



# First-In-Human Administration of Allogeneic Amnion Cells in Premature Infants With Bronchopulmonary Dysplasia: A Safety Study

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**Key Words.** Adult stem cells • Cell transplantation • Cellular therapy • Clinical trials • Clinical translation • Lung • Placenta

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## ABSTRACT

**Bronchopulmonary dysplasia (BPD) is a chronic lung disease that mainly affects premature babies who require ventilator support. The pathogenesis of BPD is complex but includes vascular maldevelopment, alveolarization arrest, and lung inflammation. There is no cure for BPD. Clinical care is limited to supportive respiratory measures. A population of stem-like cells derived from placental membranes, human amnion epithelial cells (hAECs), has shown therapeutic promise in preclinical models of BPD. With a view to future efficacy trials, we undertook a first-in-human clinical trial of hAECs in babies with BPD to assess the safety of these cells. In a single-center, open-label phase I trial, we administered allogeneic hAECs ( $1 \times 10^6$  per kilogram bodyweight) by intravenous infusion to six premature babies with BPD. The primary outcomes of the study were focused on safety, including local site reaction, anaphylaxis, infection, features of rejection, or tumor formation. Outcomes to discharge from neonatal unit were studied. The hAECs were well tolerated. In the first baby, there was transient cardiorespiratory compromise during cell administration consistent with a pulmonary embolic event. Following changes to cell administration methods, including introduction of an inline filter, and reducing the cell concentration and the rate of cell infusion, no such events were observed in the subsequent five babies. We did not see evidence of any other adverse events related to cell administration. Allogeneic hAECs can be safely infused into babies with established BPD. Future randomized clinical trials to assess efficacy in this patient population are justified.**  
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## SIGNIFICANCE STATEMENT

The authors believe that testing the efficacy of prophylactically administered hAECs is now warranted. Future studies in infants at risk of developing chronic lung disease of prematurity are necessary to assess efficacy. This study paves the way for cellular therapy as a mainstay in the prevention and treatment of a variety of diseases that claim many lives of prematurely born babies.

## INTRODUCTION

Advances in perinatal medicine have led to significantly better survival rates for premature babies, particularly those born extremely prematurely [1]. However, bronchopulmonary dysplasia (BPD) remains an all too common complication for very premature (28–32 weeks) and extremely premature (<28 weeks) babies, affecting about 30% of the latter [2]. Survivors of moderate-to-severe BPD have increased risks of longer-term morbidity, including impaired lung function and exercise intolerance into adulthood [3] and significant neurodevelopmental impairment [4].

The pathogenesis of BPD is complex. It includes vascular maldevelopment, impaired or arrested

alveolarization, and lung inflammation [5]. Once established, there is no targeted cure for BPD. Instead, clinical care is limited to respiratory supportive measures, such as postnatal corticosteroids and supplemental oxygen, until sufficient lung volume to maintain oxygenation unsupported is achieved through growth. Many babies with BPD are discharged home on supplemental nasal oxygen to maintain sufficient oxygenation until that occurs [6]. The development of targeted therapies for BPD has been identified as a research priority [7].

A variety of stem cells have been reported to improve lung architecture, compliance, and function in preclinical animal models of BPD and have been suggested as a promising therapy [8]. However, to date, clinical testing of cell

therapies in human babies has been limited. Only one clinical trial has been completed, reporting on the safety and tolerability of allogeneic human umbilical cord-derived mesenchymal stromal cells (MSCs) in extremely premature babies at risk of developing BPD [9]. There have been no reports on the safety and feasibility of delivering other cell types or of any stem cell as a treatment for established BPD.

There are established methods for scalable manufacturing of MSCs that retain biophysical properties of adult- and fetal-tissue-derived MSCs including their clonogenicity and functional signatures of multipotency [10]. However, human amnion epithelial cells (hAECs) can be sourced readily from discarded placentae and in sufficiently high numbers that do not require culture expansion, thereby reducing the cost of goods and risk of contamination during cell production. Accordingly, we sought to assess the utility of allogeneic hAECs. It has been long known that the placental amniotic membrane is an immune-privileged tissue. Stem and stem-like cells derived from the amniotic membrane are both immune privileged and immunomodulatory. Specifically, hAECs express low levels of HLA and exert profound immunomodulatory effects in preclinical models of neonatal lung disease [11].

