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14. ABSTRACT The acute respiratory distress syndrome (ARDS) is a life-threatening medical condition in which the lung is injured or inflamed to the degree that it cannot properly exchange gases and oxygenate the body. ARDS can be caused by a variety of conditions including trauma, severe blood loss, multiple or large volume blood transfusions, burns, and infections. The development of therapeutics that can limit the severity and/or progression of lung injuries that lead to ARDS and death is an immediate clinical need in both military and civilian sectors. Experimental studies carried out in small and large animals have demonstrated that specialized cells called mesenchymal stromal cells (MSC) can effectively reduce inflammation in multiple diseases including ARDS. The overall objective of this proposal is to carry out a randomized, blinded, placebo-controlled, multicenter phase 2b trial to test the therapeutic potential of allogeneic bone-marrow derived MSC for treating ARDS, with a major focus on civilian trauma patients. The specific aims of this project are: Specific Aim 1. To test the clinical efficacy of intravenously delivered allogeneic human MSC in patients with ARDS. Specific Aim 2. To test the mechanisms by which MSC reduce acute lung injury in trauma patients with ARDS.					
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Table of Contents

1. INTRODUCTION	4
2. KEYWORDS.....	4
3. ACCOMPLISHMENTS	4
4. IMPACT	14
5. CHANGES/PROBLEMS.....	14
6. PRODUCTS	16
7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS.....	16
8. SPECIAL REPORTING REQUIREMENTS	20
9. APPENDICES.....	20

1. INTRODUCTION:

The acute respiratory distress syndrome (ARDS) is a life-threatening medical condition in which the lung is injured or inflamed to the degree that it cannot properly exchange gases and oxygenate the body. ARDS can be caused by a variety of conditions including infections, trauma, severe blood loss, multiple or large volume blood transfusions, burns, and the inhalation of chemical poisons or smoke. According to the National Heart Lung and Blood Institute, approximately 190,000 people in the U.S. will develop ARDS each year, with a death rate ranging from 25–40%. Recent studies from the Department of Defense Iraq Trauma Registry (DoDTR) reported that ARDS developed in a large number of severely wounded warfighters and was associated with higher death rates. To date, there have been few advances in the treatment of major trauma related conditions such as ARDS. The development of therapeutics that can limit the severity and/or progression of lung injuries that lead to ARDS and death is an immediate clinical need in both military and civilian sectors. Experimental studies carried out in small and large animals have demonstrated that specialized cells called mesenchymal stromal cells (MSC) can effectively reduce inflammation in multiple diseases including ARDS. The overall objective of this proposal is to carry out a randomized, blinded, placebo-controlled, multicenter phase 2b trial to test the therapeutic potential of allogeneic bone-marrow derived MSC for treating ARDS, with a major focus on civilian trauma patients. The specific aims of this project are: **Specific Aim 1.** To test the clinical efficacy of intravenously delivered allogeneic human MSC in patients with ARDS. **Specific Aim 2.** To test the mechanisms by which MSC reduce acute lung injury in trauma patients with ARDS. Subsequently emerging evidence suggests that the incidence of ARDS following trauma has declined, probably related to changes in resuscitation practices with the reduced use of crystalloid fluids and balanced transfusion of blood products. In order to study all patients with ARDS after trauma as well as to meet enrollment goals over the defined study period, after the discussion with the Department of Defense, the inclusion criteria for this phase 2b trial have been broadened to include all causes of ARDS for testing MSCs for both trauma and medical causes of ARDS.

2. KEYWORDS: Acute respiratory distress syndrome, pulmonary edema, trauma, pneumonia, sepsis

3. ACCOMPLISHMENTS:

- **What were the major goals of the project?**

During Year 2, we had the major goals as follows:

1. Revise the protocol with changes in the inclusion criteria and the CMC for MSC production
2. Finalize Statistical Analysis Plan and submit to FDA and then to the DoD HRPO
3. Submit the revised protocol with the changes in the inclusion criteria and the CMC for MSC production to the FDA
4. Submit the final HRPO DoD approved protocol to the central IRB
5. Submit local consent context for all sites to the central IRB at Vanderbilt University
6. Respond to Nancy Englar's detailed requests from September 11, 2018 regarding regulatory issues
7. Upon the approval by central IRB, submit the revised final protocol with changes in the inclusion criteria and the CMC for MSC production to the DoD HRPO for final approval
8. Submit the final HRPO DoD approved protocol to the central IRB
9. Complete the laboratory and study manual of operations
10. Finalize and beta test the case report forms with the data management system, including systems for bar coding and storing biological samples, as well as new data validation and query features
11. Finalize production plans for MSC for the trial, with timeline for delivery of the first batch of MSCs and run the necessary testing at local sites prior to the initiation of the trial

12. Discuss trial-related issues and training of coordinators and investigators by site visit and with a monthly conference call
13. Webinar educational sessions to introduce the data management system to investigators, BMT staff (who are responsible for randomization) and the study coordinators
14. Run mock screening and recruitment to be familiar with the recruitment & monitoring work flow
15. Set up electronic consent for all sites through Vanderbilt RedCap
16. Send the final approved protocol to the DSMB
17. Potentially begin enrollment of patients at one site - UCSF

- **What was accomplished under these goals?**

Goal #1: Revise the protocol with changes in the inclusion criteria and the CMC for MSC production, and other related study documents – completed

We have completed the major revisions of the Clinical Protocol and updated related study documents for this Phase 2b trial, which include:

Clinical Protocol:

After the discussion and the approval from the DoD, the inclusion criteria for this phase 2b trial were broadened to include all causes of ARDS for testing MSCs, and we revised the Clinical Protocol (Protocol No: UCSF-hMSC-ARDS-P1P2-10, dated May 14, 2019). The updated protocol includes the following changes:

1. We will continue to recruit patients with moderate-to-severe ARDS, which is the same as our completed Phase 1 and Phase 2a trials (funded by the NIH). However, we have expanded the inclusion criteria so we will continue to include all causes of ARDS. In terms of broadening the inclusion criteria for ARDS, this was a major focus of our August 30, 2018 conference call, and we had a subcommittee (chaired by Dr. Matthay) to finalize these inclusion and exclusion criteria.
2. The updated protocol involves a new source (AllCells, LLC) of human bone marrow for producing the allogeneic mesenchymal stromal cells for our phase 2b trial. The previous bone marrow producer (Lonza) stopped providing bone marrow. We have also slightly modified the hMSCs preparation and processing at the University of Minnesota and at local recruitment sites in order to optimize cell viability. All these changes are reflected in the updated Clinical Protocol (Section 8) and Study Product Preparation Protocol in the Appendix E.
3. We have also made some minor changes in the Investigational Product Infusion Protocol (Appendix H of Clinical Protocol) that reflect the changes in hMSC processing at the local sites.
4. We have added a new recruitment facility – University of California San Francisco Medical Center.
5. We have revised the specimen collection scheme in the protocol: reduced the volumes of blood collection at each timepoint; reduced urine collection from 6 times to 3 times; added blood collection for DNA/RNA on day 2.
6. We have made some minor modifications throughout the Clinical Protocol, to respond to the thoughtful review from the DoD (email by Nancy Englar, the officer of HPRO at DoD, dated June 25, 2018), as well as the co-investigators from all participating sites.

Investigator Brochure:

The Investigator Brochure has been updated to reflect the changes of the Clinical Protocol. The current version is Version 10, dated May 14, 2019.

Data and Safety Monitoring Board (DSMB) Charter:

The DSMB has been identified (Chair, Taylor Thompson, MD), a Critical Care Surgeon (Dr. Jason Sperry, MD) and a biostatistician (Dr. Michael Harhay, PhD). We have finalized the DSMB Charter (dated July 26, 2019). Dr. Taylor Thompson, the Chair of the DSMB, has reviewed and signed the document on August 16, 2019.

Consent Form:

We are using the Vanderbilt IRB as the central IRB for this Phase 2b trial. Per the Vanderbilt IRB's guidelines, the consent forms at each site includes two parts: main consent (Part 1) to be used for all six sites, and local consent context form (Part 2) which has site-specific language. The main consent has been finalized and approved by the central IRB at Vanderbilt University on April 11, 2019, and an updated main consent with minor changes has been submitted and approved by central IRB on July 25, 2019.

All recruitment sites have developed site-specific consent forms. The local consent form for UCSF Medical Center was submitted and approved by the central IRB at Vanderbilt University on April 11, 2019. The local consent forms for four sites (Zuckerberg San Francisco General Hospital & Trauma Center, Harborview Medical Center, Oregon Health & Science University, and the University of Texas Health Sciences Center at Houston/Memorial Hermann-Texas Medical Center), have been pre-reviewed by their local IRB and have been submitted to the central IRB at Vanderbilt University and currently are under review. Vanderbilt University Medical Center, the 6th recruitment site, has submitted their local consent form to the Vanderbilt IRB directly per Vanderbilt IRB's guideline.

Case Report Form:

We have updated the case report forms to reflect the new changes in the Clinical Protocol. The current version is dated July 3, 2019.

Goal #2: Finalize Statistical Analysis Plan and submit to FDA and then to the DoD HRPO - completed

After reviewing our initial FDA submission for this pending Phase 2b trial under the IND 15331 Amendment #0016 in November 2017, the FDA responded in the email dated on January 10, 2018, quoted as below:

The statistical team has indicated there are no hold or non-hold issues for the Phase 2b study. However, the statistical team wishes to encourage you to write a more detailed statistical plan before too far into the clinical study.

Therefore, we have developed the Statistical Analysis Plan (Version 1.0, date May 14, 2019). We have submitted this document to the FDA under the IND amendment #0018. The FDA reviewed and had no questions about this statistical analysis plan.

Goal #3: Submit the revised protocol with the changes in the inclusion criteria and the CMC for MSC production to the FDA - completed

We have submitted the Clinical Protocol and other study documents, and received the FDA approval for the initiation of this Phase 2b trial on August 15, 2019.

During the past reporting period, we have done the following communications with the FDA:

1. On September 11, 2018, we emailed the Project Manager at the FDA (Tal Salz) regarding the plan to identify and qualify the new source of bone marrow.
2. On November 30, 2018, we had a phone discussion and email communications with FDA (Tal Salz and Sukhanya Jayachandra – new Project Manager at FDA) in CBER regarding the new source (AssureImmune) of human bone marrow for production of the MSCs for the trial and strategies for enhancing MSC viability for the trial. During the teleconference call with the FDA, Dr. Matthey (study PI) and Dr. McKenna (PI of University of Minnesota University MSC production site) provided detailed information regarding the MSC source and MSC production methodologies.
3. On December 11, 2018, we emailed the FDA (Tal Salz and Sukhanya Jayachandra) to inform them that the University of Minnesota NIH/NHLBI Program (Dave McKenna, PI) had to disqualify AssureImmune because of concerns about their donor screening process, and we were working with Dr. McKenna at the University of Minnesota to qualify AllCells (California, CA), a well established cell acquisition laboratory. We have an established collaboration with the NIH sponsored Production Assistance for Cellular Therapy (PACT) Group in the University of Minnesota (Principal Investigator: David McKenna, MD) for the production of cGMP clinical grade, cryopreserved hMSCs.
4. On May 17, 2019, we submitted an IND amendment (#0018) to the FDA under IND 15331. This IND amendment included the following study documents:
 - 1) Form 1571
 - 2) Revised Form 1572 with Site Investigator's CVs (6 sites)
 - 3) IND Section 2 (revised): Introductory Statement
 - 4) IND Section 3 (revised): General Investigational Plan
 - 5) IND Section 5 (revised): Investigator Brochure, dated 05/14/2019
 - 6) IND Section 6 (revised): Clinical Protocol, dated 05/14/2019
 - 7) IND Section 7 (revised): Chemistry, Manufacturing and Controls (CMC)
 - 8) Appendices for CMC (revised)
 - 9) Statistical Analysis Plan (new), dated 05/14/2019

On June 20, 2019, Sukhanya Jayachandra, the Project Manager of the FDA, replied to us by email. There were no concerns regarding any study documents we had submitted in this amendment. However, the FDA requested minor additional information and clarifications regarding the CMC section.

5. On July 29, 2019, we submitted a single document as the IND amendment #0019 under the IND 15331, which included our point-by-point responses to the questions from FDA regarding our plans for manufacturing of the human MSCs for the phase 2b clinical trial. On August 15, 2019, the FDA reviewed our CMC responses and found them to be acceptable, and there were no additional CMC concerns.
6. On September 21, 2019, we submitted the annual report for IND 15331 to the FDA. In this report, we have described the study design of the Phase 2b trial, as well as the study progress.

Goal #4: Submit the HRPO DoD Clinical Protocol to the central IRB – Completed

Goal #5: Submit local consent context for all sites to the central IRB – Approval pending

The central IRB at Vanderbilt University Medical Center (PI – Todd Rice, MD) is the central IRB, and all of the six sites (UCSF, ZSGH, OHSU, U. Texas, U. Washington, and Vanderbilt) have agreed to this plan and obtained the reliance approval from their institutional IRBs. The central IRB at Vanderbilt has experience managing multi-center NIH trials, including the current NHLBI Prevention and Treatment of Acute Lung Injury Trials Network (about 50 hospitals). UCSF IRB is responsible for the regulatory issues for two recruitment sites: UCSF Moffitt-Long Hospital and Zuckerberg San Francisco General Hospital and Trauma Center (San Francisco, CA).

During the past reporting period, we have conducted the following regulatory activities with the central IRB at Vanderbilt University:

1. **IRB Amendment 1:** **Submission date:** 12/13/2018 **Approval date:** 12/21/2018
Description: We added five enrollment sites in this multi-center Phase 2b trial: 1. Zuckerberg San Francisco General Hospital & Trauma Center (San Francisco, CA); 2. UCSF Moffitt-Long Hospital (San Francisco, CA); 3. Harborview Medical Center (Seattle, WA); 4. Oregon Health & Science University (Portland, OR); 5. The University of Texas Health Sciences Center at Houston/Memorial Hermann-Texas Medical Center (Houston, TX). All these sites have obtained the reliance approval from their local IRBs. The sixth recruitment site - Vanderbilt University Medical Center (Nashville, TN) will submit their IRB application separately. The application form was revised to include these sites. This was done after all reliance agreements were completed and approved in December 2018.
2. **IRB Amendment 2:** **Submission date:** 01/24/2019 **Approval date:** 03/21/2019
Description: We submitted the revised protocol (dated 01/22/2019) and Investigator Brochure (01/22/2019) to the central IRB at Vanderbilt. During the pre-review process by the central IRB at Vanderbilt University in February and March of 2019, we submitted an updated Clinical Protocol ((Protocol No: UCSF-hMSC-ARDS-P1P2-09, dated 02/21/2019) and revised master consent (dated 02/21/2019) regarding the revision of sample collection scheme, and the new site-specific consent for UCSF-Moffitt hospital (dated 02/21/2019). All regulatory documents, which include the updated Clinical Protocol, Investigator Brochure, main master consent for all six sites, and the local consent context for UCSF Medical Center, were approved by central IRB at Vanderbilt University on March 21, 2019.
3. **IRB Renewal:** **Submission date:** 03/28/2019 **Approval date:** 04/11/2019
Description: After receiving IRB amendment 2 approval, we immediately submitted a study renewal without any changes on March 28, 2019. The renewal application was approved by the central IRB at Vanderbilt on April 11, 2019. The study will be active until April 10, 2020.
4. **IRB Amendment 3:** **Submission date:** 06/20/2019 **Approval date:** 07/26/2019
Description: We have submitted the updated Clinical Protocol (Protocol No: UCSF-hMSC-ARDS-P1P2-10, dated 05/14/2019), revised Investigational Brochure (dated 05/14/2019), revised main consent (dated 06/17/2019) with very minor language changes, as well the Statistical Plan. On July 25, 2019, we received one stipulation from the central IRB, requesting that we delete the following sentence in the master consent, "These cells have been used in the treatment of patients with ARDS and showed no safety issues in clinical trials." We have revised the master consent form per the central IRB's request, and this amendment was approved on July 26, 2019.

