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FULL-LENGTH ARTICLE
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Repeated intravenous doses of human umbilical cord-derived mesenchymal stromal cells for bronchopulmonary dysplasia: results of a phase 1 clinical trial with 2-year follow-up

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ABSTRACT

Background: Currently, there is a lack of effective treatments or preventive strategies for bronchopulmonary dysplasia (BPD). Pre-clinical studies with mesenchymal stromal cells (MSCs) have yielded encouraging results. The safety of administering repeated intravenous doses of umbilical cord tissue-derived mesenchymal stromal cells (UC-MSCs) has not yet been tested in extremely-low-gestational-age newborns (ELGANs).
Aims: to test the safety and feasibility of administering three sequential intravenous doses of UC-MSCs every 7 days to ELGANs at risk of developing BPD. Methods: In this phase 1 clinical trial, we recruited ELGANs (birth weight ≤1250 g and ≤28 weeks in gestational age [GA]) who were on invasive mechanical ventilation (IMV) with FiO2 ≥ 0.3 at postnatal days 7–14. Three doses of 5×10^6/kg of UC-MSCs were intravenously administered at weekly intervals. Adverse effects and prematurity-related morbidities were recorded.
Results: From April 2019 to July 2020, 10 patients were recruited with a mean GA of 25.2 ± 0.8 weeks and a mean birth weight of 659.8 ± 153.8 g. All patients received three intravenous UC-MSC doses. The first dose was administered at a mean of 16.6 ± 2.9 postnatal days. All patients were diagnosed with BPD. All patients were discharged from the hospital. No deaths or any serious adverse events related to the infusion of UC-MSCs were observed during administration, hospital stays or at 2-year follow-up.
Conclusions: The administration of repeated intravenous infusion of UC-MSCs in ELGANs at a high risk of developing BPD was feasible and safe in the short- and mid-term follow-up.

Introduction

Bronchopulmonary dysplasia (BPD) is still the most common respiratory complication of preterm birth. Moreover, in recent years, with an increasing survival rate among extremely-low-gestational-age newborns (ELGANs), the incidence of BPD is rising and remains

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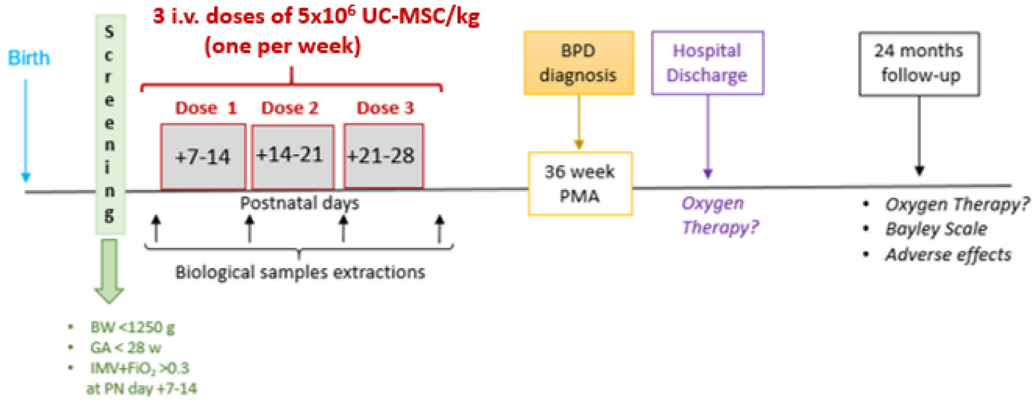

Figure 1. Scheme of the trial and administration of UC-MSCs. BW, birth weight; GA, gestational age; i.v., intravenous; PN, postnatal; UC-MSC, human umbilical cord-derived mesenchymal stromal cells. (Color version of figure is available online.)

the leading cause of morbidity and mortality in the ELGAN population [1]. The pathophysiology of BPD is complex, with inflammation, oxidative stress, abnormal vasculogenesis and impaired lung repair as the main factors [2]. Preventive strategies, such as antenatal steroids, rescue surfactant therapy, and non-invasive positive-pressure ventilation or new modes of invasive mechanical ventilation (IMV), have barely reduced its incidence [3].

In addition, survivors of BPD have a higher risk of developing pulmonary and neurodevelopmental disorders in adolescence and even in adulthood [4,5]. Therefore, it is crucial to find an effective therapeutic or preventive measurement for BPD.

Several studies have evidenced a reduction in resident stem cells in the lungs of premature infants with BPD [6–8]. These stem cells have a disrupted function due to the effects of hyperoxia, contributing to the pathogenesis of BPD [6–9]. The use of external mesenchymal stromal cells (MSCs) could play a role in the prevention or treatment of this disease. Pre-clinical studies using murine models, with MSCs or an MSC-conditioned medium, have shown promising results, showing changes in the lung architecture with restoration of angiogenesis and alveolarization, and decreasing fibrosis and inflammation [10–12]. Möbius et al. [8] observed that exogenous umbilical cord tissue-derived mesenchymal stromal cells (UC-MSCs) partially restore the oxygen-impaired fetal lung MSC secretory function. In view of these promising results in pre-clinical studies, phase 1 clinical trials in preterm infants have been initiated.

The first studies in this area were performed by the Korean research team of Chang et al. These researchers developed a phase 1 and a phase 2 clinical trial using umbilical cord blood-derived MSCs (UCB-MSCs), which were administered intratracheally [13,14]. They observed an improvement in BPD rate in the subgroup of patients with a lower GA (24–26 weeks) [14]. Powell [15] also conducted a phase 1 trial in 12 patients proving safety of a single intratracheal administration of 5×10^6 UCB-MSCs in pre-terms at high risk of BPD, administering the cells at 10 postnatal days. Xia [16] conducted in China a phase 1, dose escalating trial, trial testing safety of 1×10^6 and 5×10^6 iv delivered single doses of UC-MCSs in infants with established BPD. Nguyent et al. [17] tested the safety of two doses of 1×10^6 UC-MSCs in infants with established BPD.

In our first off-label clinical experience, we explored the feasibility and safety of repeated and increasing intravenous doses of bone marrow-derived MSCs in two ELGAN infants with established and severe BPD [18]. The intravenous administration of MSCs was associated with a decrease in serum inflammatory molecules and biomarkers of lung injury.