Unlike MSCs, hAECs can be isolated in abundance and with relative ease. Each term placenta will yield over 150 million cells following a simple enzymatic digest and purification [12]. Expansion or manipulation of the cells is not required. Exploring therapeutic utility for a variety of diverse conditions including lung disease, liver disease, and stroke, hAECs have been shown to be well tolerated following xenogeneic administration to immunocompetent small and large animals, including mice [13, 14], sheep [15], and monkeys [16]. More relevant to BPD, in preclinical models of the disease, hAECs have been shown to prevent alveolar simplification and pulmonary inflammation [15], two of the hallmarks of BPD. We undertook this first-in-human phase I trial to assess the safety and tolerability of allogeneic hAECs in premature babies with established BPD.

## MATERIALS AND METHODS

### Donor Screening Criteria

All human tissues were obtained with informed patient consent and approved by the Monash Health Human Research Ethics Committee (No. 13324B). Healthy women with an uncomplicated pregnancy undergoing an elective cesarean section at term pregnancy completed a two-page risk assessment questionnaire to assess donor suitability. Blood was obtained by venipuncture on the day of delivery for serological testing for HIV, hepatitis C virus (HCV), hepatitis B surface antigen (HBsAg), human T lymphotropic virus (HTLV), and syphilis, and nucleic acid testing for HIV, hepatitis B virus, and HCV by an independent National Association of Testing Authorities (NATA) Australia accredited laboratory (National Reference Laboratory, Fitzroy, VIC, Australia).

### Cell Preparation

Human placentae were collected at the time of surgery. Initial processing commenced in a sterile field within the operating theater. The amnion was peeled away from the underlying chorion, rinsed in sterile saline for 2 minutes, and then transferred to an antibiotic-antimycotic solution (cefazoline 1 g/l, AFT Pharmaceutical, North Ryde, NSW, Australia, <https://www.aftpharm.com>; gentamicin 80 mg/l, Pfizer, New York City, NY, <https://www.pfizer.com>;

amphotericin B 50 mg/l, Bristol-Myers Squibb, Mulgrave, VIC, Australia, <https://www.bms.com>) for 2 minutes. The amnion was then transferred into Dulbecco's Modified Eagle's Media (Cat. No. 10566016, ThermoFisher Scientific, Scoresby, VIC, Australia, <https://www.thermofisher.com/us/en/home.html>) supplemented with antibiotic-antimycotic solution (Cat. No. 15240062, ThermoFisher Scientific) for transport to the cell isolation facility within the Monash Health Translational Precinct's Cell Therapy and Regenerative Medicine Platform. There, amniotic epithelial cells were isolated and cryopreserved within a GMP-grade Biospherix Xvivo system, as previously described [12].

### Product Release Criteria

Cells were released for clinical use when the cell viability, as determined by trypan blue exclusion, was >80% at time of cryopreservation in CryoStor CS10 (07930, STEMCELL Technologies, Tullamarine, VIC, Australia, <https://www.stemcell.com>) and when a cell isolate was proved free of microbial contamination after 14 days of culture in aerobic and anaerobic conditions. Cell viability was assessed in-house by two independent technicians. Microbial testing was performed by an independent NATA-accredited microbiology laboratory (St Vincent's Hospital Pathology, Fitzroy, VIC, Australia, <https://path.svhm.org.au>). Preparations of hAECs were only used when the cell isolates were >96% EpCAM+, <1% CD105, <1% CD45+, and <1% CD90 as determined by flow cytometry. These criteria were previously established [12].

### Cell Preparation and Administration

On the day of cell infusion, hAECs were retrieved from vapor-phase liquid nitrogen storage and thawed using a prewarmed heat block for approximately 2 minutes, when only a small ice crystal remained. The cells were then washed in sterile saline with centrifugation at 350g for 5 minutes prior to resuspension in saline at the final concentration. This step includes a repeated cell count and assessment of cell viability by trypan blue exclusion, by two independent operators. For the first baby, hAECs were resuspended as 2 million cells per ml. For subsequent babies, hAECs were resuspended at 0.325 million cells per ml to provide a postfilter infusion concentration of 0.25 million cells per ml (see Results). All babies received the cells via a peripheral intravenous infusion. The first baby received cells by a slow, hand-delivered injection. Subsequently, babies received hAEC infusions delivered over 30 minutes by a syringe pump on a platform rocker. The dose of hAECs administered to all babies was 1 million/kg body weight at the time of cell delivery.

