Online Data Supplement

Stem Cell for Bronchopulmonary Dysplasia in Preterm Infants: A Randomized Controlled Phase 2 Trial

Supplementary Materials

Cytokine and factor analysis in tracheal aspirate fluid

Tracheal aspirate fluid (TAF) samples were obtained twice (before and 7 days after transplantation) by suctioning the major airways after 0.5 mL of saline had been instilled into the endotracheal tube. The following cytokines and growth factors were measured: interleukin (IL)-1α, IL-1β, IL-6, IL-8, tumor necrosis factor (TNF)-α, matrix metalloproteinase (MMP)-9, vascular endothelial growth factor (VEGF), and hepatic growth factor (HGF). The supernatant of tracheal aspirate fluid was frozen at -70°C after centrifugation at 15,000 rpm for 10 min. Quantities of hepatic growth factor and MMP-9 were calculated by enzyme immunoassay with the Quantikine kit (R&D Systems, Inc., Minneapolis, MN, USA). IL-1α/β, IL-6, IL-8, TNF-α, and VEGF were measured with the Milliplex MAP ELISA Kit (Millipore, Billerica, MA, USA), according to the manufacturer's specifications.

1 Summary of the ClinicalTrial Protocol

Protocol number	MP-CR-009
Phase	Phase 2
Test drug	PNEUMOSTEM® (Mesenchymal stem cells derived from allogeneic cord blood)
Design	Multi-center, randomized, double-blind, placebo, phase 2 clinical trial
Title	Randomized, double-blind, multi-center phase 2 clinical trial to evaluate the efficacy and safety of pneumostem® compared with placebo groups in the treatment of infant bronchopulmonary dysplasia
Objective	**Primary objective**: To evaluate the efficacy of one-time intratracheal administration of pneumostem® in high-risk patients with infant bronchopulmonary dysplasia compared to the placebo group **Secondary objective**: To evaluate the safety of one-time intratracheal administration of pneumostem® in high-risk patients with infant bronchopulmonary dysplasia compared to the placebo group
Subjects	Patients that are less than 29 weeks of gestational age, have a birth weight of 1,250 g or less, are premature infants within 2 weeks after birth, and need ventilator support as their ventilation rate is > 12 times/min and oxygen demand > 25%
Expected test period	45 months from the date of approval of the clinical trial protocol by the Ministry of Food and Drug Safety
Institute and investigator	1) Coordinator: Prof. Won Soon Park Professor at Sungkyunkwan University School of Medicine, Pediatric Adolescent Center, Samsung Medical Center 2) Clinical trial investigators ① Samsung Medical Center Principal Investigator: Prof. Won Soon Park, Sungkyunkwan University School of Medicine, Pediatric Adolescent Center Co-researchers: Prof. Yun Sil Chang, Sungkyunkwan University School of Medicine, Pediatric Adolescent Center ② Asan Medical Center Principal Investigator: Prof. Ae Ran Kim, University of Ulsan College of Medicine, Pediatric Adolescent Center 3) Institute ① Samsung Medical Center, 81 Irwon-Ro Gangnam-gu. Seoul ② Asan Medical Center, 88 Olympic-ro 43-gil, Songpa-gu, Seoul A total of two institutes

Criteria for selecting subjects	1) Age within 5 to 14 days of birth 2) Patients within 23 to 29 weeks of gestational age 3) Patients with a birth weight of 500g to 1,250 g
Criteria for excluding subjects	1) Patients with a cyanotic congenital heart malformation causing cyanosis or heart failure, except infants with patent ductus arteriosus 2) Patients with severe lung malformations, such as pulmonary hypoplasia, congenital diaphragmatic hernia, and congenital cystic diseases 3) Patients with chromosomal abnormalities (e.g., Edward syndrome, Patau syndrome, and Down syndrome) accompanied by severe malformations and severe congenital anomalies (e.g., Hydrocephalus, Encephalocele) 4) Patients with severe congenital infections (e.g., Herpes, Toxoplasmosis, Rubella, Syphilis, and AIDS) 5) Patients with C-reactive protein (CRP) > 30 mg/dl and severe sepsis or shock due to active infection that has not been properly treated with antibiotics 6) Patients who performed surgery or are expected to have surgery within 72 h before and after administration of the test drug 7) Patients who were administered a surfactant 24 h before administration of the test drug 8) Patients with intracranial hemorrhage of bilateral grade 3/4 9) Patients with active pulmonary hemorrhage or active air leak syndrome at the time of screening 10) Patients who have participated in other clinical trials 11) Patients who are allergic to gentamicin antibiotics Patients deemed inappropriate by other researchers to participate in clinical trials
Administration method and dosage	For subjects assigned to the Pneumostem® group, one-time intracheal administration of Pneumostem® at $1.0x10^7$ cells/kg
Method	This is a randomized, double-blind, multi-center, and phase 2 clinical trial which administers a placebo or Pneumostem® once in intracheally to those with ventilation rate > 12 times/min and oxygen demand > 25% in patients who were receiving ventilator support for 5 to 14 days after birth and are premature infants less than 29 weeks of gestational age, with a birth weight of 1,250g or less. Premature infants who agree to participate in this trial, who meet the selection criteria, and do not meet the exclusion criteria are randomized to either $1.0x10^7$ cells/kg Pneumostem® or the placebo group. At this time, the subjects' gestational age is classified into two strata, and stratified randomization is performed to eliminate an imbalance between the Pneumostem® and placebo group. The strata are divided into two strata: less than 25 weeks of gestational age and more than 25 weeks of gestational age. Subjects assigned to the Pneumostem® group are evaluated for the incidence of moderate or severe bronchopulmonary dysplasia (BDP) or death at 36 weeks postmenstrual age (PMA) after one intracheal administration of the test drug.

