Safety of Allogenic Human Cord Blood Derived Mononuclear Cells for Extreme Preterms Infants at High Risk of Death: A Descriptive Study

Jia Chen
7th Medical Center of Chinese PLA General Hospital
Yabo Mei
7th Medical Center of Chinese PLA General Hospital
Xue Du
7th Medical Center of Chinese PLA General Hospital
Qiuping Li
7th Medical Center of Chinese PLA General Hospital
Zizhen Wang
7th Medical Center of Chinese PLA General Hospital
Xing Zhang
7th Medical Center of Chinese PLA General Hospital
Yuhan Chen
7th Medical Center of Chinese PLA General Hospital
Yanping Zhang
7th Medical Center of Chinese PLA General Hospital
Zhichun Feng (zhichunfeng81@163.com)
7th Medical Center of Chinese PLA General Hospital

Research

Keywords: safety, effect, allogenic, extreme preterm infant, human cord blood, mononuclear cells, preterm complication

Posted Date: September 15th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-72840/v1

Abstract

Backgroud Extreme preterm infants are at a high risk for developing preterm complications and death. Despite advances in medical care, many survivors face a lifetime of disability.

Objective To assess the short term safety of and four-year follow-up outcomes of allogenic, human umbilical cord blood (hUCB) derived mononuclear cells(MNCs) infusion to extreme preterm infants with high risk potential of death.

Method This study was a phase I, open-label, single-arm, single-center trial to evaluate the safety of allogenic, hUCB-MNCs infusion for extreme preterm infants with high risk potential of death. HUCB MNCs characteristics, pre- and postinfusion vital signs and laboratory investigations were recorded. Temporal profiles of cytokines and growth factors from blood were test. Clinical data including mortality rates, preterm complications and follow-up outcomes were recorded.

Results After processing, relatively MNCs mean (1.9±0.8) $\times 10^6$/kg; volume mean (11.25±2.12)ml/kg were infused to 10 extremely preterm infants with high risk of death. No adverse effects were noticed during treatment. 40% received extubation and weaned to nasal CPAP successfully; 30% received lower FiO_2; no infants suffered from late onset sepsis; 30% received poor response to MNCs infusion. 40% infants suffered from ROP and only one infant needed laser surgery. No patients suffered from NEC after MNCs infusion. All ten infants who received hUCB MNCs infusion survived inhospital and prevent deterioration of clinical features, but 4 infants discharged against the advice of the doctor by their parents and lost connection. Regarding the rest 6 infants, no home oxygen therapy and rehospitalization, no suffered from other long-term respiratory complications at visit 1～visit 3. One infant showed cerebral palsy at visit 1, no clinical evidence associated this with MNCs infusion. Blood level of HGF significantly increased, but MMP-9, IL-6, IL-8, TNF-α and TGF-β levels were significantly lower at 24h post infusion compared with baseline ($P < 0.05$).

Conclusions Collection, preparation, and infusion of allogenic hUCB MNCs to extreme preterm infants is feasible and safe.

Trial registration The study was registered on Chinese Clinical Trials.gov (NO. ChiCTR–OPN - 15006932). Registered 17 August 2015, http://www.chictr.org.cn/edit.aspx?pid=11662&htm=4.

Background

Preterm birth is a leading cause of infant morbidity and mortality worldwide[1]. Extreme preterm infants are at a high risk for developing preterm birth complications [2], which extends to later life, leading to enormous physical, psychological, and economic costs. Inflammation, ischemia and free radical toxicity resulted in multi-organ damage in extreme preterm infants by reducing numbers of tissue cells, blood vessels, and progenitor cell [3] [4] [5]. Though current management strategies have been shown to improve relatively complications and overall morbidity, many survivors still face a lifetime of disability, including mental and physical retardation, and chronic lung disease[4] [6]. Studies reported that among infants born with gestational ages less than 28 weeks, 16% are complicated with severe intraventricular hemorrhage(IVH), 36% with late- onset sepsis(LOS), and 68% with bronchopulmonary dysplasia(BPD)[7]. Therefore, developing novel and efficient therapies to reduce overall morbidity and improve lifelong quality in extreme preterm infants are of great significance.

Human umbilical cord blood derived mononuclear cells(hUCB-MNCs) are source of stem cell reservior containing high level of primitive multi-potent stem cells, progenitor cells and regulatory T cells[8]. The cells can home into damaged tissues, produce anti-inflammatory and immune-modulatory factors by paracrine effects, and differentiate into tissue cells[9]. Potential effects on adult respiratory distress syndrome(RDS) and hypoxic-ischemic brain damage have been suggested in animal models[10] [11] [12] [13]. Recently, these potential effects have been proved to be safe and feasible in clinical applications.

Based on these evidences, we hypothesized that hUCB MNCs infusion was safe for preterm infants. We report the short term and four-year follow-up outcomes of the infusion of allogeneic, hUCB MNCs cell to 10 extremely preterm infants with high risk of death.

Methods

This study was a phase I, open-label, single-arm, single- center trial to evaluate the safety of allogeneic (Figure 1), hUCB MNCs(10^6 cells/kg) infusion for extreme preterm infants ≤ 28 weeks gestational age with high risk potential of death.

