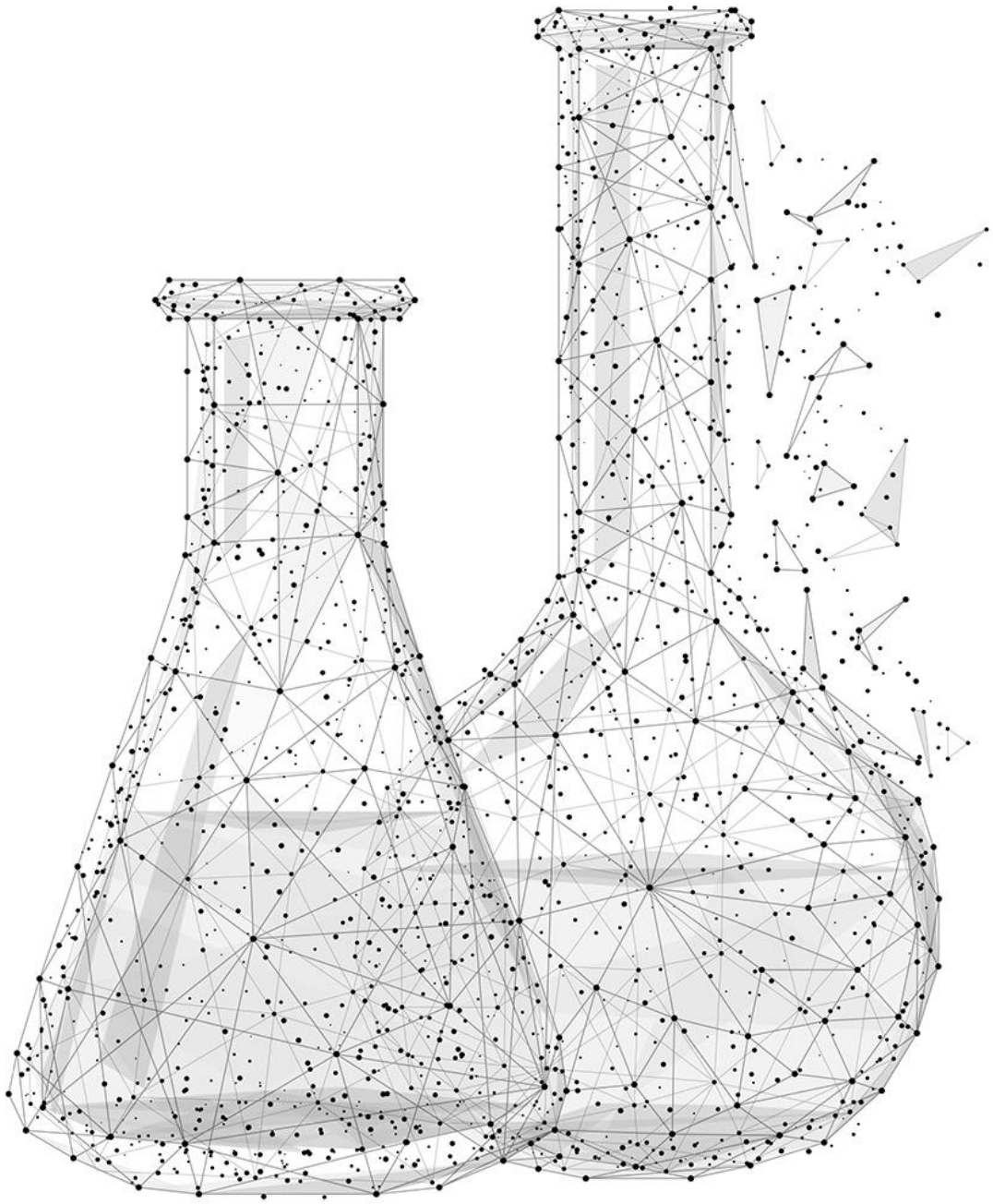

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TRANSLATIONAL AND CLINICAL RESEARCH

Treatment of Severe Adult Traumatic Brain Injury Using Bone Marrow Mononuclear Cells

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Key Words. traumatic brain injury • bone marrow stromal cells • cellular therapy • clinical trials • diffusion tensor imaging • adult stem cells • adult human bone marrow

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Received August 1, 2016; accepted for publication October 16, 2016; first published online in STEM CELLS *EXPRESS* November 1, 2016.

1066-5099/2016/$30.00/0

http://dx.doi.org/10.1002/stem.2538

ABSTRACT

Preclinical studies using bone marrow derived cells to treat traumatic brain injury have demonstrated efficacy in terms of blood–brain barrier preservation, neurogenesis, and functional outcomes. Phase 1 clinical trials using bone marrow mononuclear cells infused intravenously in children with severe traumatic brain injury demonstrated safety and potentially a central nervous system structural preservation treatment effect. This study sought to confirm the safety, logistic feasibility, and potential treatment effect size of structural preservation/inflammatory biomarker mitigation in adults to guide Phase 2 clinical trial design. Adults with severe traumatic brain injury (Glasgow Coma Scale 5–8) and without signs of irreversible brain injury were evaluated for entry into the trial. A dose escalation format was performed in 25 patients: 5 controls, followed 5 patients in each dosing cohort (6, 9, 12 $\times 10^6$ cells/kg body weight), then 5 more controls. Bone marrow harvest, cell processing to isolate the mononuclear fraction, and re-infusion occurred within 48 hours after injury. Patients were monitored for harvest-related hemodynamic changes, infusional toxicity, and adverse events. Outcome measures included magnetic resonance imaging-based measurements of supratentorial and corpus callosal volumes as well as diffusion tensor imaging-based measurements of fractional anisotropy and mean diffusivity of the corpus callosum and the corticospinal tract at the level of the brainstem at 1 month and 6 months postinjury. Functional and neurocognitive outcomes were measured and correlated with imaging data. Inflammatory cytokine arrays were measured in the plasma pretreatment, posttreatment, and at 1 and 6 month follow-up. There were no serious adverse events. There was a mild pulmonary toxicity of the highest dose that was not clinically significant. Despite the treatment group having greater injury severity, there was structural preservation of critical regions of interest that correlated with functional outcomes. Key inflammatory cytokines were downregulated. Treatment of severe, adult traumatic brain injury using an intravenously delivered autologous bone marrow mononuclear cell infusion is safe and logistically feasible. There appears to be a treatment signal as evidenced by central nervous system structural preservation, consistent with previous pediatric trial data. Inflammatory biomarkers are downregulated after cell infusion. STEM CELLS *2017;35:1065–1079*

SIGNIFICANCE STATEMENT

This phase I/IIa study of adult severe TBI sought to confirm the safety and logistic feasibility of autologous bone marrow mononuclear cells for severe TBI, as well as determine a potential treatment effect size of white matter structural preservation. Our primary outcomes were MRI-based measurements of supratentorial and corpus callosal volumes as well as DTI-based measurements of fractional anisotropy (FA) and mean diffusivity (MD) of the corpus callosum and corticospinal tract at the level of the brainstem. One limitation of our study is that since it was phase I/IIa it was not randomized and our treatment groups had a higher injury severity profile than our control groups. Despite this, we found that the FA and MD in the corticospinal tract are clearly improved by bone marrow mononuclear cell (BMMNC) treatment, and the improvement in FA in the corpus callosum has a treatment effect significant enough for us to calculate power for a future phase II study.

Introduction

Approximately 1.5 million people suffer traumatic brain injury (TBI) yearly in the United States. The annual mortality approaches 50,000 with the remaining patients suffering from varying levels of long-term sequelae[1]. Overall, 6.5 million patients are burdened by the physical, cognitive, and psychosocial deficits associated with TBI leading to a total economic impact of approximately 60 billion dollars[2].

Numerous preclinical studies have shown that autologous bone marrow derived mononuclear cells (BMMNCs) improve outcomes after TBI with a positive effect on early outcomes (blood brain barrier permeability and cerebral edema) as well as late functional outcomes such as improved spatiotemporal memory. These positive effects on functional outcomes have been replicated in various animal stroke models as well[3]. Similarly, in both injury models, BMMNCs dampen the secondary neuroinflammatory response to injury[4–6]. Finally, there appears to be a consistent treatment window of 24–72 hours[7]. In terms of translational feasibility, autologous BMMNCs have shown potential benefit in preclinical proof-of-concept studies in TBI and stroke, and have an excellent safety profiles in Phase 1 trials of pediatric TBI and adult stroke[8–10].

We have pursued the use of autologous BMMNCs for a number of reasons: (a) well-developed preclinical proof of concept and toxicity data, (b) Phase 1 trial without observed serious adverse events or toxicity, (b) no immune barrier considerations, (d) biologically sound route of delivery due to the 5–8 micron cell size (vs. 13–19 micron size for mesenchymal stromal cells) obviates pulmonary sequestration, or the "pulmonary first pass effect" making intravenous delivery more practical[11], (e) no in vitro culture/scaling issues for autologous application, (f) ready availability, (g) no issues with uncontrolled replication as with embryonic stem cells or fetal cells, and (h) no ethically objectionable issues with cell type.

The purpose of this study was to evaluate the safety, logistical feasibility, and potential signals of a treatment effect in a prospective, single center, dose escalation trial in adult patients with acute TBI. Safety was assessed by organ injury related to infusional toxicity. The efficacy outcome measure was based upon serial imaging data obtained early postinjury and at 6 months, evaluating global white matter volume and metrics of white matter integrity using diffusion tensor magnetic resonance imaging. Exploratory analysis of circulating markers of the inflammatory response to injury was evaluated sequentially before and after injury as well as at 6 months (representing the patient's endogenous baseline preinjury).

