

Autologous Bone Marrow Mononuclear Cell Therapy for Severe Traumatic Brain Injury in Children

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BACKGROUND: Severe traumatic brain injury (TBI) in children is associated with substantial long-term morbidity and mortality. Currently, there are no successful neuroprotective/neuroreparative treatments for TBI. Numerous preclinical studies suggest that bone marrow-derived mononuclear cells (BMMNCs), their derivative cells (marrow stromal cells), or similar cells (umbilical cord blood cells) offer neuroprotection.

OBJECTIVE: To determine whether autologous BMMNCs are a safe treatment for severe TBI in children.

METHODS: Ten children aged 5 to 14 years with a postresuscitation Glasgow Coma Scale of 5 to 8 were treated with 6×10^6 autologous BMMNCs/kg body weight delivered intravenously within 48 hours after TBI. To determine the safety of the procedure, systemic and cerebral hemodynamics were monitored during bone marrow harvest; infusion-related toxicity was determined by pediatric logistic organ dysfunction (PELOD) scores, hepatic enzymes, Murray lung injury scores, and renal function. Conventional magnetic resonance imaging (cMRI) data were obtained at 1 and 6 months postinjury, as were neuropsychological and functional outcome measures.

RESULTS: All patients survived. There were no episodes of harvest-related depression of systemic or cerebral hemodynamics. There was no detectable infusion-related toxicity as determined by PELOD score, hepatic enzymes, Murray lung injury scores, or renal function. cMRI imaging comparing gray matter, white matter, and CSF volumes showed no reduction from 1 to 6 months postinjury. Dichotomized Glasgow Outcome Score at 6 months showed 70% with good outcomes and 30% with moderate to severe disability.

CONCLUSION: Bone marrow harvest and intravenous mononuclear cell infusion as treatment for severe TBI in children is logistically feasible and safe.

KEY WORDS: Cellular therapy, Clinical trial, Mononuclear cell, Pediatric, Stem cell, Traumatic brain injury

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ABBREVIATIONS: **BFU-E**, burst forming unit-erythroid; **BMMNC**, bone marrow-derived mononuclear cells; **CFU**, colony forming unit; **GM**, granulocyte, macrophage; **GEMM**, granulocyte, erythrocyte, monocytes, megakaryocyte; **cMRI**, conventional magnetic resonance imaging; **CPP**, cerebral perfusion pressure; **DTI**, diffusion tensor imaging; **FFP**, fresh frozen plasma; **GCS**, Glasgow Coma Score; **GOS**, Glasgow Outcome Score (**EC**, expanded, children); **hUCB**, human umbilical cord blood cells; **ICP**, intracranial pressure; **MAPC**, multipotent adult progenitor cells; **MHC**, major histocompatibility complex; **MNC**, mononuclear cell; **MSC**, mesenchymal stromal cell; **P:F**, PaO₂:FIO₂; **PELOD**, pediatric logistic organ dysfunction; **TBI**, traumatic brain injury

More than 2.5 million Americans live with the devastating consequences of a traumatic brain injury (TBI). These injuries frequently leave patients with acute or chronic deficits in motor, cognitive, behavioral, or social function. The hospitalization rate for moderate (Glasgow Coma Scale [GCS] 9-12) and severe (GCS <9) TBI in children has not changed over the past 10 years, and approximately one-third of patients with severe TBI have unfavorable outcomes (death or severe/moderate disability).¹⁻³ Furthermore, the impairment from moderate injuries is substantial. Mortality and long-term morbidity have not improved significantly over the past 3 decades.^{4,5}

Acute treatment of TBI is limited to controlling intracranial pressure (ICP) and optimizing cerebral perfusion pressure (CPP) to prevent secondary brain injury, while chronic treatment centers on motor, cognitive, and behavioral rehabilitation. Pharmacologic neuroprotective therapies for TBI have been unsuccessful, and postinjury hypothermia for TBI in children was associated with worse outcomes in a recent multicenter trial.^{2,6} No current therapy alters the underlying pathologic processes via cellular salvage, repair, or replacement.

In preclinical and clinical studies, the intravascular delivery of bone marrow-derived cells has shown significant promise. Their mechanisms of action on injured cells may include cell replacement/fusion or cell salvage via cellular/local milieu alterations in systemic and/or regional inflammation, growth factor(s) production, angiogenesis, or unknown microenvironment modification.⁷⁻⁹ More than 50 clinical trials evaluating adult progenitor cell therapy for acute neuropathologic insults are completed or ongoing. The most common cell population used in these trials is the bone marrow-derived mononuclear cell (BMMNC). The BMMNC fraction has many advantages, including easy and rapid isolation with little cell processing, ample cell number, and a very low risk, minimally invasive bone marrow harvest procedure. Using an autologous approach, the potential complications of cell rejection, graft vs host disease, and blood-borne disease transfer are avoided.