## PATIENTS

Ex preterm infants (born  $\leq 28^{+0}$  weeks gestation) with established BPD at 36 weeks postconceptional age, according to NIH classification [17], were eligible if they were dependent on mechanical ventilation or continuous positive airway pressure (CPAP) support in 0.3–0.5 FiO<sub>2</sub>. Infants with an active bacterial or viral infection, necrotizing enterocolitis, patent ductus arteriosus, or known severe brain injury were excluded. A condition of Human Research Ethic Committee (HREC) approval was that cells were to be given successfully to three babies who were intubated and on mechanical respiratory support before administering cells to any baby on CPAP. Written, informed

parental consent was obtained prior to enrollment with clear explanations provided, including that this was a safety trial and that no benefit to infant's health condition was expected.

## OUTCOMES

The primary outcome of this phase I trial was safety. This included absence of acute adverse events during and after the administration, and adverse events during follow-up for a period of up to 2 years following cell therapy. Possible adverse events included local site reaction (change in color and/or appearance, swelling around site of administration), anaphylaxis (as evidenced by change in physiological parameters—heart rate, peripheral oxygen saturation, blood pressure accompanied by airway and breathing difficulty), infection (growth of bacterial or viral pathogen on cultures taken from sterile sites within 28 days of therapy), features of rejection (including unexplained fever, weight loss, change in vital organ [kidney, liver, heart, lung] function), and tumor formation (appearance of solid tissue growth on physical examination or focused imaging). Serial blood tests, chest x-rays, echocardiograms, cranial and abdominal ultrasounds, and brain magnetic resonance imaging (MRI) were done according to a predefined trial protocol (ACTRN12614000174684). An independent data safety monitoring board (DSMB) comprising two neonatologists, one each in two other tertiary hospitals in Melbourne, was established to inform and advise on the continuation/discontinuation of the trial after each recruit. Secondary outcomes studied included change in respiratory support requirements following cell therapy. This was an open-label safety study. There was no control group. Here, we present data until the time of discharge from the neonatal unit.

## RESULTS

Six preterm infants (five boys, one girl) with a median (range) gestation at birth of 26 (24–28) weeks and birth weight of 795 (450–990) grams were administered cells at 89 (59–187) postnatal days between August 2015 and August 2017. Fetal growth restriction was present in four infants at birth. Median (range) Apgar score at 5 minutes of life was 6 (0–9). The first three infants were dependent on invasive mechanical ventilation with a median mean airway pressure of 18 (11–23) cm H<sub>2</sub>O and FiO<sub>2</sub> of 0.4 (0.35–0.4) at the time of cell administration. The next three infants were dependent on CPAP with a median positive end-expiratory pressure (PEEP) of 10 (8–11) cm H<sub>2</sub>O and FiO<sub>2</sub> of 0.45 (0.36–0.5) at cell administration. hAECs were administered at a dose of 1 million per kg body weight at the time of administration. Characteristics of the infants are summarized in Table 1.

The first infant received cells via a slow manual intravenous infusion at a cell suspension of 2 million per ml saline. This infant had transient cardiorespiratory compromise during cell administration involving sudden acute hypoxia and bradycardia without change in blood pressure (Fig. 1). Cell administration was discontinued midway through the infusion with subsequent recovery. The event was thought to be a cell-related microembolic phenomenon. Following this event, and with the written approval of the HREC and the DSMB, we changed the protocol of cell administration. Specifically, we (a) changed cell delivery to a 30-minute infusion using a syringe-driver on a rocking platform; (b) included an

inline 200- $\mu$ m pediatric transfusion filter to provide a cell-filtration step; and (c) reduced the final (postfilter) cell infusion concentration eightfold to 0.25 million live hAECs per ml saline. To correct for cell entrapment and loss in the filter, using five different hAEC cell preparations, we evaluated cell loss rates following filtration of several trial hAEC suspensions through the filter. We observed that cell filtration resulted in a loss of 30%–35% of cells. To give a postfilter cell concentration of 0.25 million cells per ml, prefilter cell suspensions were made to 0.325 million live hAECs per ml saline.

Given the immune-privileged nature of hAECs, we did not anticipate rejection of the allogeneic cells. However, we did observe for acute adverse events including cardiopulmonary compromise and organ failure, and long-term adverse outcomes such as tumor formation by chest x-ray, MRI, and cranial and abdominal ultrasounds. This is in keeping with recent clinical trials utilizing allogeneic stem cells, in the absence of a definitive assay for allogeneic cell rejection [9, 18]. Additionally, serum tryptase levels were measured to assess for allograft rejection. No acute adverse events were observed during or after cell administration in any of the other five infants (Fig. 1). Monitoring during the remainder of neonatal intensive care unit admission, including serial blood tests for the first 3 days after cell therapy (full blood examination, C-reactive protein [CRP], liver function tests, coagulation screen, urea, creatinine, and electrolytes), routine cardiorespiratory monitoring, daily physical examination, cranial and abdominal ultrasound, and echocardiogram 1 month after cell delivery, was all satisfactory (Fig. 2; Table 2). A chest x-ray performed 24 hours following cell administration and monthly until discharge revealed no new respiratory findings or features of tumor formation. One infant (baby 2) died 1 month after cell administration due to multiorgan failure related to a cardiorespiratory collapse related to accidental extubation. This was an infant who had never been successfully extubated since birth. The DSMB and HREC both found that the death was not related to cell administration and advised continuation of the study.