Materials and Methods

The Treatment of Severe Adult Traumatic Brain Injury Using Bone Marrow Mononuclear Cells (NCT 0157540) trial was an open label, nonrandomized, single center clinical trial. This phase I/IIa trial was performed under the authority of the Federal Investigational New Drug Application BB 12620. The trial was approved by the University of Texas Health Sciences Center at Houston Committee for the Protection of Human Subjects and by the Memorial Hermann Office of Research. Patients were enrolled into the trial from March of 2012 through October of 2014. The trial was funded by the U.S. Army Medical Research and Materials Command grant number W81XWH-11-1-0460. Safety monitoring during the trial was provided by an independent medical monitor and a medical safety monitor in coordination with the data safety monitoring board. The study was planned to proceed until completing enrollment or after one of the following stopping rules: 4 deaths; PaO_2:$FiO_2 < 70$ with a $PaCO_2 > 90$ mmHg within 48 hours of infusion; Alanine Transferase/Aspartate Transferase > 900 U/dL within 24 hours of infusion in any subject; Grade 4–5 central nervous system cerebrovascular ischemia event or Grade 4–5 seizure event as defined by version 4 of the National Cancer Institute Common Terminology Criteria for Adverse Events within 12 hours of infusion; Any Grade 4–5 adverse event as defined by the National Cancer Institute Common Terminology Criteria for Adverse Events that is determined to be temporally-related by the data safety monitoring board.

Study Population

Trauma patients between the ages of 18 and 55 years of age presenting to the trauma Emergency Department were screened for enrollment into the study. Eligible patients were those with nonpenetrating head trauma, a postresuscitation Glasgow Coma Scale (GCS) [12] of 5–8 and injury occurring less than 24 hours prior to consent. Patients were excluded on the basis of pre-existing serious medical comorbidities or infection as revealed by patient history: prior moderate-severe brain injury, major psychiatric disorders, or any neurologic impairment or seizure disorder. In addition, patients were excluded if there was evidence of hypoxic-ischemic insult, opening intracranial pressure > 40 mmHg, continued hemodynamic instability at the time of consent, uncorrected coagulopathy at the time of harvest, pelvic fracture requiring operative fixation, spinal cord injury, American Association for the Surgery of Trauma grade IV solid or hollow visceral organ injury, prolonged hypoxia, weight ≥ 300 lbs. (magnetic resonance imaging [MRI] limitation), pregnancy, and unwillingness to return for follow up visits (Table 1).

Study personnel approached the patient's legally authorized representative (LAR) with information about study participation, and the PI/Co-I obtained informed consent. A research intermediary interviewed each LAR to confirm consent and avoid therapeutic misconception. A total of 25 patients were enrolled into the study. Patients were enrolled sequentially into one of five different groups: control group 1($n = 5$; sham intervention), low-dose treatment group ($n = 5$; 6×10^6 cells/k), medium dose treatment group ($n = 5$; 9×10^6 cells/kg), high-dose treatment group ($n = 5$; 12×10^6 cells/kg), and control group 2 (n = 5; sham intervention). The additional controls were added to include patients across the time spectrum of the study.

Patient Management

Patients were admitted to the Shock Trauma or Neurotrauma Intensive Care Units at Memorial Hermann Hospital, which is an American College of Surgeons Verified/Texas Department of Health approved Level 1 Adult Trauma Center. Patients initially resuscitated according to Advanced Trauma Life Support guidelines, and subsequently in accordance with the *Guidelines for the Management of Severe Traumatic Brain Injury,*

Table 1. Trial inclusion and exclusion criteria

Inclusion criteria
Between 18 and 55 years of age on the day of injury
Postresuscitation Glasgow Coma Scale of 5–8
Initial injury occurring less than 24 hours prior to consent
English speaking
Exclusion Criteria
Known history of: prior brain injury, psychiatric disorder, neurological impairment and/or deficit,
Seizure disorder requiring anti-convulsant therapy, recently treated infection, renal disease,
Hepatic disease, cancer, substance abuse or positive urine drug screen at admission, cancer,
Immunosuppression, human immunodeficiency virus
Obliteration of perimesencephalic cistern on initial head computed tomography suggesting prolonged hypoxic ischemic
Insult
Opening intracranial pressures >40 mmHg
Hemodynamic instability at the time of consent with ongoing fluid resuscitation and/or inotropic
Support[a]
Uncorrected coagulopathy at the time of bone marrow harvest defined as international normalized ratio > 1.6, partial thromboplastin time > 36s, platelet <
100k, Fibrinogen < 100 mg/dL
Unstable pelvic fracture requiring operative fixation
Pulmonary contusions defined as a chest X-ray with nonanatomic opacification and/or partial pressure arterial oxygen: fraction of inspired oxygen
< 250 associated with mechanism of injury
Greater than American Association for the Surgery of Trauma Grade III solid or hollow visceral injury of the abdomen and/or pelvis
Spinal cord injury
Weight ≥ 300 lbs
Any contraindication to magnetic resonance imaging
Positive urine pregnancy test
Participation in a concurrent interventional study
Unwillingness to return for follow-up visits

The inclusion and exclusion criteria had the intent of including patients with acute, severe TBI without signs of irreversibility. Also, severe other organ injury was excluded as defined in the exclusions with most of these being excluded due to the presence of hemorrhagic shock.
[a]Does not include cerebral perfusion pressure-based inotropic support.
Abbreviation: TBI, traumatic brain injury.

3rd Edition[13]. Central vascular catheter placement was performed via either the subclavian or femoral route, as well as radial or femoral arterial cannula placement according to standard techniques. Coagulopathy was corrected with fresh frozen plasma titrated based on thromboelastography results on arrival to the hospital and to the intensive care unit (ICU). All patients received an intraparenchymal pressure monitor or ventriculostomy for continuous monitoring of intracranial pressures (ICP) and drainage of cerebrospinal fluid (CSF). ICPs were managed in accordance with Memorial Hermann Trauma Guidelines with a goal ICP < 20. If ICP was refractory to mechanical measures and respiratory measures (minimal stimulation, head of bed elevation, $PaCO_2$ 35–40), hyperosmotic therapy was initiated with a goal sodium 145–155. Sedation was managed singly or in combination with infusions of midazolam and fentanyl or propofol and titrated according to the Richmond Agitation and Sedation Scale and maintaining ICP below 20. For refractory ICP spikes a 30 ml bolus of 23.4% NaCl was infused. Unexpected neurologic changes, ICP spikes, or refractory-elevated ICP prompted repeat computed tomography (CT) imaging of the brain. Each ICU-treated patients with seven days of phenytoin for posttraumatic seizure prophylaxis.

Study Intervention

Bone Marrow Harvest. Patients enrolled into the low, medium, and high-dose treatment arms underwent bone marrow harvest within 36 hours of injury. The harvest was performed aseptically using an 11 or 15 gauge trocar from the anterior superior iliac crest of each patient. A total volume of 3 to 5 ml per kg of body weight was aspirated from each patient with a 30 ml syringe. Pain was controlled through the use of local anesthesia (1% lidocaine without epinephrine) and infusions of fentanyl, midazolam, and/or propofol.

Blood pressure, heart rate, oxygen saturation, and intracranial pressure were measured at 5 minute intervals for the duration of the procedure. Recordings of these parameters were continued every 15 minutes postharvest for 1 hour, then every 30 minutes for 2 hours, and every hour until the time of cell product infusion. Hemoglobin and hematocrit were measured at 4 hour intervals for 12 hours after the harvest. Harvest-related adverse events were defined as a 20% decrease in either cerebral perfusion pressure (CPP) or mean arterial pressure (MAP) that was sustained for at least 10 minutes.

Cell Processing. Following completion of the procedure, the bone marrow sample was transferred in an anticoagulant-containing blood collection bag at ambient temperatures to the processing facility in a validated cooler using a professional courier service in compliance with requirements dictated by the Code of Federal Regulations Titles 29 and 49 for both Occupational Safety and Health Administration and Health Insurance Portability and Accountability Act Medical Courier and Biohazard training. The cell processing facility was located at the University of Texas Health Science Center at Houston-Medical School, Evelyn H. Griffin Stem Cell Therapeutics Research Laboratory. The facility is Food and Drug Administration-registered, Foundation for the Accreditation of

Cell Therapy-accredited, and compliant with current Good Manufacturing Practice (cGMP).

Upon its arrival at the cGMP facility, the bone marrow was filtered through a 170–260 μm filter to remove aggregates and/or spicules. The bone marrow was sampled for aerobic, anaerobic, and fungal sterility as well as nucleated cell count and multiparameter flow cytometric analysis. The MNC-enriched fraction was obtained by density gradient centrifugation of the bone marrow on Ficoll-Paque PREMIUM (GE Healthcare Life Sciences, Pittsburgh, PA, USA) using the Biosafe Sepax 2 RM cell processor, the NeatCell protocol, and the CS-900.2 kit for regenerative medicine applications (Biosafe America, Houston, TX, USA). Risk management methods used for the production of clinical-grade cells were performed, and all reagents used had the manufacturer's certificate of analysis on file and maintained according to applicable regulation. Any residual Ficoll-Paque PREMIUM was removed during the automated washing step of the Sepax 2 RM cell enrichment procedure and no other infusion-incompatible reagents were added during manufacturing.