This study presents results of a phase I clinical trial to evaluate early, intravenous administration of 6×10^6 autologous BMMNC/kg body weight in children with a TBI. This study was designed to evaluate the logistics, feasibility, and safety of autologous BMMNCs in this patient population. Also, we sought to estimate potential treatment effect size in terms of efficacy and structural outcomes measures for future trial planning.

METHODS

This study was conducted under Federal Investigational New Drug Application BB 12620 and was approved by the University of Texas Health Sciences Center at Houston Committee for the Protection of Human Subjects and approved by the Children’s Memorial Hermann Office of Research.

Patient Enrollment

Patients aged 5 to 14 years with a TBI and a postresuscitation GCS of 5 to 8 were screened for enrollment into the study. These ages were chosen because 14 years of age was the cutoff for triage to the adult trauma service in our trauma center, and below age 5, there is an increasing incidence of nonaccidental trauma that could be a confounder in future follow-up evaluation. Patients were then screened for exclusion criteria, which are listed in Table 1. Patients with preexisting serious medical conditions and/or infections were excluded. Furthermore, patients with significant concomitant injuries were excluded. Finally, patients with evidence of transtentorial herniation, or initial ICP over 40 mm Hg were excluded. Families were approached regarding the study, and informed consent was obtained with the assistance of a research intermediary from the Institutional Review Board. Initial evaluation and the protocol timeline are shown in Figure 1. Patients were

TABLE 1. Inclusion and Exclusion Criteria for Patients With Severe TBI Who Were Screened for the Trial^a

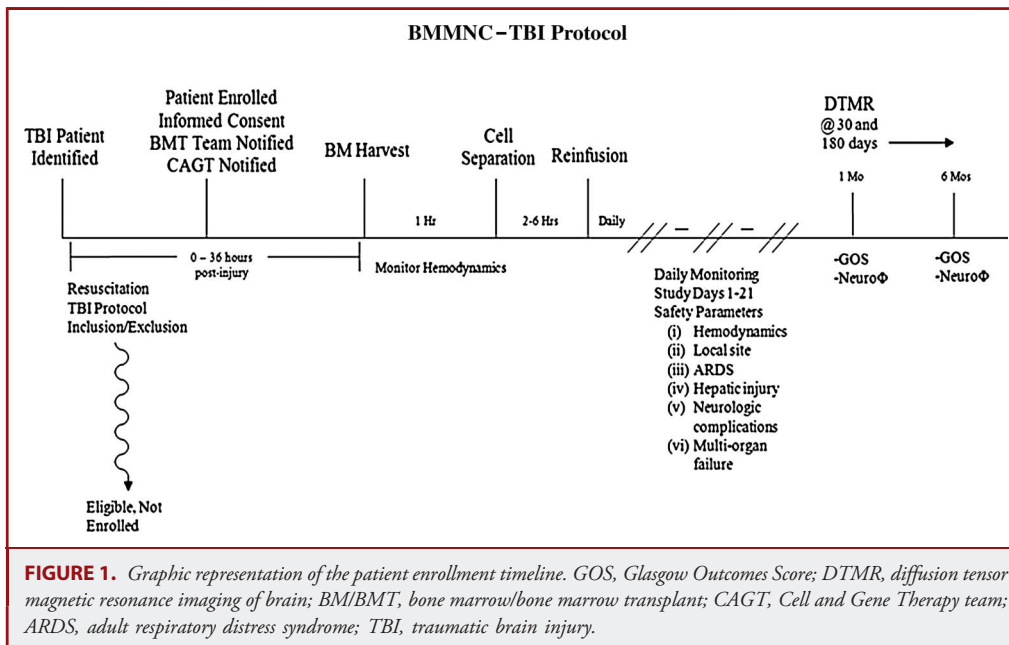
Inclusion Criteria	
Age, 5-14 y	
Admission GCS 5-8	
Injury occurring <24 h within enrollment	
Exclusion Criteria	
Initial ICP >40	
Findings on head CT/MRI suggestive of prolonged hypoxic ischemic insult	
Hemodynamic instability	
Uncorrected coagulopathy at the time of harvest	
Pulmonary contusions	
Solid or hollow visceral injury of the abdomen/pelvis	
Spinal cord injury	

^aGCS, Glasgow Coma Scale; ICP, intracranial pressure; TBI, traumatic brain injury.

enrolled within 24 hours after injury, and cell infusion was completed before 48 hours after injury. Patients could only be enrolled sequentially, and after review of their initial postinjury/treatment course by an independent data safety monitoring board. All data were audited by an external clinical monitor.