Study design and patients.

We initiated this pilot study in January 2015. Extreme preterm infants admitted to the Neonatal Intensive Care Unit (NICU) of Bayi Children's Hospital were eligible if they were gestation ≤ 28 weeks with high risk potential of death such as long time invasive respiratory support and severe preterm complications. Ten patients underwent hUCB MNCs infusion. The study protocol was approved by the Ethics Committee of Seventh Medical Center of Chinese PLA General Hospital, Beijing, China (No.2015111) and obtained the informed consent in writing from the legal guardian. All infants in the study were given an intensive care therapy in accordance with the departmental guidelines which included therapies including positive pressure mechanical ventilation, noninvasive respiratory support, oxygen therapy, exogenous surfactant replacement, moderately active pharmacology including introduction of caffeine citrate, supplementation of intramuscular vitamin A and administration of corticosteroids. Physical examination and chest radiographs with blood gas were performed before and after hUCB MNCs admission in the patients. Blood gas was monitored every 24 hours until weaning from ventilation. All clinical diagnoses were defined according to a standard reference. When the extreme preterm infant was needed continuous invasive respiratory support more than 28 days and with severe complications, written consent was signed by the parents, and allogeneic cord blood MNCs infusion was applied to the infant

HUB MNCs preparation

Beijing Cord Blood Bank (Beijing Jlachenhong Biotech CO.LTD) is a public provincial blood bank. HUCB-MNCs were supplied Human Umbilical cord was obtained from uncomplicated full-terms by Beijing Cord Blood Bank following approval of the informed consent guidelines by the Ethical Review Board of the seventh medical center of the PLA general hospital. Written informed consent was obtained from parents. HUCB-MNCs were allogenic and were prepared according to the protocol of the Beijing Cord Blood Bank. Cell quality control and quality assurance tests were conducted in accordance with AABB and Net Cord-FACT (website:http://www.factwebsite.org/uploadedFiles/Standards/NetCord%20FACT %206th%20Ed%20Standards%20Draft.0 9.01.15_Redlined. pdf) standards. Briefly, less than 30ml hUCB units were collected by phlebotomy from full-term infants. Red blood cells were reduced by centrifugation to obtain hUCB MNCs. Cryopreserved hUCB was quickly put into a 37°C water bath to rapidly recover. All the hUCB was transferred into a plasma transfer bag(Nagel Biotechnology Co., Ltd, 200ml) through a sterile feeder, centrifuged at 300 x g at 4°C for 15 min(Eppendorf Centrifuge 5810/5810), put into the plasma paste clamp to remove the supernatant, and then suspended in 200 ml of 0.5% human albumin phosphate buffer. The mononuclear layer was isolated by density gradient centrifugation (1000g, 30 min, Eppendorf Centrifuge 5810/5810), then was remove the supernatant, and again suspended in 200 ml of 0.5% human albumin phosphate buffer. 3 ml of the prepared hUCB MNCs was used for clinical efficacy and toxicity analysis. Excessive nucleated cell-poor plasma was expelled. Meanwhile, MNC count, CD34 cell, CFU-GM, and sterility detection were performed. Cell viability was measured detection kit through flow cytometry analysis (Beckman Coulter, Inc.). All infusions were administered in Bayi Children's Hospital. Infusate and subject identities were double-checked by the research and clinical nursing staff. Infusions were also monitored by the research and clinical staff. Cells were infused over 30 minutes, followed by a 2 ml saline flush to clear the intravascular line.

Assessment of Safety.

Shortly before, during, and until 24 hours after transfusion, heart rate, systolic, diastolic, and mean arterial blood pressure and arterial blood oxygen saturation level were monitored in peripheral blood continually and documented. Moreover, laboratory investigations in peripheral blood were monitored and kept stable during the whole treatment period. Infusion reactions and signs of circulatory overload were checked.

Six infants discharged alive were prospectively followed up until 48 months of corrected age (CA). The primary goal was to determine the long-term safety of MNCs transplantation in the enrolled infants up to 48 months of CA. Safety was defined as the absence of treatment-related serious adverse events, which defined as an untoward medical occurrence that resulted in death, or life-threatening, required inpatient hospitalization or prolongation of existing hospitalization, or resulted in persistent or significant disability. The secondary goal was to evaluate the potential long-term effects of MNCs transplantation on growth, respiratory outcomes, and neurodevelopmental outcomes up to 48 months of CA. Infants were evaluated at 12months, 24months and 48months of CA in the Neonatal Follow-up Clinic of Health. At each visit, infants were examined with standard outcome assessments, including ascertainment of growth, respiratory health status, and neurodevelopmental outcomes by using a standardized follow-up interview, growth measurements, and thorough physical and neurologic examinations. In the standardized interview with parents, all medication use (including bronchodilators, inhaled/systemic steroid and home oxygen therapy), the occurrence of wheezing or whistling sounds in the chest, and rehospitalization since the last visit were documented. The infants' height, body weight, and head circumference were measured for growth assessment, and these values were according to the database for 2009 Chinese National Growth Charts [14]. Catch-up growth was defined as >10th percentile weight, height, or head circumference according to the 2009 Chinese National Growth Charts. At 12~48 months of CA, the neurodevelopmental outcome was evaluated with the Neuropsychological Development Sale (NDS) for Children, which made by Children's Hospital Capital Institute of Pediatrics of China. Neuropsydevelopmental delay was defined as NDS score <70. Blindness was defined as no useful vision in either eye. Deafness was defined as requiring hearing amplification aids in both ears. Cerebaral palsy was defined as a Palisano gross motor function score[15]≥2. The score on the NDS was based on the parent's report of language skill attainment and the child's performance with the administered items. The scores from the NDS were detailed in the supplemental materials.