After this preliminary clinical experience, we designed and conducted a phase 1 clinical trial testing the feasibility and safety of repeated intravenous doses of Wharton's jelly UC-MSCs in ELGAN infants, which were administered in the second to fourth weeks after birth.

Materials and Methods

Study design and objectives

PULMESCELL-1 was a phase 1, open-label, single-arm, multi-center clinical trial. The primary endpoint was to evaluate the safety parameters of repeated intravenous administration of UC-MSCs in ELGANs at a high risk of BPD. The secondary endpoint was to evaluate the feasibility of this therapy.

The target sample size was 10 patients. The patients were enrolled at four different hospitals of the Spanish National Health System: Hospital Universitario La Paz (Madrid), Hospital Universitario Clínico San Carlos (Madrid), Hospital Universitari i Politècnic La Fe (Valencia) and Complexo Hospitalario Universitario A Coruña (A Coruña). The patients were recruited between April 2019 and June 2020.

Tracheal aspirates (TA) and serum samples were collected the before the first administration of UC-MSCs, prior to the administration of the second and third doses, and the week after the administration of the third dose (Figure 1) to determine changes in inflammation biomarkers. In this study, we assessed changes in cytokines and growth factors known to be involved in the development of BPD: IL-6 and IL-10. Tracheal aspirates for bronchoalveolar (BALF) were obtained only while the patients were intubated.

Table 1
Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
GA ≤ 28 weeks	Severe congenital and chromosomal anomalies[b]
Birth weight ≤ 1250 g	Severe septic shock or hemodynamic instability
IMV with $FiO_2 \geq 0.3$ at postnatal days 7–14[a]	Severe intraventricular hemorrhage (grade ≥III according to the Volpe classification [20])
	Necrotizing enterocolitis (grade >II according to the Bell classification [21])
	Major surgery 72 h before inclusion
	Active pulmonary bleeding
	HIV infection

GA, gestational age; HIV, human immunodeficiency virus; IMV, invasive mechanical ventilation.

[a] During the first 6 months of the trial, we enrolled patients on IMV at postnatal day 14. To ease recruitment and to be in accordance with the results of Hunt et al. [19], we received approval from the AEMPS (Spanish Medicine Agency) for a protocol amendment, allowing the inclusion of ELGANs on IMV with $FiO_2 \geq 0.3$ at postnatal day 7 onward during the last 8 months of the trial recruitment period.

[b] Severe congenital anomalies include lung hypoplasia, renal malformations, congenital heart disease and multiple malformative syndromes.

To compare the incidence of prematurity comorbidities, we retrospectively collected data from a cohort of patients of the same age and at a high risk of BPD (birth weight ≤1250 g and GA ≤28 weeks, on IMV with FiO2 ≥ 0.3 at postnatal days 7–14) born in 2019–2020 in five Spanish referral neonatal intensive care units, who did not receive the UC-MSC therapy. A total of 20 patients met the inclusion criteria and were included as the comparison group.

Inclusion and exclusion criteria

Patients with a gestational age ≤28 weeks, a birth weight ≤1250 g, and on respiratory support were included. More details on the inclusion and exclusion criteria are shown in Table 1.

BPD definition

At the time the trial was approved, the National Institutes of Child Health and Human Development (NICHD)'s 2001 definition of BPD was the definition in use [22]. Therefore, we defined BPD according to these criteria, at 36 weeks' postmenstrual age (PMA). This definition establishes different clinical stages of severity (mild, moderate and severe) depending on either the days of oxygen therapy and oxygen dependency at 36-week PMA in newborns with GA < 32 weeks or oxygen dependency at 56 days of life in newborns with GA ≥ 32 weeks.

Production and administration of the cell therapy

All patients were assigned to receive three consecutive doses of UC-MSCs (5×10^6 cells/kg/dose), at weekly intervals (Figure 1).

The doses of UC-MSCs were prepared in compliance with good manufacturing practices at the Gene and Cell Therapy Laboratory of the Hospital Universitario Niño Jesús (Madrid), which is accredited by the Spanish Medicine Agency (AEMPS) to produce UC-MSCs for human use. UC-MSCs were extracted from Wharton's jelly, expanded and frozen (see Appendix 1 for more detail). The UC-MSCs were suspended in sterile syringes and administered slowly through a peripheral vein. Once a patient was recruited, the laboratory thawed the UC-MSCs and manufactured the dose according to the patient's weight. The dose was provided in a sterile syringe, which was labeled for the patient and ready to be injected. The syringes were sent to the recruiting neonatal intensive care units, with control and registration of the temperature of the vials (data logger) throughout the entire transportation.

Assessment of safety

Safety was defined primarily as the absence of serious adverse events (SAEs) related to the administration of UC-MSCs according to the Consolidated Standards of Reporting Trials [23]. Second, it was defined as the absence of dose-related toxicity, defined as death at 6 h after a UC-MSC administration or anaphylactic shock related to a UC-MSC injection. After each intravenous UC-MSC injection, all patients were continuously monitored for blood pressure, oxygen saturation, heart rate and ventilation parameters. Blood, urine and tracheal aspirate samples were obtained before and 7 days after each UC-MSC administration. The patients were followed until 2 years of age for further safety evaluation. The AEMPS (Spanish Medicine Agency) and SCReN (Spanish Clinical Research Network) acted as the external monitors. The incidence of prematurity-related morbidities was registered and compared with the incidence of these morbidities in a coetaneous comparison group.

Assessment of feasibility

Feasibility was defined as the ability to administer UC-MSCs intravenously within the stipulated time frame. The first dose was to be administered within the second and the fourth day after screening. During the interval between screening and administration of the first dose, UC-MSCs had to be prepared, thawed, packed, labeled, transported and infused to the patients. The remaining doses were to be administered at weekly intervals (±3 days) after the first dose.

Study variables

Clinical, echocardiographic and analytical variables were recorded in an *ad hoc* online database (www.pulmescell.org). At 2-year follow-up, respiratory and neurological outcomes were recorded.

