PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Human amnion cells for the prevention of bronchopulmonary dysplasia: a protocol for a phase 1 dose escalation study
AUTHORS	Baker, Elizabeth; Malhotra, Atul; Lim, Rebecca; Jacobs, Susan; Hooper, Stuart; Davis, Peter; Wallace, Euan

VERSION 1 – REVIEW

REVIEWER	Chung-Ming Chen Department of Pediatrics Taipei Medical University Hospital Taipei 110, Taiwan
REVIEW RETURNED	15-Sep-2018

GENERAL COMMENTS	Comments: The investigators extend their previous human trial to assess the safety of higher doses of human amnion epithelial cells in preterm infants at risk of bronchopulmonary dysplasia. Concerns: 1. One of six subjects developed bradycardiac and hypoxic during the intravenous infusion of 1 million cells/kg in previous trial. This trial will inject two- to ten-fold dose of cells. How to prevent the adverse event in this trial? 2. Please define dose-limiting toxicity. 3. Do the investigators intend to determine the maximum tolerable dosage?

REVIEWER	Bernard Thébaud Ottawa Hospital Research Institute Canada
REVIEW RETURNED	04-Oct-2018

GENERAL COMMENTS	This is a study protocol for a phase I dose-escalation study of human amnion epithelial cells (hAECs) for bronchopulmonary dysplasia (BPD). The chronic lung disease BPD remains the main complication of extreme prematurity. The rationale for this study is based on (1) a decade of in vitro and in vivo studies in experimental models demonstrating the safety and lung protective potential of hAECs and (2) a phase I trial in 6 preterm infants showing feasibility and no short-term toxicity of iv infusion of hAECs in this patient population. While the previous phase I tested a single dose of 1 million cells/kg, this current phase I trial proposes a dose-escalation study in doses of up to 30 million cells/kg. This study is a logical continuum of a step-wise and careful approach to translate hAECs into the clinic. The group should be commended for their efforts. The strengths and limitations of this phase I study are appropriately

	listed. The current challenge of predicting and defining BPD is less of a problem for this current safety trial, but will be particularly important to address in subsequent phase II and III trials. Major comments: The term “mesenchymal stromal cell” should be preferred to mesenchymal “stem” cell (Dominic et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2016). The statement about the comparative risk of tumor growth between hAECs and mesenchymal stroma cells (MSCs) should be nuanced. A systematic review and meta-analysis, currently the most robust method to assess evidence, of MSC clinical trials has not found an increased risk of tumor formation in over 1000 adult patients treated with MSCs (Lalu et al, PLoS One 2012). Such a systematic review has not been performed for hAECs. Of course, larger scale controlled clinical trials with rigorous reporting of adverse events are required to further define the safety profile of MSCs, hAECc and other upcoming cell therapies. Sample size: Please clarify in this paragraph that there is a total of 6 dose-escalation regimens. Please also state a rationale for increasing the sample size from 3 to 6 patients in the last two dose-escalation regimens. Intervention: - Please provide details regarding the cell administration process (fresh vs frozen cell product, thawing device/procedure, cell counting, cell viability assessment pre- or post-infusion, etc), as this can affect product activity and thus study results. - Please provide a rationale for several dose administrations in infants receiving 20 and 30 million cells/kg. - Please define “Escalation of respiratory support”. - Please provide a rationale for the choice of cytokines. Please clarify potential conflict of interest. Please state the starting date of the trial and expected date of trial completion. Minor comments: “The trajectory into adulthood of infants born in the era of surfactant use remains unknown, but it is likely that these infants will be burdened with a greater risk of chronic obstructive pulmonary disease in mid-life.” This statement is appropriately nuanced, but merits citations that corroborate this concept of developmental origin of adult diseases for BPD (Wong et al, Eur Respir J 2008 for example), but also the great repair capability of the lung using novel techniques (Narayanan et al, Am J Respir Crit Care Med 2012), which could be useful for assessing long term effects of cell therapies.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1 concerns:
1. One of six subjects developed bradycardiac and hypoxic during the intravenous infusion of 1 million cells/kg in previous trial. This trial will inject two- to ten-fold dose of cells. How to prevent the adverse event in this trial?

Thank you for highlighting this point. The changes that were made to the infusion protocol in the previous trial in response to this adverse event are outlined in paragraph 3 of our section titled 'Intervention'. Our infusion protocol for this next trial has adopted these changes in full, as outlined in paragraph 3, 'Intervention' section.

2. Please define dose-limiting toxicity.

'Dose limiting toxicity' will be defined by the occurrence of adverse events during or in the 72 hours post the hAECs infusion. This has been included in a new sub-section of 'Primary Outcome' titled 'Dose Limiting Toxicity'

3. Do the investigators intend to determine the maximum tolerable dosage?

No, we do not intend to determine the maximum tolerable dose. We intend to evaluate the safety of a dose which has been efficacious in our pre-clinical models and which, based on trials of other cell types, we believe will be therapeutic. We have added a statement to this effect at the end of our introduction.