Patient Management

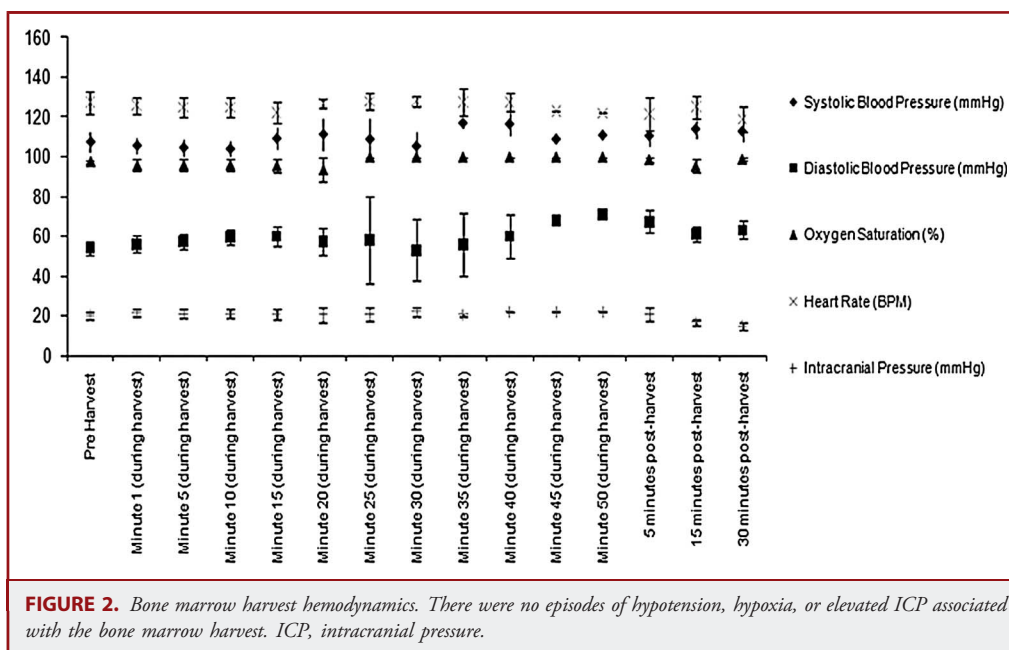
All patients were admitted to the Children’s Memorial Hermann Hospital, which is an American College of Surgeons Verified/State of Texas Department of Health Approved Level I Pediatric Trauma Center. Patients were fluid resuscitated initially according to Advance Trauma Life Support guidelines, followed by fluid titration to their age-appropriate physiological norms. Central vascular catheter placement was performed via either the femoral or subclavian route, and this access was used for cell infusion (smallest internal diameter, 20 gauge). Radial or femoral arterial cannula placement was performed according to standard techniques. Coagulopathy was corrected with fresh frozen plasma (FFP) and/or recombinant factor VIIa if resistant to FFP infusions or clinical bleeding was persistent. Initial imaging was performed using CT (Siemens Somatom-40) and conventional magnetic resonance imaging (cMRI) and diffusion tensor imaging (DTI) and were obtained when clinically stable, at 1 and 6 months postinjury. Patients were managed according to the *Guidelines for the Acute Medical Management of Severe Traumatic Brain Injury in Infants, Children and Adolescents*.¹⁰ In the Pediatric Intensive Care Unit, all patients received an intracranial pressure monitor/ventriculostomy for ICP measurement and CSF drainage. Intracranial hypertension was managed with an ICP-driven intervention algorithm based on published guidelines. However, hypertonic saline was used as the primary osmotherapeutic as opposed to mannitol, as we described previously.¹¹ Patients received an initial 3% NaCl bolus (10 mL/kg body weight) followed by 1 mL/kg per h continuous infusion. The initial goal was to achieve a serum sodium concentration of 150 to 155 mEq/L. Further adjustment to the infusion rate was to titrate to an ICP < 20 mmHg with peak sodium of 160 mEq/L and/or a serum osmolarity of 355 mosm/L. For ICP spikes resistant to sedation, etc., a bolus of 7.3% NaCl (2 mL/kg per dose) was infused. Unexpected or refractory elevations in the ICP prompted repeat CT imaging of the brain; routine