Statistical analysis

Data were collected prospectively using the PULMESCELL online database. Since this was a phase 1 clinical trial on safety, and we had performed two previous off-label treatments with repeated intravenous doses in BPD infants, we decided to recruit a sample of 10 patients. All statistical analyses were descriptive. Categorical variables are expressed as frequencies and percentages, and continuous variables are presented as mean ± standard deviation (SD) and median (inter-quartile range [IQR]). The dataset containing clinical variables obtained from serum and TA was explored to examine the distributions and summary statistics of the variables of interest. Missing values of the type missing completely at random were imputed using multiple imputation. Statistical analysis was performed using the R programming language (version 4.3.0) and relevant packages.

Ethical aspects

The protocol was approved by the AEMPS and by the CEIm-R on January 31, 2017 (reference number 07/140183.9/17).

In order to be included in the trial, written informed consent was obtained from both parents after confirming their full understanding, with a particular emphasis that the main study outcome was safety and there was no expectation or promise of therapeutic benefit.

Results

Between April 2019 and July 2020, 10 patients (6 males and 4 females) were recruited (see consort chart in Figure 2), with a mean GA of 25.2 ± 0.8 weeks (median = 25; IQR = 24.6–25.4) and a birth weight of 659.9 ± 153.8 g (median = 665; IQR = 492–780).

All patients received three doses of 5×10^6 cells/kg of UC-MSCs. The first UC-MSC dose was administered at a mean postnatal day of 16.6 ± 2.9 (median = 17; IQR = 13.8–17.8). The intravenous administration of all UC-MSC doses took less than 10 min in all cases. All patients tolerated the infusion without complications. No events occurred in the 24 h after the UC-MSC administration. No respiratory or cardiovascular compromise was observed during the administration or in the following day. The 10 patients included in the trial were discharged from the hospital. No deaths were reported during the treatment period or at follow-up. No adverse effects related to UC-MSC administration were observed at follow-up, with a mean follow-up period of 24.7 ± 1.9 months (median = 24.5; IQR = 23–27). The baseline patient characteristics are summarized in Table 2. The clinical characteristics, outcomes at 36 weeks' PMA and at the end of the 2 years' follow-up of each individual patient are shown in the Supplementary Table S1.

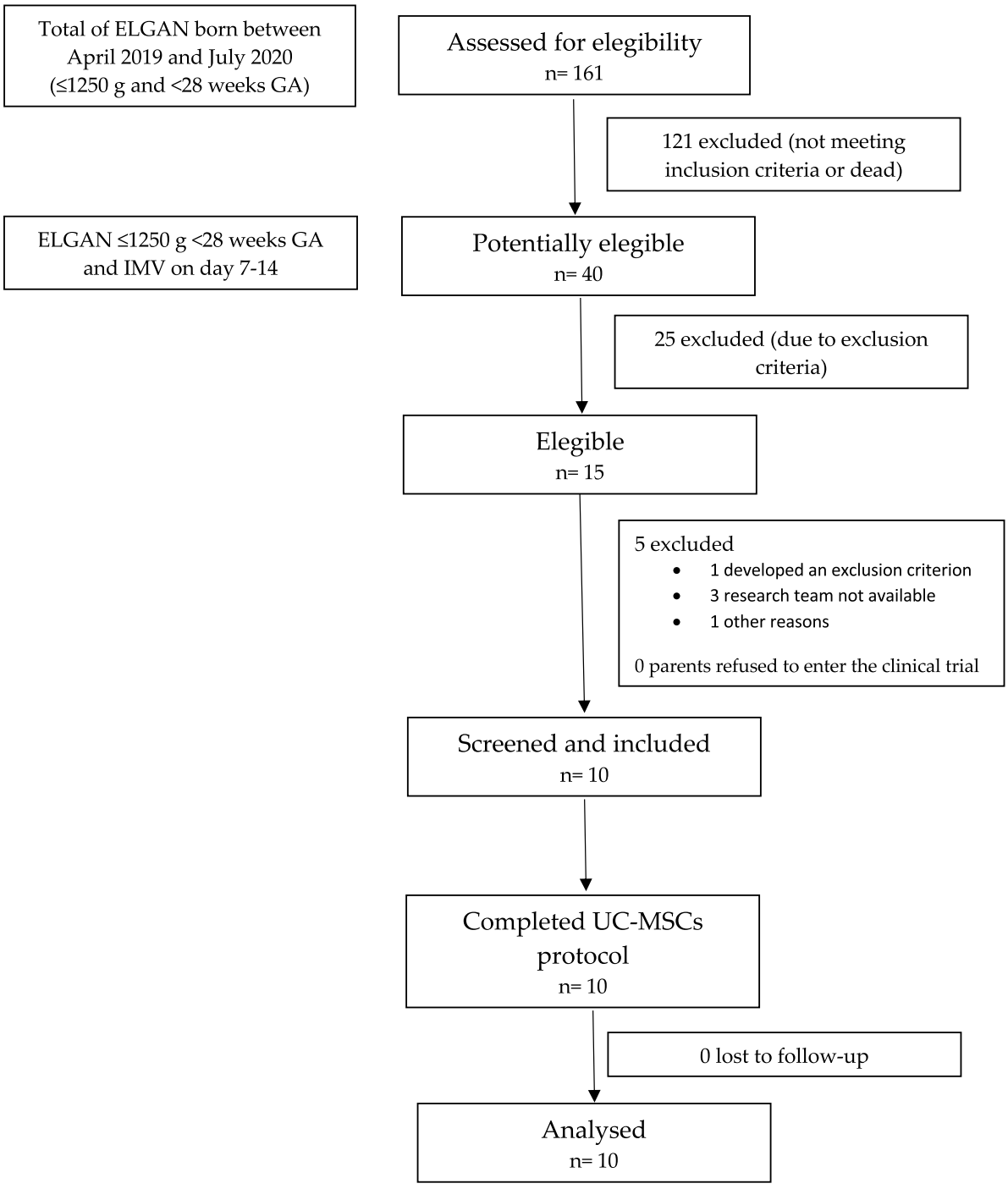

Figure 2. Consort chart of the clinical trial.

The baseline characteristics of the comparison group used to compare the incidence of prematurity-related morbidities are described in Supplementary Tables S2–S4.