Prior to product release, aliquots of the final product were taken for quality-control testing (nucleated cell count, viability, gram stain, 14 day aerobic and anaerobic bacterial and 28 day fungal cultures, endotoxin and mycoplasma content, colony forming unit assays and multiparameter flow cytometry for an extended differential cell count and to assess cell population identity). Only products that passed the release acceptance criteria (negative Gram stain; $>$70% viability determined with the Trypan Blue exclusion method and endotoxin levels $<$5.0 EU/Kg measured using the Endosafe PTS system by Charles River Laboratories) were authorized by the quality team for infusion. Upon meeting the release criteria, the final dose consisting of 6×10^6 cells/ml/kg, 9×10^6 cells/ml/kg or 12×10^6 cells/ml/kg in 0.9% saline containing 5% volume/volume human serum albumin was prepared in one or two sterile syringes and transported to the infusion site at ambient temperature in a validated cooler using a professional courier service.

Cell Product Infusion. The patients enrolled into the low dose, medium dose, and high dose groups were to receive target doses of 6×10^6 BMMNC/kg, 9×10^6 BMMNC/kg, and 12×10^6 BMMNC/kg, respectively. The dosing range was derived from our preclinical laboratory data[6] and our prior phase I pediatric trial which used a similar protocol[8]. The BMMNC infusion was performed through either peripheral or central venous catheters approximately 7–8 hours after harvest. Postinfusion monitoring of postharvest hemodynamics was performed every 15 minutes for the first hour, every 30 minutes for hours 2–3, and 1 hour for hours 3–7.

Flow Cytometry. A four-color direct immunofluorescent "lyse/no wash" labeling method was used in the evaluation of progenitor cells and lymphocyte subsets. Samples of bone marrow starting material and final product (MNCs) were stained with the following four panels: (a) 7AAD to assess overall viability; (b) CD45/CD14 to identify lymphocytes, lymphoblasts, monocytes, and granulocytes; (c) CD45/CD19/CD3/CD16 + 56 to identify T, B, NK, and NKT subsets; (d) Lin1/CD34/CD45/CD133 to identify hematopoietic stem cells and other progenitor cells. The fourth panel was done in TruCount tubes (BD Biosciences, San Jose, CA) to allow calculation of absolute cell counts. The following antibodies were used at saturated concentrations: CD45 clone 2D1 conjugated to PerCP-Cy5.5 (BD Biosciences), CD14 clone M5E2 conjugated to FITC (BD Biosciences), MultiTest CD3/CD16 + 56/CD45/CD19 conjugated to FITC/PE/PerCP/APC (BD Biosciences), CD34 clone 561 conjugated to APC (BioLegend, San Diego, CA), CD133/1 clone AC133 conjugated to PE (Miltenyi Biotech, San Diego, CA), and Lineage 1 cocktail ("Lin-1" consisting of CD3, CD14, CD16, CD19, CD20 and CD56) conjugated to FITC (BD Biosciences). Immediately after lysing samples for 15 minutes with PharmLyse (BD Biosciences), data were acquired with an LSRII cytometer equipped with FACSDiva software (BD Biosciences). For the four-part differential panel 10,000 singlet cells were acquired, for the lymphocyte subsets 10,000 singlet CD45+ lymphs were acquired, and (where possible) 100,000 singlet CD45+ cells were acquired for progenitor cells. Analysis was performed with FlowJo (Tree Star Inc., Ashland, OR) and FCS Express 4 (De Novo Software, Glendale, CA). The differential was analyzed using CD45 and light scatter gates, with CD14 to confirm monocytes. Lymphocyte subsets were gated using the CD3, CD19, and CD16 + 56 markers within the low side-scatter CD45+ lymphocyte population. The progenitor cells were evaluated using ISHAGE gating strategy for total CD34+ progenitors, with additional gating to identify the Lin-1[neg]CD34 + CD133+, Lin-1[neg]CD34 + CD133[neg], and Lin-1[neg]CD34[neg]CD133+ subsets. Process controls CD-Chex Plus and CD-Chex CD34 (Streck, Omaha, NE) were used to ensure assay consistency.

Study Outcomes. The primary aims of this study were to evaluate safety of bone marrow harvest/infusional toxicity of BMMNC after severe TBI, compare changes in white matter metrics longitudinally and investigate potential changes in the inflammatory cytokine response.

Harvest procedure safety was measured by measuring systemic and cerebral hemodynamic responses to bone marrow withdrawal as well as harvest site complications. To assess for multiorgan dysfunction the Sequential Organ Failure Assessment (SOFA) was calculated prospectively for each patient which has been validated for use in trauma patients[14]. Evidence of pulmonary infusion-related toxicity was determined by using the Murray score which is based upon PaO_2:FiO_2, chest radiograph, lung compliance, and positive end expiratory pressure[15]. A basic metabolic profile and complete blood count with differential were obtained each day in the intensive care unit to monitor for renal or hematologic insults. Hepatic transaminases were followed daily to assess for any potential hepatic injury as a result of microthrombosis. Finally, neurologic status was also assessed daily while in the ICU. Intracranial pressure was recorded hourly by the nursing staff and entered into the hospital's electronic medical record. With the exception of bone marrow harvest and BM-MNC infusion monitoring, all ICP data used for analysis were obtained from the hospital's electronic medical record.

Whole blood was obtained at the time of consent and in the acute period every 12 hours for 7 days following infusion by study staff. Chronic samples were obtained at 1 month and 6 months. Blood samples were collected in EDTA tubes

and immediately centrifuged to obtain a platelet poor plasma sample. All samples were then stored at −80°C until biomarker analyses were performed. Cytokine levels for interleukin 1 beta (IL-1β), interleukin 4, interleukin 6, interleukin 10, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) were then assessed by enzyme-linked immunosorbent assay (Legend Max™, BioLegend, San Diego, CA, USA).

Imaging Protocols. All imaging data were acquired using a General Electric 3.0 Tesla Sigma HD scanner with an eight channel parallel imaging head coil. Imaging was performed at 1 month and 6 months from injury to acquire data for volumetric and diffusion tensor imaging (DTI) comparisons. MRI acquisition parameters for the high-resolution anatomical and 30-direction DTI sequences quantitatively analyzed in this study are as follows:(a) Sagittal isotropic 3D T1-weighted spoiled gradient echo ($1 \times 1 \times 1$ mm^3); repetition time (TR) = 7.236 ms; echo time (TE)=2.968 ms; flip angle = 11. 2) Axial 30-direction single-shot spin-echo diffusion sensitized echo-planar (with additional 3 b_0 volumes) ($2.73 \times 2.73 \times 2.70$ mm^3): TR = 14900 ms; TE = 85.6 ms; flip angle = 90. The high-resolution anatomical MRI sequence from each MRI session was processed using Freesurfer software version 5.3.0 (www.surfer.nmr.mgh.harvard.edu) to obtain global measures of supratentorial and total white matter volumes. The isotropic 30-direction DTI sequence from each imaging session was processed with FMRIB's Software Library version 5.0.7 (FSL, Oxford, UK) to correct for eddy currents and motion before using DTIFIT to estimate the diffusion tensor at each voxel. Based on prior neuropathological and imaging studies reporting major impact of TBI on corpus callosum (CC) and corticospinal tract[16], we selected these regions for analysis. To obtain DTI metrics of white matter integrity from the CC, a binary mask was manually created on three adjacent midsagittal slices. Additionally, DTI metrics from five subregions of the CC were obtained by creating individual binary masks for each of five equally-spaced segments of the CC. To obtain DTI metrics from a region of interest at the level of the brainstem, a single axial slice was selected for probing the cortical spinal tract (CST) at the midpons level where the pontine crossing tract was distinctly visible[17].

Neurocognitive Outcome Testing. A licensed neuropsychologist blinded to treatment groups performed neurobehavioral testing as well as functional outcome assessments at 1 month and 6 months following injury. Assessments were determined by direct assessment of each patient and patient or caregiver interviews. The measures are consistent with recommendations supported by the NIH, National Institute of Disability and Rehabilitation Research, National Institue of Mental Health, and DoD for the use of common outcome measures in TBI research in adults[18] as well as recommendations for assessment of outcome in clinical trials of TBI[19]. Functional outcomes included ratings of the global level of functioning, independence in daily functioning, adjustment, and community participation. Neuropsychological outcome measures targeted psychological adjustment, declarative memory, working memory, attention, fine motor skills, processing speed, and verbal fluency. The neurocognitive testing matrix in the Supporting Information figures provides descriptive information and lists dependent variables of interest for each measure in italics.

Data Analysis

Data are presented as mean ± SEM. Neuroimaging metrics were evaluated using repeated measures ANOVA used to evaluate within subject change over time in each group and test for group*time interaction. Correlations of fractional anisotropy (FA) of the CC and neurocognitive outcomes were assessed using Spearman's rho. Biomarker data were analyzed in both a pooled and dose-dependent format using ANOVA and post hoc nonparametric analysis with Wilcoxon Rank-Sum after confirming nonnormal distribution of data. Cohen's d was calculated to estimate treatment effect size between control and treated groups.