Assessment of follow-up

This follow-up study protocol was approved by the ethics committee of The Seventh Medical Center of PLA General Hospital, Beijing, China (No.2020-037) on May 18, and the study was registered on Chinese Clinical Trials.gov (NO. ChiCTR – OPN - 15006932). Informed consent for the study was obtained from the parents of each child after review with the principal investigator or study staff.

Statistical analysis.

Data were all mean ± SD. Clinical data were performed by unpaired Student's t-test. All statistical analyses were performed with SPSS version 13.0 (SPSS Inc., Chicago, IL) and graphed by Prism software program (version 7.03; GraphPad Software, San Diego, CA). Data are representative of three independent biological replicates.

Results

Characteristicsof the infants

From January 1, 2015 till December 31, 2015, ten infants were enrolled for the treatment, gestational age ranged from 26 5/7 to 28 3/7 (27.1 ± 0.7) weeks and birth weight ranged from 730 to 1180 (1003.0± 154.3) grams; 5/10 (50%) were delivered by cesarean section. All infants presented with tachypnea and grunting soon after birth. The infants were all diagnosed with RDS, 6/10 (60%) cases were grade III, and 4/10 (40%) cases were grade IV; and 1/10 (10%) received four dose PS replacement, 1/10(10%) received three dose PS replacement, 2(20%) received two dose PS replacement, and 6/10(60%) received one dose PS replacement. All infants received intubated mechanical ventilation before MNCs infusion; the median duration was 36.8±14.7days. All infants suffered from BPD, and chest radiographs were in line with BPD change. 6/10(60%) had birth asphyxia and among them four suffered from IVH(grade IV) before MNCs infusion. 7/10(70%) had maternal chorioamnionitis. 2/10(20%) had early onset sepsis, of which one was cases of Klebsiella and one was Candida albicans. 4/10(30%) suffered from ventilation-associated pneumonia (VAP), of which one were cases of Klebsiella and Candida albicans pneumonia,

one was a case of Pseudomonas aeruginosa pneumonia, and one was a case of Acinetobacter baumannii and Klebsiella pneumonia before MNCs infusion. 1/10(10%) diagnosed NEC and 2/10(20%) diagnosed gastrointestinal bleeding before MNCs infusion(Table 1).

Characteristics of Cord Blood Processing.

Cord blood volume post processing ranged from 7 to 13 ml, mean (11.25 ± 2.12) ml; cells collected ranged from1.87 to 4.12 ($\times10^8$)/ ml, mean(2.67±0.65)$\times10^8$/ ml; cells postprocessing ranged from 8.10 to 24.35 ($\times10^8$)/ ml, mean (11.7 ±4.9)$\times10^8$/ ml; TNC cells range 8.18 to 24.35 ($\times10^8$)/ ml, mean (11.5 ±4.9)$\times10^8$/ ml; CFU-GM ranged from 0.699 to 2.764 ($\times10^6$)/ml, mean (1.57 ±0.65)$\times$ 10^6/ml; amount of $CD34^+$ cells in units ranged from 0.765 to 3.71($\times$ 10^6), mean (2.34 ± 1.02) $\times$ 10^6/ml. (Table 2.)

Infusion. Infused relatively MNCs ranged from 0.51 to 3.07 ($\times10^6$)/kg, mean (1.9 ±0.8 $\times10^6$/kg); infused volume ranged from 7 to 13ml, mean (11.25 ± 2.12) ml/kg. (Table 2.)

Cord Blood Safety. The patient's vital signs and laboratory investigations were monitored during the whole treatment period. No significant infusion reactions were noted, except two infants monitored transient hypertension. No signs of circulatory over- load and graft-versus-host disease (GVHD) were detected. Heart rate, mean arterial pressure, and oxygen saturation did not vary significantly before and after infusion(Table 3).

Clinical Presentation and Complications inhospitalization

Mortality. The ten extremely infants who received the cord blood infusion survived in hospital, but 4 infants discharged against the advice of the doctor by their parents who thought their infants would face a lifetime of disability including mental and physical retardation, and chronic lung disease. The duration of hospitalization ranged from 44 to 129 (86.1 ± 28.3) days (Table 1).