SAEs and prematurity-related morbidities

The details regarding SAEs and prematurity-related morbidities, recorded for up to 2 years after the UC-MSC administration, are presented in Table 3. The most common event was patent ductus arteriosus ligation, occurring in 5 of the 10 patients (50%). All patients received postnatal corticosteroids. Nine of the 10 patients received systemic corticosteroids simultaneously (partially or totally) during the 3 weeks of administration of the UC-MSC (6 of the patients received hydrocortisone, and the remaining 3 patients received dexamethasone) (see Table S1 for more detail). Prematurity-related events and comorbidities were not considered adverse events unless their frequency or severity was unexpected for this type of patients. This was evaluated by the neonatologists in charge of the patients. The incidence and severity of observed prematurity-related comorbidities were compared with those in the comparison group of coetaneous preterm newborns born in the same period of time.

Table 2
Baseline patient characteristics.

Characteristics	Values
Maternal age: median (IQR), in yr	34.5 (31–38)
mean ± SD, in yr	34.7 ± 6.9
Spontaneous conception: no./total (%)	8/10 (80%)
Single gestation: no./total (%)	5/10 (50%)[c]
Prenatal steroids: no./total (%)	10/10 (100%)
Complete prenatal steroid protocol: no./total (%)[a]	3/10 (30%)
Oligohydramnios: no./total (%)	2/10 (20%)
Chorioamnionitis: no./total (%)[b]	3/7 (43%)
Gestational age: median (IQR), in wk	25 (24.6–25.4)
mean ± SD, in wk	25.2 ± 0.8
Birth weight: median (IQR), in g	665 (492–780)
mean ± SD, in g	659.8 ± 153.8
Male: no./total (%)	6/10 (60%)
IUGR ($P < 3$): no./total (%)	1/10 (10%)
Apgar score: median (IQR)/mean ± SD	
• Minute 1	4 (3–7)/4,8 ± 2.8
• Minute 5	7 (3–9)/6.3 ± 2.8
Intubation and IMV at birth: no./total (%)	6/10 (60%)
Surfactant administration: no./total (%)	10/10 (100%)
• 1 dose	5/10 (50%)
• 2 doses	2/10 (20%)
• 3 doses	3/10 (30%)
SNAPPE-II: median (IQR)	46.5 (40–69)
mean ± SD	51.3 ± 17.1

IQR, interquartile range; IUGR, intrauterine growth retardation; IMV, invasive mechanical ventilation; RSS, respiratory severity score (mean airway pressure x FiO2); SD, standard deviation.
[a] Complete prenatal steroid protocol included 2 doses separated by 12 h and administered 48 h before labor.
[b] Data are available for 7 patients.
[c] One of the single gestation was multiple until week 12.

BPD incidence was evaluated at 36 weeks' GA, as it is shown in Table 4, with 5 patients (50%) being diagnosed of severe BPD at this time.

At the 2-year follow-up, no patient required respiratory support, and only one patient was on inhaled steroid treatment. The remaining nine patients were not receiving respiratory treatment. One patient, born at 24 weeks of GA, was diagnosed with cerebral palsy at 18 months, which was considered not related to the UC-MSC administration by the neonatologist in charge. A Bayley score was performed at 2 years of age in 3 of 10 patients; 3 patients only had telephone follow-up at age 2 (parents moved to other countries), and in the remaining 4 patients, linguistic barriers precluded the application of the Bailey score.

Table 3
Incidence of prematurity-related morbidities in the patients included in the trial.

Prematurity-related morbidities	Values	No./total (%)
NEC grade IB[a]: no./total (%)		1/10 (10%)
Nosocomial sepsis: no./total (%)		8/10 (80%)
IVH grade II[b]: no./total (%)		1/10 (10%)
ROP: no./total (%)		7/10 (70%)
• Grade I		1/10 (10%)
• Grade II		1/10 (10%)
• Grade III		3/10 (30%)
• Grade >III		2/10 (20%)
ROP treatment: no./total (%)		
• No treatment		2/7 (29%)
• Bevacizumab/Ranivizumab		5/7 (71%)
• Surgery		1/7 (14%)
PDA: no./total (%)		9/10 (90%)
PDA treatment: no./total (%)		
• NSAIDs		9/10 (90%)
• Percutaneous/surgical closure		5/10 (50%)

NEC, necrotizing enterocolitis; NSAIDs, non-steroidal anti-inflammatory drugs; ROP, retinopathy of prematurity; IVH, intraventricular hemorrhage; PDA, persistent ductus arteriosus.
[a] According to the Bell classification.
[b] According to the Volpe classification.

Table 4
Respiratory outcomes of the patients included in the trial.

Respiratory outcomes	Values	No./total (%)
BPD at 36 weeks PMA (NICHD 2001 definition [19]): no./total (%)		10/10 (100%)
• Mild		1/10 (10%)
• Moderate		4/10 (40%)
• Severe		5/10 (50%)
Death before discharge: no./total (%)		0/10 (0%)
BPD or death before discharge: no./total (%)		10/10 (100%)
Death or severe BPD (NICHD 2001 definition): no./total (%)		5/10 (50%)
Postnatal steroids[a]: no./total (%)		10/10 (100%)
Respiratory condition at 36 wk PMA: no./total (%)		
• Room air		1/10 (10%)
• NC		1/10 (10%)
• HFNC		4/10 (40%)
• NIV		4/10 (40%)
Supplemental oxygen at discharge: no./total (%)		6/10 (60%)
Supplemental oxygen at 24-month follow-up		0/10 (0%)
Need for steroids at 24-month follow-up		1/10 (10%)

BPD, bronchopulmonary dysplasia; HFNC, high-flow nasal cannula; NA, not available; NC, nasal cannula; NIV, nasal intermittent ventilation; PMA, postmenstrual age; wk, week.
[a] 90% received steroids during the 3 weeks period of UC-MSC administration.