Results

Enrollment and Patient Characteristics

Through the course of the study 320 patients were screened for enrollment with 90 in the control phase and 230 in the treatment phase (Fig. 1). During the control phase, 11 patients were found to meet eligibility requirements with the LAR agreeing to consent in 10 cases. Twenty-two out of 230 patients screened during the treatment phase met eligibility requirements. In four cases, the patient's LAR declined enrollment, one patient had no available LAR, and patients were unable to be enrolled due to a safety review in two cases. The most frequent reason for not meeting study eligibility was a postresuscitation GCS outside of the 5–8 range.

Seven of 10 patients in the control group and 14 of 15 in the treatment group were able to complete all phases of study follow-up. One patient in the control group was lost to follow-up prior to the 1 month postinjury assessment. Additionally, three control patients and one treated patient were unable to complete the 6-month time point imaging study. No patient fatalities were recorded during the 6-month study follow up period.

Sex distribution was similar between control and treatment groups with approximately 70% of patients enrolled male. Injury severity scores and the head component of the abbreviated injury scale (AIS-head) were calculated and provided by the hospital trauma registry. These scores in addition to postresuscitation GCS were similar across all patient cohorts. With the exception of one patient in the control arm of the study, all AIS-head scores were greater or equal to 3 and thus consistent with alternative measures of severe TBI.

Table 2 describes the patient characteristics. In the control arm, 1 out of 10 patients underwent surgical decompression *on presentation* for their injury-not after or a consequence of treatment. Four out of 15 patients in the treated arm (one low dose, one medium dose, two high dose) required decompressive craniectomy for ICP management on presentation. No patients in the control arm had ventriculostomy placement on initial presentation or for management of refractory ICP. In the treated arm of the study, two patients underwent ventriculostomy placement for initial management of ICP and three patients underwent ventriculostomy placement after several days of admission for refractory ICP issues. Additionally, the peak ICP and Therapeutic Intensity Level recorded prior to treatment and over the first 24 hours of injury was

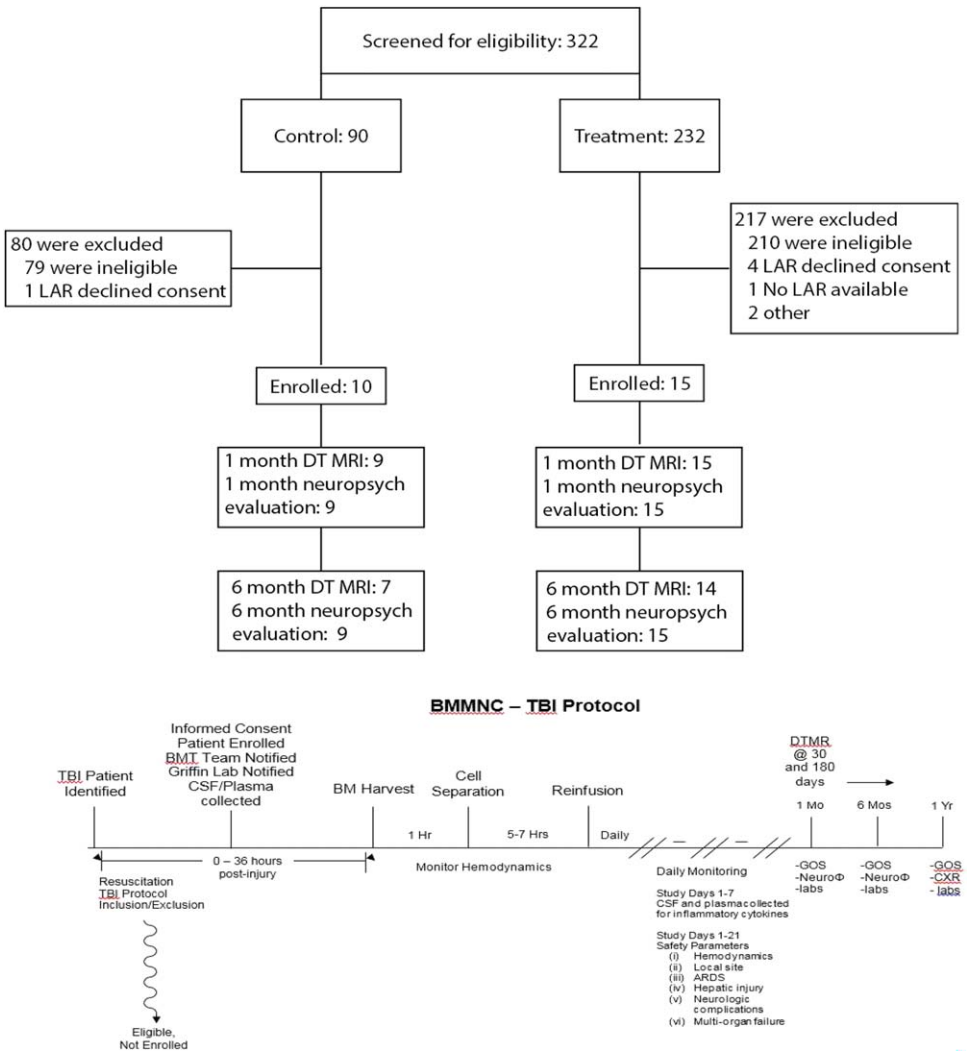

Figure 1. CONSORT diagram for trial enrollment/participation. Patients were screened with a ×greater than 10:1 ratio of screened to be enrolled. This has logistical implications for trial design. Long-term follow-up was exceptionally strong for an acute trauma study (normally approximately 30–50%). The flow diagram shows how patients are identified, enrolled, and treated within 48 hours, with a measured mean of approximately 36 hours. Abbreviations: BMMNC, bone marrow mononuclear cell; CSF, cerebrospinal fluid LAR, legally authorized representative, TBI, traumatic brain injury.

significantly higher in treated patients than controls (Supporting Information Fig. S1).

Bone Marrow Harvest

Bone marrow harvest was performed at an average of 28 hours following admission. Harvest was performed at bedside in 14 of 15 cases and in the operating room following a decompressive craniectomy in one case. No major adverse events were recorded during bone marrow harvest for any of the patients and there were no significant hemodynamic changes during postharvest monitoring. These data are graphically displayed in Supporting Information Figure S3.

Cell Dose Characterization

The immunophenotypic characterization of the infused cell product by dose group is shown in Supporting Information Table S1 and Figure S2, as well as the underlying graphics regarding progenitor cell dose infused per kilogram body weight.

Infusion-Related Toxicity/Safety Outcomes

Adverse Events. The adverse events are tabulated in Supporting Information Table S2 and grouped according to type and control versus treatment groups. There were no events that triggered the stopping rules and no serious adverse events related to the study protocol.

Hemodynamics: Central and Cerebrovascular. Intraharvest and postharvest hemodynamic monitoring are shown in Supporting Information Figure S3. There was no drop in the MAP or the CPP or increase in ICP.

SOFA Scoring. No statistically significant difference in SOFA scores was present over a 21-day follow-up period when comparing pooled or individual (Supporting Information Fig. S4A) treatment groups against control patients. There was a trend towards increased organ dysfunction in the high-dose treatment group.

Table 2. Patient characteristics

Variable	Control	Low dose	Medium dose	High dose	Treated combined
Males (%)	70(7)	80(4)	80(4)	60(3)	73(11)
Mean age	34 ± 5	25 ± 4	31 ± 4	34 ± 3	30 ± 2
Injury severity score	28 ± 3	28 ± 2	27 ± 4	27 ± 5	28 ± 2
Postresuscitation Glasgow Coma Scale	7 ± 0	7 ± 1	7 ± 0	7 ± 0	7 ± 0
Craniectomies (prior to infusion) (%)	10(1)	20(1)	20(1)	40(2)	27(4)
External ventricular Drain (%)	0(0)	0(0)	40(2)	60(3)	33(5)
Abbreviated injury scale-Head	4 ± 0	4 ± 0	4 ± 0	4 ± 0	4 ± 0
1 month GOS-E	3 ± 0.1	3 ± 0.2	3 ± 0	3 ± 0	3 ± 0
6 month GOS-E	5 ± 1	4 ± 1	5 ± 1	3 ± 0	4 ± 0
Time of infusion (hours)	-	33 ± 3	39 ± 2	36 ± 2	36 ± 1
Time of harvest (hours)	-	25 ± 3	30 ± 2	28 ±1	28 ± 1
Time of enrollment (hours)	16 ± 2	13 ± 1	15 ± 0	19 ± 1	16 ± 1

There were no differences in the injury demographics. However, treated patients had a greater therapeutic intensity with decompressive craniectomy and ventricular drain placement for ICP management compared to control patients. This is shown in subsequent figures as well.
Abbreviation: ICP, intracranial pressures; GOS, glasgow outcome score.

Pulmonary Function-Murray Scoring. Treated patients were found to have a significant increase in Murray scores in the pooled treated arm of the study when compared to control patients ($p < .05$) (Supporting Information Fig. 4B). On subgroup analysis, the high-dose treatment group remained with a significant increase ($p < .01$) in Murray scores [Supporting Information Fig. 4C).

Imaging Data and Functional Outcome Correlation. Global measures of volumetric changes over time. Quantitative analyses of the high-resolution 3D T1-weighted images yielded longitudinal volumetric measures of the supratentorium and cerebral white matter at 1 month and 6 months postinjury in 20 of 25 patients. These data are graphically summarized in Figure 2. In the treated group of patients, these data were available for all but 1 of 15 subjects; one subject who was in the high-dose group of treated patients did not complete the 6 month imaging session. In the untreated group of patients, these data were not available for 4 of 10 subjects; 3 subjects did not complete the 6 month imaging session and 1 subject had significant artifacts distorting the images at the 6 month time-point.