5. **Site Addition:** **Submission:** 07/19/2019 **Approval date:** 07/19/2019
Description: The central IRB at Vanderbilt requested that we add the five non-Vanderbilt sites in their DISCOVER-e system (electronic system for IRB submission) to reflect current performance site activity for the single IRB. Please note that the central IRB at Vanderbilt University has approved these single-IRB performance sites in the submission of IRB Amendment 1 (listed above), and this is an administrative review only as no revisions were made to the study relative to approved study documents or study design.

6. **IRB Amendment 4:** **Submission date:** 08/20/2019 **Approval date:** Pending

Description: We have submitted the revised local consent document for UCSF site for two minor changes: (1) Fixing a telephone typo; (2) Adding the Bills of Rights, which is required for the California site. We have also submitted the new local consent for four sites: 1. Zuckerberg San Francisco General Hospital & Trauma Center; 2. Harborview Medical Center; 3. Oregon Health & Science University (Portland, OR); 4. The University of Texas Health Sciences Center at Houston/Memorial Hermann-Texas Medical Center. The Vanderbilt team, per central IRB's guideline, has submitted their local consent context separately.

Goal #6: Respond to Nancy Englar's detailed requests from September 11, 2018 regarding regulatory issues – Completed

Goal #7: Upon the approval by central IRB, submit the revised final protocol to the DoD HRPO for final approval - Completed

Goal #8: Submit the final HRPO DoD approved protocol to the central IRB – Not applicable

During the past reporting period, we have had the following communications with the DoD:

1. On December 20, 2018, we responded to Nancy Englar's detailed requests in the emails dated 06/25/2018 and 09/11/2018 regarding regulatory issues. All the revision requests by the DoD HRPO on the Clinical Protocol and Consent Form have been addressed in the later version of the Clinical Protocol and the updated Consent Form.
2. On January 31, 2019, we emailed Nancy Englar with the following study documents:
 - 1) Vanderbilt cIRB approval letters for initial submission (dated 04/30/2018) and the amendments 1 & 2.
 - 2) CITI training certificate for investigators and co-investigators
 - 3) Conflict of interest statements for investigators
 - 4) Initial approval notice for IND 15331 (email dated 01/18/2013) and the email communications for IND amendment for this Phase 2b trial.
 - 5) Form 1572 for all sites
 - 6) The DSMB Charter
 - 7) Clinical Protocol (dated 01/17/ 2019)
 - 8) Investigator Brochure (dated 01/22/2019)
 - 9) Master Consent Form (dated 01/22/2019)
 - 10) Registration of Clinical Trial, the identifier is NCT03818854
3. On February 13, 2019, we emailed Nancy Englar the reliance agreements for five non-Vanderbilt sites. Vanderbilt does not require a Reliance Agreement because the central IRB is their own IRB.
4. On August 16, 2019, after we obtained the approval from the central IRB at Vanderbilt University (dated 07/26/2019) and from the FDA (dated 08/15/2019) for the revised Clinical

Protocol and related study documents, we submitted the following documents to HRPO of the Department of Defense (Nancy Englar) for review:

- 1) Updated Clinical Protocol: Protocol No: UCSF-hMSC-ARDS-P1P2-10, dated 05/14/2019
- 2) IRB Application Forms with their IRB approval letters
 - a. Initial submission
 - b. Amendment 1: adding 5 non-Vanderbilt enrollment sites after obtaining the reliance approvals from their local IRBs
 - c. Amendment 2: submitting updated Clinical Protocol, Investigator's Brochure, revised master consent form, local context consent section at UCSF site
 - d. Study annual renewal
 - e. Site additions for single IRB review requested by central IRB at Vanderbilt
 - f. Amendment 3: submitting updated Clinical Protocol, Investigator's Brochure, revised master consent form
- 3) Approved Informed Consent Document(s) and HIPAA Authorization Forms:
 - (a) Master consent for all six recruitment sites: Version 1.3, dated 7/25/2019
 - (b) Local context consent with HIPAA Authorization Form at UCSF site: dated 2/21/2019
- 4) Required documents for Medical Monitor – Taylor Thompson, MD
 - (a) a. Curriculum vitae
 - (b) b. Human subjects training certificate
- 5) Documentation of human subjects training for the Principal Investigator, Co-Investigator, Associate Investigator(s)
- 6) Study instruments - Telephone interview script at day 60
- 7) Conflict of Interest forms
- 8) Additional committee and regulatory committee approvals: institutional biosafety committee approval for Dr. Matthay's repository lab
- 9) FDA approval by email regarding the IND amendment #0018 submission
- 10) Updated Investigational Brochure: Version 10, dated 5/14/2019
- 11) Form FDA 1572
- 12) Case Report Forms (dated 7/3/2019)
- 13) Statistical Analysis Plan (dated 5/14/2019)
- 14) Updated DSMB Charter (dated 7/26/2019)
- 15) Delegation of Duty Log for all research sites

On September 5, 2019, Nancy Englar responded and agreed that we had sufficient supporting evidence for a potential benefit for all participants. On September 12, we received the initial HRPO approval for the updated protocol of this Phase 2b trial.

Because the DoD HRPO approved the central IRB approved Clinical Protocol (dated 05/14/2019) without any change, there was no need to resubmit this protocol to the central IRB at Vanderbilt University. Once we obtain the central IRB's approval for site-specific consent context forms for all other performance sites, we will submit these documents to the DoD HRPO.

We will also submit the DoD approval letter to the central IRB, compliant with the reporting central IRB's guideline at Vanderbilt University. Currently, we will not be able to submit this approval letter because the IRB amendment #4 is still under review.

Goal #9: Complete the laboratory and study manual of operations – Completed

We have created a laboratory manual of standard operating procedures (SOPs), as led by Lizette Caballero at UCSF in the bone marrow transplant laboratory. The cell and bone marrow facilities

for processing of the cryopreserved MSCs (or placebo) at each participating clinical site will be responsible to develop site-specific SOPs and submit to the Clinical Coordinating Center (CCC) at UCSF to review and approve.

Goal #10: Finalize and beta test the case report forms with the data management system, including systems for bar coding and storing biological samples – In progress (90% completion)

The case report form has been finalized to reflect the new changes in the protocol.

The electronic data base system was developed by Quesgen. We have completed several rounds of database revisions to reflect the Clinical Protocol updates. The CCC at UCSF has completed two rounds of beta-testing. New data management features for data validation and query have been recently built in the system per our Data Manager's request. The development of these brand new data management features is time-consuming, and we are working very closely with QuesGen to optimize these features. Please note that the development of these new database features will not affect the data entry by the study coordinators. We estimate that the data management system will be finalized in the 9th quarter (October 1-December 31, 2019).

We have also set up the tracking system using de-identified barcodes to track and store biological samples.

Goal #11: Finalize production plans for MSC for the trial, with timeline for delivery of the first batch of MSCs and run the necessary testing at local sites prior to the initiation of the trial – Production plan completed; MSC delivery and MSC testing at local sites are pending

As we reported earlier, the prior bone marrow source supporting Phase 1 and Phase 2a trials (Lonza) stopped supplying healthy bone marrows. Therefore, we have been working to qualify a new bone marrow source for the production of the allogeneic human mesenchymal stromal cells at the University of Minnesota NIH/NHLBI production center.

During the past reporting period, we have performed the following activities in order to achieve this goal:

1. On November 27-28, 2018, Dr. Matthay made an in person visit to the University of Minnesota MSC production site (Dave McKenna, MD, PI) to review plans for MSC production for the trial revised to include a new source of MSCs and to maximize MSC viability for the trial, based on the findings in our published Phase 2a trial.
2. In November 2018, the University of Minnesota MSC production site completed the MSC production plan with the new source AssureImmune and the study Chemistry, Manufacturing and Controls, was revised effective on November 9, 2018.
3. On November 30, 2018, we had a phone discussion and email communications with FDA in CBER regarding the new source, AssureImmune, of human bone marrow for production of the MSCs for the trial and strategies for enhancing MSC viability for the trial.
4. In early December 2018, the University of Minnesota MSC production site disqualified AssureImmune due to concerns regarding eligibility of the donors. Another new source of

bone marrow was identified (AllCells) and qualified by the University of Minnesota (Dave McKenna, MD, PI).

5. We established measures of MSC cell dose evaluation, quality and potency, conducted by the Pati Lab. To establish our evaluation assays, we used MSC donors from the Phase 2A START trial. We have standard operating procedures in place for measurement of cell viability post-thaw, plating efficiency, recovery post thaw, growth curves, metabolic assays, and markers of potency including functional assays such as the pulmonary endothelial cell assays of permeability and measures of MSC protein secretion tested by Luminex and ELISA. These tests will be used on the doses produced by the University of Minnesota. These are not intended to be release criteria but be used at the end of trial to help interpret the results of the clinical trial.
6. The updated CMC (CCMC-0019, Rev:E; effective date 05/06/2019) has been finalized, including the new source of bone marrow (AllCells) and the correct MSC preparation procedures for maximizing viability with the cryoprotectants and related details. It also includes the additional monitoring that the FDA requested on the teleconference on November 30, 2018. We have updated the Clinical Protocol and related documents to reflect the CMC updates. We have also submitted the updated CMC to the FDA under the amendment submission under IND 15331, and provided CBER at FDA the requested details of CLIA certification of the lab that does infectious disease testing and the plans for monitoring MSC viability and potency during the trial.
7. The University of Minnesota MSC production site is now completing the production of the clinical-grade MSCs. Currently, there are approximately 2.3 billion MSCs that have been expanded from our donor 3030192 which are still in quarantine for final quality control assessments. It is anticipated that these MSCs will be ready for shipment to UCSF the week of November 18, 2019. This would make it possible for us to start screening and enrolling at UCSF Parnassus Medical Center.

The goal of MSC delivery at local sites has not yet been met due to two reasons: 1. The process of disqualifying AssureImmune and qualifying AllCells as a new source of bone marrow for the production of MSCs delayed our timeline. 2. Based on our published Phase 2a trial, Dr. McKenna (PI at the University of Minnesota) has worked with his group to modify the production process to maximize cell viability.

Goal #12: Discuss trial-related issues and training of coordinators and investigators with site visits and a monthly conference call – In progress

Dr. Matthay and the CCC at UCSF have communicated on a regular basis with the research team at each site to report the study preparation progress and discuss the trial-related issues (e.g. protocol clarifications, regulatory documents preparation). We will plan the training of coordinators and investigators in the eighth quarter with a conference call.

Dr. Matthay made in person site visits to two of the participating sites (The University of Texas site in Houston on April 3, 2019 and Oregon Health Science center on April 9, 2019) to review the protocol and trial implementation details with the investigators; the coordinators, and the Cell therapy personnel. Both visits were productive and informative.

At this stage, we are focusing on the preparation of initiating the trial. We think that email communication and personal site visit will be most efficient and productive. We plan to schedule a monthly conference call for all sites prior to study initiation in the 9th quarter.

Goal #13: Webinar educational sessions to introduce the data management system to investigators, BMT staff (randomization) and study coordinators – Pending

We have not set up the webinar educational sessions and plan to schedule both training and practice in November 2019, when we approach the initiation of the Phase 2b trial at all sites.

We have not set up the webinar educational sessions for two reasons: (1) We are still working on data query feature in the Data Capture System; (2) We plan to start study enrollment at the UCSF site only, then expand to the other sites. We plan to schedule the webinar education session for all sites after first patient has been enrolled at UCSF, and prior to expanding to other sites. In this case, with the real-time data entry experience by the study coordinators at UCSF, we will have a good opportunity to optimize the system and develop related FAQ for training purpose.

Goal #14: Run mock screening and recruitment to be familiar with the recruitment & monitoring work flow – Completed at UCSF

We have completed the mock screening and recruitment for UCSF teams (including UCSF Moffitt-Long Hospital and Zuckerberg San Francisco General Hospital & Trauma Center). The same training will be conducted at other four sites prior to the initiation of study enrollment at their sites.

Goal #15: Set up electronic consent for all sites through Vanderbilt RedCap – In progress

RedCap is the only system approved for E-consenting by central IRB at Vanderbilt University. The data manager of the Clinical Coordinating Center at UCSF had a teleconference with the RedCap support team at Vanderbilt University on June 19, 2018, in order to discuss the best strategies to build user-friendly E-consent feature in Vanderbilt RedCap. The Vanderbilt RedCap team also provided the instruction of creating Vanderbilt RedCap accounts for all participating sites.

The local consent contexts for five sites (except UCSF) are currently under the review by the central IRB at Vanderbilt University. Once we obtain the approvals for consent documents for all participating sites, we will upload the approved consent forms into Vanderbilt RedCap and set up the access for electronic consenting.

Goal #16: Send the final approved protocol to the DSMB - Completed

A DSMB teleconference was held on October 11, 2019 to review the DSMB charter, the protocol, and the statistical analysis plan. The DSMB has approved the initiation of the trial (see Appendix).

Goal #17: Potentially begin enrollment of patients at one site – UCSF

We are in the final preparation stage for initiating the Phase 2b trial. Due to the delay of identifying new source of bone marrow, the University of Minnesota is currently still working on the production of clinical grade level of MSCs and post-production QA tests. We are waiting for

the first batch of MSC being delivered to UCSF in November 2019 which would allow us to begin enrollment at UCSF Parnassus Medical Center in late November or December 2019.