No significant differences related to GA and birth weight were found between the treated patients and the comparison group. Postnatal corticosteroid use was similar between the UC-MSC treatment group and the comparison group. All patients in the comparison group were also diagnosed with BPD according to the NICHD 2001 definition, with a similar distribution of severity. A similar percentage of ELGANs in both groups needed supplemental oxygen at discharge. The incidence of prematurity-related morbidities did not differ in the treatment group versus the comparison group (Table S3). There were three deaths (15%) in the comparison group, while all patients in the UC-MSC treatment group were discharged

Inflammatory biomarkers

Blood and tracheal aspirate samples were obtained before and a week after each UC-MSC administration to analyze inflammation biomarkers. The levels of IL-6, IL-10 and IL-6/IL-10 ratio in blood and BALF aspirates before and after the MSCs are shown in Figures 3 and 4, respectively.

We found a trend toward a decrease in the levels of IL6, both in blood and in BALF, and decrease in ratio of IL-6/IL-10 after the UC-MSC injections that run parallel to the improvement in the respiratory severity score.

The urine samples could only be collected in a few patients, and with missing samples, so were finally not included in the analysis.

Discussion

This is the first trial exploring repeated intravenous administration of allogenic UC-MSCs, produced from Wharton's jelly, in extremely preterm infants at a high risk of developing BPD, as preventive strategy. Our results showed that the administration of these cells is safe and feasible. No adverse events related to the therapy were observed neither acutely after the cell infusion nor at 2-year follow-up. This study opens new research lines with UC-MSCs as a preventive or therapeutic strategy for preterm infants at a high risk of developing BPD, although several issues will have to be addressed in future trials: ideal route of administration, timing, protocol, dose and number of doses.

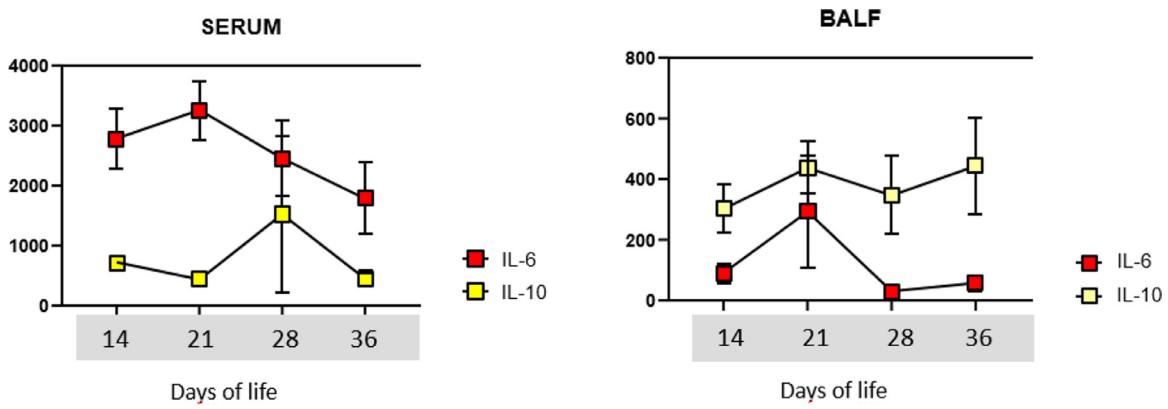

Figure 3. Serum and BALF determination of IL-6 and IL-10. BALF, bronchoalveolar aspirate. (Color version of figure is available online.)

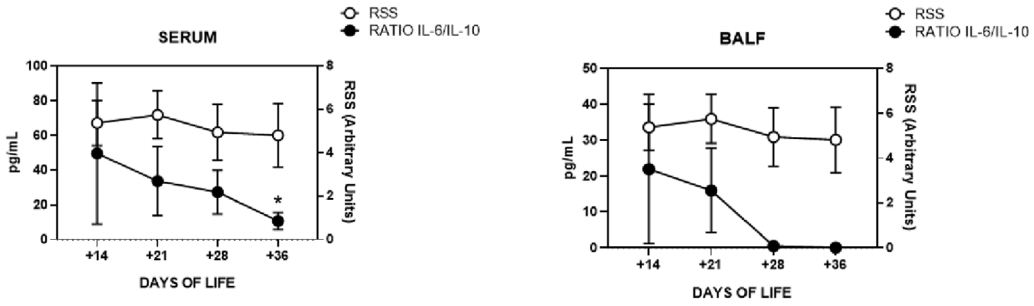

Figure 4. Serum and BALF determination of IL-6/IL-10 ratio. BALF, bronchoalveolar aspirate; RSS, respiratory severity score.

With the exception of two phase 1 clinical trials being conducted MSCs in patients with established BPD [16,17], the rest of trials testing MSCs in preventive models had used a single dose, either intravenously or intratracheally. In this study, the decision to use repeated doses was motivated by our previous off-label experience [18] and the high immaturity of eligible patients (ELGANs ≤ 28 weeks of GA). At this age, the lung is still being subjected to inflammatory stimuli and oxidative stress for several weeks; therefore, these infants will continue to be at risk for alveolo-capillary damage and progression of BPD [24]. In a pre-clinical study conducted by O'Reilly in 2020 with rat models exposed to hyperoxia with established alveolo-capillary damage, the intratracheal administration of four doses of MSCs showed superiority versus a single dose in restoring lung architecture, demonstrating pulmonary reverse remodeling and increased vessel density [25].

Other reason for the use of repeated doses is the lifespan of the MSCs once infused. Guess et al. [26] showed that inoculated MSCs in mice did not remain in the lungs 7 days after the administration [26]. Similarly, we performed a pre-clinical biodistribution study in mice, observing that inoculated MSCs reached the lungs but were no longer detectable 4–7 days after the infusion [27]. This experiences together with the absence of engraftment in tissues and the positive results obtained in animal models using either intravenous [13,14], intraperitoneal [28,29] intranasal [30] MSCs administration or MSC-derived extracellular vesicles administration [31–34] support the hypothesis of a paracrine mechanism of action of MSCs, with systemic effects [35].

Regarding the optimal dose of MSCs, in our study, we used three intravenous doses of 5×10^6 UC-MSC/kg so as to not exceed the maximum dose used in the phase 1 study by Chang et al. [13] published in 2014, which tested the safety and feasibility of UCB-MSCs in ELGANs with a single intratracheal dose of 10×10^6 or 20×10^6/kg. Also, we have published our previous off-label study in which we administered MSCs to two ELGAN patients with severe BPD [18]. In the first patient, we administered increasing doses of bone marrow-derived MSCs, from 1.1×10^6 in the first dose to up to 13.9×10^6 MSCs/kg [15]. The second patient received three sequential doses of 5×10^6 /kg [18]. The treatment was well tolerated in both patients. Inflammatory markers and biomarkers of lung damage decreased after the treatment with bone marrow-derived MSCs in both cases and increased again after the therapy was stopped.