As indicated in Figure 2 (Supratentorial Volume), pooled comparisons of all treated versus untreated patients showed similar supratentorial volumes at 1 month. Whereas the treated group demonstrated a 1.87% decrease in supratentorial volume, the untreated group showed a 3.9% reduction. When treated patients #19 and #20 were excluded from the analyses, there was a significant interaction between group and time ($p = .0294$) as the treated group demonstrated well-preserved supratentorial volume (<0.15% decrease) at the 6 month time-point. In Figure 2, similar cerebral white matter volumes are evident in comparisons of treated versus untreated patients at the 1 month time-point. At the 6 month time-point, the treated group demonstrated a 2% decrease in cerebral white matter volume while the untreated group showed a 3.6% reduction. After reviewing unique aspects of patients #19 and 20, we decided to analyze the data with and without these patients. One had a massive intracerebral contusion/hematoma and the other had a middle cerebral artery distribution stroke as imaging confounders (Fig. 2B). Representative CT scans are shown in Figure 5B. When treated patients #19 and #20 were excluded, there was a significant group*time interaction ($p = .0049$) as the treated group exhibited well-preserved cerebral white matter volume (<0.65% decrease) at the 6 month time-point. Although statistical comparisons across dose levels did not reach significance, a trend for greater white matter volume preservation is evident in the low and middle dose-treated groups relative to the untreated group of patients (see Fig. 2A).

ROI-based measures of white matter integrity changes over time. Quantitative analyses of the DTI data yielded measures of white matter integrity which included FA, and mean diffusivity (MD). These data were available longitudinally in 21 of 25 patients as four subjects (one treated and three untreated) did not complete the 6-month neuroimaging session.

Corpus callosum. As shown in Figure 3, FA values of the CC in the untreated group were lower at both imaging time-points relative to the low and middle dose groups of treated patients. The high-dose group of treated patients exhibited the lowest FA values of the CC at both imaging time-points (CC-FA Fig. 3A). Inspection of individual subject's data in the high-dose-treated group of patients (CC-FA Fig. 3B) revealed exceptionally low FA values of the CC at both imaging time-points for patients #19 and #20. Pooled comparisons of all treated versus untreated patients demonstrated a trend for better FA values at both imaging time-points in the treated group (CC-FA Fig. 3C). When patients #19 and #20 were excluded, group differences approached significance ($p = .055$) with the treated patients exhibiting higher FA values (mean = 0.59 and 0.57 at 1 month and 6 months, respectively) relative to the untreated patients (mean = 0.54 and 0.53).

As shown in Figure 3, MD values of the CC in the untreated group were higher at both imaging time-points relative to the low and middle dose groups of treated patients. The high-dose group of treated patients showed the highest MD values of the CC at both imaging time-points (CC-MD Fig. 3A). Evaluation of individual subject's data in the high-dose-treated group of patients identified exceptionally high MD values of the CC for patients #19 and #20 (CC-MD Fig. 3B). Pooled comparisons of all treated versus untreated patients indicated lower MD values of the CC at both imaging time-points (CC-MD Fig. 3C). When patients #19 and #20 were excluded, group differences were significant ($p = .008$) with the treated patients exhibiting lower MD values (mean = 0.897 $\times 10^{-3}$ mm^2/s and 0.938 $\times 10^{-3} mm^2/s$) at 1 and 6 months, respectively, relative to the untreated patients (mean = 0.975 $\times$ $10^{-3} mm^2/s$ and 0.986 $\times$ $10^{-3} mm^2/s$).

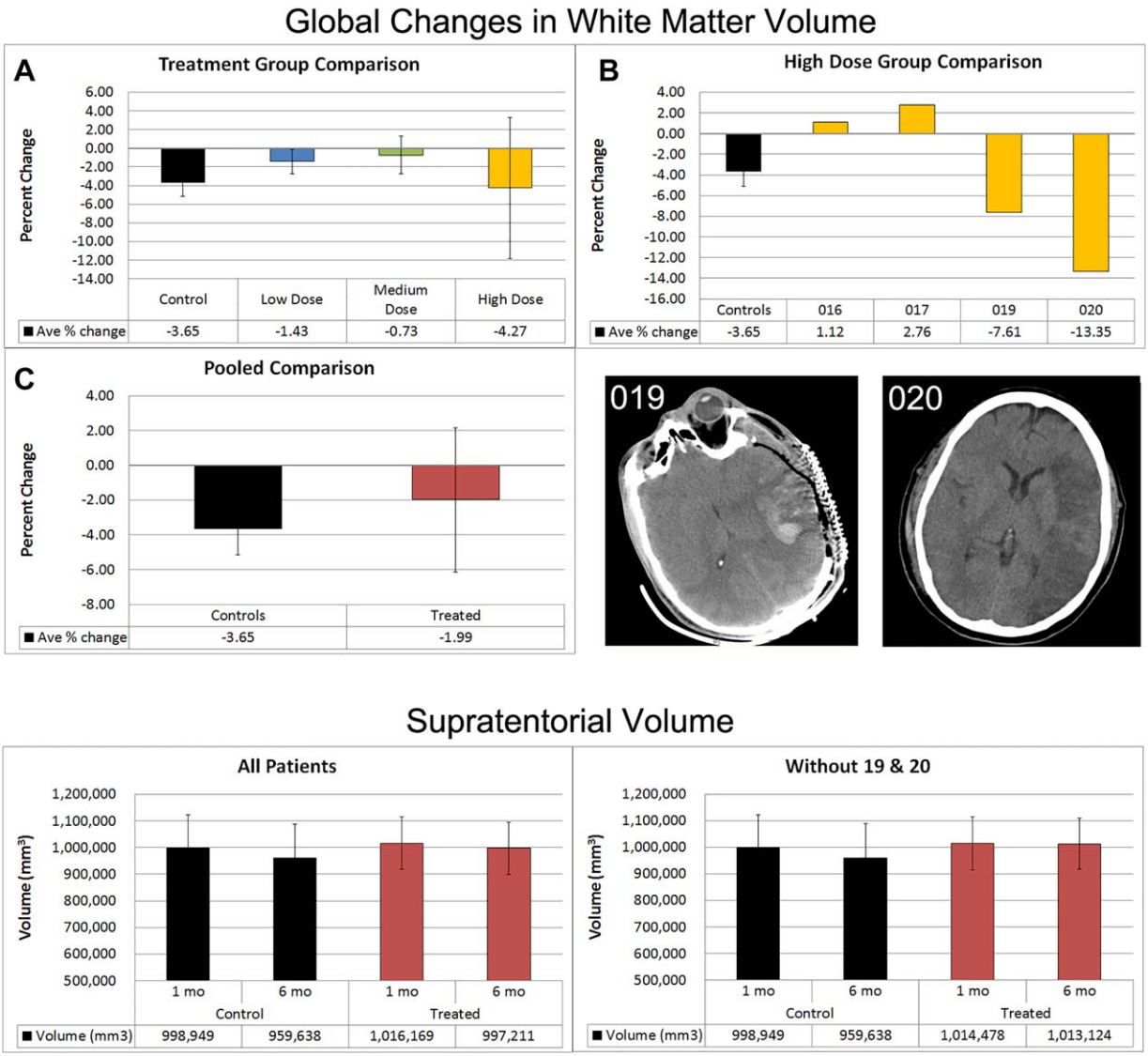

Figure 2. Global change in white matter volume. These data graphically represent the changes in WM volume by treatment group **(A)**, the individual patients in the high dose group with their corresponding computed tomography images explaining their outlier status **(B)**, and the pooled comparisons of treated versus controls **(C)**. Supratentorial white matter volume is demonstrated with and without the outlier patients 19 and 20. Treated patients show greater supratentorial WM volume preservation compared to controls. Abbreviation: WM, white matter.

CST at the midpons level of the brainstem. As summarized in Figure 4, DTI metrics extracted from a region of interest of the CST at the level of the brainstem exhibited characteristics of better white matter integrity in the pooled group of treated patients relative to the untreated group of patients. A significant interaction between group and time was evident for FA ($p = .0137$). While FA values decreased over time in the untreated group, FA values increased over time in the treated group of patients (Fig. 4A). Additionally, a significant group*time interaction for analyses of radial diffusivity (RD) ($p = .033$) indicated well-preserved RD values over time in the treated group of patients whereas RD values increased over time in the untreated group of patients (Fig. 4B). Analyses of MD showed a significant group difference in MD values ($p = .0137$). The treated group of patients demonstrated stable MD values over time, which was lower than the untreated group at both imaging time-points (Fig. 4C). These data include patients 19–20 as the imaging was below the anatomic level of the injury.