Respiratory System. After MNCs infusion, 4/10（40%）received extubation and weaned to nasal continuous positive airway pressure (CPAP) successfully; the duration ranged from 1 to 17 days; however, one patient needed reintubation. 3/10(30%) received lower FiO_2; decreased FiO_2 ranged from 5% to 30%. 3/10(30%) received poor response to MNCs infusion. The duration of oxygen therapy was (14.5± 5.1) days(Table 4). The infants demonstrated reduction of inflammatory shadow in chest x-ray examination(Figure 2).

Nervous System. None of the rest six infants developed abnormal clinical features of central nervous system disorders such as convulsions, apnea, or dysphagia (Table 1).

Infection. No infants suffered from late onset sepsis, VAP, and infected with Klebsiella, Candida albicans, or Pseudomonas aeruginosa pneumonia proved by blood culture after MNCs infusion (Table 1).

Complications. 4/10(40%) infants suffered from ROP and 1/10(10%) received laser surgery. No patients suffered from NEC after MNCs infusion. 4/10 infants suffered from from IVH, and there were no further developmental after MNCs infusion (Table 1).

Body weight during inhospitalization. 2/10(20%) infants were at at the 50~75% of the same gestational age child and 8/10(80%) infants were below the 30% of the same gestational age child (Figure 3).

Temporal profiles of cytokines and growth factors from blood

Levels of HGF from the blood at 24h after infusion were significantly increased compared with baseline (P＜0.05). MMP-9, IL-6, IL-8, TNF-α, and TGF-β levels were significantly lower at 24h post infusion compared with baseline ($P < 0.05$) (Figure 4)

Follow up Presentation and Complications

Mortality and Rehospitalization. For 4/10(40%) were discharged against the neonatologist's advice and lost connection. 1/6(16.7%) surviving infants who received MNCs infusion was rehospitalized one times because of pneumonia at visit 1. No infants who received MNCs infusion was rehospitalized because of respiratory infection and suffered from other long-term respiratory complications such as wheezing, asthma, or bronchial hyperresponsiveness at each visit.

Nervous System. Neurodevelopmental outcomes were assessed at visit 1～3 in the 6 surviving infants who received MNCs transplantation. 1/6(16.7%) surviving infants who received MNCs infusion showed cerebral palsy at visit 1, no clinical evidence associated this with MNCs infusion. Because the infant is in vitro fertilization (IVF) (his father has asthenospermia), and had birth with fetal distress, asphyxia, chorioamnionitis and early onset Candida albicans sepsis. No other 5 infants who received MNCs infusion were diagnosed with cerebral palsy, blindness and deafness. Findings of brain MRI obtained at 40 weeks and 9 months of CA (Figure 3). Five infants' NDS score＞70(Table 5).

Body weight and height. All infants catched up the same weight and height at visit 1~visit 3(Figure 3).

Discussion

In our study, we treated 10 extremely preterm infants with allogenic, hUCB-MNCs. The treatment was started when the infants received intubated mechanical ventilation for a long time and were diagnosis BPD. Most of the infants were observed complications such as NEC, ROP and IVH before cells infusion. No adverse effect of cell therapy was noticed. No infants died during treatment and inhospitalization. No preterm complications such as IVH, NEC, or ROP worse were observed. Although 4 out of the 10 infants discharged against neonatologist's advice and lost connection, no serious adverse events (SAEs) associated

with MNCs infusion were observed in the remaining 6 infants up to 12 months, 24 months and 48 months. 1 out of the 6 infants diagnosed cerebral palsy at 1 visit, which was not judged to be related to MNCs infusion.

The infants with severe BPD can continue to require supplemental oxygen after discharge adding to lifelong affliction, our data of no home oxygen therapy in the infants who received MNCs infusion and discharged according to neonatologist advice. Other long-term respiratory complications such as wheezing, asthma, or bronchial hyperresponsiveness were not observed in this study. Overall, to prove further safety and effect, larger phase II and III trials will be needed (ChiCTR2000035227).

Previous studies showed that inflammatory responses mediated by proinflammatory cytokines play a pivotal role in the development of BPD[16] [17] [18]. Furthermore, the protective effects of stem cell therapy against neonatal hyperoxic lung injury are mediated primarily by paracrine antinflammatory, antioxidative, and antifibrotic effects, rather than by the cells' regenerative capacity[10, 19] [20] [21]. In this study, we found the protective effects of MNCs may increase the concentrations of IL-1, IL-6, IL-8, MMP-9, TNF-α, TGF-β1 and HGF in the blood rather than increase the IL-10. Thus, without a control group, whether our data showing decreased blood inflammatory cytokines are related to immunomodulatory effects of MNCs or simply reflect the natural course of inflammation is difficult to ascertain. In the study, VEGF tended to be reduced after MNCs transplantation, but not HGF; the results contradict MSCs study showing significant down-regulation of both hyperoxia-induced growth factors[22].

In the present study, during hospitalization only 2 infants catch up the weight of the same gestational age infants. The reason might be the severe condition of the infants. But the infants catch up the same wight and height of the same month of the child at visit 1(12 months of CA), visit 2(24 months of CA) and visit 3(48 months of CA). Our data support that MNCs infusion may not be harmful and might even be beneficial for later somatic growth. But our data did not support that standard weight gain in infancy was associated with improved neurodevelopment up to months of CA. Further studies will be necessary to demonstrate the associations of later weight gain with better long-term neurodeveopmental outcomes.