Deciding the ideal route of administration, a single intratracheal administration of UCB-MSCs was the first published in human babies [13], but with a non-significant decrease in BPD incidence in the subgroup of patients under 26 weeks of GA in the phase 2 trial [14]. The intratracheal route has the advantage of MSCs being delivered directly into the lungs, but the increasing use of non-invasive mechanical ventilation [36] makes this route less feasible, especially for multiple doses protocols.

The ideal time to administer MSCs is another issue that requires further research. The inclusion criteria of this study included ELGANs at a high risk of developing BPD (birth weight ≤1250 g and ≤28 weeks in GA, on IMV with FiO_2 ≥0.3 at postnatal day 14, and immediate extubation was not foreseeable). Six months after the recruitment had begun, the AEMPS authorized a protocol amendment to start screening patients on IMV at day +7 (instead of day +14) for UC-MSC administration to ease recruitment and to administer the UC-MSC therapy at earlier stages of lung injury. Several publications have shown that ELGANs on IMV at postnatal day +7 are at a high risk of moderate/severe BPD [19,37]. Also, in newborn Sprague Dawley rats exposed to hyperoxia, a decrease in inflammatory markers and oxidative stress was achieved only in rats when MSCs were given early (postnatal day 3 versus day 10) [38]. After this protocol amendment to ease the inclusion criteria, the window for the administration of the first dose of MSCs was anticipated in our trial

from postnatal days +18 to +21 to postnatal days +11 to +14 (taking into account the delay from patient screening to cell administration).

Patients who will probably benefit most from UC-MSC administration will be those at a higher risk of developing moderate or severe BPD. Therefore, the selection of patients at early stages is mandatory to allow an early administration of MSC. However, the identification of these patients is not yet well established. In an attempt to perform early detection in the high-risk population, several risk scores are under investigation. In a clinical trial by Hunt et al., the need for IMV at postnatal day +7 showed a sensitivity of 99% and a specificity of 67% for predicting the development of BPD, and a sensitivity of 63% and a specificity of 92% for predicting severe BPD [19]. Jung et al. [39] reported an 11-fold increase in the risk of developing severe BPD in patients with a respiratory severity score $>$ 3 at postnatal day +14 in a sample of 138 ELGANs. Leigh et al. [40] developed a machine-learning BPD-free survival model based on five perinatal factors and respiratory support requirement at the time of assessment (postnatal days 1, 7 and 14). The five perinatal factors taken into account in the development of the model were gender, self-reported race/ethnicity, birth gestation, birth weight category and maternal smoking during pregnancy. These five factors obtained the best prediction of risk when they were measured together with respiratory support between postnatal days 1 and 14, showing an area under the curve (AUC) of 0.921 (0.848–0.949)

Several patients in our study received concomitant steroids and UC-MSCs. Steroids are potent anti-inflammatory agents that reduce vascular permeability and pulmonary edema [41], shortening the time of IMV in ELGANs or extremely-low-birth-weight infants [42]. Nevertheless, its routine use is controversial due to short- and long-term effects [43]. In our clinical trial, all patients received postnatal steroids; 9 of the 10 patients received postnatal steroids coinciding, totally or partially, with the administration of UC-MSCs. Little is known about the potential interactions of corticosteroids in the immunomodulatory function of UC-MSCs. In vitro studies, Salimiyan et al. [45] have shown in cultures that hydrocortisone long-term treatment can have effect on immunomodulatory properties of human adipose-derived mesenchymal stromal/stem cells, depending on the dose and length of the treatment. Nevertheless, there are no data regarding the potential interactions in human preterm lungs by the simultaneous use of systemic corticosteroids and UC-MSCs. The results of our trial, in which some patients received both therapies at the same time, suggest that this combination was not related to adverse effects.

This phase 1 clinical trial was designed to evaluate safety and feasibility of UC-MSC administration in preterm infants at risk of BPD. The comorbidities of prematurity and respiratory outcomes were compared with a peer group of similar characteristics who did not receive the treatment, just to evaluate safety of the MSCs, and we found that prematurity-related complications were neither more frequent nor more severe in the intervention group. Nevertheless, as the comparison group data were collected retrospectively (after the phase 1 recruitment had finished), and the number of both groups small, we consider any other difference in respiratory outcomes or survival, observational data that will have to be properly evaluated in a phase 2 trial that has already been approved by the Spanish drug Agency.

As a strength of the study, it should be noted that despite the need to thaw and prepare UC-MSCs once a patient was randomized and the fact that several of the participating hospitals are located far away from the producing laboratory, it was possible to administer the UC-MSC doses within the stipulated time interval, which reinforces the feasibility of the therapy.

Regarding safety, no SAES attributable to the UC-MSC administration were observed. The case of cerebral palsy diagnosed at 18 months of corrected age in a patient born at 24 weeks of GA was reported as being “not related” to the intervention; the incidence of this SAE in our trial was similar to that reported in the literature for ELGANs [44].

Conclusions

In conclusion, this phase 1 clinical trial showed the feasibility and safety of repeated intravenous UC-MSC administration in ELGAN infants at a high risk of BPD. Based on these results, a new phase 2 clinical trial to investigate intravenous administration of UC-MSCs in pre-term infants is currently ongoing with active recruitment (NCT06270199). Further studies are needed to determine the optimal UC-MSC dose, number of doses, timing of therapy and route of administration, and possibly the ideal target population for this strategy.

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Declaration of Competing Interest

Dr. Manuel Ramírez Orellana received research grants from Orgenesis. The other authors declare no conflicts of interest. The funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Author Contributions

Conceptualization: MJC, AGN, MAF, PLO, AAA, LAG, MLB, LM, GM, MR, MV and MJC. Methodology: AGN, PLO, AAA, LAG, MLB, MV and

MJC. Investigation: LM, GM and MR. Writing—original draft preparation: IGO and MJC. Writing—review and editing: MAF, AAA, LAG, TST, AP and MV. Supervision: MJC. Funding acquisition: MJC. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of HOSPITAL RAMÓN Y CAJAL (protocol NTC02443961, approved on January 31, 2017).