- **What opportunities for training and professional development has the project provided?** Nothing to report.
- **How were the results disseminated to communities of interest?** Nothing to report.
- **What do you plan to do during the next reporting period to accomplish the goals?**
 1. Obtain the central IRB approvals for local consent context for all sites
 2. Submit the local consent context to the DoD HRPO to obtain the approval from the DoD
 3. First batch of MSC product be delivered to UCSF and complete the local testing by the BMT lab
 4. Each site to finalize their site-specific MSC preparation and MSC infusion protocol, based on the approved MSC preparation and infusion protocol which are included in the current Clinical Protocol
 5. Set up a plan for monthly teleconference with the study investigators and study coordinators at all participating sites
 6. Create Data Capture System accounts for all sites, including BMT staff
 7. Webinar educational sessions to introduce the data management system to investigators, BMT staff (randomization) and study coordinators
 8. Run mock screening and recruitment to be familiar with the recruitment & monitoring work flow
 9. Set up electronic consent for all sites through Vanderbilt RedCap
 10. Potentially begin enrollment of patients at one site – UCSF

4. IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?** Nothing to report.
- **What was the impact on other disciplines?** Nothing to report.
- **What was the impact on technology transfer?** Nothing to report.
- **What was the impact on society beyond science and technology?** Nothing to report.

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**

The study design of this Phase 2b trial includes the following major changes:

1. **Change:** We will continue to recruit patients with moderate-to-severe ARDS, which is the same as our completed Phase 1 and Phase 2a trials (funded by the NIH). However, we have changed the inclusion criteria so we will continue to include all causes of ARDS. In terms of broadening the inclusion criteria for ARDS.

Reason: Subsequently emerging evidence suggests that the incidence of ARDS following trauma has declined, probably related to changes in resuscitation practices with the reduced use of crystalloid fluids and balanced transfusion of blood products. In order to study patients with ARDS after trauma as well as to meet enrollment goals over the defined study period, after the discussion with the Department of Defense, the inclusion criteria for this phase 2b trial have been broadened to include all causes of ARD for testing MSCs for both trauma and medical causes of ARDS.

2. **Change:** The updated protocol involves a new source (AllCells, LLC) of human bone marrow for producing the allogeneic mesenchymal stromal cells for our phase 2b trial.

Reason: The previous bone marrow producer (Lonza) stopped providing bone marrow.

3. **Change:** We have also slightly modified the hMSCs preparation and processing at the University of Minnesota and at local recruitment sites in order to optimize the cell viability.

Reason: The *Post hoc* analysis of our previous Phase 2a trial revealed that the viability of the MSCs by trypan blue exclusion prior to administration varied. Why did MSC viability vary in this trial? There are two explanations. First, a new study from the University of Minnesota MSC production center shows a 14% reduction in eventual MSC viability by flow cytometry when the MSCs are produced from unfractionated bone marrow rather than fresh bone marrow. Second, our procedure in the trial to remove the DMSO from the thawed MSCs resulted in a further reduction in MSC viability. Therefore, in this Phase 2b trial, our plan will be to adjust the number of MSCs supplied by the University of Minnesota to clinical sites to 100% viable MSCs. We will also eliminate the washing step to remove the DMSO.

4. **Change:** We have added a new recruitment facility – University of California San Francisco Medical Center.

Reason: This performance site has been added due to the inclusion criteria broadening to include the ARDS patients from trauma and non-trauma causes.

5. **Change:** We have revised the specimen collection scheme in the protocol: reduced the volumes of blood collection at each timepoint; reduced urine collection from 6 times to 3 times; added blood collection for DNA/RNA on day 2.

Reason: This will provide valuable biological information on the effects of MSC versus placebo treatment.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

As explained above, the primary reason for the delay in starting the trial was the need to find a new source of human bone marrow since the prior vendor (Lonza) unexpectedly announced in May 2018 that they would no longer supply human bone marrow to private or public entities. The University of Minnesota MSC production site then chose AssureImmune to be the new source of bone marrow but later disqualified them because the donor failed the screening eligibility. This caused the further delay of MSC production.

Currently, the University of Minnesota MSC production site has received the human bone marrow from another source (AllCells), and is now finalizing on the production of clinical-grade MSC. It would typically be 3 weeks of culture and then historically about 6 weeks to get all testing back after company QA sign-off. We expect the first batch of

MSC will be delivered to UCSF in November 2019.

- **Changes that had a significant impact on expenditures**

Expenditures during the second year have been minimal at each of the four subaward sites since the clinical trial has not started.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

As mentioned above, we have broadened the inclusion criteria to include ARDS patients for all causes, medical or trauma. This revision has been discussed and approved by the DoD. We have revised the Clinical Protocol to reflect this change. The updated protocol has been approved by the central IRB at Vanderbilt, the FDA under IND 15331, and by the DoD HRPO.

There is nothing to report for human subjects, vertebrate animals, biohazards, or select agents.

6. PRODUCTS:

- **Publications, conference papers, and presentations:** Nothing to report.
- **Website(s) or other Internet site(s):** Nothing to report.
- **Technologies or techniques:** Nothing to report.
- **Inventions, patent applications, and/or licenses:** Nothing to report.
- **Other Products:** Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

- **What individuals have worked on the project?**

UCSF - UCSF Moffitt-Long Hospital and Zuckerberg San Francisco General Hospital & Trauma Center (San Francisco, CA):

Michael A. Matthay MD

Project Role: Principal Investigator

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 4.80

Contribution to Project: Revised the FDA approved Clinical Protocol and Investigator Brochure for submission to the Vanderbilt cIRB; communicated with all of the sites (4 sites in addition to UCSF) by conference calls and by emails and phone calls; supervised the preparation of the case report form and submitted to our data management firm (Quesgen); working on the plans for a central IRB at Vanderbilt with Hanjing Zhuo and Kathleen Liu; worked with UCSF Research Management Services (Sara Yturralde) on the budget for UCSF and the other sites; communicated with FDA by email and by conference call (November 30, 2018 – Tal Salz, CBER); communicated and visited in person with the Dave McKenna, MD at the University of Minnesota (November 21-22, 2018) regarding details for production of the mesenchymal stromal cells for the trial; worked on selecting the DSMB for the trial.

Kathleen D. Liu, MD, PhD, MAS

Project Role: Co-Investigator

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 0.60

Contribution to Project: Worked with Dr. Matthay to write and edit the Clinical Protocol;

advised Dr. Matthay on the plans for a central IRB at Vanderbilt and spoke directly with the Head of that cIRB, Dr. Todd Rice; helped plan completion of Statement of Work Tasks and the case report form; helped to oversee Hanjing Zhou, the project manager. Dr. Liu also worked with Lizette Caballero on finalizing the laboratory SOPs for preparing the MSC and placebo infusion products.

Carolyn Calfee, MD MAS

Project Role: Co-Investigator

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 0.30

Contribution to Project: Worked with Dr. Matthay on editing the clinical protocol, the screening form, and Case Report Form.

Carolyn Hendrickson, MD, MAS

Project Role: Co-Investigator

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 1.00

Contribution to Project: Worked with Dr. Matthay to prepare the Clinical Protocol and the case report form and the screening form and to initiate mock screening.

Rachael Calcutt, MD, MAS

Project Role: Co-Investigator

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 0.60

Contribution to Project: Worked with Dr. Matthay to write and edit the Clinical Protocol and to edit the case report form for the cIRB and for mock screening.

Jeffrey Gotts, MD, PhD

Project Role: Co-Investigator

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 0.58

Contribution to Project: Worked with Dr. Matthay to write and edit the Clinical Protocol and to edit the case report form for the cIRB.

Hanjing Zhuo, MD, MPH

Project Role: Project Manager

Research Identifier 0000-0003-3039-8155:

Nearest person month worked: 0.0

Contribution to Project: Worked with Dr. Matthay to write and edit the Clinical Protocol and the Investigator Brochure, and to edit the case report form and to set up the cIRB with Vanderbilt for this trial.

Serena Ke, BS

Project Role: Coordinator

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 1.71

Contribution to Project: Worked on preparing the case report form and screening form for the trial and also the laboratory and study manuals for the trial.

Brian Daniel

Project Role: Coordinator

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 0.19

Contribution to Project: Worked on the clinical protocol and case report form development.

Kevin Delucchi, BS, PhD

Project Role: Statistician

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 0.87

Contribution to Project: Prepared the statistical plan for the clinical protocol with attention to the FDA-requested expansion of the statistical plan with more details.

Shibani Pati, MD, PhD

Project Role: Co-Investigator

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 1.08

Contribution to Project: Working on standardizing laboratory assays for the MSCs for this trial including plans to test the viability and other features of the bone marrow derived MSCs from the University of Minnesota.

Jason Abbott, BS

Project Role: Laboratory Manager

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 0.00

Contribution to Project: Organizing specimen tubes and bar coding for this trial and working with Dr. Pati on the MSC laboratory assays

Xiaohui Fang

Project Role: Laboratory analysis of MSCs for viability and functional characteristics

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 0.10

Contribution to Project: Testing properties of MSCs with in vitro assays and potency assays

Stuart Gibb, PhD

Project Role: Research assistant

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 0.00

Contribution to Project: In the past he worked with Dr. Pati on standardizing laboratory assays for the MSCs for this trial but he has left UCSF for another position

Alpa Mahuvakar

Project Role: Research assistant

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 3.12

Contribution to Project: Worked with Dr. Pati on laboratory assays for the MSCs for this trial.

Erin E Ross

Project Role: Coordinator

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 1.00

Contribution to Project: Prepare and test the screening forms and practicing obtaining consents with Dr. Matthay.

Kathryn Mary Vessel

Project Role: Coordinator

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 1.00

Contribution to Project: Prepare and test the screening forms and practicing obtaining

consents with Dr. Matthay.

Maribeth Ruperto

Project Role: Post award grant manager

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 0.65

Contribution to Project: Worked with Dr. Matthay for organizing the personnel contributions to this grant and preparing the quarterly report.

Margaret Luk

Project Role: Post award grant manager

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 0.40

Contribution to Project: Worked with Dr. Matthay for organizing the personnel contributions to this grant and preparing the quarterly report.

University of Harborview Medical Center (Seattle, WA)

Bryce Robinson, MD

Project Role: Site Principal Investigator

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 0.60

Contribution to Project: communicated with overall PI, Michael A. Matthay, MD and Clinical Coordinating Center at UCSF by conference calls and by emails and phone calls; supervised the reliance to a central IRB at Vanderbilt, and the preparation of site-specific regulatory documents for submission to the central IRB at Vanderbilt University, the FDA and the Sponsor, the Department of Defense.

Oregon Health & Science University (Portland, OR)

Martin Schreiber, MD

Project Role: Site Principal Investigator

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 0.60

Contribution to Project: communicated with overall PI, Michael A. Matthay, MD and Clinical Coordinating Center at UCSF by conference calls and by emails and phone calls; supervised the reliance to a central IRB at Vanderbilt, and the preparation of site-specific regulatory documents for submission to the central IRB at Vanderbilt University, the FDA and the Sponsor, the Department of Defense.

University of Texas Health Sciences Center at Houston/Memorial Hermann (Houston, TX)

Laura Moore, MD

Project Role: Site Principal Investigator

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 0.60

Contribution to Project: communicated with overall PI, Michael A. Matthay, MD and Clinical Coordinating Center at UCSF by conference calls and by emails and phone calls; supervised the reliance to a central IRB at Vanderbilt, and the preparation of site-specific regulatory documents for submission to the central IRB at Vanderbilt University, the FDA and the Sponsor, the Department of Defense.

Vanderbilt University Medical Center (Nashville, TN)

Lorraine Ware, MD

Project Role: Site Principal Investigator

Research Identifier: 0000-0003-3039-8155

Nearest person month worked: 0.60

Contribution to Project: communicated with overall PI, Michael A. Matthay, MD and Clinical Coordinating Center at UCSF by conference calls and by emails and phone calls; supervised the reliance to a central IRB at Vanderbilt, and the preparation of site-specific regulatory documents for submission to the central IRB at Vanderbilt University, the FDA and the Sponsor, the Department of Defense.

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?** – The PI is Dr. Matthay and his updated Other Support is included in this report.
- **What other organizations were involved as partners?** Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS:

- **COLLABORATIVE AWARDS:** Not applicable.
- **QUAD CHARTS:** Not applicable.

9. APPENDICES:

1. **DoD (STAT) trial DSMB meeting memo**
2. **Active other support of the PI: Michael A. Matthay, MD**
3. **Journal article**

Matthay MA, Calfee CS, Zhuo H, Thompson BT, Wilson JG, Levitt JE, et al. Treatment with allogeneic mesenchymal stromal cells for moderate to severe acute respiratory distress syndrome (START study): a randomised phase 2a safety trial. *Lancet Respir Med.* 2019;7(2):154-62



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Boyd Taylor Thompson M.D.
*Medical Director, PETAL CCC
Director, Translational Research
Division of Pulmonary and Critical Care Medicine,
Massachusetts General Hospital
Professor of Medicine, Harvard Medical School*

DoD (STAT) Trial DSMB Meeting
October 9, 2019 10 AM – 11 AM PST/1 PM – 2 PM EDT

Attendees:

DSMB:

B. Taylor Thompson, MD, DSMB Chair
Jason Sperry, MD, MPH
Michael Harhay, PhD

Michael A. Matthay, MD, Principal Investigator
Kathleen D. Liu, MD, PhD, Co-Investigator
Hanjing Zhuo, MD, MPH, Project Manager
Kevin Delucchi, PhD, Study Biostatistician
Kimberly Yee, BS, Clinical Research Coordinator

New Conflicts of Interest: None reported.

Review of study progress in the past year by Drs. Matthay and Liu:

Delay in starting the trial: This trial has been delayed due to issues regarding the bone marrow source used in producing allogeneic mesenchymal stromal cells (MSCs). The previous bone marrow source (Lonza) stopped supplying bone marrow, so a new bone marrow source (AllCells) is being used. The production of the first round of clinical grade MSCs is expected to be completed by November 2019.

Dr. Matthay noted that we may not have been able to start this trial before this Fall of 2019 anyway due to the time that was required to obtain regulatory approval.

Approvals: This trial has been approved by the Department of Defense and the cIRB.

Change to inclusion criteria: Although the original grant was written for ARDS after trauma, the inclusion criteria has been broadened to include all causes of ARDS. The Department of Defense approved this change.

Trial design: This is a 120 patient, phase 2b, placebo-controlled, double-blind trial. The primary endpoint is oxygenation index over 36 hours. Data will be collected on non-pulmonary organ failure, ventilator-free days, mortality, and other variables.

Reference to START phase 2a trial: The phase 2a START trial was a safety trial that enrolled 60 patients. 40 patients were in the MSCs arm and 20 patients were in the placebo arm. The FDA requested a 2:1 randomization to determine any safety issues with MSCs, but there were none (*Lancet Resp Med*, 2018). In that trial, the viability of MSCs was much lower than anticipated in over half of the patients for two reasons. First, the unfractionated bone marrow was cryopreserved after receiving it in Minnesota. It was later discovered by the Minnesota group that MSC viability improved if the bone marrow was advanced to P1 before cryopreservation. Second, the process of washing the MSCs and reconstituting them reduced viability.