In our study, 1 out of the 6 infants diagnosed cerebral palsy at 12 months of CA, the complication was not judged to be related to MNCs infusion, while the other 5 infants were not diagnosed as cerebral palsy or developmental delay. Overall, the findings suggest that MNCs infusion in extreme preterm infants with high potential risk of death was not detrimental to neurodevelopment. For severe BPD an independent risk factor of adverse neurodevelopmental and psychosocial outcome[23], we speculate that improvement in BPD after MNCs infusion might reduce neurodevelopmental morbidities.

Taken together, the results observed in the present study provide an important first step toward the safety of human allegenic MNCs infusion for extreme preterm infant. Safety is presented by our preliminary data. But, there are limitations of phase I trials, such as small sample size and lack of control group, the extreme preterm infants are high potential risk of death, the time point of MNCs infusion is late, etc., further evaluation with double blind randomized phase II/III clinical trials and long-term follow up of these infants to clarify the safety and efficacy of MNCs infusion for extreme preterm infants with BPD.

Conclusions

In summary, infusion of MNCs to treat extreme preterm infants of high risk of death appears safe and potentially effective up to 48 months CA. This warrants a phase II trial in more infants to further evaluate safety and efficacy (ChiCTR2000035227).

List Of Abbreviations

hCUB, human umbilical cord blood

MNCs, mononuclear cells

RDS, respiratory distress syndrome

IVH, intraventricular hemorrhage,

ROP, retinopathy of prematurity

LOS, late- onset sepsis

BPD, bronchopulmonary dysplasia

NEC, Necrotizing enterocolitis

CA, corrected age

GVHD, graft-versus-host disease

NDS, Neuropsychological Development Sale

CPAP, nasal continuous positive airway pressure

NICU, the neonatal intensive care unit

PS, surfactant

IVF, in vitro fertilization

SAE, serious adverse events

VAP, ventilation-associated pneumonia

Declarations

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The trial was approved by the Ethics Committee of Seventh Medical Center of Chinese PLA General Hospital, Beijing, China (No.2015111).

Consent for publicatication

The parents of the patients provided informed consent to publish this manuscript, including medical data and images. Proof of consent to publish from study participants can be requested at any time and a copy of it is available to the journal.

Competing interests

The authors declare that they have no competing interests.

Funding

This research was jointly granted by National Key R&D Program of China (2017YFC1001701, 2018YFC1002701) and Chinese National Natural Science Foundation Projects (30973210、30772036、81901518、81170602)

Author's contributions

JC, YM and ZF paricipated in the design and coordination of the trial. JC, YM, XD, QP and ZF paricipated in the operation of the trial. XD, ZW XZ and YC paricipated in the collected the data. JC, YM, XD, QP and ZF paricipated in analyzed the data. The authors read and approved the final manuscript.

Acknowledgements

Available.

Reference

1. L. Liu, S. Oza, D. Hogan, Y. Chu, J. Perin, J. Zhu, J.E. Lawn, S. Cousens, C. Mathers, R.E. Black, Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals.
2. J.E. Lawn, J. Cousens S Fau - Zupan, J. Zupan, 4 million neonatal deaths: when? Where? Why?
3. D. Niver, Bronchopulmonary dysplasia: structural challenges and stem cell treatment potential.
4. M.A.-O. Möbius, D. Freund, A. Vadivel, S. Koss, S. McConaghy, R.K. Ohls, M. Rüdiger, B. Thébaud, Oxygen Disrupts Human Fetal Lung Mesenchymal Cells. Implications for Bronchopulmonary Dysplasia.
5. K.K. Ballen, H.E. Gluckman E Fau - Broxmeyer, H.E. Broxmeyer, Umbilical cord blood transplantation: the first 25 years and beyond.
6. J.A.-O. Yang, Z. Ren, C. Zhang, Y. Rao, J. Zhong, Z. Wang, Z. Liu, W. Wei, L. Lu, J. Wen, G. Liu, K.A.-O. Liu, Q.A.-O.X. Wang, Safety of Autologous Cord Blood Cells for Preterms: A Descriptive Study.
7. J. Walter, L.B. Ware, M.A. Matthay, Mesenchymal stem cells: mechanisms of potential therapeutic benefit in ARDS and sepsis.
8. E.B. Peters, B. Liu, N. Christoforou, J.L. West, G.A. Truskey, Umbilical Cord Blood-Derived Mononuclear Cells Exhibit Pericyte-Like Phenotype and Support Network Formation of Endothelial Progenitor Cells In Vitro.
9. S. Neuhoff, M. Moers J Fau - Rieks, T. Rieks M Fau - Grunwald, A. Grunwald T Fau - Jensen, R. Jensen A Fau - Dermietzel, C. Dermietzel R Fau - Meier, C. Meier, Proliferation, differentiation, and cytokine secretion of human umbilical cord blood-derived mononuclear cells in vitro.
10. A. Borghesi, D. Cova C Fau - Gazzolo, M. Gazzolo D Fau - Stronati, M. Stronati, Stem cell therapy for neonatal diseases associated with preterm birth.
11. S.Y. Ahn, Y.S. Chang, D.K. Sung, H.S. Yoo, S.I. Sung, S.J. Choi, W.S. Park, Cell type-dependent variation in paracrine potency determines therapeutic efficacy against neonatal hyperoxic lung injury.
12. D. Monz, C. Tutdibi E Fau - Mildau, J. Mildau C Fau - Shen, M. Shen J Fau - Kasoha, M.W. Kasoha M Fau - Laschke, T. Laschke Mw Fau - Roolfs, A. Roolfs T Fau - Schmiedl, T. Schmiedl A Fau - Tschernig, K. Tschernig T Fau - Bieback, L. Bieback K Fau - Gortner, L. Gortner, Human umbilical cord blood mononuclear cells in a double-hit model of bronchopulmonary dysplasia in neonatal mice.
13. D.R. Mills, Q. Mao, S. Chu, K. Falcon Girard, M. Kraus, J.F. Padbury, M.E. De Paepe, Effects of human umbilical cord blood mononuclear cells on respiratory system mechanics in a murine model of neonatal lung injury.