Informed Consent Statement

Informed consent was obtained from all parents of the patients involved in this study. Written informed consent was also obtained from the parents to publish this paper.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jcyt.2024.02.028.

References

[1] Siffel C, Kistler KD, Lewis JFM, Sarda SP. Global incidence of bronchopulmonary dysplasia among extremely preterm infants: a systematic literature review. J Matern Fetal Neonatal Med 2021;34:1721–31.
[2] Sahni M, Bhandari V. Recent advances in understanding and management of bronchopulmonary dysplasia. F1000Res 2020;9. F1000 Faculty Rev-703.
[3] Thébaud B, Goss KN, Laughon M, Whitsett JA, Abman SH, Steinhorn RH, et al. Bronchopulmonary dysplasia. Nat Rev Dis Primers 2019;5:78.
[4] Sriram S, Schreiber MD, Msall ME, Kuban KCK, Joseph RM, O' Shea TM, et al. Cognitive development and quality of life associated with BPD in 10-year-olds born preterm. Pediatrics 2018;141:e20172719.
[5] Hove MV, Prenzel F, Uhlig HH, Robel-Tillig E. Pulmonary outcome in former preterm, very low birth weight children with bronchopulmonary dysplasia: a case-control follow-up at school age. J Pediatr 2014;164:40–5.
[6] Möbius MA, Thébaud B. Bronchopulmonary dysplasia: where have all the stem cells gone? Origin and (potential) function of resident lung stem cells. Chest 2017;152:1043–52.
[7] Fujinaga H, Baker CD, Ryan SL, Markham NE, Seedorf GJ, Balasubramaniam V, et al. Hyperoxia disrupts vascular endothelial growth factor-nitric oxide signaling and decreases growth of endothelial colony-forming cells from preterm infants. Am J Physiol Lung Cell Mol Physiol 2009;297:L1160–9.
[8] Möbius MA, Freund D, Vadivel A, Koss S, McConaghy S, Ohls RK, et al. Oxygen disrupts human fetal lung mesenchymal cells. Implications for bronchopulmonary dysplasia. Am J Respir Cell Mol Biol 2019;60:592–600.
[9] Chia WK, Cheah FC, Abdul Aziz NH, Kampan NC, Shuib S, Khong TY, et al. A review of placenta and umbilical cord-derived stem cells and the immunomodulatory basis of their therapeutic potential in bronchopulmonary dysplasia. Front Pediatr. 2021;9:615508.
[10] Curley GF, Hayes M, Ansari B, Shaw G, Ryan A, Barry F, et al. Mesenchymal stem cells enhance recovery and repair following ventilator-induced lung injury in the rat. Thorax 2012;67:496–501.
[11] Hansmann G, Fernández-González A, Aslam M, Vitali SH, Martin T, Alex Mitsialis S, et al. Mesenchymal stem cell-mediated reversal of bronchopulmonary dysplasia and associated pulmonary hypertension. Pulm Circ. 2012;2:170–81.
[12] Van Haaften T, Byrne R, Bonnet S, Rochefort GY, Akabutu J, Bouchentouf M, et al. Airway delivery of mesenchymal stem cells prevents arrested alveolar growth in neonatal lung injury in rats. Am J Respir Crit Care Med 2009;180:1131–42.
[13] Chang YS, Ahn SY, Yoo HS, Sung SI, Choi SJ, Oh WI, Park WS. Mesenchymal stem cells for bronchopulmonary dysplasia: phase 1 dose-escalation clinical trial. J Pediatr 2014;164:966–72.
[14] Ahn SY, Chang YS, Lee MH, Sung SI, Lee BS, Kim KS, et al. Stem cells for bronchopulmonary dysplasia in preterm infants: a randomized controlled phase II trial. Stem Cells Transl Med 2021;10:1129–37.
[15] Powell SB, Silvestri JM. Safety of intratracheal administration of human umbilical cord blood derived mesenchymal stromal cells in extremely low birth weight preterm infants. J Pediatr 2019;210:209e213.e2.
[16] Xia Y, Lang T, Niu Y, Wu X, Zhou O, Dai J, et al. Phase I trial of human umbilical cord-derived mesenchymal stem cells for treatment of severe bronchopulmonary dysplasia. Genes Dis 2022;10:521–30.
[17] Nguyen LT, Trieu TTH, Bui HTH, Hoang VT, Nguyen ATT, Trinh NTH, et al. Allogeneic administration of human umbilical cord-derived mesenchymal stem/stromal cells for bronchopulmonary dysplasia: preliminary outcomes in four Vietnamese infants. J Transl Med 2020;18:398.
[18] Álvarez-Fuente M, Arruza L, López-Ortego P, Moreno L, Ramírez-Orellana M, Labrandero C, et al. Off-label mesenchymal stromal cell treatment in two infants with severe bronchopulmonary dysplasia: clinical course and biomarkers profile. Cytotherapy 2018;20:1337–44.
[19] Hunt KA, Dassios T, Ali K, Greenough A. Prediction of bronchopulmonary dysplasia development. Arch Dis Child Fetal Neonatal Ed 2018;103:F598–9.
[20] Volpe JJ. Perinatal brain injury: from pathogenesis to neuroprotection. Ment Retard Dev Disabil Res Rev 2001;7:56–64.
[21] Gregory KE, Deforge CE, Natale KM, Phillips M, Van Marter LJ. Necrotizing enterocolitis in the premature infant: neonatal nursing assessment, disease pathogenesis, and clinical presentation. Adv Neonatal Care 2011;11:155–64. quiz 165-6.
[22] Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med 2001;163:1723–9.
[23] Ioannidis JP, Evans SJ, Gotzsche PC, O'Neill RT, Altman DG, Schulz K, et al. Better reporting of harms in randomized trials: an extension of the CONSORT statement. Ann Intern Med 2004;141:781–8.
[24] Pierro M, Ionescu L, Montemurro T, Vadivel A, Weissmann G, Oudit G, et al. Short-term, long-term and paracrine effect of human umbilical cord-derived stem cells in lung injury prevention and repair in experimental bronchopulmonary dysplasia. Thorax 2013;68:475–84.
[25] O'Reilly M, Möbius MA, Vadivel A, Ionescu L, Fung M, Eaton F, et al. Late rescue therapy with cord-derived mesenchymal stromal cells for established lung injury in experimental bronchopulmonary dysplasia. Stem Cells Dev 2020;29:364–71.
[26] Guess AJ, Daneault B, Wang R, Bradbury H, La Perle KMD, et al. Safety profile of good manufacturing practice manufactured interferon γ-primed mesenchymal stem/stromal cells for clinical trials. Stem Cells Transl Med 2017;6:1868–79.
[27] Gambera S, del Cerro MJ, Álvarez-Fuente M, Prieto R, Melen Frajilich G, González Murillo A, et al. Biodistribution of umbilical cord-derived mesenchymal stem cells in a mouse model. In: 11th International Conference Neonatal & Childhood Pulmonary Vascular Disease, San Francisco, USA; 19-21 April 2018. (unpublished).
[28] Zhang Z, Wang H, Shi Y, Peng W, Zhang S, Zhang W, et al. Role of bone marrow-derived mesenchymal stem cells in the prevention of hyperoxia-induced lung injury in newborn mice. Cell Biol Int 2012;36:589–94.
[29] Braun RK, Chetty C, Balasubramaniam V, Centanni R, Haraldsdottir K, Hematti P, et al. Intraperitoneal injection of MSC-derived exosomes prevent experimental bronchopulmonary dysplasia. Biochem Biophys Res Commun 2018;503:2653–8.
[30] Moreira Á, Winter C, Joy J, Winter L, Jones M, Noronnha M, et al. Intranasal delivery of human umbilical cord Wharton's jelly mesenchymal stromal cells restores lung alveolarization and vascularization in experimental bronchopulmonary dysplasia. Stem Cells Transl Med 2020;9:221–34.
[31] Willis GR, Fernandez-Gonzalez A, Anastas J, Vitali SH, Liu X, Ericsson M, et al. Mesenchymal stromal cell exosomes ameliorate experimental bronchopulmonary dysplasia and restore lung function through macrophage immunomodulation. Am J Respir Crit Care Med 2018;197:104–16.
[32] Willis GR, Reis M, Gheinani AH, Fernández-González A, Taglauer ES, Yeung V, et al. Extracellular vesicles protect the neonatal lung from hyperoxic injury through the epigenetic and transcriptomic reprogramming of myeloid cells. Am J Respir Crit Care Med 2021;204:1418–32.
[33] Chaubey S, Thueson S, Ponnalagu D, Alam MA, Gheorghe CP, Aghai Z, et al. Early gestational mesenchymal stem cell secretome attenuates experimental bronchopulmonary dysplasia in part via exosome-associated factor TSG-6. Stem Cell Res Ther 2018;9:173.
[34] You J, Zhou O, Liu J, Zou W, Zhang L, Tian D, et al. Human umbilical cord mesenchymal stem cell-derived small extracellular vesicles alleviate lung injury in rat model of bronchopulmonary dysplasia by affecting cell survival and angiogenesis. Stem Cells Dev 2020;29:1520–32.
[35] Kim YE, Park WS, Sung DK, Ahn SY, Sung SI, Yoo HS, et al. Intratracheal transplantation of mesenchymal stem cells simultaneously attenuates both lung and brain injuries in hyperoxic newborn rats. Pediatr Res 2016;80:415–24.
[36] Ávila-Álvarez A, Zozaya C, Pértega-Díaz S, Sánchez-Luna M, Iriondo-Sanz M, Elorza MD, et al. Temporal trends in respiratory care and bronchopulmonary dysplasia in very preterm infants over a 10-year period in Spain. Arch Dis Child Fetal Neonatal Ed 2022;107:143–9.
[37] Álvarez-Fuente M, Moreno L, López-Ortego P, Arruza L, Ávila-Álvarez A, Muro M, et al. Exploring clinical, echocardiographic and molecular biomarkers to predict bronchopulmonary displasia. PLoS One 2019;14:e0213210.

