PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Autologous transplantation of umbilical cord blood derived stem cells in extreme preterm infants: protocol for a safety and feasibility study
AUTHORS	Malhotra, Atul; Novak, Iona; Miller, Suzanne; Jenkin, Graham

VERSION 1 – REVIEW

REVIEWER	Anup Katheria Sharp Mar Birch Hospital for Women & Newborns
REVIEW RETURNED	23-Dec-2019

GENERAL COMMENTS	This was a well written paper with clearly defined hypothesis, aims, adverse events and good long term followup. One question for the authors with whether mri should be included to rule out the concern of stem cell emboli? Otherwise I have no concerns.

REVIEWER	Paolo Rebulla Department of Transfusion Medicine and Hematology, Foundation IRCCS Ca' Granda Ospedale Maggiore Policolinico, via Francesco Sforza 35, 20122 Milan, Italy
REVIEW RETURNED	26-Dec-2019

GENERAL COMMENTS	This is a well designed safety and feasibility protocol addressing an important clinical issue. The quality of the protocol can be improved by addition of some methodological details. Specific comments: 1) Page 8, lines 16-18. Please provide details on 'size appropriate needle and collection containers(bags' that will be used. 2) Page 8, line 22. Infants with collected cord 'blood' volume ... 3) Page 8, lines 24-28. Please provide details on cord blood processing, in particular on the separation, characterization and quality control of the UCBCs. 4) Page 8, line 45. Please clarify the meaning of 'impurities'. 5) Please shortly describe the red cell, plasma and platelets transfusion protocols for these extremely preterm newborns (thresholds, volumes, etc). 6) PLease check references format (pages are missing in ref 6, 11, 12, 15 and 17; report abbreviated journals names).

REVIEWER	Maria Pierro Fondazione Poliambulanza Istituto Ospedaliero
REVIEW RETURNED	07-Jan-2020

GENERAL COMMENTS	MAJOR COMMENT

	Malhotra and colleagues presented an interesting study protocol aiming to test the feasibility of umbilical cord blood cells (UCBC) collection at birth and the safety of autologous intravenous UCBC transplantation in preterm infants. The protocol is enrolling infants born before 28 weeks of gestation, in case of absence of severe brain injury on neonatal cranial ultrasound, performed during the first week of life. Although the purpose of this study protocol is to assess the safety of the treatment, rather than the efficacy, it should be the precursor of a future study aiming to prevent severe neurodevelopmental impairment in preterm infants. Preterm brain injury, leading to cognitive damage, is mainly caused by: (i) intraventricular haemorrhage (IVH), which is always detected on head ultrasound and it is exceptional after the first week of life, and (ii) periventricular leukomalacia (PVL), a much rarer complication that develops after the first 2-3 weeks of life, although in some cases can be suspected much earlier on the basis of head ultrasound. The incidence of cerebral palsy or major long-term neurological disabilities in infants born above 24 weeks gestation with normal head ultrasound is extremely low. Approximately 90-98% of the the infants born between 25 to 28 weeks with normal head ultrasound in the first week of life will survive with no major neurodevelopmental complications. This poses an ethical question, since these babies should probably be considered as “healthy” cases, as their likelihood of developing a negative outcome is not significantly high. Several regulatory authorities and organisations approve studies in healthy children and newborn only if the possible risk is not greater that minimal, and given the lack of knowledge, we cannot include cord blood cells in this group of treatments. On the contrary, infants below 24 weeks’ gestation are at higher risk of moderate to severe neurodevelopmental damage, even in case of negative early head ultrasound, although the incidence of IVH in these patients may be up to 50%. In order to avoid unethical experimental treatments in fragile newborns, it would be better to enrol infants between 24 weeks up to 28 weeks that had a neurological complication at head ultrasound (early treatment rather than prophylactic) or treat extremely preterm infants (below 24 weeks) with and without normal early head ultrasound that are higher risk of long-term problems independently from the neuroimaging results (prophylactic and early treatment). A second step could be done later, in case safety and efficacy is proven in affected infants, to treat preterm infants at higher gestational ages with perinatal or neonatal risk factors for lower cognitive scores and cerebral palsy and no abnormalities on early cranial ultrasound. MINOR COMMENTS 1. Throughout the manuscript an adverse long-term neurological outcomes is rightly associated to the development of IVH. However, the onset IVH (detected on head ultrasound) is exceptional after the first week of life and this is not mentioned in the protocol. As a consequence, a non-expert reader may be misled to think that the infants with a normal head ultrasound after the first week of life, are still at high risk of developing IVH later on,