14. H. Li, X.-N. Ji Cy Fau - Zong, Y.-Q. Zong Xn Fau - Zhang, Y.Q. Zhang, [Height and weight standardized growth charts for Chinese children and adolescents aged 0 to 18 years].
15. R.A.-O. Palisano, L. Avery, J.A.-O. Gorter, B. Galuppi, S.W. McCoy, Stability of the Gross Motor Function Classification System, Manual Ability Classification System, and Communication Function Classification System.
16. Y.S. Chang, S.J. Oh W Fau - Choi, D.K. Choi Sj Fau - Sung, S.Y. Sung Dk Fau - Kim, E.Y. Kim Sy Fau - Choi, S. Choi Ey Fau - Kang, H.J. Kang S Fau - Jin, Y.S. Jin Hj Fau - Yang, W.S. Yang Ys Fau - Park, W.S. Park, Human umbilical cord blood-derived mesenchymal stem cells attenuate hyperoxia-induced lung injury in neonatal rats.
17. Y.S. Chang, S.Y. Choi Sj Fau - Ahn, D.K. Ahn Sy Fau - Sung, S.I. Sung Dk Fau - Sung, H.S. Sung Si Fau - Yoo, W.I. Yoo Hs Fau - Oh, W.S. Oh Wi Fau - Park, W.S. Park, Timing of umbilical cord blood derived mesenchymal stem cells transplantation determines therapeutic efficacy in the neonatal hyperoxic lung injury.
18. L.W. Doyle, R.A. Halliday Hl Fau - Ehrenkranz, P.G. Ehrenkranz Ra Fau - Davis, J.C. Davis Pg Fau - Sinclair, J.C. Sinclair, Impact of postnatal systemic corticosteroids on mortality and cerebral palsy in preterm infants: effect modification by risk for chronic lung disease.
19. A. Lodha, R. Entz, A. Synnes, D. Creighton, K. Yusuf, A. Lapointe, J. Yang, P.S. Shah, Early Caffeine Administration and Neurodevelopmental Outcomes in Preterm Infants. LID - e20181348 [pii] LID - 10.1542/peds.2018-1348 [doi].
20. W. Onland, A.P. De Jaegere, M. Offringa, A. van Kaam, Systemic corticosteroid regimens for prevention of bronchopulmonary dysplasia in preterm infants.
21. K. Dalamagkas, M. Tsintou, A. Seifalian, A.A.-O. Seifalian, Translational Regenerative Therapies for Chronic Spinal Cord Injury. LID - 10.3390/ijms19061776 [doi] LID - 1776.
22. Y.S. Chang, D.K. Choi Sj Fau - Sung, S.Y. Sung Dk Fau - Kim, W. Kim Sy Fau - Oh, Y.S. Oh W Fau - Yang, W.S. Yang Ys Fau - Park, W.S. Park, Intratracheal transplantation of human umbilical cord blood-derived mesenchymal stem cells dose-dependently attenuates hyperoxia-induced lung injury in neonatal rats.
23. H.A. Frey, M.A. Klebanoff, The epidemiology, etiology, and costs of preterm birth.