The higher viability MSCs in that phase 2a trial did have a statistically significant effect on reducing plasma ANG-2 and a trend for improving oxygenation index (*Lancet Resp Med*, 2018).

MSC Viability: In this trial, we anticipate having high viability MSCs. We are currently working with Lizette Caballero, who is the head of the BMT lab at UCSF, on testing MSC viability at UCSF and UT Health. An SOP on viability testing will be sent out to other sites. In this new trial, when production of MSCs is completed in Minnesota, MSC viability will be measured and more MSC cells will be added to increase the number of viable cells in each bag. For example, if the viability of MSC cells is 80% in a bag with 100 million cells, 20 million viable cells will be added to increase the number of viable cells to approximately 100 million cells. Preliminary data has shown that this plan is working well. During the trial, the viability of cells will be monitored closely through prespecified criteria for the BMT labs.

The DSMB emphasized the importance of ensuring that the highest proportion of MSCs are viable.

Timeline: The goal is to start the trial by November 2019 when the first round of MSCs are produced and to enroll the first patient at UCSF or Zuckerberg San Francisco General Hospital by November or December 2019. When the next round of MSCs is produced around January 2020, the remaining sites will begin enrolling patients.

Logistical Issues: For Zuckerberg San Francisco General Hospital and Harborview Medical Center, the bone marrow production sites are not located at the same hospital, so the MSCs will need to be delivered from the BMT labs to these sites. To address this issue, additional funding will be used to keep the BMT lab open on Saturday's from 8 AM – 4 PM and until 6 PM on the weekdays. This will increase the number of days possible to enroll patients.

Two stratifications for the trial: This trial will stratify for $\text{PaO}_2/\text{FiO}_2 < 150$ mmHg and trauma versus non-trauma. The DSMB suggests that these two variables should be

adjusted for in the primary analysis, which is confirmed to be already included in the Statistical Analysis Plan for the adjusted mixed linear model for the primary analysis, but not the unadjusted model. Dr. Harhay recommends that if these two variables are true stratification factors, they need to be in the primary analysis because the unadjusted analysis may be biased if they are not included. Therefore, the unadjusted analysis will have those 2 variables included in the unadjusted analyses.

Serious Adverse Event Reporting by Dr. Thompson:

Serious, unexpected, and study-related adverse events: According to the protocol, all unexpected and study-related serious adverse events will be reported in an expeditious manner to the cIRB and DSMB for review. Dr. Thompson agrees that this is reasonable.

Serious and study-related adverse events: The charter states that serious and study-related adverse events will be reported to the DSMB on an ongoing basis. This includes the list of pre-specified infusion associated events in the protocol (section 6.2.2). Dr. Thompson recommends reporting all serious and study-related adverse events to him on a monthly basis for review. If further examination is needed, Dr. Thompson will contact Dr. Harhay and Dr. Sperry to schedule a call or conduct an email review. If not, the DSMB will review these adverse events by treatment (treatment A versus B) every 6 months. This plan was approved by the DSMB.

Potential number of early deaths in the trial: Dr. Harhay brought up the potential impact of a large number of early patient deaths on the primary data analysis. However, in the phase 2a START trial, there were only three patient deaths in the first 48 hours. Additionally, with the inclusion and exclusion criteria and the time it takes to enroll a patient, this study is designed to select patients who are expected to survive long enough to measure a treatment effect.

Next DSMB Meeting: The DSMB decided that the next DSMB meeting will take place 6 months after the first patient has been enrolled.

Respectfully Submitted,

A handwritten signature in black ink, appearing to read 'B. Taylor Thompson'.

B. Taylor Thompson MD
Chair, DoD (STAT) Trial DSMB

These minutes were approved by the DSMB on October 15, 2019.

SUPPORT
MATTHAY, MICHAEL A.

Current

Title: Prevention and Early Treatment of Acute Lung Injury

Time Commitments: 0.45 Calendar

Supporting Agency: NIH/NHLBI, U01 HL123004

Address:

NHLBI Health Information Center

P.O. Box 30105

Bethesda, MD 20824-0105

Contracting/Grants Officer: Gayle Jones

Performance period: 6/17/2014-04/30/2021

Level of funding: \$289,070

Project Goals: Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal

To test new treatments for acute lung injury in patients enrolled in the Emergency Department and in the Intensive Care Unit.

Specific Aims: The specific aim is to test new therapeutic approaches to testing the preventative or early treatment value of novel treatments in patients admitted to the Emergency Department at risk for ARDS or new treatments for ARDS in patients in the intensive care unit in primarily phase 3 designs.

Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal

Title: Mechanistic roles of Cytochrome P4501A enzymes in hyperoxic lung injury

Time Commitments: 0.45 Calendar

Supporting Agency: NIH/NHLBI R01HL129794/ Baylor College of Medicine

Address:

Baylor College of Medicine

One Baylor Plaza, BCM310

Houston, TX 77030-3411

Contracting/Grants Officer: Leanne B. Scott, Ph.D

Performance Period: 04/01/2016-03/31/20

Level of funding: \$12,080

Project Goals: Mechanistic roles of cytochrome P4501A enzymes in hyperoxic lung injury

These analyses will specifically relate to the mouse studies with the metabolomics data and the planned proteomic studies.

Specific Aims: To study biomarkers as reliable indices of acute lung injury.

Overlap: None

Title: Mesenchymal Stem Cells for Treatment of ARDS Following Trauma

Time Commitments: 4.80 Calendar

Supporting Agency: Department of Defense W81XWH-17-1-0631

Address:

US Army Medical Research Acquisition Activity

820 Chandler ST

Fort Detrick MD 21702-5014

Contracting/Grants Officer: Kevin R. Moore

Performance Period: 9/15/2017-9/14/2021

Level of funding: \$2,118,476

Project Goals: The overall objective of this proposal is to carry out a randomized, double-blind, placebo-controlled multicenter phase 2b trial to test the therapeutic potential of allogenic bone-marrow derived MSC for treating ARDS in trauma patients.

Specific Aims: *Specific Aim 1.* To test the clinical efficacy of intravenously delivered allogeneic human MSC in trauma patients with ARDS. *Specific Aim 2.* To test the mechanisms by which MSC reduce acute lung injury in trauma patients with ARDS

Overlap: None

Title: Novel Paracrine Mechanism for Cell-Based Therapy of Injured Lungs

Time Commitments: 0.45 Calendar

Supporting Agency: University of Texas Health Science Center at Tyler/NIH R01 HL134828

Address:

University of Texas Health Science Center at Tyler

11937 U.S. Highway 271

Tyler, TX 75708-3154

Contracting/Grants Officer: Dena Walton

Performance Period: 9/1/2017-8/31/2021

Level of funding: \$70,493

Project Goals: The results of these experiments will provide novel insights into how mesenchymal stem (stromal) cells enhance the resolution of alveolar edema in human lungs harvested from brain dead donors, an important scientific and clinical question.

Overlap: None

Title: University of California, San Francisco (UCSF) CIRM Alpha Stem Cell Clinic

Time Commitments: 1.80 calendar

Supporting Agency: CIRM/CHORI

Address:

UCSF Benioff Children Hospital Oakland

5700 Martin Luther King Jr. Way

Oakland, CA 94609

Contracting/Grants Officer: Michael Worden

Performance Period: 10/01/2017-09/30/2021

Level of funding: \$1,072,396

Project Goals To address these gaps and expand clinical trial activity in cell therapies, the specific aims for an Alpha Stem Cell Clinic at UCSF at the above locations will be designed to accelerate the tempo of pre-award planning, clinical trial activation, patient accrual and trial completion, expand access to these therapies by under-represented populations with disorders in the Alpha Stem Cell Network, and to establish a disease team approach that promotes participation in the CIRM Alpha Stem Cell Network trials

Overlap: None

Title: Picetannol entrapped albumin nanoparticles (PANPs) to combat ALI/ARDS

Time Commitments: 0.30 Calendar

Supporting Agency: Nano Biotherapeutics / NIH

Address:

Cell Biologics, Inc

2201 W Campbell Park Dr

Chicago, IL 60612

Contracting/Grants Officer: Jeanne Chang

Performance Period: 09/15/2017-06/31/2020

Level of funding: \$100,000

Project Goals: This project is being done at UCSF in order to carry out the goals of specific aim 3, which is to test the efficacy of picetannol, an anti-inflammatory compound that is prepared as part of entrapped albumin nanoparticles (PANPs), to treat acute lung injury that occurs in the acute respiratory distress syndrome.

Overlap: None

Title: Precision Medicine in the Acute Respiratory Distress Syndrome

Time Commitments: 0.30 Calendar

Supporting Agency: NHLBI/R35HL140026 (Calfee)

Address:

NHLBI Health Information Center

P.O. Box 30105

Bethesda, MD 20824-0105

Contracting/Grants Officer: Manda C Richards

Performance Period: 1/16/18-12/31/2024

Level of funding: \$637,937

Project Goals: To identify molecular endotypes of ARDS with distinct clinical and biological profiles, including integration of environmental exposure data and identification of differential treatment responses; to develop practical models for endotype identification; and to test biological mechanisms in an experimental human lung model.

Role: Co-Investigator

Overlap: None

Title: Angiopoietin/Tie signaling regulation of vascular leakage in lung inflammation

Time Commitments: 0.30 Calendar

Supporting Agency: NHLBI/R01 HL143896 (McDonald)

Address:

NHLBI Health Information Center

P.O. Box 30105

Bethesda, MD 20824-0105

Contracting/Grants Officer: Tammi Simpson

Performance Period: 7/1/2018-5/31/2022

Level of funding: \$393,001

Project Goals: To determine the contributions of angiopoietin-1 (Ang1) and angiopoietin-2 (Ang2), and their receptors, Tie1 and Tie2 (Tek), to the regulation of vascular leak in lung injury and inflammation.

Role: Co-Investigator

Overlap: None

Title: The GOLD STUDY: Goal of open lung ventilation in donors

Time Commitments: 0.45 Calendar

Supporting Agency: NIH/NHLBI, R01HL126176

Address:

NHLBI/VUMC

3319 West End Avenue, STE 100

Nashville TN 37203

Contracting/Grants Officer: Libby D. Salberg

Performance period: 5/1/2016-5/31/2021

Level of funding: \$79,238

Project Goals: Dr. Matthay has laboratory will be responsible for processing the human lungs collected and studied in Aim 2 of this application. Dr. Matthay himself will also oversee the conduct of the trial as described in Aim 1 in conjunction with Dr. Ware at Vanderbilt.

Overlap: None

Title: Integrated Health, Behavioral and Economic Research on Current and Emerging Tobacco Products **Time**

Commitments: 0.60 Calendar

Supporting Agency: NIH/NHLBI, U54HL147127 (Glantz)

Address:

NHLBI Health Information Center
P.O. Box 30105
Bethesda, MD 20824-0105

Contracting/Grants Officer: Judy Sint**Performance period:** 9/1/2013-8/31/2023**Level of funding:** \$327,681 (Project 1: Calfee)**Project Goals:** To provide a comprehensive assessment of the impact of varying e-cigarette characteristics on acute lung injury by combining data from cell culture, mouse models, and humans, including testing different device and e-liquid characteristics**Role:** Co-Investigator**Overlap:** None**Title:** Aerosol Delivery of Surfactant for ARDS**Commitments:** 0.12 Calendar**Supporting Agency:** KAER Biotherapeutics Corp/NIH**Address:**

926 S. Andreasen Dr., Ste 105
Escondido, California 92029

Contracting/Grants Officer: Donovan B. Yeates (CEO)**Performance Period:** 9/15/2018-6/30/2020**Level of Funding:** \$26,216**Project Goals:** I will work closely with Dr. Donovan Yeates (CEO of KAER) and his team to adapt this technology for delivery to patients with acute hypoxemic respiratory failure from acute lung injury, most commonly from severe bacterial or viral pneumonia.**Overlap:** None**Pending****Title:** ARREST RESPIRATORY FAILURE DUE TO PNEUMONIA (ARREST PNEUMONIA)**Time Commitments:** 0.60 Calendar**Supporting Agency:** NIH/Stanford University**Address:**

Stanford University
3172 Porter Drive
Palo Alto CA 94304

Contracting/Grants Officer: Sharon Collum**Performance period:** 9/1/19-3/31/2024**Level of funding:** \$41,566**Project Goals:** Dr. Michael Matthay will serve as an Executive Committee Member providing expertise in the design and conduct of multicenter clinical trials in patients at risk for lung injury.**Overlap:** No scientific or budgetary overlap. If all the pending applications are funded, the PI will adjust his efforts accordingly to stay in compliance.**Title:** Bacterial Pneumonia and PAR-Modified Mesenchymal Stem Cells**Commitments:** 0.12 Calendar**Supporting Agency:** UC San Diego/NIH**Address:**

UC San Diego
10300 North Torrey Pines Road
La Jolla, CA 92093

Contracting/Grants Officer:

Performance period: 4/1/2022-3/31/2025

Level of funding: \$25,000

Project Goals: We will be able to determine with greater precision whether alteration of PA1 and/or PAR2 on human MSCs can boost their therapeutic potency against important pathogens such as MRSA and Pseudomonas.

Overlap: No scientific or budgetary overlap. If all the pending applications are funded, the PI will adjust his efforts accordingly to stay in compliance.

Title: Traumakine Overpowering Capillary Breach in ARDS

Commitments: 1.20 Calendar

Supporting Agency: Faron Pharmaceuticals

Address:

Faron Pharmaceuticals Oy

Joukahaisenkatu 6

FIN-20520

Finland

Contracting/Grants Officer: Jenni Hurme

Performance period: 11/1/2019-10/31/2021

Level of funding: \$181,075

Project Goals: Prior clinical trial data suggested that a beta-interferon therapy might be effective in reducing the severity of acute respiratory failure in patients with the acute respiratory distress syndrome (ARDS). This trial will test the efficacy of this therapy in patients with ARDS in a randomized double blind placebo controlled trial.

Overlap: No scientific or budgetary overlap. If all the pending applications are funded, the PI will adjust his efforts accordingly to stay in compliance.

Title: Molecular profiling of ARDS edema fluid: a window to an injured lung.

Commitments: 0.12 Calendar

Supporting Agency: Stanford/NIH

Address:

Stanford University

3172 Porter Drive

Palo Alto CA 94304

Contracting/Grants Officer: Anitra Johnson

Performance period: 4/1/2020-3/31/2021

Level of funding: \$41,556

Project Goals: We will deliver clinical data and biologic data on 20 patients with ARDS each year to be included in the studies and analyses that Dr. Rogers will do on mechanisms of acute lung injury in ARDS.

Overlap: No scientific or budgetary overlap. If all the pending applications are funded, the PI will adjust his efforts accordingly to stay in compliance.