	which is not the case. This should better explained and the criteria of enrolment should be revised as per major comment. 2. A previously mentioned, although this study is only assessing safety, it should be a precursor of an efficacy trial. The population enrolled in an eventual efficacy trial should be the same enrolled in this safety study, in order to avoid unnecessary experimental treatments in the neonatal population. However, it is unlikely that these enrolment criteria would suit an efficacy trial, given the low risk of adverse neurological outcome in the population selected. 3. The standard cord blood processing operating procedures, since they are not detailed, should be at least referenced.

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1

Reviewer Name: Anup Katheria

Institution and Country: Sharp Mar Birch Hospital for Women & Newborns

Please state any competing interests or state ‘None declared’: I have no competing interests.

Please leave your comments for the authors below

This was a well written paper with clearly defined hypothesis, aims, adverse events and good long term followup. One question for the authors with whether mri should be included to rule out the concern of stem cell emboli? Otherwise I have no concerns.

Thank you for the comment. Most of these infants will be having a term equivalent MRI as per unit practice. However, we are not sure a late MRI (term age) will actually be helpful to pick up “stem cell emboli” in the brain as these cells do not engraft in brain tissue.

Reviewer: 2

Reviewer Name: Paolo Rebulla

Institution and Country:

Department of Transfusion Medicine and Hematology, Foundation IRCCS Ca' Granda Ospedale Maggiore Policolinico, via Francesco Sforza 35, 20122 Milan, Italy

Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below

This is a well designed safety and feasibility protocol addressing an important clinical issue. The quality of the protocol can be improved by addition of some methodological details.

Specific comments:

1) Page 8, lines 16-18. Please provide details on 'size appropriate needle and collection containers bags' that will be used.

Thank you. We have added details as suggested.

2) Page 8, line 22. Infants with collected cord 'blood' volume ...

Thank you for picking up the error. Edited as suggested.

3) Page 8, lines 24-28. Please provide details on cord blood processing, in particular on the separation, characterization and quality control of the UCBCs.

Thank you. Standard procedures have been referenced where relevant.

4) Page 8, line 45. Please clarify the meaning of 'impurities'.

Thank you for picking up the error. It was meant to be "microbes". Edited now.

5) Please shortly describe the red cell, plasma and platelets transfusion protocols for these extremely preterm newborns (thresholds, volumes, etc).

We have briefly described the transfusion/ infusion protocols as suggested.

6) Please check references format (pages are missing in ref 6, 11, 12, 15 and 17; report abbreviated journals names).

References have been double checked and edited. Thank you

Reviewer: 3

Reviewer Name: Maria Pierro

Institution and Country: Fondazione Poliambulanza Istituto Ospedaliero

Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below

MAJOR COMMENT

Malhotra and colleagues presented an interesting study protocol aiming to test the feasibility of umbilical cord blood cells (UCBC) collection at birth and the safety of autologous intravenous UCBC transplantation in preterm infants. The protocol is enrolling infants born before 28 weeks of gestation, in case of absence of severe brain injury on neonatal cranial ultrasound, performed during the first week of life.

Although the purpose of this study protocol is to assess the safety of the treatment, rather than the efficacy, it should be the precursor of a future study aiming to prevent severe neurodevelopmental impairment in preterm infants. Preterm brain injury, leading to cognitive damage, is mainly caused by: (i) intraventricular haemorrhage (IVH), which is always detected on head ultrasound and it is exceptional after the first week of life, and (ii) periventricular leukomalacia (PVL), a much rarer complication that develops after the first 2-3 weeks of life, although in some cases can be suspected much earlier on the basis of head ultrasound.