Tables

Table 1
Clinical findings previous and post infusion

No.	Sex	GA (W)	BW (g)	Delivery mode	Test-tube baby	Genetic abnormality	Weight □correct GA□	Intrauterine infection	Dexamethasone times	Apgar score 1 min	Apgar score 5 min	Apgar score 10 min	RDS grade	PS time	Reintubation	Complication BPD	Complication VAP	Complication IVH	Complication ROP
1	M	27	1180	VD	0	1	2300	0	1	-	-	-	3	3	N	1	1	1	1
2	M	28+3	1010	CS	0	0	2400	0	1	10	10	10	0	1	Y	1	0	0	1
3	F	27+4	1150	CS	1	0	3510	1	1	6	7	8	3	1	N	1	1	0	0
4	M	27+5	1120	VD	1	0	2680	1	1	5	7	-	0	1	N	1	0	0	0
5	M	26+5	850	VD	1	1	2500	1	1	9	9	9	3	2	N	1	1	0	1
6	M	26+5	880	VD	1	0	2590	1	1	6	10	10	3	2	N	1	1	0	1
7	M	27+1	730	CS	0	0	1700	1	2	2	8	9	3	4	N	1	0	1	0
8	M	26+5	950	CS	0	0	-	0	0	5	7	9	0	1	N	1	0	0	0
9	M	26+5	980	CS	0	0	-	0	1	6	9	9	0	1	N	1	0	1	0
10	M	28	1180	VD	0	0	2180	0	2	9	9	9	3	1	N	1	0	0	0
MEAN±SD		27.1±0.7	1003.0±154.3				2482.5±514.2												

Table 2
Characteristics of the HCB-MNCs

No.	Volume postprocessing	Cells Collected (*10^8)	Cells Postprocessing (*10^8)	TNC cells □*10^8□	Number of coordination sites	CFU-GM (*10^6)	$CD34^+$ (*10^6)	Infused relatively Cells (*10^6/kg□	Infused age (D)	Infused volume
1	20.80	2.02	8.94	8.94	4	1.34	2.3	1.7	34	10.5
2	22.00	2.13	10.38	9.81	4	0.961	0.765	0.51	61	10
3	23.00	2.41	10.98	9.88	4	1.422	1.15	1	20	12
4	22.00	1.87	8.10	8.18	4	2.764	3.71	1.86	64	13
5	22.00	3.12	11.59	11.54	6	0.8308	1.2	1.2	34	14
6	20.80	2.83	15.21	15.21	6	0.699	2	2	34	13
7	26.00	2.75	9.75	9.75	4	1.969	2.93	2.93	35	10
8	24.00	2.48	8.54	8.54	4	1.827	3.07	3.07	24	10
9	24.00	2.93	9.22	9.22	5	1.7149	3.28	2.52	38	7
10	20.90	4.12	24.35	24.35	5	2.191	3.02	2.32	24	13
MEAN±SD	22.5±1.7	2.67±0.65	11.7±4.9	11.5±4.9	-	1.57±0.65	2.34±1.02	1.9±0.8	36.8±14.7	11.25±2.12

Table 3
Laboratory investigations previous and post infusion

Serial number	Bood routine								Blood gas					
	HB		HCT		WBC		PLT		PH		PO2		PCO2	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	129	104	0.41	0.34	13.29	10.31	245	259	7.38	7.39	105	63	51	46
2	163	112	0.49	0.32	9.5	8.23	399	385	-	-	-	-	-	-
3	139	119	0.43	0.36	15.16	14.02	359	287	7.32	7.38	101	192	55	46
4	115	123	0.33	0.37	6.43	13.59	386	412	7.37	7.38	117	51	35	55
5	159	123	0.48	0.38	12.27	8.23	368	286	7.39	7.51	84	64	51	24
6	145	142	0.43	0.42	7.67	10.76	206	239	7.29	7.37	35	63	57	51
7	146	129	0.42	0.40	12.07	15.62	125	375	7.39	-	62	-	50	-
8	107	97	0.29	0.26	42.02	38.53	400	461	7.12	7.31	29	41	90	43
9	102	135	0.31	0.39	15.98	16.52	100	63	7.38	7.41	67	66	48	37
10	121	119	0.37	0.35	18.4	16.36	404	422	7.339	7.405	32	42.5	67	51

Table 4
Outcomes of ventilator parameters before and after MNCs infusion

Serial number	Ventilator parameters													
	pattern		FiO2(%)		PIP		PEEP		Mechanical ventilation		Oxygen therapy time		Duration of hospitalization	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	SIMV	SIMV	28	23	22	12	6	6	34	17	0	9	34	37
2	SIMV	Oxygen therapy	21	-	22	-	6	6	61	6	0	9	61	22
3	SIMV	SIMV	30	23	22	13	5	5	20	42	0	20	20	57
4	SIMV	SIMV	30	25	23	13	6	6	64	10	0	22	64	55
5	SIMV	SIMV	35	28	22	14	6	6	34	33	0	18	34	65
6	SIMV	SIMV	30	25	22	17	5	6	34	42	0	19	34	95
7	SIMV	SIMV	47	33	24	19	5	4	35	63	0	0	35	63
8	HFO	HFO	60	30	-	-	15	15	24	20	0	0	24	20
9	SIMV	SIMV	31	28	24	18	5	5	38	6	0	0	38	6
10	SIMV	SIMV	100	95	24	24	5	5	24	73	0	0	24	73
MEAN±SD									36.8±14.7	30.6±24.4		14.5±5.1		49.3±27.4

Table 5
Neuropsychological development sale (NDS) score

Serial number	Visit 1		Visit 2		Visit 3	
	score	grade	score	grade	score	grade
Patient 1	104	average	102	average	108	average
Patient 2	102	average	105.5	average	106	average
Patient 3	110	average	118	good	121	good
Patient 4	98	average	103.5	average	107	average
Patient 5	118	good	120	good	123	good
Patient 6	Cerebral palsy					