The immediate respiratory response to therapy, mean airway pressure and FiO<sub>2</sub> requirements, for each infant for the first 7 days after cell therapy are shown in Figure 2. There was no significant change in respiratory support requirements after hAEC therapy. All five surviving infants were discharged home on supplemental oxygen (median low-flow oxygen rate 0.25 l/minute) at a median of 174 (155–388) days of life. All five of these infants had complications of prematurity and BPD in the form of systemic or pulmonary hypertension. Serum levels of CRP (an acute systemic inflammatory response protein) either decreased or remain unchanged in the 48 hours following hAEC administration (Fig. 3). The longer-term safety outcomes will be analyzed and reported after completion of 2 years of follow-up of the five infants, which are expected in 2019.

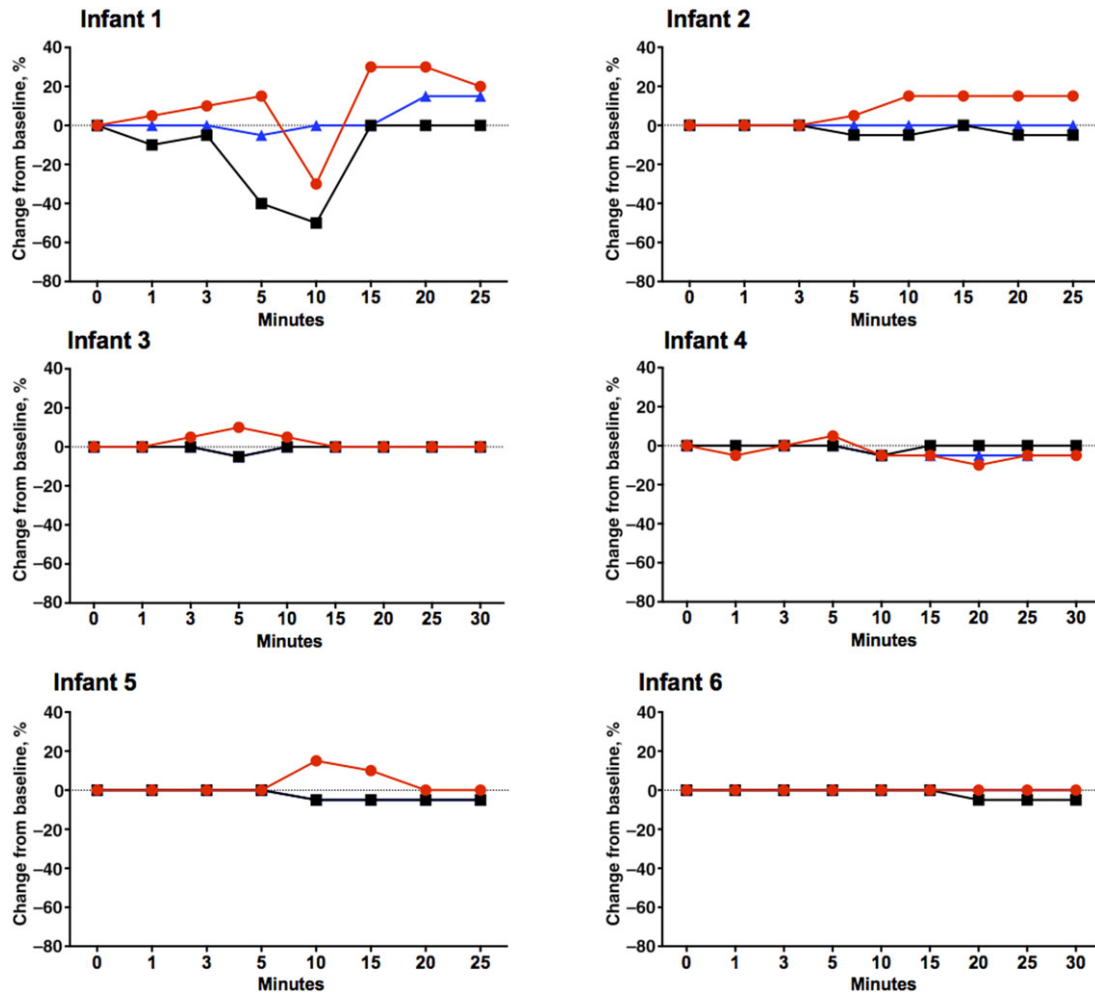
## DISCUSSION

This is the first-in-human report of systemically delivered hAECs. Six infants with established severe BPD were given hAECs at a dose of 1 million per kg bodyweight. Apart from transient cardiopulmonary instability during cell delivery in the first baby, the administration of allogeneic hAECs appears to be feasible, well tolerated, and safe. Indeed, the first baby was

**Table 1.** Infant characteristics

Characteristics	Infant 1	Infant 2	Infant 3	Infant 4	Infant 5	Infant 6
Birth gestation, weeks	27 <sup>+6</sup>	28 <sup>+0</sup>	25 <sup>+1</sup>	25 <sup>+0</sup>	27 <sup>+4</sup>	24 <sup>+5</sup>
Birth weight, grams	814	450	870	990	730	775
Sex	Male	Male	Male	Male	Male	Female
Fetal growth restriction	Yes	Yes	Yes	No	Yes	No