Correlation of Callosal and Neurobehavioral Outcomes. Callosal fibers are generally topographically organized relative to the cortical regions that they connect. Our parcellation system is based on dividing the callosal fibers into five equal segments roughly corresponding to the following cortical termination sites: CC1-rostrum and genu, CC2-rostral and anterior midbody, CC3-posterior midbody, CC4-isthmus, and CC5-splenium. Based on our prior studies, FA was selected as the most sensitive DTI metric in relation to posttraumatic changes in cognitive and motor abilities[20]. Consequently, neuropsychological outcomes for the total sample from the 6 month follow-up were examined in relation to FA values from the

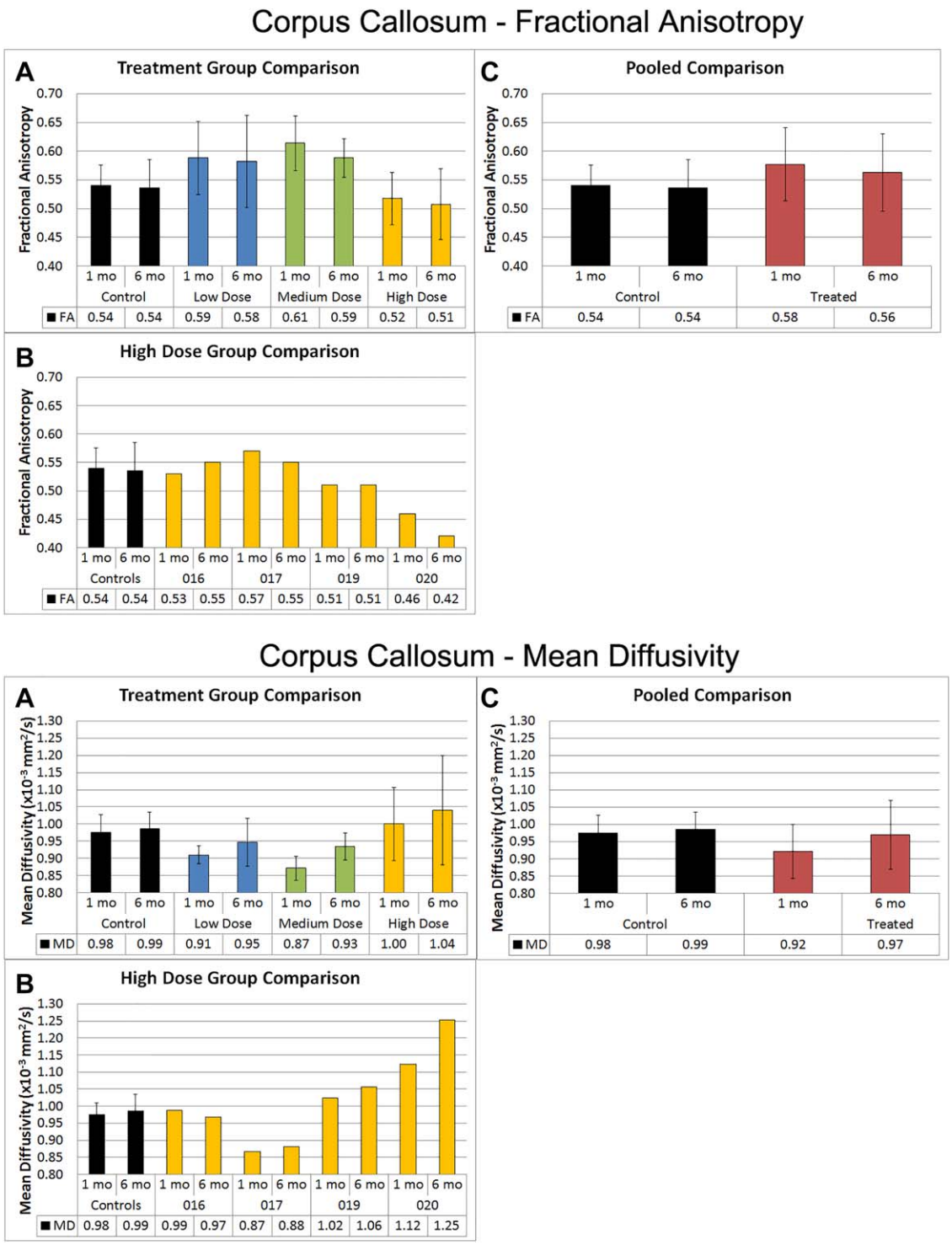

Figure 3. Corpus callosum FA and MD. These data graphically display the changes in FA and MD in the control and treated groups in a dose-dependent manner **(A)**, High dose individual patients **(B)** and pooled comparisons **(C)**. FA is a summary measure of microstructural integrity. Oversimplified, high FA values are a surrogate measure of coherent, tightly packed, and myelinated fibers. Mean diffusivity is an inverse measure of cell membrane density and is sensitive to edema and necrosis (higher FA is "good," and lower MD is "good"). FA is higher in the treated groups except in the outliers as demonstrated in Panel B. MD is lower in the low and medium dose groups, and again the higher dose outliers skew those results. Abbreviations: FA fractional anisotropy; MD, mean diffusivity.

regional and total CC using Spearman rho or Pearson r depending on the variable distribution (Supporting Information Table S3). Due to the small sample size, control and treated cases were pooled ($n = 21$). FA of the splenium (CC5) and/or the whole CC were significantly related to all major functional outcomes, confirming that more favorable outcomes were seen in patients with greater tissue integrity. Similarly, all neuropsychological outcomes were significantly

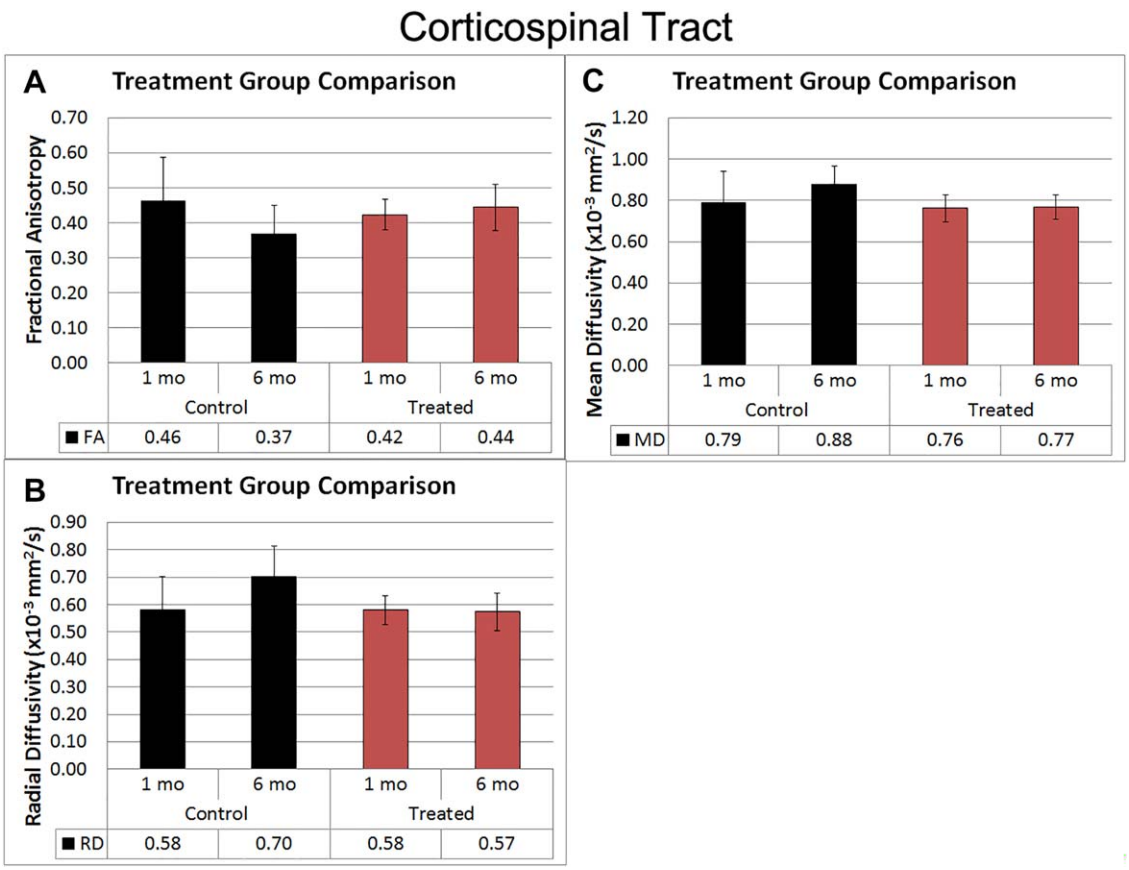

Figure 4. Corticospinal tract FA, MD, and radial diffusivity. These data demonstrate the increase in FA over time in the treated group compared to the decrease over time in the control group. (A) Conversely, the diffusivity measurements (Radial Diffusivity-Panel B; Mean Diffusivity-Panel C) show progression in the control group and improvement in the treated group. Abbreviations: FA fractional anisotropy; MD, mean diffusivity.

correlated with the total CC FA. Tasks with a major motor speed component (Trail Making B, Processing Speed, Grooved Pegboard), were significantly related to FA of CC2-5, reflecting the adverse impact of generalized white matter injury on tasks requiring speed and efficiency. In addition, some test scores were significantly related to specific CC regions; verbal fluency and working memory were related to CC2 and CC3. Memory of a word list was related to CC1 and CC2; delayed recall of the same list was related to CC4 and CC5.the same list was related to CC4 and CC5.

Biomarker Analysis

The plasma concentrations of cytokines were analyzed in both a pooled and dose-dependent format. These are graphically represented in Figure 5. The data are shown as baseline (we used the 6-month value that should represent the patient's endogenous level remote from injury/trauma/operation/infection/stress) to the peak value obtained within the first 96 hours as well as a postinjury/pretreatment to 96 hour peak. There is a dose-dependent trend for TNF-α suppression and a statistically significant, reduction in IL-1β, IL-10, and IFN-γ in the high dose group.