Title: ENaC- α mediates lung fluid clearance and capillary barrier function in pneumonia

Commitments: 0.19 Calendar

Supporting Agency: Augusta University/ NIH

Address:

1120 15th Street

Augusta, GA 30912

Contracting/Grants Officer: Sandy Ferguson

Performance period: 7/1/2020-6/30/2020

Level of funding: \$44,043

Project Goals: The experiments in the ex vivo perfused human lung preparation will provide valuable data for evaluating the efficacy of these agonists of sodium transport and barrier function in a clinically relevant model

of bacterial pneumonia in the ex vivo perfused human lung.

Overlap: No scientific or budgetary overlap. If all the pending applications are funded, the PI will adjust his efforts accordingly to stay in compliance.

Title: Novel small-molecule therapies for CF

Commitments: 0.24 Calendar

Supporting Agency: NIH-NIDDK

Address:

9000 Rockville Pike

Bethesda, MD 20892

Contracting/Grants Officer:

Performance period: 6/1/2020-5/31/2021

Level of funding: \$817,476

Project Goals: This is a proposal to continue our Cystic Fibrosis (CF) Research and Translation Core Center at the University of California, San Francisco and collaborating institutions. The focus of our Core Center remains the discovery and evaluation of novel small-molecule therapies for CF.

Role: Co-Investigator

Overlap: No scientific or budgetary overlap. If all the pending applications are funded, the PI will adjust his efforts accordingly to stay in compliance.

Title: Lung endothelial microRNA-1, a novel therapeutic target in ARDS

Commitments: 0.24 Calendar

Supporting Agency: Yale/NIH

Address:

New Haven

CT, 06520

Contracting/Grants Officer: Teresa Bohan

Performance period: 7/1/2022-6/30/2024

Level of funding: \$134,859

Project Goals: The proposed studies in this NIH R01 application with Dr. Takyar will test a promising new therapeutic approach in our clinically relevant ex vivo perfused human lung preparation

Overlap: No scientific or budgetary overlap. If all the pending applications are funded, the PI will adjust his efforts accordingly to stay in compliance.

Previous

Title: Pulmonary Hypertension in ARDS study (To define the clinical and biological correlates of pulmonary hypertension and increased pulmonary dead space in patients with ARDS.)

Time Commitments: 1.2 calendar

Supporting Agency: Bayer AG

Address:

Bayer AG

Aprather Weg 18a

D-42113 Wuppertal

GDWRC/building WUP 431 2 223

Contracting/Grants Officer: Hubert Trübel

Performance Period: 7/1/17-6/30/19

Level of funding: \$395,998

Project Goals: To determine the relationship of elevated pulmonary arterial pressures and elevated dead space to respiratory outcomes, 28 day mortality and biological markers of lung and systemic injury

Specific Aims: To determine incidence of pulmonary hypertension in ARDS patients and whether it identifies

patients with a higher mortality along with measurement of pulmonary dead space and biologic markers of inflammation and lung injury.

Overlap: None

Title: Targeting Angiopoietin-2 in ARDS

Time Commitments: 0.21 calendar

Supporting Agency: NHLBI/University of Pennsylvania (R01 HL137006)

Address:

Office of Research Services

3451 Walnut St, 5th Floor Franklin Building

Philadelphia PA 19104-6205

Contracting/Grants Officer: Amy Camilleri

Performance Period: 2/1/18-2/28/18

Level of funding: \$27,131

Project Goals: The major goals are to test the role of angiopoietin-2 (ANG2) as a predictor of acute respiratory distress syndrome (ARDS) risk and evaluate early anti-ANG2 therapy to decrease lung leak in an ex vivo lung perfusion model of human disease.

Overlap: None

Title: Quantification and Biomarkers of Short-Term Pulmonary Effects of Tobacco Smoke Exposure: Infection-Related Acute Lung Injury

Time Commitments: 0.60 calendar

Supporting Agency: NIH/FDA

NCI Contact Center

BG 9609 MSC 9760

9609 Medical Center Drive Bethesda, MD 20892-9760

Contracting/Grants Officer: Rebecca Brightful

Performance period: 09/01/2013-08/31/2018

Level of funding: \$23,601

Project Goals: To quantify the association between cigarette smoke exposure and the development of acute lung injury in patients with severe infection and in mouse models of infection-related ALI, and to develop new biomarkers for tobacco-related acute lung injury

Specific Aims: The specific aims are to test the biological and clinical predictors of developing ARDS in patients at risk for developing ARDS who smoke cigarettes versus those who do not and identifying biomarkers that may be associated with the increased risk. One aim also tests the effects of cigarette smoke exposure in mice to determine if they are more susceptible to acute lung injury from endotoxin or bacterial lung infection.

Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal

Title: Resolution of Clinical Lung Injury

Time Commitments: 0.12 calendar (NO COST EXTENSION)

Supporting Agency: NIH/NHLBI, R37 HL051856

Address:

NIH/NHLBI Information center

P.O Box 30105

Bethesda, MD 20824-0105

Contracting/Grants Officer: Charmaine Prasad

Performance Period: 04/01/2011-03/31/2018

Level of funding: \$263,019

Project Goals: To study the pathogenesis of acute lung injury and ARDS, with an emphasis on alveolar epithelial fluid clearance, through the use of clinical studies.

Specific Aims: The specific aims are to study the the pathogenetic and prognostic value of biomarkers in

patients with ARDS, to test the effect of human edema fluid from ARDS patients in both an in vitro model of cultured human alveolar epithelial type 2 cells and new therapeutics for acute lung injury in an isolated perfused human lung preparation.

Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal

Title: Allogeneic Human Mesenchymal Stem Cells for the Treatment of Acute Lung Injury

Time Commitments: 0.12 calendar (NO COST EXTENSION)

Supporting Agency: NIH/NHLBI, U01 HL108713

Address:

NHLBI Health Information Center

P.O. Box 30105

Bethesda, MD 20824-0105

Contracting/Grants Officer: Kimberly Stanton

Performance period: 09/01/2011-06/30/2018

Level of funding: \$1,587,045

Project Goals: To test the safety and efficacy of human mesenchymal stem cells for the treatment of severe acute lung injury.

Specific Aims: The specific aim is to test the therapeutic value of intravenous human bone marrow derived mesenchymal stem cells for the treatment of 60 patients with moderate to severe ARDS for safety and limited efficacy endpoints, using a 2:1 randomization with a double blind design. There is also an aim to study the biologic markers of injury that may be altered in the plasma and bronchoalveolar lavage in the placebo versus treated patients.

Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal

Title: Genetic risks for ALI in ARDSnet and the iSPAAR Consortium

Time Commitments: 0.6 calendar

Supporting Agency: NIH/NHLBI RC2 HL101779/University of Washington

Address:

NHLBI Health Information Center

P.O. Box 30105

Bethesda, MD 20824-0105

Contracting/Grants Officer: Michael Blackwell (University of Washington)

Performance Period: 9/30/2009-8/31/2012

Level of Funding: \$50,000

Project Goal: To identify genetic factors contributing to the pathogenesis of ARDS.

Specific Aims: To study DNA and plasma for biological factors that predict outcomes in ARDS patients.

Overlap: None

Title: Treatment of Pulmonary Edema in Organ Donors

Time Commitments: 0.6 calendar

Supporting Agency: NIH/NHLBI R01 HL088263/VUMC (subcontract)

Address:

NHLBI Health Information Center

P.O. Box 30105

Bethesda, MD 20824-0105

Contracting/Grants Officer: Libby Salberg (VUMC)

Performance Period: 2/01/2008 -01/31/2013

Level of Funding: \$11,982

Project Goal: To test aerosolized albuterol a beta agonist to improve lung function in brain dead subjects.

Specific Aims: To carry out a randomized trial of inhaled albuterol versus placebo to increase lung utilization for lung transplantation.

Overlap: None

Title: Sedation Management in Pediatric Patients with Acute Respiratory Failure

Time Commitments: 0.6 calendar

Supporting Agency: NIH/NHLBI U01HL086622 /University of Pennsylvania (subcontract)

Address:

NHLBI Health Information Center

P.O. Box 30105

Bethesda, MD 20824-0105

Contracting/Grants Officer: Sheila R. Atkins (University of Pennsylvania)

Performance Period: 4/1/2008-3/31/2013

Level of Funding: \$11,269

Project Goal: To test a sedation strategy to improve clinical outcomes in children with acute respiratory failure who were being mechanically ventilated.

Specific Aims: To use a cluster design to test a protocolized sedation strategy to increase ventilator free days in pediatric patients with acute respiratory failure.

Overlap: None

Title: Lung Fluid Balance and Mesenchymal Stem Cells

Time Commitments: 2.4 calendar

Supporting Agency: NIH/NHLBI R01HL051854

Address:

NHLBI Health Information Center

P.O. Box 30105

Bethesda, MD 20824-0105

Contracting/Grants Officer: Dianna Jessee (GMO)

Performance Period: 9/30/2008-6/30/2013

Level of Funding: \$382,388

Project Goal: To study the mechanisms by which mesenchymal stem cells reduce lung injury in experimental models.

Specific Aims: To study the efficacy and mechanisms of mesenchymal stem cells in mouse models of acute lung injury.

Overlap : None

Title: Stromal stem cells of human placenta for the treatment of Acute Lung Injury

Time Commitments: 0.6 calendar

Supporting Agency: NIH/NHLBI R43HL108327/Plasalus LLC

Address:

NHLBI Health Information Center

P.O. Box 30105

Bethesda, MD 20824-0105

Contracting/Grants Officer: Frans A Kuypers (Plasalus)

Performance Period: 8/1/12-5/31/2014

Level of Funding: \$72,342

Project Goal: To test the efficacy of human placental mesenchymal stem cells for reducing lung injury in both in vitro and in vivo models of lung injury.

Specific Aims : To use human type 2 cells and the ex vivo perfused human lung preparation to test the efficacy of human placental stem cells for reducing lung injury from endotoxin.

Overlap: None

Title: Clinical Research Network for the Treatment of Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS)

Time Commitments: 0.6 cal

Supporting Agency: NIH/NHLBI HHSN268200536166C

Address: NHLBI, NIH

Rockledge II building, Rm 6016

6701 Rockledge Drive MSC 7902

Bethesda MD 20892-7902

Contracting/Grants Officer: Scott Bredow (NHLBI)

Performance Period: 12/1/2011-6/30/2014

Level of Funding: \$33,122

Project Goal: To test in phase 3 trials new treatments for acute lung injury and ARDS.

Specific Aims: To enroll patients in randomized clinical trials in the NHLBI ARDS Network.

Overlap: None

Title: Metabolic Response to Acute Injury in Alveolar Epithelium and ARDS

Time Commitments: 0.12 calendar

Supporting Agency: Stanford /American Thoracic Society, 60995841-117524

Address:

Stanford University Office of Sponsored Research 3160 Porter Drive, Suite 100

Palo Alto, CA 94304-8445

Contracting/Grants Officer: Teresa Tom

Performance Period: 11/30/14-11/29/15

Level of Funding: \$10,000

Project Goal: To study the metabolic factors released by human alveolar epithelial type 2 cells in culture and to supply pulmonary edema fluid for metabolomics studies.

Specific Aims: The specific aim is to determine the metabolic abnormalities that may have pathogenetic or prognostic significance in cultured human epithelial type 2 cells exposed to cytomix (pro-inflammatory stimulus) and to test the metabolic abnormalities in undiluted edema fluid from patients with hydrostatic versus acute lung injury (ARDS).

Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal

Title: Gene-modified mesenchymal stem (stromal) cells for Treatment of the Acute Respiratory Distress Syndrome A125202

Time Commitments: 0.6 calendar

Supporting Agency: NIH/NHLBI U54HL119893/UCLA

Address:

NHLBI Health Information Center

P.O. Box 30105

Bethesda, MD 20824-0105

Contracting/Grants Officer: Mary Haskins (UCLA)

Performance Period: 3/1/15-2/29/2016

Level of Funding: \$100,000

Project Goal: Our primary objective will be to carry out proof of principle studies to determine which combination of genes for KGF, Ang-1, and TIMP3 transfected into MSCs will produce the most therapeutically effective conditioned media (CM) for treating ARDS using pre-clinical models of pneumonia and sepsis in mice and severe pneumonia and lung injury in our novel ex vivo perfused human lung.

Specific Aims: Specific aim is to determine to potential therapeutic efficacy of an enriched conditioned media from transfected MSCs for reducing in vitro lung endothelial and epithelial injury and then test the conditioned media in an endotoxin model of lung injury in mice.

Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal

Title: The inflammasome: A Novel Biomarker in ALI/ARDS

Time Commitments: .12 calendar

Supporting Agency: NIH/NHLBI R01 HL112747/Brigham & Women's Hospital

Address: NHLBI Health Information Center

P.O. Box 30105

Bethesda, MD 20824-0105

Contracting/Grants Officer: Stephanie Redfield (Brigham & Women's Hospital)

Performance Period: 5/15/2012-4/30/2016

Level of Funding: \$6,577

Project Goal: To determine the predictive value of biomarkers of the inflammasome in acute lung injury.

Specific Aims: To test the predictive value of plasma levels of biomarkers of the inflammasome on developing ARDS in at risk patients plus to determine the modifying effect if any on these biomarkers of treatment with statins.

Overlap: None

Title: Recipient Epidemiology and Donor Evaluation Study-III *REDS-III) –Domestic Sites

Time Commitments: 1.8 calendar

Supporting Agency: NIH/NHLBI, HHSN268110005I

Address:

NIH/NHLBI Information center

P.O Box 30105

Bethesda, MD 20824-0105

Contracting/Grants Officer: Michael Spears

Performance period: 03/15/2011-08/31/2016

Level of funding: \$637,636

Project Goals: To assure safe and effective blood banking and transfusion medicine practices through a comprehensive, multi-targeted strategy involving basic, translational, and clinical research to improve the benefits of transfusion while reducing its risks.

Specific Aims: The specific aim is to test clinical criteria for determining if patients who have blood product transfusions who develop pulmonary edema have TACO or TRALI or ARDS from a usual risk factor (not blood products) by reviewing specific patient cases from three hospitals with a consensus panel.

Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal

Title: Gene-modified mesenchymal stem (stromal) cells for Treatment of the Acute Respiratory Distress Syndrome

Time Commitments: 0.3 calendar

Supporting Agency: UC/CAI grant, 20130924SFM

Address:

11000 Kinross Avenue, Suite 211 Los Angeles, CA 90051

Contracting/Grants Officer: Susan Waelder

Performance period: 03/01/2015-02/28/2017

Level of funding: \$100,000

Project Goals: Our primary objective will be to carry out proof of principle studies to determine which combination of genes for KGF, Ang-1, and TIMP3 transfected into MSCs will produce the most therapeutically effective conditioned media (CM) for treating ARDS using pre-clinical models of pneumonia and sepsis in mice and severe pneumonia and lung injury in our novel ex vivo perfused human lung.