Apgar score, 5 minutes	9	4	3	0	8	8
Birth defect	Hypospadias	NA	Short long bones	NA	Hypospadias	Polydactyly
Culture proven sepsis	Yes	Yes	No	No	No	Yes
IVH, maximum grade	0	0	0	0	0	1
Periventricular leucomalacia	No	No	No	No	No	No
PDA, requiring treatment	Yes	Yes	Yes	Yes	No	Yes
Necrotizing enterocolitis, confirmed	No	Yes	Yes	No	No	No
Retinopathy of prematurity, requiring treatment	No	No	No	No	No	No
BPD, severity	Severe	Severe	Severe	Severe	Severe	Severe
Postnatal steroid use	Yes	Yes	Yes	Yes	Yes	Yes
Pulmonary hypertension	Yes	Yes	Yes	Yes	No	No
Postnatal age at hAEC, days	187	98	122	78	59	80
Gestation at hAEC, weeks	54 <sup>+4</sup>	42 <sup>+0</sup>	41 <sup>+0</sup>	36 <sup>+1</sup>	36 <sup>+0</sup>	36 <sup>+0</sup>
Weight at hAEC, grams	4,163	1,600	2,500	2,368	1,325	2,430
hAEC dose, million cells/kg	0.5	1	1	1	1	1
Death during NICU stay	No	Yes	No	No	No	No
Serum tryptase	Negative	Negative	Negative	Negative	Negative	Negative
Postnatal age at discharge, days	388	NA	168	238	174	155
Home oxygen	Yes	NA	Yes	Yes	Yes	Yes
Other major diagnoses in NICU	Acute renal failure, hypothyroidism, rectal prolapse, inguinal hernia	Meckel's diverticulum, refractory thrombocytopenia, conjugated hyperbilirubinemia, pleural effusion, acute renal failure, inguinal hernia	Malabsorption, conjugated hyperbilirubinemia, inguinal hernia	Systemic hypertension, inguinal hernia	Systemic hypertension, adrenal insufficiency, inguinal hernia	

Abbreviations: BPD, bronchopulmonary dysplasia; hAEC, human amnion epithelial cells; IVH, intraventricular hemorrhage; NA, not applicable; NICU, neonatal intensive care unit; PDA, patent ductus arteriosus.