Discussion

This is the first controlled trial to test BMMNC as a treatment for TBI, confirming safety in a prior trial in pediatric TBI, and now providing signals of a treatment effect on structural preservation and the global neuroinflammatory response[8]. Specifically, (a) early, autologous BMMNC harvest and infusion are safe and logistically feasible within a 36 hour time window of treatment, (b) there is a treatment signal of brain tissue preservation measureable on DT-MRI in a clinically relevant setting, (c) functional outcomes correlate with brain tissue preservation, and (d) BMMNC infusion may be altering the global inflammatory response to injury as measured by cytokine profiles. As a Phase 1/2a trial in TBI with small patient numbers, this study suffers from numerous limitations: (a) injury heterogeneity as exemplified by the higher severity of injury in the treatment group as measured by initial ICPs, and statistically greater numbers of patients requiring decompressive craniectomy prior to BMMNC infusion, (b) "early" DT-MRI may actually be too late to capture much of the FA/MD effects, (c) a nonrandomized/unblinded design impacting patient injury severity heterogeneity and potential for observer bias.

Rationale for Cell Type/Dosing and Route

Numerous cell types have been proposed for the treatment of TBI: BMMNC, mesenchymal stromal cells/multipotent adult stem cells, as well as neural stem cells from either fetal or embryonic derivation; preclinical studies have used all of these, as well as a variety of routes of administration (intracranial, intrathecal, intra-arterial, and intravenous). At the

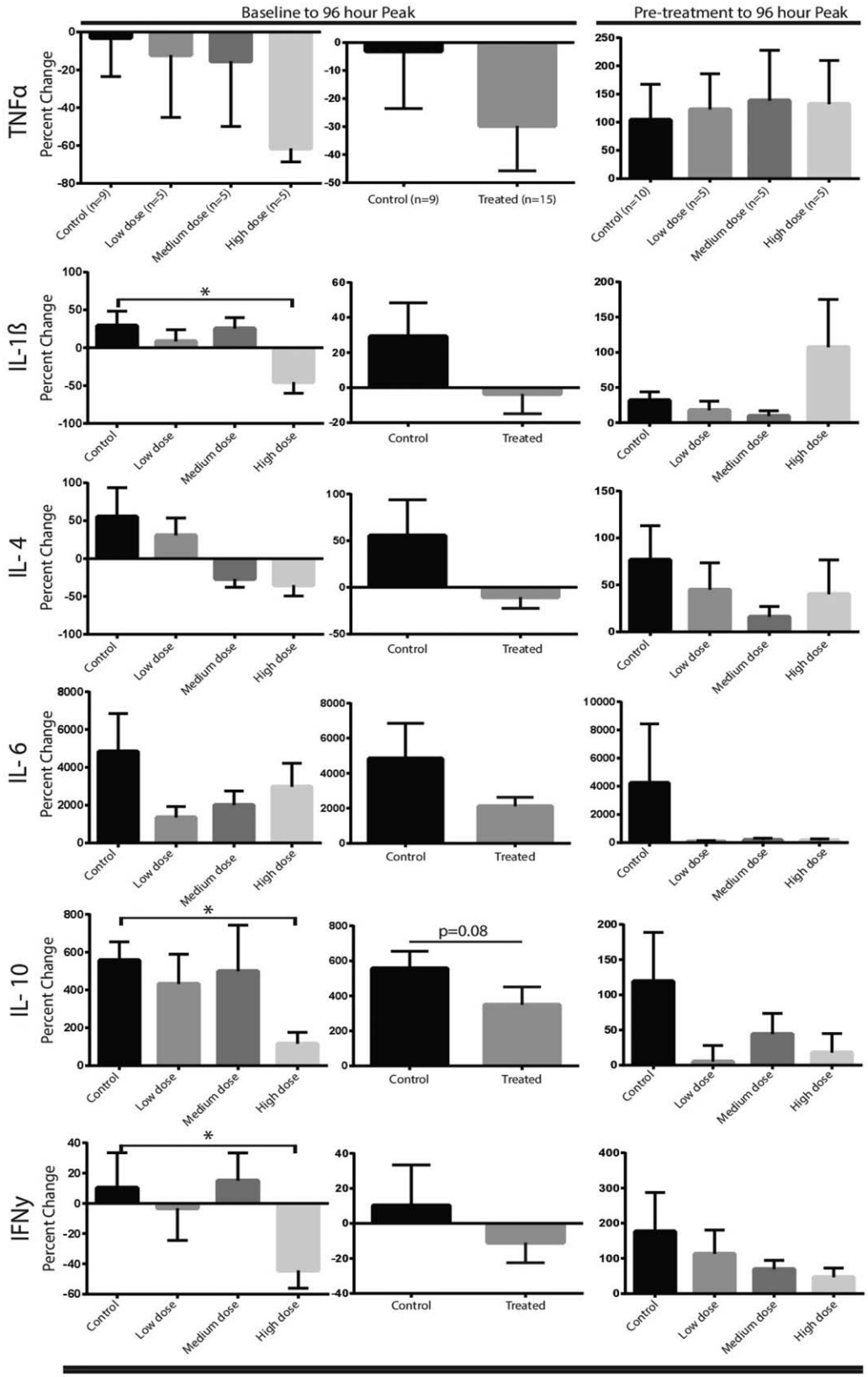

Figure 5. Inflammatory cytokine array. A panel of pro and anti-inflammatory cytokines were measured in plasma. Peak levels within the first 96 hours postinjury were considered the physiological maximal response, and the 6 month follow-up levels were considered "baseline." True uninjured baseline/pretreatment cytokine data cannot be obtained. Pretreatment and peak levels were also analyzed and presented. The data show a significant decrease in IL-1β, and IFN-γ in the high dose group and an apparent dose-dependent downward trend in those cytokines as well as TNF-α. The anti-inflammatory cytokine IL-10 was decreased in the high dose group with a dose-dependent downward trend in IL-4. Abbreviations: IFN-γ, interferon gamma; IL-1β, **Interleukin-**1β**;** TNF-α, **tumor necrosis factor-**α.

pathophysiologic level, the majority of traumatic brain injuries are multifocal, and even focal injuries have areas exacerbated by secondary neuroinflammatory processes. Thus, localized cell replacement is impractical for a diffuse process. Additionally, we have not been in favor of localized injection strategies since these strategies require a craniotomy or multiple burr-holes (possibly causing more damage), and also depend on stereotactic implantation and cell migration followed by differentiation and engraftment. Our preclinical data in both stroke and TBI demonstrate efficacy with intravenous delivery, thus we chose this low risk approach with equivalent efficacy[6, 9]. Previously, we and others attempted to track numerous cell types post-IV infusion after TBI in preclinical models[11, 21]. Not surprisingly, most cell types lodge at least transiently in the pulmonary circulation but later reside in the reticuloendothelial system, specifically the spleen[22]. We did not track engraftment location in our current clinical study, though this could be attempted in future studies using superparamagnetic iron particles.

Numerous preclinical studies demonstrate the efficacy of mesenchymal stromal cells in TBI[23–36]. When infused intravenously, bone marrow derived cells (either allogeneic or autologous) seem to have similar mechanism of action and similar efficacy dampening the innate immune response to injury. We and others have shown that infusion of bone marrow derived cells preserves the blood-brain barrier and improves long-term outcomes by dampening the microglial activation that occurs in response to the primary injury[3]. Of course, the logistical constraints of the current paradigm for infusion of autologous bone marrow mononuclear cells limits the application of this technique to major centers. The advantages of an allogeneic MSC treatment would include an "off-the-shelf" availability and a product that has already been thoroughly characterized and tested for release criteria and potency. This would obviate the need for an invasive bone marrow harvest in the acute period as well as cGMP cell processing. However, there are potential risks with MSC administration and careful expansion is required to maintain potency. Moll et al., have described an "instant blood-mediated inflammatory reaction" to MSC infusion[37]. This could be of greater theoretical concern in acutely injured trauma patients in whom the endothelial/coagulation cascade are upregulated to promote thrombosis[38]. Further, Moira et al. reported loss of immune regulatory potency of MSCs after freeze-thaw cycles, but this has not been universally replicated[39]. With this knowledge in mind, we proceeded with the safest and most readily available treatment strategy: intravenous autologous BMMNC administration.

Safety

This study demonstrates that early BMMNC harvest and infusion is safe. There were no episodes of hypotension, hypoxia, or exacerbation of ICP/CPP parameters associated with harvest of the bone marrow at up to 5 ml/kg body weight or infusion of the cell product. These parameters were prospectively collected and evaluated as a safety read-out. There were no serious events in terms of organ failure. However, there did appear to be a dose-dependent pulmonary toxicity with an increase in the Murray score suggesting a low-level lung injury. While a Murray score of less than 1.5 is not a clinically critical/serious event, it highlights that patients with underlying lung disease or concomitant lung injury may need to be excluded from these protocols/treatments. No patients developed hypoxia related to the infusion, however, since hypoxia adversely affects TBI outcomes, it may be prudent to avoid any potential intervention that exacerbates pulmonary function.