Specific Aims: Specific aim is to determine to potential therapeutic efficacy of an enriched conditioned media from transfected MSCs for reducing in vitro lung endothelial and epithelial injury and then test the conditioned media in an endotoxin model of lung injury in mice.

Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal

Title: TIMP-3 For Viral Induced Acute Lung Injury

Time Commitments: 0.8 calendar

Supporting Agency: Amgen, 2013583306

Address:

Extramural Research Alliances (ERA)

Amgen, Inc.

One Amgen Center Drive Thousand Oaks, CA 91320

Contracting/Grants Officer: Scott Simonet

Performance period: 12/03/2013-06/02/2017

Level of funding: \$174,275

Project Goals: To test a new therapy with TIMP-3 for influenza pneumonia and lung injury.

Specific Aims: To evaluate the potential therapeutic value of inhibiting TIMP-3 to reduce acute lung injury from PR8 H1N1 influenza in mice.

Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal

Title: Identification of Patients at High Risk for the Development of ALI with Clinical and Biological Predictors

Time Commitments: Effort as needed

Supporting Agency: U Penn Subcontract/Glaxo Smith Kline, Galaxy ALI (subcontract)

Address:

Glaxo Smith Kline

709 Swedeland Road

King of Prussia, PA 19406

Contracting/Grants Officer: Susan Russell

Performance period: 06/26/2012-07/31/2017

Level of funding: \$39,681

Project Goals: To identify clinical and biological predictors of ALI in a cohort of patients with sepsis

Specific Aims: The aim is to determine the biological predictors of ARDS in the plasma of sepsis patients in the Emergency department at risk for developing ARDS.

Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal

Title: Cigarette Smoke Exposure and Acute Lung Injury After Severe Blunt Trauma

Time Commitments: 0.30 calendar

Supporting Agency: NIH/NHLBI, R01 HL110969

Address:

NHLBI Health Information Center

P.O. Box 30105

Bethesda, MD 20824-0105

Contracting/Grants Officer: Charmaine Prasad

Performance period: 12/15/2011-11/30/2017

Level of funding: \$250,000

Project Goals: To determine the biologic effects of cigarette smoke exposure that increase susceptibility to acute lung injury after severe trauma.

Specific Aims: The specific aim is to determine the effect of cigarette smoke on increasing the risk of ARDS in major trauma patients, including accounting for passive versus active cigarette smoke exposure and alcohol use. There is also one aim designed to test the relationship of the microbiome in the airways at baseline and on days 2-4 sampled by bronchoalveolar lavage to cigarette smoke exposure and to the development of ARDS in major trauma patients.

Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal

Title: Molecular Endotypes of ARDS: Identification, Biology, and Differential Response to Therapy

Time Commitments: 0.6 calendar

Supporting Agency: NIH/NHLBI R01 HL131621

Address:

NHLBI Health Information Center

P.O. Box 30105

Bethesda, MD 20824-0105

Contracting/Grants Officer: Sunshine Wilson

Performance Period: 3/15/2016-1/15/18

Level of funding: \$12,328

Project Goals: To identify endotype-specific treatment responses and differences in endotype biology within ARDS

Specific Aims: To test biologic and clinical variables in ARDS patients to identify clinically meaningful phenotypes that would be more specific for therapeutic targets.

Overlap: None



Treatment with allogeneic mesenchymal stromal cells for moderate to severe acute respiratory distress syndrome (START study): a randomised phase 2a safety trial

Michael A Matthay, Carolyn S Calfee, Hanjing Zhuo, B Taylor Thompson, Jennifer G Wilson, Joseph E Levitt, Angela J Rogers, Jeffrey E Gotts, Jeanine P Wiener-Kronish, Ednan K Bajwa, Michael P Donahoe, Bryan J McVerry, Luis A Ortiz, Matthew Exline, John W Christman, Jason Abbott, Kevin L Delucchi, Lizette Caballero, Melanie McMillan, David H McKenna, Kathleen D Liu

Summary

Background Treatment with bone-marrow-derived mesenchymal stromal cells (MSCs) has shown benefits in preclinical models of acute respiratory distress syndrome (ARDS). Safety has not been established for administration of MSCs in critically ill patients with ARDS. We did a phase 2a trial to assess safety after administration of MSCs to patients with moderate to severe ARDS.

Methods We did a prospective, double-blind, multicentre, randomised trial to assess treatment with one intravenous dose of MSCs compared with placebo. We recruited ventilated patients with moderate to severe ARDS (ratio of partial pressure of oxygen to fractional inspired oxygen <27 kPa and positive end-expiratory pressure [PEEP] ≥ 8 cm H₂O) in five university medical centres in the USA. Patients were randomly assigned 2:1 to receive either 10×10^6 /kg predicted bodyweight MSCs or placebo, according to a computer-generated schedule with a variable block design and stratified by site. We excluded patients younger than 18 years, those with trauma or moderate to severe liver disease, and those who had received cancer treatment in the previous 2 years. The primary endpoint was safety and all analyses were done by intention to treat. We also measured biomarkers in plasma. MSC viability was tested in a post-hoc analysis. This trial is registered with ClinicalTrials.gov, number NCT02097641.

Findings From March 24, 2014, to Feb 9, 2017 we screened 1038 patients, of whom 60 were eligible for and received treatment. No patient experienced any of the predefined MSC-related haemodynamic or respiratory adverse events. One patient in the MSC group died within 24 h of MSC infusion, but death was judged to be probably unrelated. 28-day mortality did not differ between the groups (30% in the MSC group *vs* 15% in the placebo group, odds ratio 2.4, 95% CI 0.5–15.1). At baseline, the MSC group had numerically higher mean scores than the placebo group for Acute Physiology and Chronic Health Evaluation III (APACHE III; 104 [SD 31] *vs* 89 [33]), minute ventilation (11.1 [3.2] *vs* 9.6 [2.4] L/min), and PEEP (12.4 [3.7] *vs* 10.8 [2.6] cm H₂O). After adjustment for APACHE III score, the hazard ratio for mortality at 28 days was 1.43 (95% CI 0.40–5.12, *p*=0.58). Viability of MSCs ranged from 36% to 85%.

Interpretation One dose of intravenous MSCs was safe in patients with moderate to severe ARDS. Larger trials are needed to assess efficacy, and the viability of MSCs must be improved.

Funding National Heart, Lung, and Blood Institute.

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Introduction

Acute respiratory distress syndrome (ARDS) is associated with protein-rich pulmonary oedema and acute respiratory failure characterised by acute inflammation and injury to the lung and epithelia.^{1,2} In a multinational observational study, ARDS occurred in 10% of patients admitted to intensive-care units, and mortality among these patients was increased by more than 30% despite improvements in supportive care and ventilator management.³

In several preclinical studies using animal models of ARDS, treatment with mesenchymal stromal cells (MSCs) derived from bone marrow, umbilical cord, or adipose tissue has reduced the severity of acute lung injury from infectious and non-infectious causes.^{4,5} In

a 24 h model of severe lung injury from bacterial pneumonia in sheep, human bone-marrow-derived MSCs improved oxygenation and reduced extravascular lung water compared with a placebo control, without adverse haemodynamic effects or respiratory compromise.⁶ In addition, allogeneic MSCs derived from human bone marrow were effective in models of acute lung injury in ex-vivo perfused human lung preparations injured with endotoxin or Gram-negative bacteria.^{7,8} In some studies the MSCs were administered intravenously and in others they were administered by the intratracheal or intrabronchial routes. The preclinical data overall indicate reduced lung vascular and epithelial permeability to protein, increased clearance of alveolar oedema fluid, and anti-inflammatory and antimicrobial properties,

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Research in context

Evidence before this study

We searched PubMed for studies published between Jan 1, 2003, and Jan 1, 2013, with the search string "(acute respiratory distress syndrome OR acute lung injury) AND (marrow-derived mesenchymal stem cells) AND ('2003/01/01'[Date-Publication]: '2013/01/01' [Date-Publication])" and no language restrictions. Preclinical studies of ARDS in small and large animals and perfused ex-vivo human lung preparations have shown therapeutic benefits after treatment with bone-marrow-derived mesenchymal stromal cells (MSCs). Based on these findings, clinical studies were done to assess the effects of MSCs to treat ARDS. One dose-escalation trial of 1, 5, and 10×10^6 MSCs/kg predicted bodyweight in nine patients with moderate to severe ARDS indicated that the highest dose could be well tolerated. We obtained regulatory approval from the US Food and Drug Administration to do a phase 2a multicentre, randomised, double-blind trial of MSCs to assess safety.

Added value of this study

In patients with moderate to severe ARDS based on prespecified haemodynamic and respiratory parameters, MSC therapy was well tolerated. Clinical outcomes did not differ significantly between groups, but post-hoc analyses showed a trend for improvement in oxygenation index in the MSC group. Post-thaw viability of MSCs emerged as a potentially important factor in the biological and clinical effects of this treatment for ARDS.

Implications of all the available evidence

Our results support the finding that single-dose intravenous MSC therapy is well tolerated in patients with moderate to severe ARDS. Measurement of biomarkers suggested that endothelial injury is significantly reduced, and that the effect is associated with higher viability of MSCs after thawing. Further well powered trials are needed to assess efficacy.

which support the therapeutic potential of bone-marrow-derived MSCs for ARDS.⁹

Several clinical trials have suggested that patients with moderate to severe ARDS (ratio of partial pressure of oxygen to fractional inspired oxygen [$\text{PaO}_2:\text{FiO}_2$] <27 kPa), in whom lung inflammation and diffuse alveolar damage are increased,¹⁰ have differential and greater responses to treatments than patients with mild ARDS ($\text{PaO}_2:\text{FiO}_2$ 27–40 kPa). Given this difference in response, the increased mortality risk in patients with moderate to severe ARDS (29–48%),¹¹ and the multipotent effects of cell-based therapies,¹² we tested MSC-based treatment in patients with moderate to severe ARDS.

As a first step in testing safety, we did a phase 1, open-label, dose-escalation trial in nine patients with moderate to severe ARDS, using 1, 5, and 10×10^6 MSCs/kg predicted bodyweight. Infusions at all doses were well tolerated.¹³ Therefore, we obtained approval from the US Food and Drug Administration (FDA) to do a randomised, double-blind, placebo-controlled, phase 2a trial to test the safety of intravenous treatment with the highest dose in patients with moderate to severe ARDS.

Methods

Study design and patients

START was a double-blind, randomised, placebo-controlled, phase 2a trial that was done in five university medical centres in the USA (University of California San Francisco, Stanford University, University of Pittsburgh Medical Center, Ohio State University, and Massachusetts General Hospital). Eligible patients were endotracheally intubated, had a $\text{PaO}_2:\text{FiO}_2$ less than 27 kPa, were mechanically ventilated with at least 8 cm H_2O positive-end expiratory pressure (PEEP), had bilateral pulmonary infiltrates consistent with pulmonary oedema on chest radiographs, and had no clinical evidence

of left-heart failure or volume overload as the primary cause of the pulmonary oedema. A protocol amendment was made to allow enrolment of patients with PEEP of 5 cm H_2O if they had evidence of barotrauma.

Exclusion criteria (appendix) included age younger than 18 years, ARDS present for more than 96 h, pregnancy or breastfeeding, being an inmate in the prison system, having received treatment for cancer in the past 2 years (except non-melanoma skin cancer), having an underlying medical status with life expectancy less than 6 months, moderate to severe liver disease (Child-Pugh score >12), severe chronic lung disease with the use of home oxygen, or partial arterial pressure of carbon dioxide greater than 7 kPa, and not being committed to full support (ie, had do not resuscitate or limit on life support orders).

The trial was approved by the institutional review boards at each medical centre, the FDA (15331), and the National Heart, Lung, and Blood Institute (U01HL108713). The clinical protocol is available in the appendix. A data safety monitoring board approved the trial design and reviewed serious adverse events and the results after 20 and 40 patients had been enrolled. Written informed consent was obtained from patients or authorised surrogates.

Randomisation and masking

Patients were randomly assigned (2:1) to receive either 10×10^6 MSC/kg predicted bodyweight or placebo (Plasma-Lyte A, Baxter, Deerfield, IL, USA). The randomisation had a variable block design, was stratified by site, and the sequence was generated by computer. The allocation sequence could be accessed by each cell laboratory through a dedicated website. Personnel in the cell laboratories were not masked, but patients, clinical staff, and investigators were unaware of treatment assignment. To maintain masking of the investigators and clinicians, bags containing

See Online for appendix

the study products and intravenous tubing had opaque coverings applied in the cell laboratories. No measures were taken to check the success of masking. For data analysis, investigators were unaware of treatment allocation until the database was fully cleaned and locked.

Procedures

Allogeneic MSCs derived from human bone marrow were prepared from three donors (aged 18–45 years, one woman and two men) with support from the National Heart, Lung, and Blood Institute Production Assistance for Cellular Therapies Program at the University of Minnesota (Minneapolis, MN, USA). MSCs were manufactured from fresh (four lots) or cryopreserved (eight lots) bone marrow. The mononuclear cell fraction was enriched with Ficoll. Mononuclear cells were tested for nucleated cell count, differential, viability, flow cytometry (CD105, CD73, CD90, CD45, CD34, CD14, CD19, and HLA-DR), and sterility before seeding at 100 000 cells/cm² for culture. At 70% confluence, MSCs were lifted and passaged at a low density (40–50 cells/cm²) into 24 cell factories. At 70–80% confluence, the MSCs were harvested, washed, resuspended, and cryopreserved. Karyotyping (G banding) was done for all lots of MSCs and was normal. The criterion for release of MSCs from the University of Minnesota was at least 70% viability.

The cryopreserved MSCs were shipped to the clinical sites frozen at a maximum temperature of –150°C, in a validated liquid nitrogen dry shipper with a continuous temperature monitoring device. Upon receipt, the cellular product was inspected and stored in a controlled, continuously monitored liquid nitrogen storage tank. Before administration, the MSCs were thawed, washed to remove dimethyl sulfoxide (DMSO), and resuspended in Plasma-Lyte A by a local cell therapy laboratory. DMSO was removed because studies in sheep with *Pseudomonas aeruginosa* pneumonia suggested that efficacy of MSCs would be decreased without this step before intravenous administration.⁶ The total volume of the MSC or placebo infusion was 100 mL. Viability of infused MSCs was determined by trypan blue exclusion after the MSCs had been thawed and prepared for infusion at each of the clinical sites. The time to measurement of viability after MSC preparation was approximately 1 h, although no time range was specified in the standard operating procedures.