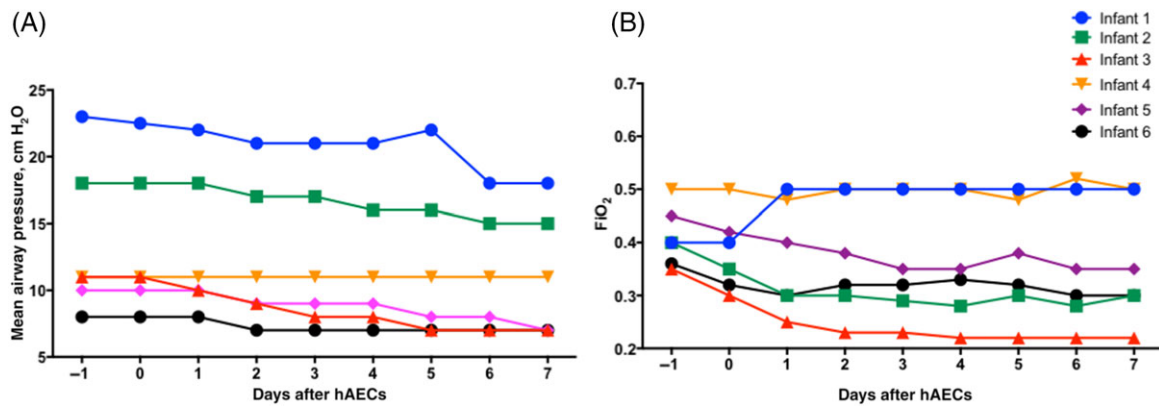


**Figure 1.** Changes in physiological parameters in babies 1–6 from baseline observations during cell administrations. Heart rate (red circles), peripheral oxygen saturation (black squares), mean blood pressure (blue triangles).

discharged at 1 year of age. At his 2-year post-hAEC follow-up, we were able to confirm that he has not experienced any long-term consequences from the acute adverse event. As of the writing of this manuscript, he is spontaneously breathing in air during the day and only requiring low-flow oxygen during his nighttime sleep. We suggest that the observations from this study provide both the reassurance necessary to assess the potential efficacy of hAECs as a treatment for BPD and a cell delivery protocol suitable for any future trials. Specifically, for the first time we present a protocol for amnion cell delivery including cell density, route, and infusion rate of administration that could be the basis of future efficacy trials.

Although it would have been useful to have included a dose-escalation arm to this trial, we had to consider a number of issues when planning this first-in-human trial of hAECs. This was not only for this trial but also with future efficacy trials in mind. We considered the dose of cells that would be tolerated, likely effective dose, route of administration, cell suspension density and delivery volumes, and timing of cell administration. Necessarily, we erred on the side of safety in this phase I trial but with a clear view to informing stepwise progress to a trial design able to assess the effectiveness of hAECs in the prevention and treatment of BPD.

With regard to the likely effective cell dose and maximally tolerated doses, some guidance was afforded by preclinical studies and other human stem cell trials. In newborn mice, hAECs were effective at preventing BPD-like lung injury and associated pulmonary hypertension at a dose of about 50 million cells per kg but not at 25 million per kg [19]. In a sheep model of BPD, hAECs given intravenously to 110-day-old fetal lambs were effective in preventing ventilation-induced lung injury at a dose of 30 million cells per kg [20]. Considering all of the preclinical studies in rodent and ovine models of BPD-like injury, we estimate that the likely effective therapeutic dose of hAECs will be within the range of 30–60 million cells per kg. Broadly consistent with this, albeit in a very different disease, administration of doses of 20 million umbilical cord blood cells per kg was associated with improved brain connectivity and motor function in children with cerebral palsy [21]. Such doses of cells are far greater than we have proved safe in the current phase I trial. Achieving cell doses of upward of 20 million cells per kg will require a careful escalation plan in any future trials. This takes us to consider the likely maximum tolerated single dose of cells. Estimating this will determine whether single-dose therapy or multiple cell infusions will likely be required to deliver therapeutic dose of cells without



**Figure 2.** Graphs showing respiratory support requirements for 7 days after hAEC therapy. **(A):** Mean airway pressure. **(B):** FiO<sub>2</sub>. Abbreviation: hAECs, human amnion epithelial cells.

compromising patient safety. Here, we believe that human trials of other cell therapies are probably most informative.

First, in a trial of MSCs for acute adult respiratory distress syndrome, 10 million cells/kg was proved safe [22]. Similarly, in a 19-month-old toddler receiving allogeneic MSCs for the treatment of osteogenesis imperfecta, an intravenous infusion of 10 million MSCs per kg was well tolerated [18]. However, if 10 million cells per kg was the ceiling cell dose per infusion, then three to six infusions would be required to achieve what we estimate will be a likely therapeutic dose for BPD. In that regard, in a recent phase II trial of autologous cord blood stem cells in 63 children with cerebral palsy intravenous infusions of 20 million nucleated cells per kg were shown to be safe in children as young as 1 year of age [23]. In adults, intravenous infusions of 25 million per kg of multipotent adult progenitor cells were found to be safe in a phase I MultiStem phase I trial for prophylaxis of graft-versus-host disease when administered as five weekly doses of 5 million per kg [19]. Together, these studies suggest that multiple infusions may serve as an effective way to safely achieve therapeutic doses. For example, it might be necessary to administer two to three infusions of up to 20 million hAECs per kg in order to safely evaluate efficacy.