Reviewer: 2

The chronic lung disease BPD remains the main complication of extreme prematurity. The rationale for this study is based on (1) a decade of in vitro and in vivo studies in experimental models demonstrating the safety and lung protective potential of hAECs and (2) a phase I trial in 6 preterm infants showing feasibility and no short-term toxicity of iv infusion of hAECs in this patient population. While the previous phase I tested a single dose of 1 million cells/kg, this current phase I trial proposes a dose-escalation study in doses of up to 30 million cells/kg. This study is a logical continuum of a step-wise and careful approach to translate hAECs into the clinic. The group should be commended for their efforts.

Thank you for your thoughtful words and considered review of our manuscript.

Major comments:

The term "mesenchymal stromal cell" should be preferred to mesenchymal "stem" cell (Dominic et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2016).

We have edited the manuscript to refer to mesenchymal stromal cells rather than mesenchymal stem cells.

The statement about the comparative risk of tumor growth between hAECs and mesenchymal stroma cells (MSCs) should be nuanced. A systematic review and meta-analysis, currently the most robust method to assess evidence, of MSC clinical trials has not found an increased risk of tumor formation in over 1000 adult patients treated with MSCs (Lalu et al, PLoS One 2012). Such a systematic review has not been performed for hAECs. Of course, larger scale controlled clinical trials with rigorous reporting of adverse events are required to further define the safety profile of MSCs, hAECc and other upcoming cell therapies.

We have better qualified our statement regarding the potential for tumour growth and have included the above reference in the 'Introduction', paragraph 3.

Sample size: Please clarify in this paragraph that there is a total of 6 dose-escalation regimens. Please also state a rationale for increasing the sample size from 3 to 6 patients in the last two dose-escalation regimens.

A further explanation of the dose cohorts and the rationale for their size has been include in the 'Sample Size' section, paragraph 2. We hope this is clearer.

Intervention:

- Please provide details regarding the cell administration process (fresh vs frozen cell product, thawing device/procedure, cell counting, cell viability assessment pre- or post-infusion, etc), as this can affect product activity and thus study results.

We have added a new section titled 'Human Amnion Epithelial Cell Infusion Preparation' to address these concerns. This includes subsections titled Donor Screening, Cell Collection, Product Release, Infusion Preparation and Post Infusion Testing.

- Please provide a rationale for several dose administrations in infants receiving 20 and 30 million cells/kg.

The intervention section has been edited to include our rationale in paragraph 2.

- Please define "Escalation of respiratory support".

A definition has been provided in the description of adverse events.
- Please provide a rationale for the choice of cytokines.
Rationale for the choice of cytokines has been provided in the section 'Cytokine Profiling' in 'Secondary Outcomes'.

Please clarify potential conflict of interest.
Our competing interests statement can be found at the end of the manuscript. It has been edited for clarity.

Please state the starting date of the trial and expected date of trial completion.
Our trial started recruiting in August 2018 and is expected to complete follow-up in mid 2022.
These dates have been included in the abstract under the section 'Ethics and Dissemination'.

Minor comments:
"The trajectory into adulthood of infants born in the era of surfactant use remains unknown, but it is likely that these infants will be burdened with a greater risk of chronic obstructive pulmonary disease in mid-life." This statement is appropriately nuanced, but merits citations that corroborate this concept of developmental origin of adult diseases for BPD (Wong et al, Eur Respir J 2008 for example), but also the great repair capability of the lung using novel techniques (Narayanan et al, Am J Respir Crit Care Med 2012), which could be useful for assessing long term effects of cell therapies.
Thank-you. A number of references (3,4,5,6) have been added to support the statement regards infants with BPD being at higher risk of COPD.

VERSION 2 – REVIEW

REVIEWER	Chung-Ming Chen Department of Pediatrics Taipei Medical University Taipei, Taiwan
REVIEW RETURNED	08-Nov-2018

GENERAL COMMENTS	The authors have addressed the concerns.

REVIEWER	Bernard Thébaud Professor of Pediatrics | University of Ottawa Senior Scientist | Ottawa Hospital Research Institute Division of Neonatology | Children's Hospital of Eastern Ontario University of Ottawa Partnership Research Chair in Regenerative Medicine General Campus 501 Smyth Road CCW Room W6120 Ottawa, ON K1H 8L6
REVIEW RETURNED	22-Nov-2018

GENERAL COMMENTS	The authors were very responsive to the reviewer's comments. I would like to commend them once more for their efforts. The authors declare no conflict of interest. The editor should verify whether the authors hold intellectual property and clarify whether this requires to be disclosed.