After a patient had been clinically stable for 2 h (transcutaneous oxygen saturation 88–95% without the need for FiO₂ >0.8 or PEEP >20 cm H₂O and use of maximum two vasopressors, <0.1 µg/kg per min epinephrine, or both, for blood pressure support) in the supine position, infusion was started with a standard blood filter tubing set with a pore size of 170 µm. The study product was infused by gravity over 60–80 min. The infusion rate, based on the droplet count, was controlled by a physician investigator who remained at the patient's bedside for the duration of the infusion and in the intensive care unit for 6 h after the start of infusion

to monitor for any signs of adverse reaction. All patients were ventilated according to the modified ARDS Network lower tidal volume protocol.¹⁴

Theoretically, MSC infusion could increase pulmonary vascular resistance in the presence of injured pulmonary microcirculation, potentially by aggregating or clumping in capillaries or small arterioles. Subsequent potential risks are acute right heart failure, impaired cardiac output, and haemodynamic instability. Additionally, infusion of MSCs could potentially worsen ventilation–perfusion mismatch and result in further impairment of oxygenation or carbon dioxide excretion. All patients, therefore, were monitored closely for any changes in a prescribed list of respiratory or cardiovascular parameters (panel). The incidence and nature of all serious adverse events were reviewed and independently assessed by the data safety monitoring board to determine whether they were related to MSC administration, with special focus on events that would be unexpected in a critically ill patient with ARDS. Additionally, we measured creatinine, total bilirubin, and alanine aminotransferase concentrations in serum on days 3, 7, and 14 after infusion if patients were still in hospital. Methods for data collection and in-study measurements are described elsewhere.¹⁵

Clinical and biological outcomes

The primary outcome of the study was the safety of the MSC infusion, assessed with prespecified infusion-associated adverse events focused on acute haemodynamic or respiratory compromise (panel). The secondary outcomes were all-cause mortality at

Panel: Prespecified infusion-associated adverse events

Within 6 h of study product infusion

- Increase in vasopressor dose to the following values or higher:
 - Norepinephrine 10 µg/min
 - Phenylephrine 100 µg/min
 - Dopamine 10 µg/kg per min
 - Epinephrine 0.1 µg/kg per min
- or addition of a third vasopressor
- New ventricular tachycardia, ventricular fibrillation or asystole
- New cardiac arrhythmia requiring cardioversion
- Hypoxaemia requiring an increase in FiO₂ of 0.2 or more and an increase in PEEP of 5.0 or more to maintain SpO₂ in the target range of 88–95%
- Clinical scenario consistent with transfusion incompatibility or transfusion-related infection (eg, urticaria, new bronchospasm)

Within 24 h of study product infusion

- Any cardiac arrest or death

FiO₂, fractional concentration of oxygen. PEEP=positive end-expiratory pressure.
SpO₂=peripheral capillary oxygen saturation.

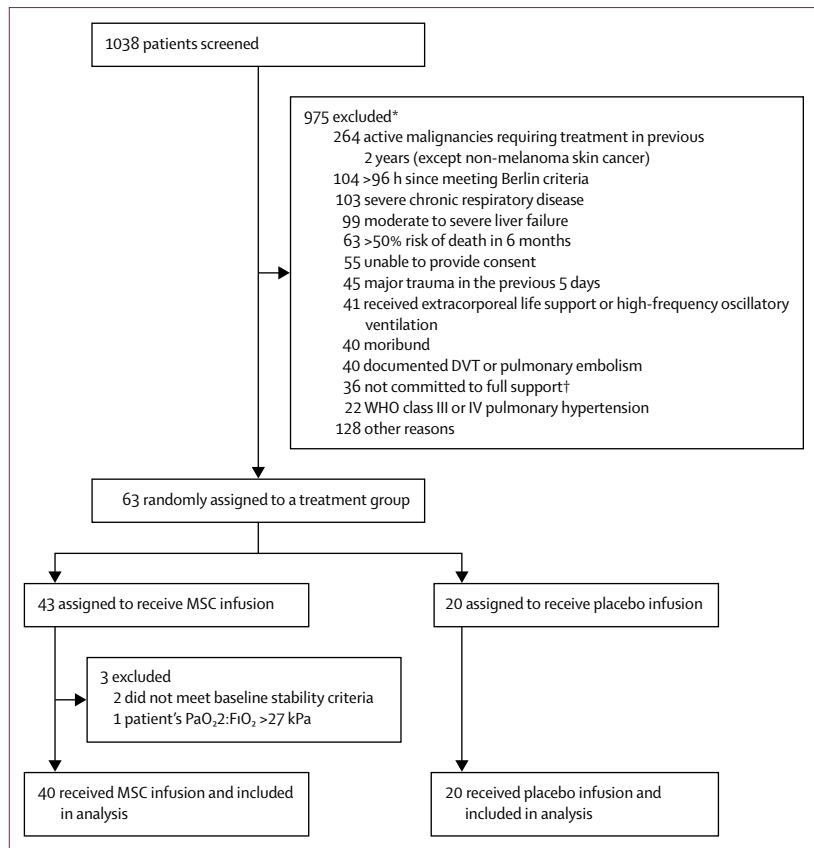


Figure 1: Trial profile

DVT=deep-vein thrombosis. MSC=mesenchymal stromal cell. PaO₂:FiO₂=ratio of partial pressure of oxygen to fractional inspired oxygen. *Numbers total more than 975 because some patients met multiple exclusion criteria. †Do not resuscitate or limit on life support order in place.

day 28 and day 60, number of ventilator-free days to day 28, duration of ventilation in patients alive at day 28, number of intensive-care-free days to day 28, number of days free from organ failure to day 28 (cardiovascular, coagulation, hepatic, and renal), and the sequential organ failure assessment (SOFA) score.¹⁶ For respiratory parameters, we calculated the oxygenation index with the following validated measure of respiratory function,^{17,18}

$$\text{oxygenation index} = \frac{\text{FiO}_2(\%) \times \text{mean airway pressure}}{\text{PaO}_2}$$

and with the lung injury score.¹⁹ Biological markers of endothelial injury (angiopoietin 2), inflammation (interleukin [IL]-6 and IL-8), and alveolar epithelial injury (receptor for advanced glycation end products [RAGE]) were measured in plasma collected at baseline, 6 h, and 24 h after infusion, and were measured with ELISA kits (R&D Systems, Minneapolis, MN, USA).

Post-hoc study of MSC viability

After the trial was completed, MSC viability after thawing was found to be variable. Therefore, the

University of California, San Francisco bone marrow transplant laboratory assessed the effects of washing to remove DMSO between thawing and resuspension of the MSCs in Plasma-Lyte A for infusion compared with simple thawing and dilution. We thawed MSCs that had originally been prepared for the trial but were not used. We measured the proportions of viable MSCs with trypan blue exclusion at 0, 30, 60, 120, and 180 min after removal from cryopreservation to simulate the time from before thawing to completing clinical infusion.

Statistical analysis

Because safety was the primary endpoint, we did not calculate sample size on the basis of an efficacy endpoint. We therefore used the FDA's recommendation that 60 patients randomly assigned in a ratio of 2:1 would be enough to assess the primary endpoint. Analyses were done by intention to treat. Descriptive analyses of the variables were expressed as mean (SD), median (IQR), or number (%). All-cause mortality at 28 days and 60 days was analysed with Cox proportional hazard models and reported as hazard ratios (HRs) with 95% CIs. We also report HRs after adjustment for Acute Physiology and Chronic Health Evaluation III (APACHE III) scores to resolve imbalances in severity of illness at baseline. To investigate the effects of MSC treatment on biomarker concentrations in plasma, we used the Mann-Whitney-Wilcoxon test on the original scale or ANCOVA after log transformation to compare values in the MSC and placebo groups at baseline (0 h) with results at 6 and 24 h after the start of infusion. In a post-hoc analysis, MSC viability at the time of administration was separated into tertiles and biomarker changes within 24 h from the start of infusion were compared between tertiles and between the MSC and placebo groups.

Two-sided *p* values less than 0.05 were significant. All statistical analyses were done with Stata software, version 12.0. The independent data safety monitoring board reviewed safety data after 20 and 40 patients were enrolled. This trial is registered with ClinicalTrials.gov, number NCT02097641.

Role of the funding source

The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of this report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

From March 24, 2014, to Feb 9, 2017, 1038 patients were screened for eligibility, of whom 975 were excluded, and 63 were randomly assigned to a treatment group (figure 1). Three patients were not eligible for treatment after randomisation and, therefore, 40 patients received MSCs and 20 patients received placebo (figure 1). All

	Mesenchymal stromal cell group (n=40)	Placebo group (n=20)
Patients' characteristics		
Age (years)	55 (17)	55 (20)
Men/women	23 (58%)/17 (42%)	10 (50%)/10 (50%)
Cause of ARDS		
Sepsis with pneumonia	19 (48%)	12 (60%)
Sepsis without pneumonia	5 (13%)	2 (10%)
Pneumonia without sepsis	11 (28%)	5 (25%)
Aspiration only	4 (10%)	1 (5%)
Other	1 (3%)	0
Arterial pressure (mm Hg)	75 (10)	76 (9)
Taking vasopressors at the time of infusion	24 (60%)	9 (45%)
SOFA score	8.1 (3.3)	6.9 (2.7)
APACHE III score	104 (31)	89 (33)
Respiratory characteristics		
Minute ventilation (L/min)	11.1 (3.2)	9.6 (2.4)
Respiratory rate (breaths/min)	27.8 (6.6)	24.5 (6.3)
Tidal volume (mL/kg PBW)	6.3 (0.9)	6.1 (0.7)
Mean airway pressure (cm H ₂ O)	17.8 (4.9)	16.4 (3.6)
Plateau airway pressure (cm H ₂ O)	26.4 (5.7)	23.7 (5.1)
PEEP (cm H ₂ O)	12.4 (3.7)	10.8 (2.6)
Driving pressure (cm H ₂ O)	14.0 (4.1)	12.5 (4.3)
PaO ₂ :FiO ₂ (kPa)	18.1 (4.3)	19.1 (5.2)
Oxygenation index (kPa)*	98.7 (78.1–123.3)	95.6 (71.6–113.8)
Lung injury score	3.1 (0.4)	3.0 (0.5)
Ventilation mode		
Volume control	37 (93%)	17 (85%)
Pressure-regulated volume control	1 (3%)	2 (10%)
Other	2 (5%)	1 (5%)

Data are n (%), mean (SD), or median (IQR); percentage values might not sum 100% due to rounding. ARDS=acute respiratory distress syndrome; SOFA=sequential organ failure assessment. APACHE III=Acute Physiology and Chronic Health Evaluation III score. PBW=predicted bodyweight. PEEP=positive end-expiratory pressure. PaO₂:FiO₂=ratio of partial pressure of oxygen to fractional inspired oxygen. Oxygenation index=FiO₂ × mean airway pressure / PaO₂. *Unavailable for three patients in the mesenchymal stromal cell group and one in the placebo group because mean airway pressure was not recorded.

Table 1: Baseline characteristics

patients received their allocated treatments and were included in all outcome analyses.

Age, sex, and cause of ARDS were similar in the two groups at baseline (table 1). Scores were numerically higher in the MSC group than in the placebo group for severity of illness (SOFA and APACHE III) and respiratory parameters (minute ventilation and PEEP; table 1).

No patients had any of the prespecified adverse haemodynamic or respiratory safety events during study product infusion or within 6 h from the start of infusion (one-sided 97.5% CI 0–0.09). One patient in the MSC group had a fatal cardiopulmonary arrest 20 h after the

	Mesenchymal stromal cell group (n=40)	Placebo group (n=20)	Odds ratio* or median difference (95% CI)	p value
Mortality to day 28	12 (30%)	3 (15%)	2.4 (0.5 to 15.1)	0.34
Mortality to day 60	15 (38%)	5 (25%)	1.8 (0.5 to 7.6)	0.40
Number of ventilator-free days to day 28	2 (0 to 23)	17 (0 to 24)	0 (–12 to 0)	0.28
Number of ICU-free days to day 28	2 (0 to 19)	14 (7 to 20)	–5 (–11 to 0)	0.05
Number of organ-failure-free days to day 28				
Cardiovascular	18 (7 to 24)	24 (19 to 27)	–4 (–10 to 0)	0.09
Coagulation	28 (17 to 28)	28 (28 to 28)	0 (–3 to 0)	0.10
Hepatic	28 (16 to 28)	28 (25 to 28)	0 (0 to 0)	0.43
Renal	28 (7 to 28)	28 (25 to 28)	0 (–5 to 0)	0.25
Non-pulmonary organs	12 (2 to 24)	22 (13 to 25)	–4 (–13 to 0)	0.10
Duration of ventilation in survivors to day 28 (days)	12 (4 to 24)	8 (4 to 15)	2 (–3 to 10)	0.51

Data are n (%), odds ratio (95% CI), or median (IQR) with median difference (95% CI calculated by Hodges-Lehmann method). Fisher's exact test was used to compare categorical variables, and Mann-Whitney U test for continuous variables. ICU=intensive-care unit. *Calculated for mortality.

Table 2: Main clinical outcomes

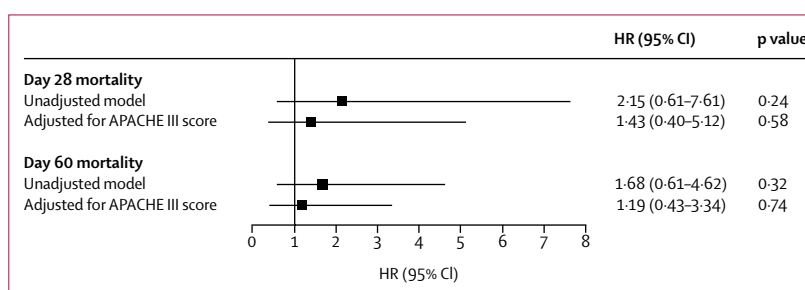


Figure 2: Mortality to day 28 and day 60 for mesenchymal stromal cell therapy versus placebo. APACHE III=Acute Physiology and Chronic Health Evaluation III score; HR=hazard ratio.

start of product infusion and following a sustained fiberoptic bronchoscopy for clinical indications. This patient had a pre-existing history of coronary artery disease. The DSMB judged that the death was probably not related to the MSC infusion. All other deaths occurred after 24 h, and most were attributable to multiorgan failure after withdrawal of life support. The 6-month and 12-month follow-up data are provided in the appendix.

Mortality at 28 and 60 days was non-significantly higher in the MSC group than in the placebo group (table 2). The number of ventilator-free and organ-failure-free days were all numerically fewer in the MSC group than in the placebo group, but the differences were not significant (table 2). The number of intensive-care-free days was higher in the placebo group than in the MSC group (table 2). Further data on the prespecified secondary endpoints and the SOFA score at 3, 7, and 14 days are provided in the appendix. After adjustment for APACHE III score, the HRs for 28-day and 60-day mortality remained non-significant but moved towards 1 (figure 2).