[38] Chang YS, Choi SJ, Ahn SY, Sung DK, Sung SI, Yoo HS, et al. Timing of umbilical cord blood derived mesenchymal stem cells transplantation determines therapeutic efficacy in the neonatal hyperoxic lung injury. PLoS One 2013;8:e52419.
[39] Jung YH, Jang JJ, Kim HS, Shin SH, Choi CW, Kim EK, et al. Respiratory severity score as a predictive factor for severe bronchopulmonary dysplasia or death in extremely preterm infants. BMC Pediatr 2019;19:121.
[40] Leigh RM, Pham A, Rao SS, Vora FM, Hou G, Kent C, et al. Machine learning for prediction of bronchopulmonary dysplasia-free survival among very preterm infants. BMC Pediatr 2022;22:542.
[41] Bonadies L, Zaramella P, Porzionato A, Perilongo G, Muraca M, Baraldi E. Present and future of bronchopulmonary dysplasia. J Clin Med 2020;9:1539.
[42] Doyle LW, Davis PG, Morley CJ, McPhee A, Carlin JB. DART Study Investigators. Low-dose dexamethasone facilitates extubation among chronically ventilator-dependent infants: a multicenter, international, randomized, controlled trial. Pediatrics 2006;117:75–83.
[43] Doyle LW, Ehrenkranz RA, Halliday HL. Dexamethasone treatment after the first week of life for bronchopulmonary dysplasia in preterm infants: a systematic review. Neonatology 2010;98:289–96.
[44] Agut T, Póo P, Launes C, Auffant M, Iriondo M. Incidence of cerebral palsy in a cohort of preterm infants with a gestational age of less than 28 weeks. An Pediatr (Barc) 2015;82:49–50.
[45] Salimiyan S, Mohammadi M, Aliakbari S, Kazemi R, Ali Amini A, Rahmani MR. Hydrocortisone long-term treatment effect on immunomodulatory properties of human adipose-derived mesenchymal stromal/stem cells. Interferon Cytokine Res 2022;42:72–81.