The transient cardiopulmonary event we observed in baby 1 was unexpected because we had previously shown that higher densities of hAECs could be given in animal studies without cardiopulmonary event. In ovine studies, 30 million hAECs were given intravenously to 110-day-old lamb fetuses by slow manual push in 2.5 ml saline over 5 minutes without event. This equates to a cell concentration of 12 million cells per ml, which is 6 times the cell concentration given to baby 1. However, given that fetal

pulmonary vascular resistance is far higher than in the neonate, in utero studies are not informative regarding risks of pulmonary embolic events. Nonetheless, in murine studies, up to 100,000 hAECs were given intravenously to ~2 g neonatal mice in a 10 μl saline, which equates to 10 million cells per ml [24]. In a clinical trial to treat osteogenesis imperfecta, 5.8 million MSCs per ml was safely administered intravenously to a 19-month-old child [18]. It is therefore possible that, of the cell delivery protocol changes we made in response to baby 1, the inclusion of a rocking platform and an inline cell filter would have been sufficient to prevent any further recurrence of microemboli such that the eightfold reduction in cell density may have unnecessarily increased the infusion volume.

This being said, it is important to consider the maximal tolerated volumes in premature babies, which vary according to the extremity of prematurity. Repeated transfusions up to 20 ml per kg are commonly carried out in preterm babies, mainly to address anemia of prematurity. Also, up to 80% of preterm babies weighing less than 1,500 g at birth are transfused at least once, according to the Joint U.K. Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee [20]. Indeed, transfusion volumes of 10–20 ml per kg are considered conventional [25], albeit such transfusions are often covered with frusemide to reduce risks of volume overload. With this in mind, we believe that infusions of 20 million hAECs in 10 ml saline would be well tolerated by a 1,000-g premature baby,

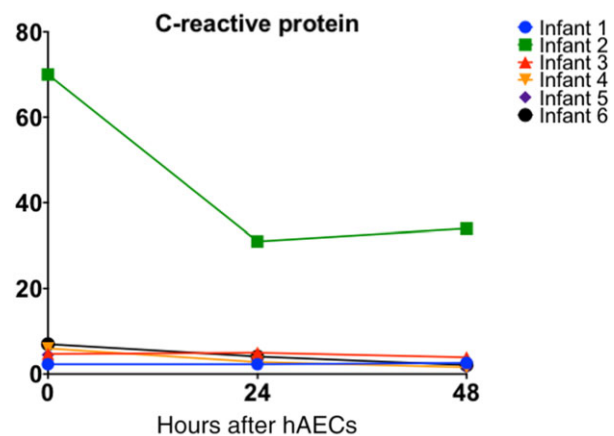
**Table 2.** Primary safety outcomes until time of discharge from neonatal intensive care unit

Safety parameter	Infant 1 <sup>a</sup>	Infant 2 <sup>b</sup>	Infant 3	Infant 4	Infant 5	Infant 6
Local reaction	NA	NA	NA	NA	NA	NA
Anaphylaxis	NA	NA	NA	NA	NA	NA
Rejection	NA	NA	NA	NA	NA	NA
Infection	NA	NA	NA	NA	NA	NA
Tumor formation	NA	NA	NA	NA	NA	NA

<sup>a</sup>Acute hypoxic event during cell administration—likely microembolic phenomenon.

<sup>b</sup>Infant died 1 month after cell administration due to unrelated causes.

Abbreviation: NA, not applicable.



**Figure 3.** Serum C-reactive protein levels (in mg/l) for the first 48 hours after hAECs. Abbreviation: hAECs, human amnion epithelial cells.

particularly if the infusion duration was extended to 2–4 hours in line with conventional transfusion protocols.

Our experience highlights the challenges of extrapolating cell dosing from animal experiments and the need to progress carefully in human trials to ensure safety. That the changes we made to cell concentration and infusion rate for the subsequent five babies were not associated with any cardiopulmonary compromise supports the likelihood that baby 1's response was embolic. Indeed, the altered infusion protocol with in-line filter appears to afford the systemic delivery of allogeneic hAECs in a manner that is well tolerated by premature born infants with established BPD and, we suggest, a protocol suitable for future efficacy trials.