The oxygenation index was numerically higher in the MSC group than in the placebo group at baseline

	Baseline		Change from baseline to 6 h		Change from baseline to 24 h	
	Mesenchymal stromal cell group (n=39)	Placebo group (n=19)	Mesenchymal stromal cell group (n=39)	Placebo group (n=19)	Mesenchymal stromal cell group (n=39)	Placebo group (n=19)
Angiopoietin 2 (pg/mL)	12 004 (9701 to 23 110)	9387 (6558 to 13 261)	-1120 (-4706 to -66)*	287 (-1005 to 2374)	-2080 (-6404 to 47)	-537 (-4294 to 1029)
Interleukin 6 (pg/mL)	167 (61 to 541)	164 (30 to 300)	-51 (-124 to 4)	-20 (-71 to 17)	-34 (-137 to 4)	-43 (-165 to 39)
Interleukin 8 (pg/mL)	56 (34 to 90)	40 (26 to 69)	-2 (-24 to 3)	-1 (-8 to 1)	-5 (-34 to 4)	-5 (-17 to -1)
RAGE (pg/mL)	2065 (994 to 4296)	1818 (1235 to 5190)	-326 (-888 to -45)	-322 (-624 to -93)	-393 (-1118 to -78)	-411 (-1057 to -110)
Protein C (pg/mL)	60 (44 to 76)†	75 (58 to 105)	2 (-3 to 6)	0 (-16 to 5)	4 (-5 to 10)	1 (-11 to 21)

Data are median (IQR). Differences in biomarker concentrations between the mesenchymal stromal cell group and control group were calculated with the Mann-Whitney-Wilcoxon test. RAGE=receptor for advanced glycation end products. *p=0.006 for change before log transformation. †p=0.052 for difference between groups.

Table 3: Comparison of biomarker concentrations in plasma at baseline and at 6 h and 24 h after start of infusion

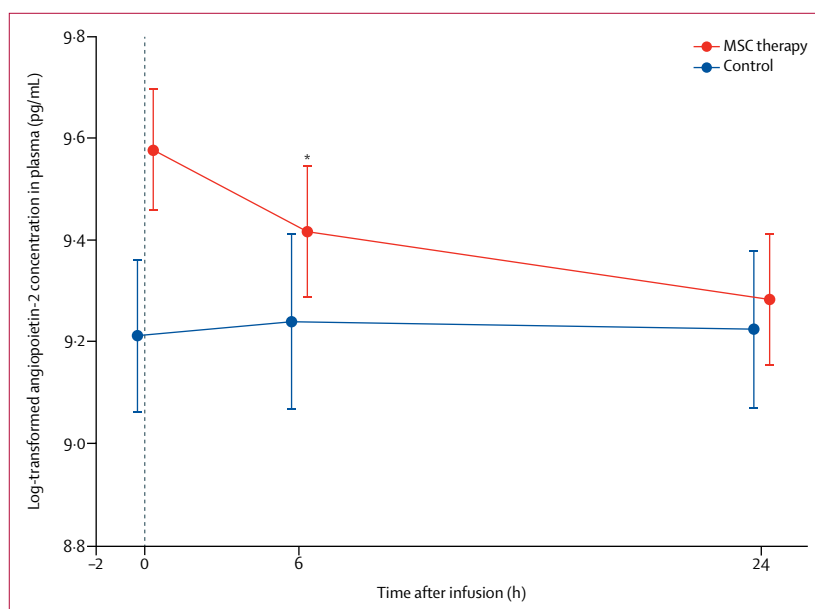


Figure 3: Changes in angiopoietin 2 concentrations in plasma from baseline to 6 and 24 h
Data are mean (SE) after log transformation. MSC=mesenchymal stromal cell. *p=0.005 compared with placebo.

(appendix). Over the following 2 days, oxygenation index in the MSC group reduced but not by significantly more than in the placebo group. A similar pattern in reduction of oxygenation index was seen in patients who remained intubated for 2 days (appendix).

At baseline, protein C concentration in plasma was numerically lower in the MSC group than in the placebo group, but no other biomarkers differed between groups at baseline (table 3). Concentrations of angiopoietin 2 had reduced by 6 h after the start of infusion in the MSC group (figure 3). No changes from baseline were seen for IL-6, IL-8, RAGE, or protein C concentrations at 6 or 24 h in either group.

After completing the study, the viability of MSCs at the time of study product preparation was found to range widely from 36% to 85%. Tertiles of viability were 36–56% (n=14 recipients), 57–69% (n=13), and 70–85% (n=13). In our post-hoc analysis of viability-dependent effects on biomarkers, we found no differences for IL-6,

IL-8, or RAGE at baseline (appendix p 5), but after adjustment for baseline biomarker values, concentrations of angiopoietin 2 decreased progressively, and in the intermediate and the highest tertiles of MSC viability were significantly lower than in the placebo group (appendix). In addition, oxygenation index seemed to improve from baseline to 2 days after the start of infusion in the intermediate and highest tertiles of MSC viability, albeit non-significantly (appendix). Further comparisons of secondary outcomes with the placebo group and between MSC tertiles are provided in the appendix.

Washing to remove DMSO during preparation was associated with a notable reduction in the proportion of viable MSCs compared with simple thawing (figure 4). We found no significant differences in MSC viability among the donors (appendix). MSC viability was higher at one preparation site than the other four (appendix), but we could identify no differences in processing of MSCs in the viability measurements.

Discussion

In this double-blind, randomised, placebo-controlled phase 2a trial, we found no MSC-related predefined haemodynamic or respiratory adverse events within 6 h of the start of infusion in 40 patients with moderate to severe ARDS. One patient died within 24 h of receiving MSCs but the DSMB judged the death unlikely to be related to administration of the cell product.

Mortality at 28 and 60 days was numerically but not statistically higher in the MSC group than in the placebo group. The numerical difference might have been driven by imbalances in the severity of illness, represented by SOFA and APACHE III scores, at baseline. Several baseline respiratory variables (minute ventilation, respiratory rate, oxygenation index, and PEEP level) indicated that respiratory failure might have been more severe in the MSC group than in the placebo group. Although neither the unadjusted nor the adjusted analyses showed significant differences in the secondary outcomes, we recognise that the higher mortality in the MSC group could represent an unfavourable effect of MSCs. Nevertheless, mortality in the placebo group was lower than anticipated,¹² whereas that in the MSC group was as

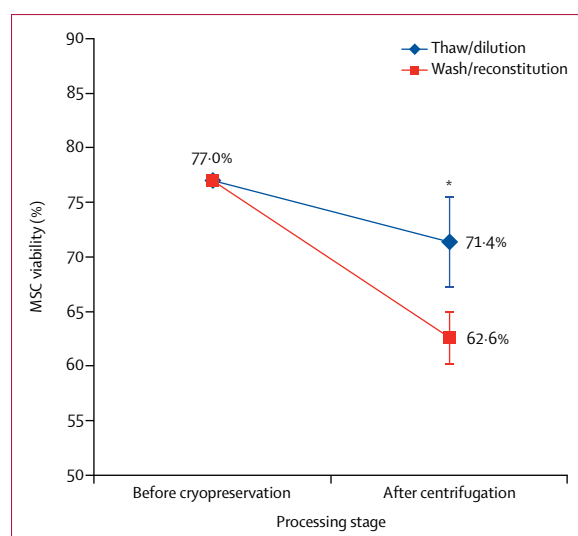


Figure 4: MSC viability measured after simple thawing and dilution versus after cell wash and reconstitution

Data are mean (SD) based on viability measurements at 0, 30, 60, 120, and 180 min after removal from cryopreservation. MSC=mesenchymal stromal cell. * $p=0.004$ compared with before cryopreservation.

anticipated. Additionally, drawing conclusions from this small clinical trial is difficult due to wide CIs for the secondary endpoints.

Post-hoc analysis indicated reductions in oxygenation index over the 2 days following infusion among patients who received MSCs compared with those who received placebo. This analysis is limited for several reasons, including death, extubation, and some missing data. We attempted to account for these effects by doing a paired analysis only in patients intubated during the 2 days after infusion, and the findings remained similar. However, the numerical improvement in oxygenation should not be overinterpreted as a favourable finding until it has been assessed further in a larger trial.

The decrease of concentrations of angiopoietin 2 in plasma was significantly greater in the MSC group than in the placebo group 6 h after the start of infusion. This decrease suggests a biological effect of the MSC treatment. Angiopoietin 2 is a well recognised mediator and biomarker of pulmonary²⁰ and systemic vascular injury.²¹ We have found previously that angiopoietin-2 concentrations have important predictive value for the development of ARDS.²² In addition, they robustly predict poor clinical outcomes in adults²³ and children²⁴ with ARDS, and are recognised as pathogenetic and prognostic factors in patients with pneumonia.²⁵ The reductions in angiopoietin-2 concentrations in this trial might have been related to the release of anti-inflammatory factors that can moderate lung injury.⁵ No significant changes were seen in concentrations of IL-6, IL-8, RAGE, or protein C.

The most unanticipated finding in this trial, found only after study completion, was the wide range of

MSC viability after preparation for infusion (36–85%). Substantial variations had not been anticipated and, therefore, we planned no checks of viability during the trial. Because of this finding, we did a post-hoc analysis, which showed a significant viability-dependent effect on angiopoietin-2 concentrations in plasma 6 h after administration. No effect on angiopoietin 2 was seen in the lowest viability tertile, but significant differences between groups were seen in the intermediate ($p=0.02$) and highest tertiles ($p=0.001$). We also saw a slight improvement in oxygenation index on day 2 in the intermediate and highest viability tertiles. No other significant differences were seen, although there was a favourable shift of the adjusted HR for mortality in the higher MSC viability tertiles. Although the differences are inconclusive, they suggest that there is potential for improved efficacy of MSCs with adequate viability. We suggest two explanations for the variation in MSC viability in this trial. First, a study from the University of Minnesota, which has a centre that produces MSCs, showed 14% less eventual MSC viability by flow cytometry with cell products derived from cryopreserved rather than fresh bone marrow.²⁶ Second, our procedure in the trial to remove the DMSO from the thawed MSCs also seemed to reduce MSC viability. The scientific justification for the wash step was based on unpublished data from our sheep studies of *P aeruginosa* pneumonia which suggested that DMSO lowered the efficacy of MSC on oxygenation.⁶ For future trials, we will obtain all MSCs from the University of Minnesota and will use those showing 100% viability before distribution. We will also eliminate the wash step for removal of DMSO.

Some questions remain about the biology and clinical testing of MSCs harvested from bone marrow, umbilical cord, or adipose tissue. An important issue is the uncertainty about the effects of live and dead MSCs.²⁷ Some data suggest that live and dead human cells evoke similar responses in mouse lungs and in some experimental studies, dead cells have produced favourable immune responses after being phagocytised by monocytes or macrophages.²⁷ More research is needed to optimise the methods for MSC cryopreservation, thawing, and production of MSCs for clinical trials.

Several other therapy-related issues deserve comment. The optimum delivery route for MSC therapy for ARDS has not been established. In our preclinical studies we used intrabronchial, intratracheal, and intravenous routes, all of which were associated with efficacy.^{7,9} Because of an excellent safety track record of MSC administration by the intravenous route in other clinical disorders, the FDA advised us to test the intravenous route in our safety trials of MSCs for ARDS, as this would probably be more reasonable for eventual regulatory approval than administration by the intrabronchial route. We aimed to give MSC therapy in the exudative phase of ARDS. In one study, 74 (90%) of 82 patients who died within 7 days of

diagnosis of ARDS had post-mortem evidence of the exudative phase of lung injury and minimal evidence of fibrosis.¹⁰ Because we treated patients within 7 days of ARDS diagnosis, it seems likely that administration of MSCs during the exudative phase was achieved.

We screened a large number of patients for this trial to be able to recruit 60 (6%). In multiple phase 3 trials done by the National Heart, Lung, and Blood Institute ARDS Network, approximately 10% of screened populations were enrolled. We have noted differences between observational studies and clinical trials of ARDS in the selection of patients and the presence of comorbidities relative to the higher mortality reported.²⁸ The results of clinical trials in which screening includes several exclusion criteria, as in this trial, are not necessarily generalisable to the broader unselected population of ARDS patients. If such trials test supportive care interventions, such as low tidal volume or fluid conservative therapy, the findings might have broader relevance,² but, for pharmacological and cell-based therapies, extrapolation of the findings might not be appropriate.

Further limitations of this trial were that the sample size was too small to reliably assess efficacy. For example, the Billiards trial,²⁹ which tested a strategy of reduced tidal volume ventilation against an established strategy in 52 patients with ARDS, showed no difference in clinical outcomes between groups. By contrast, the larger phase 3 ARMA trial of 861 patients³⁰ showed reduced in-hospital mortality with low tidal volume ventilation. The findings of phase 2 trials in ARDS, in which CIs are often wide, therefore, have limited generalisability.³¹ Additionally, some analyses in this trial were done post hoc without adjustment for multiple comparisons.

This randomised, double-blind, phase 2a trial of treatment with human bone-marrow-derived MSCs for moderate to severe ARDS in critically ill patients, including patients with vasopressor-dependent shock at baseline, showed no infusion-related haemodynamic or respiratory adverse events. The patients in the MSC group had numerically higher disease severity scores than those in the placebo group at baseline, but mortality did not differ significantly between groups. Concentrations of angiopoietin 2 in plasma were significantly reduced at 6 h in MSC recipients. An important finding was that MSC viability had a significant viability-dependent effect on angiopoietin-2 concentrations. We anticipate being able to avoid wide variability in MSC viability in future trials by changes to product preparation methods. Larger trials will be needed to test the efficacy of MSCs for ARDS. We are planning a phase 2b trial in 120 patients that will be powered to detect efficacy for respiratory endpoints, with oxygenation index as the primary endpoint.

Contributors

MAM, CSC, HZ, BTT, JGW, JEG, LAO, KLD, LC, MM, DHM, and KDL designed the study design and developed the protocol. MAM, CSC, BTT, JEL, AJR, JEG, JPW-K, EKB, MPD, BJM, ME, JWC, and KDL were

responsible for study enrolment and data collection. MAM, CSC, HZ, JEG, JA, and KDL were responsible for biorepository management and biomarker analyses. MAM, CSC, HZ, JEG, KLD, and KDL analysed the data and wrote the report. All authors revised the report and read and approved the final version before submission.

Declaration of interests

MAM has received grants from Bayer Pharmaceuticals and GlaxoSmithKline, personal fees from Boehringer Ingelheim, Cerus Therapeutics, CSL Behring, Quark Pharmaceuticals, Roche-Genentec, and Thesano Pharmaceuticals. CSC has received grants and personal fees from Bayer and GlaxoSmithKline, personal fees from Boehringer Ingelheim, CSL Behring, Prometic, and Roche/Genentec. EKB has received personal fees from Merck. BJM has received grants from Bayer Pharmaceuticals and personal fees from Vapotherm. KDL has received personal fees from Achaogen, Durect, Potrero Medical, Quark, Theravance, and Z S Pharma, and other funding or awards from Amgen. The other authors declare no competing interests.

Data sharing

No additional data are available for this Article.

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