Figures

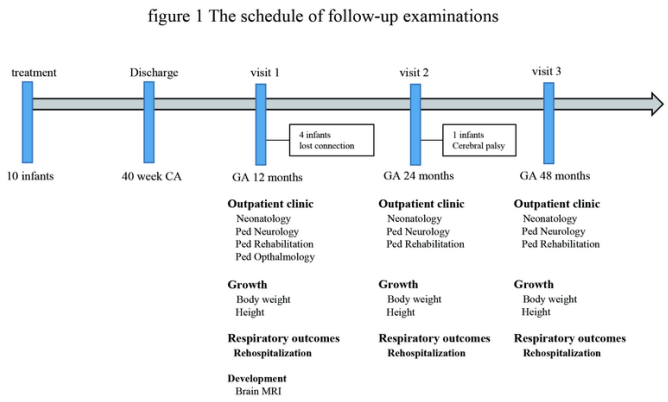

Figure 1

The schedule of follow-up examinations

figure 2 Growth profiles of infants till the CA of in hospitization 12 month、24month and 48 month

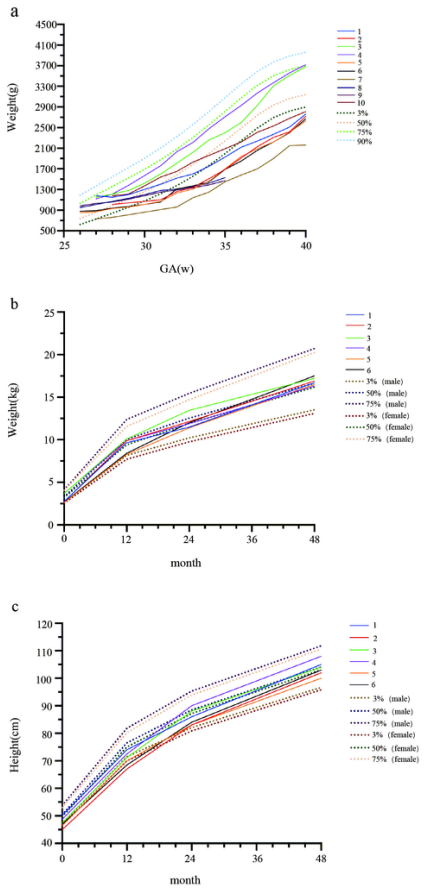

Figure 2

Growth profiles of infants till the CA of in hospitization 12 month、24month and 48 month a. Growth curve before and after MNCs infusion inhospitalization in all 10 study patients b. Weight obtained at 12,24,36 and 48 months in 6 survival study patients c. Height obtain at 12,24,36 and 48 months in 6 survival study patients

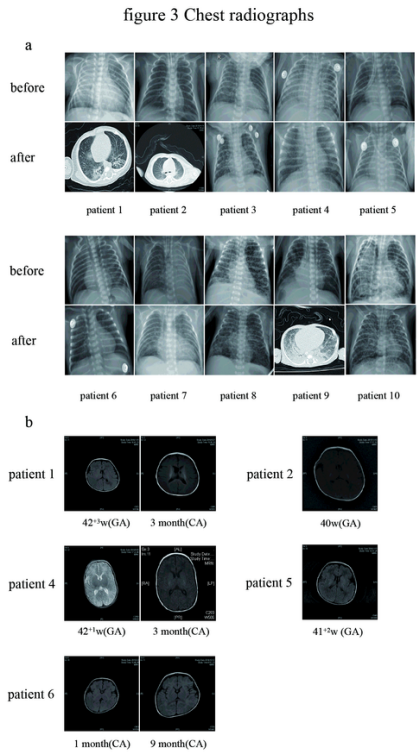

Figure 3

Chest radiographs and brain MRI a. Chest radiographs obtained before and after MNCs infusion in all 10 study patients showing comparable finding in both lung fields. b. Brain MRI obtained at 40 to 41 weeks CA in 6 survival study patients and 3 patient rechecked at 3 month and 9 month.

figure 4 Blood level of cytokine and growth factors collected before MNCs infusion and at 24 hours postinfusion.

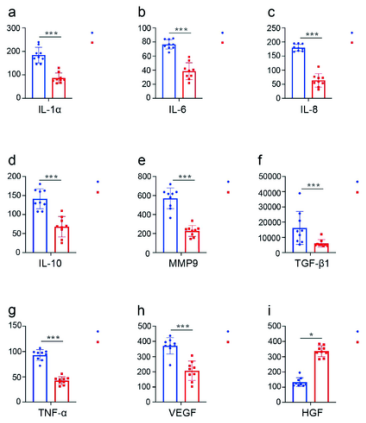

Figure 4

Blood level of cytokine and growth factors collected before MNCs infusion and at 24 hours postinfusion. Data are presented as mean±SEM. P□.05, compared preinfusion level with postinfusion level at 24 hours.