The incidence of cerebral palsy or major long-term neurological disabilities in infants born above 24 weeks gestation with normal head ultrasound is extremely low. Approximately 90-98% of the the infants born between 25 to 28 weeks with normal head ultrasound in the first week of life will survive

with no major neurodevelopmental complications. This poses an ethical question, since these babies should probably be considered as “healthy” cases, as their likelihood of developing a negative outcome is not significantly high. Several regulatory authorities and organisations approve studies in healthy children and newborn only if the possible risk is not greater that minimal, and given the lack of knowledge, we cannot include cord blood cells in this group of treatments.

On the contrary, infants below 24 weeks’ gestation are at higher risk of moderate to severe neurodevelopmental damage, even in case of negative early head ultrasound, although the incidence of IVH in these patients may be up to 50%.

In order to avoid unethical experimental treatments in fragile newborns, it would be better to enrol infants between 24 weeks up to 28 weeks that had a neurological complication at head ultrasound (early treatment rather than prophylactic) or treat extremely preterm infants (below 24 weeks) with and without normal early head ultrasound that are higher risk of long-term problems independently from the neuroimaging results (prophylactic and early treatment).

Thank you for your important comments and concern. The inclusion criteria for this feasibility and safety study were discussed at length in the departmental expert review panel and subsequently in the ethics committee meetings at an institutional level. Further, consumer feedback through the hospital ethics committee and cerebral palsy alliance (a patient advocacy non-profit organisation) consumer groups was also sought in the design of this and other cord blood trials. At the scientific and ethical review panels, clinicians did not have treatment acceptability for cell therapy for extremely premature infants, nor did they have equipoise for treatment in infants with moderate –severe brain injury, and thus required us to revert to a pure safety and feasibility trial in a lower risk group, to mitigate risk of any adverse effects of administration in infants.

Whilst cord blood cell therapy may ultimately be more useful in the infant who has moderate-severe brain injury, it was widely felt and consensus reached that in the first instance trying to establish feasibility and safety of autologous transplantation in extreme premature infants (all of who are at risk of poor neurodevelopmental outcomes, albeit not the highest risk) was of utmost importance. We have added the risks of overall adverse neurodevelopment in this population in the revised paper.

As the cells are autologous, we anticipate that the cells should be well tolerated by the infants with minimal or negligible risk of adverse effects. This has been supported by previous autologous cell therapies in older infants.

In the next phase, we intend to conduct a randomised controlled trial in infants at highest risk of brain injury/ adverse neurodevelopment as you have suggested. Thank you kindly.

A second step could be done later, in case safety and efficacy is proven in affected infants, to treat preterm infants at higher gestational ages with perinatal or neonatal risk factors for lower cognitive scores and cerebral palsy and no abnormalities on early cranial ultrasound.

Thank you. Please see response above.

MINOR COMMENTS

1. Throughout the manuscript an adverse long-term neurological outcomes is rightly associated to the development of IVH. However, the onset IVH (detected on head ultrasound) is exceptional after the first week of life and this is not mentioned in the protocol. As a consequence, a non-expert reader may be misled to think that the infants with a normal head ultrasound after the first week of life, are still at high risk of developing IVH later on, which is not the case. This should better explained and the criteria of enrolment should be revised as per major comment.

Thank you. We have edited and rephrased the discussion around IVH and its impact on neurodevelopment.

2. A previously mentioned, although this study is only assessing safety, it should be a precursor of an efficacy trial. The population enrolled in an eventual efficacy trial should be the same enrolled in this safety study, in order to avoid unnecessary experimental treatments in the neonatal population. However, it is unlikely that these enrolment criteria would suit an efficacy trial, given the low risk of adverse neurological outcome in the population selected.

Thank you. The prime intention of this trial is feasibility and through enrolling most infants less than 28 weeks, we will be able to establish this in a timely manner, to then be able to establish efficacy of cell therapy in the highest risk infants.

3. The standard cord blood processing operating procedures, since they are not detailed, should be at least referenced.

Thank you. Standard procedures have been referenced as suggested.