Another point to consider for future hAEC trials is the route of administration used. Although it may be tempting to speculate that intratracheal administration of hAECs may have resulted in observable benefits due to direct pulmonary access, a recent meta-analysis of preclinical evidence in BPD indicates that intravenous delivery of MSCs was significantly superior compared with intratracheal administration [26]. The intratracheal administration of hAECs has been previously shown to be efficacious in experimental BPD [27], and a previous report from an ovine study suggests that greatest efficacy may actually be achieved by combined intratracheal and intravenous delivery [15]. However, intravenously delivered hAECs were recently shown to be equally efficacious in normalizing alveolar simplification and preventing secondary cardiopulmonary complications when compared with intratracheally delivered hAECs [24]. With a view to future efficacy trials that will most likely involve babies on CPAP and so not suitable for intratracheal cell delivery, we elected for the intravenous route in this current study. This is in contrast to the intratracheal route used in the Pneumostem trial [9], which is to date the only other phase I trial of a cell therapy in BPD. In that trial, nine premature infants were studied ( $25.3 \pm 0.9$  weeks; birth weights  $793 \pm 127$  g) after being determined as being at high risk of developing BPD and requiring continuous ventilator support at the time of cell therapy. The investigators assessed the safety of intratracheally delivered umbilical cord-derived MSCs given at 2 weeks postnatally at doses of 10 million cells per kg in 2 ml normal saline ( $n = 3$ ) and 20 million cells per kg in 4 ml normal saline ( $n = 6$ ). No serious adverse effects associated with the MSC delivery were reported after 84 days of monitoring. Another phase I/II safety and efficacy dose escalation (10 million per kg and 20 million per kg) trial of endotracheal Pneumostem MSCs in 12 premature babies is currently underway in the U.S. (NCT02381366). A phase II randomized, double-blind, multicenter trial to further assess safety and efficacy of endotracheal Pneumostem MSCs has been recently completed, but not yet reported, in South Korea (NCT01828957). In our current study, the dose of cells was an order of magnitude lower but achieved a similar effect by way of secondary outcomes—that is, secondary mean airway pressure and fraction of inspired oxygen. We did not collect tracheal aspirates to assess inflammatory markers because half our infants were not intubated. Future trials should look to identify and assess changes to biomarkers that would inform frequency of cell administration and efficacy of treatment.

Similar to MSCs, the major mechanism of hAEC action appears to be via modulation of host immune cell responses

to injury achieved through paracrine factors such as lipid-based mediators [14] and the release of extracellular vesicles [28]. In the Pneumostem trial, the tracheal aspirate fluid of MSC-treated infants had reduced levels of matrix metalloproteinase-9, interleukins -6 and -8, tumor necrosis factor  $\alpha$ , and transforming growth factor  $\beta$  compared with the matched comparison group [9]. In preclinical studies, hAEC-mediated improvements to lung function and architecture were associated with reduced pulmonary accumulation of macrophages, natural killer cells, and dendritic cells, coincident with reduced levels of IL-1 $\beta$ , TNF $\alpha$ , MIP-2, LIF, and RANTES [23]. The reduction in pulmonary inflammation was also associated with reduced muscularization of small (<50  $\mu$ m) pulmonary arteries, and secondary pulmonary hypertension [24]. Although beyond the scope of this current study, future efficacy trials should assess immunological outcomes so as to verify mechanisms of action. Another necessary immunological evaluation to be explored in future trials is the assessment of donor recognition using recipient CD45+ cells. Thus far, all reports of hAECs indicate that they are immune privileged; however, repeated administration of the hAECs in dose-escalation studies may immunize the recipient against the hAEC donor antigen. Evaluation of donor recognition by hAEC recipients will provide conclusive data around this safety aspect of using allogeneic hAECs.

Finally, it is useful to consider the timing of cell administration in future trial design. In the current trial, we only studied infants with established BPD. Although this was appropriate for a first-in-human phase I study of hAECs, we believe that the established safety profile of allogeneic hAECs should now justify a dose-escalation study with an earlier intervention. Indeed, the safety outcomes from this study may justify the use of hAECs in preventing BPD in at-risk babies, as preclinical studies indicate that hAECs are most efficacious shortly after lung inflammation begins and is not overwhelming [24].

## CONCLUSION

The primary outcomes of our current study indicate that allogeneic hAECs are safe and well tolerated by premature babies. We believe that testing the efficacy of prophylactically administered hAECs is now warranted. Future studies in infants at risk of BPD in randomized, placebo-controlled dose-escalation trials are necessary to assess efficacy. This might pave the way for cellular therapy to be a mainstay in the prevention and treatment of neonatal chronic conditions.

## AUTHOR CONTRIBUTIONS

R.L., A.M., and E.M.W.: data analysis and interpretation, manuscript writing; R.L., J.T., S.L., S.T.C., and D.Z. were responsible for cell manufacturing and quality assurance under GMP guidelines; R.L., A.M., J.C.M., and E.M.W. obtained human ethics submission and prepared reports for the DSMB.

## DISCLOSURES

The authors indicated no potential conflicts of interest.

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