Logistical Feasibility

There are a number of critical elements required to execute this research or future treatment strategy. There must be a robust clinical neurotrauma program that is expert in critical care of the patient with severe TBI. The clinical neurotrauma critical care team must have extensive experience in care of patients with severe TBI. Standard placement of intracranial pressure monitors/ventricular drainage catheters must occur routinely without delay. The clinical trial infrastructure must support the ability to rapidly identify and screen all TBI patients, thus a research "on call" team is mandatory. The call team includes the ability to harvest and process/infuse cells every day and night including week-ends and holidays. This requires a significant increase in activity and personnel that goes beyond what is available at centers with pure oncologic stem cell laboratories. The future may allow closed system, point-of-care processing, which is not currently available. Allogeneic cell infusions using a pharmacy type "off the shelf" model would obviate the burden of the allogeneic harvest and processing, with each approach having pros and cons. A substantial amount of coordination with the clinical care team is mandatory to obtain data and to harmonize safe and timely imaging acquisition. Imaging infrastructure is another important component of the logistical requirements as these imaging data require a 3T magnet that has flexible availability. Finally, there must be a strong follow-up program with engaged social services to facilitate transport of patients back to the hospital for long-term neurocognitive and imaging evaluation. Failure of any of these points in the protocol can undermine the progress of the program.

Imaging Outcomes

Our study sought to examine whether there were any potential structural changes associated with BMMNC treatment for TBI. The rationale that this treatment may result in structural preservation is based on the proposed pleiotropic mechanisms of action described in preclinical studies and our own Phase 1 trial in children[40–42]. Preclinical studies in rodents demonstrate that there was a reduction in the degree of microglial activation with autologous BMMNC infusion in rodents and microglial activation after TBI is associated with white matter loss over time[3, 43]. Adult and pediatric TBI longitudinal imaging studies have established a chronic-phase volumetric reduction in gray and white matter[18, 44–47]. An important finding noted in this study is the treatment effect size determination (Supporting Information Table S3) that has allowed us to make the best possible sample size estimate for an upcoming Phase 2b trial. Our imaging data show that there is a protective effect that may have a dose-dependent relationship. Despite two confounding patients (19, 20) with higher injury severity requiring decompressive surgery prior to cell infusion that altered the imaging read-out, there were significant differences and trends in structural preservation in global volumetric measures of the supratentorium and cerebral white matter. Further corpus callosal tract preservation as measured by FA and MD

Table 3. Treatment effect size estimation (Cohen's d)-corpus callosal DTI metrics

	6 month: all patients					
	Untreated ($n = 9$)		Pooled treated ($n = 15$)		Untreated vs. treated	
	Mean	SD	Mean	SD	Cohen's d	95% CI
Whole CC FA	0.536	0.049	0.563	0.067	0.442	−0.394 to 1.278
Whole CC MD	0.986	0.049	0.969	0.100	−0.200	−1.028 to 0.629
Whole CC RD	0.654	0.066	0.623	0.124	−0.291	−1.121 to 0.540
Whole CC AD	1.650	0.053	1.661	0.080	0.154	−0.673 to 0.982
GOS-E	4.444	1.509	4.200	1.320	−0.175	−1.003 to 0.653
DRS	4.889	4.285	5.267	2.738	0.112	−0.715 to 0.939

	6 month: #19 & #20 excluded					
	Untreated ($n = 9$)		Pooled treated ($n = 13$)		Control vs. treated	
	Mean	SD	Mean	SD	Cohen's d	95% CI
Whole CC FA	0.536	0.049	0.579	0.054	0.846	−0.040 to −1.732
Whole CC MD	0.986	0.049	0.938	0.052	−0.944	−1.839 to −0.050
Whole CC RD	0.654	0.066	0.586	0.073	−0.968	−1.864 to −0.071
Whole CC AD	1.650	0.053	1.643	0.064	−0.117	−0.968 to 0.734
GOS-E	4.444	1.509	4.308	1.377	−0.095	−0.945 to 0.755
DRS	4.889	4.285	4.923	2.783	0.010	−0.840 to 0.860

We estimated the effect size of BMMNC treatment on DTI metrics of corpus callosal integrity as a region of interest. These data are important in planning a Phase 2b trial with CC DTI metrics as an outcome measure. BMMNC has a moderate to strong treatment effect on FA in the CC (Cohen's d 0.5–0.8). These data would allow a sample size estimation for a study powered at 0.8 with a Cohen's d between 26 (0.8) and 64. Adding an exclusion in future trials for hemispheric stroke/contusion volume threshold value that invalidates volumetric/DTI metrics would also make MD a powerful outcome measure. These data and the outcome correlates (Supporting Information Table S3) also suggest that CC DTI metrics are useful primary outcome measures.

Abbreviations: BMMNC, bone marrow mononuclear cell; DRS, disability rating scale; DTI, diffusion tensor imaging; FA, fractional anisotropy; GOS, glasgow outcome score; MD, mean diffusivity; RD, radial diffusivity.

demonstrated preservation with treatment and correlated strongly with neurocognitive outcomes. We estimated the effect size of BMMNC treatment on DTI metrics of corpus callosal integrity which is important in planning a Phase 2b trial with CC DTI metrics as an outcome measure. BMMNC treatment has a moderate to strong treatment effect on FA in the CC (Cohen's d 0.5–0.8). These data would allow a sample size estimation for a study powered at 0.8 with a Cohen's d between 26 (0.8) and 64. These data are shown in detail in Table 3. Adding an exclusion in future trials for hemispheric stroke/contusion volume threshold value that invalidates volumetric/DTI metrics would also make MD a powerful outcome measure. These data and the outcome correlates (Supporting Information Table S3) also suggest that CC DTI metrics are useful primary outcome measures.

Neurocognitive Outcomes

Despite substantial patient heterogeneity, small sample size, and the restricted range of TBI severity, callosal FA values were strongly correlated with functional and neuropsychological outcomes. The strength of this relationship provides additional rationale for using imaging variables as a primary clinical endpoint. FA of the whole CC was significantly correlated with nearly all of the functional status, motor, and cognitive measures. Moreover, neuropsychological variables were significantly related to the integrity of fibers coursing through different callosal segments. Motor and processing speed scores, which are sensitive markers of generalized injury, were correlated with FA in four of five segments. Memory and verbal fluency scores were more strongly related to FA from anterior and midcallosal regions carrying fibers terminating in frontal, parietal, and temporal cortical areas. The integration of sensitive metrics from neuroimaging and neuropsychological outcome domains will enhance the detection of intervention effects in larger samples.

Inflammatory Markers

The cytokine/biomarker data are interesting in that there appears to be a dose-dependent downregulation of the proinflammatory innate immune response to injury. Specifically, the IL-1β and IFN-γ signals show a statistically significant reduction in systemic concentrations relative to the patient's biological baseline (chronic follow-up level used as "baseline"). TNF-α demonstrates a similar trend. These data parallel preclinical data in cell therapy models of TBI (and stroke), in terms of dampening of the global inflammatory response to injury[3]. However, most of the preclinical models use some other assays such as T-cell responsiveness to stimuli, modified mixed lymphocyte reactions, etc. that are impractical for many clinical trials. These types of assays typically require processing of fresh specimens for flow cytometry-based interrogation of the cellular response to stimuli, thus requiring a constantly staffed immune function facility.

Conclusion

Autologous BMMNC infusion for adults with severe TBI is safe and logistically feasible. There is a potential signal of treatment effects in terms of structural preservation of critical CNS

architecture, mimicking preclinical data in rodents[48]. These structural data correlate with a dampening of the proinflammatory signaling. A Phase 2b trial has been planned to evaluate structural outcomes as the primary endpoint, eliminating the high-dose regimen.

Acknowledgements

Research Coordinators: Steven Kosmach, RN, MSN., Mary-Clare Day, RN, BSN; Fernando Jimenez, RN, MS. Cell Processing Team: Andrew Havens, Sufira Kiran, James Roye, Philippa Smith, Suchit Sahai, Ph.D., Marysuna Wilkerson. Technical Support: Anthony Moore10. All Cellular Processing was performed at UTHealth-Medical School, The Evelyn H. Griffin Stem Cell Therapeutics Research Laboratory, FDA Establishment Identifier 3009561521. Funding Sources: DOD Grant: W81XWH-11-1-0460 (Cox, PI); NIH 2T32 GM 0879201-11 (Holcomb, PI); Glassell Foundation Stem Cell Research Program (Cox, PI). Brown Foundation (Cox, PI).

Author Contributions

C.S.C.: Conception and design, financial support, administrative support, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; R.A.H.: Collection and assembly of data, data analysis and interpretation; G.P.L.: Collection and assembly of data, data analysis and interpretation; B.M.A.: Collection and assembly of data, data analysis and interpretation; L.E.-C.: Conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing; J.J.: Conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing; S.I.S.: Conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing; M.J.: Collection and assembly of data, data analysis and interpretation, manuscript writing; A.M.R.-P.: Collection and assembly of data, data analysis and interpretation; F.T.: Provision of study material; collection and assembly of data; P.K.D.: Collection and assembly of data, data analysis and interpretation; C.P.: Conception and design data analysis and interpretation; D.L.: Provision of study material or patients; L.W.: Provision of study material or patients; I.A.: Provision of study material or patients; H.M.C.: Provision of study material or patients, administrative report, manuscript writing; J.H.: Provision of study material or patients, administrative support; R.K.: Provision of study material or patients, administrative support, manuscript writing.

Disclosure of Potential Conflicts of Interest

Since the time of completion, review and publication of this manuscript, Dr. Cox and UTHealth have an equity interest in Cellvation, Inc.- a company which seeks to develop cellular therapies for neurological injuries.

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