of anti-inflammatory and pro-angiogenic factors compared with MSC derived from male subjects and, in animal models, MSC from female rats better mitigated hyperoxic lung injury in newborn rats compared with MSC from male rats[70]. The need of large- scale production will also be necessary for routine clinical use, and extensive ex vivo proliferation and cryopreservation are needed for commercial preparation of MSC. Both, cell senescence and cryopreservation reduce the immunomodulatory properties of MSC[71,72]. Cell-free preparations, such as conditioned media and exosomes without MSC, still remain a preclinical technology, despite their great clinical potential[45]. Additional clinical trials will be conducted to investigate the efficacy of MSC in the prevention or treatment of BPD in premature infants.

## Conclusion

The discovery of the potential benefit of the use of MSCs in BPD has opened a major door in the current research as well as a great hope in the treatment of the disease. The results of the studies carried out to date have shown that treatments on BPD were well tolerated, without serious adverse effects or dose-limiting toxicity attributable to the transplantation. The severity of BPD was also shown to be lower in the transplant recipients. However, many questions remain open, namely sources of MSCs, route, dose, and timing of administration, ex vivo culture conditions and differentiation; and donor and recipient factors.

Neonatologists look forward to the results of trials currently underway around the world, and it is very likely that the next decade will define the role of MSCs as preventive and regenerative therapy for BPD.

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### Conflicts of interest

None.

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**Protection of human and animal subjects.** The authors declare that no experiments were performed on humans or animals for this study.

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**Right to privacy and informed consent.** The authors declare that no patient data appear in this article.

**Use of artificial intelligence for generating text.** The authors declare that they have not used any type of generative artificial intelligence for the writing of this manuscript, nor for the creation of images, graphics, tables, or their corresponding captions.

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