Efficacy evaluation variable	Primary evaluation variable Incidence of moderate or severe BPD or death at 36 weeks of PMA Secondary evaluation variable Severe BPD or death Survival at 28 days of birth, at 36 weeks PMA, and discharge Duration of ventilator dependence Duration of intubation Postnatal steroid use (%) for the purpose of weaning the ventilator Cumulative duration of oxygen use Stage III or higher retinopathy of prematurity (ROP) ROP requiring treatment (avastin OR laser) Growth rate (Z-score) Period of hospitalization until the first discharge
Safety evaluation variable	Safety data identified by the following tests Adverse reactions directly related to transplantation Frequency of pneumothorax requiring chest tube insertion Frequency of moderate or severe pulmonary hemorrhage

Observation Schedule (Study Flow-Chart)

	Screening	D 1 Treatment	D 2	D 3	D 7	W 2	Birth D 28	PMA W 36	At Discharge Or Discontinuation	F/U W 24
Visit window	**-14D~**				±D1	±D1	±D2	±D4	±D4	±D7
Visit No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8[10)]**	**9**	**10[11)]**
Written consent	V									
Checking selection/exclusion criteria	V									
Demographic and delivery survey[1)]	V									
Checking medical history and medication	V									
Physical examination	V	V	V	V	V	V	V	V	V	V
Vital signs	V	V	V	V	V	V	V	V	V	V[16)]
Physical measurement (Wt, Ht, HC)	V	V (Wt)	V (Wt)	V (Wt)	V (Wt)	V (Wt)	V (Wt)	V (Wt)		V
Chest X-ray	V	V	V	V	V	V	V	V	V	V
Cranial US	V[12)]		V		V	V	V	V		
Echocardiogram	V[12)]				V		V	V		
ECG test[2)]	V							V		
Blood gas analysis[3)] (CBGA or ABGA)	V	V	V	V	V	V	V	V		
Laboratory test[4)]	V[13)]	V		V[15)]	V	V	V	V	V	V
Tracheal suction fluid test[5)]	V				V[6)]					
TTA Culture	V[14)] Blood Culture		V		V[6)]					
Test drug administration		V								
BPD evaluation								V	V	

Efficacy evaluation other than BPD[7]			V[8]	V[8]	V[8]	V[8]	V	V	V	V[9]
Checking adverse reactions		V	V	V	V	V	V	V	V	V
Checking concomitant medication		V	V	V	V	V	V	V	V	V

1) Demographic and delivery information: Factors such as gender, date of birth, Apgar score(1, 5 min), birth weight, height and gestational age, multiple birth, premature rupture of membranes, birth weight appropriateness for GA, delivery form, history of maternal gestational hypertension, pathologic chorioamnionitis (it takes about 2 weeks to confirm test results, so results are recorded later) are surveyed.
2) An ECG test is performed by selecting a lead that is easy to observe, and continuous ECG monitoring is performed from Day 1 to Day 3 when the test drug is administered.
3) Blood gas analysis (CBGA or ABGA): pH, PO_2, PCO_2, base excess (In case of CBGA, PO_2 test is excluded)
4) Laboratory tests: WBC, RBC, hemoglobin, hematocrit, platelet count, glucose, AST(SGOP), ALT(SGPT), total bilirubin, alkaline phosphatase, total protein, albumin, BUN, Creatinine, CRP
5) Tracheal suction fluid test: IL-1, IL-6, IL-8, IL-10, TNF-a, MMP-9, TGF-b, HGF, VEGF
6) If the subject's extubation occurs before Day 7, this test may be performed immediately before extubation.
7) Evaluation of efficacy other than BPD: intubation, ventilator treatment, assisted positive-pressure ventilation, oxygen administration, survival, length of hospitalization, ROP, steroid use for the purpose of weaning off the ventilator
8) Steroid use for the purpose of weaning off the ventilator
9) Survival survey, growth rate, steroid use to wean off the ventilator
10) If the visit schedule on Visit 7 (28^{th} day of birth) is included in the window of another visit, the results of the test conducted at Visit 7 are used instead.
11) If the visit schedule on Visit 8 (36 weeks of PMA) is included in the window of another visit, the results of the test conducted at Visit 8 are used instead.
12) If there is a test result within a week of screening, the test result replaces the screening result based on the researcher's judgement.
13) If there is a test result within a week of screening, the test result replaces the screening result.
14) The test results before screening can be used based on the researcher's judgment.
15) Based on the researcher's judgement, clinically necessary items can be selected and tested among laboratory test items.
16) Blood pressure can be measured at the discretion of the researcher.