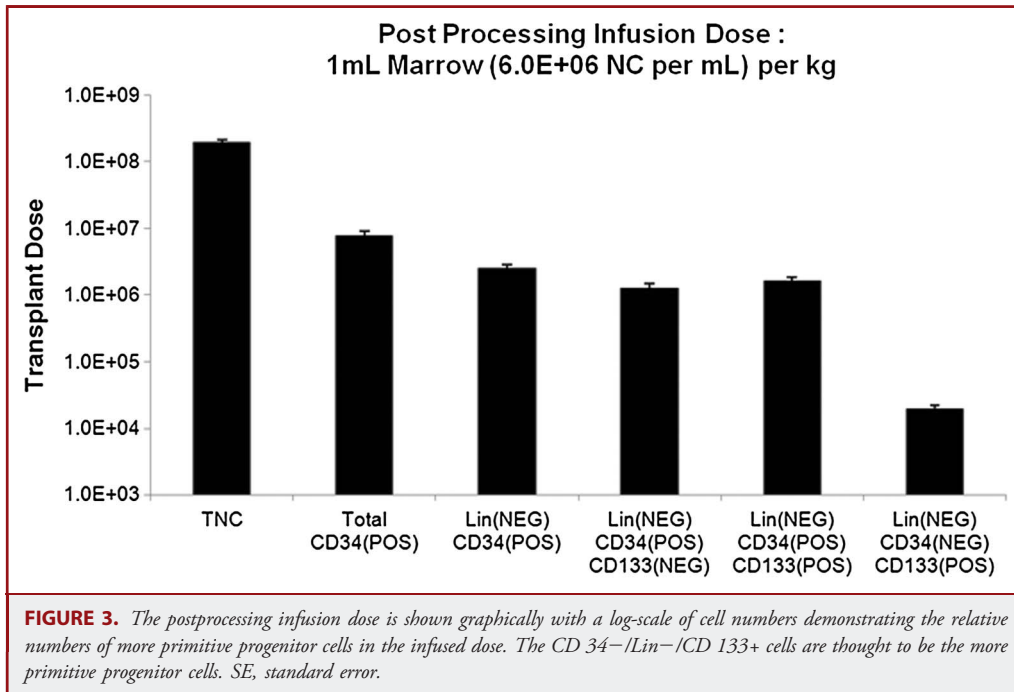


postinjury scanning was not universally used. Sedation was managed with continuous infusions of midazolam and fentanyl, titrated to the level of activity/agitation. Enteral feedings with a polymeric formula was begun within 24 hours using a nasogastric feeding tube. Hypothermia was not used as a therapeutic adjunct. Posttraumatic seizure prophylaxis was accomplished with fosphenytoin.

Bone Marrow Harvest

Bone marrow was harvested aseptically from the posterior iliac bone (from the anterior iliac crests in 1 patient) and a total volume of 3 to 5 mL/kg body weight was harvested. Local anesthesia (1% lidocaine without epinephrine) and augmented infusion of fentanyl and midazolam were used to minimize discomfort during the procedure. Nine of





10 patients were positioned in the right or left lateral decubitus and one remained supine because of skeletal traction. An 11-gauge needle was placed into the posterior iliac crest and approximately 2 to 3 mL (<40 kg body weight) to 5 to 6 mL (>40 kg body weight) was aspirated with a 60-mL syringe. Another site on the iliac crest was chosen and the procedure repeated. After aspiration of 3 to 6 sites, the first site was reentered with the needle bevel rotated. These procedures increased the yield of bone marrow cells and minimized the peripheral blood obtained. Crystalloid was infused intravenously in 10 mL/kg body weight increments if there were changes in hemodynamic parameters. Blood pressure (systolic, diastolic, and mean), heart rate, capillary refill, arterial oxygen saturation, and intracranial pressure were all measured at 5-minute intervals intraharvest and at 15-minute intervals postharvest for 1 hour. Hemoglobin and hematocrit were measured preharvest and at 4, 8, and 12 hours postharvest. A 20% decrease in blood pressure or increase in heart rate unresponsive to fluids was considered an adverse event.

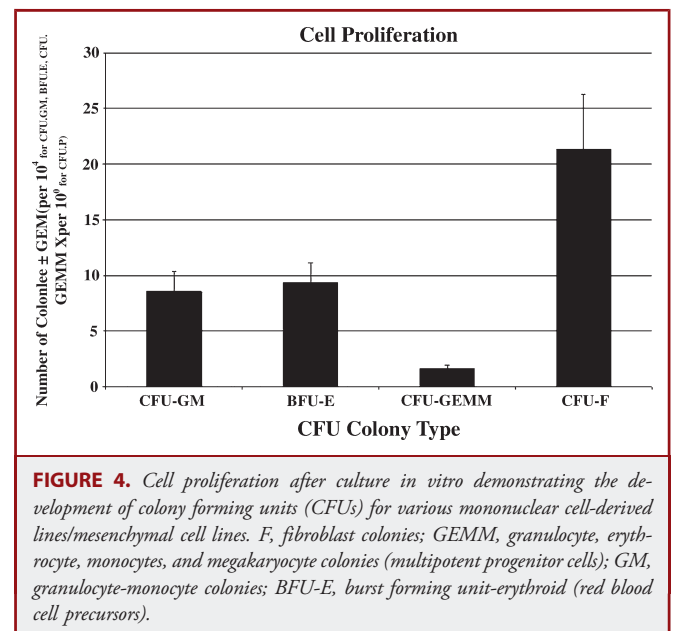
Bone Marrow Processing/Infusion

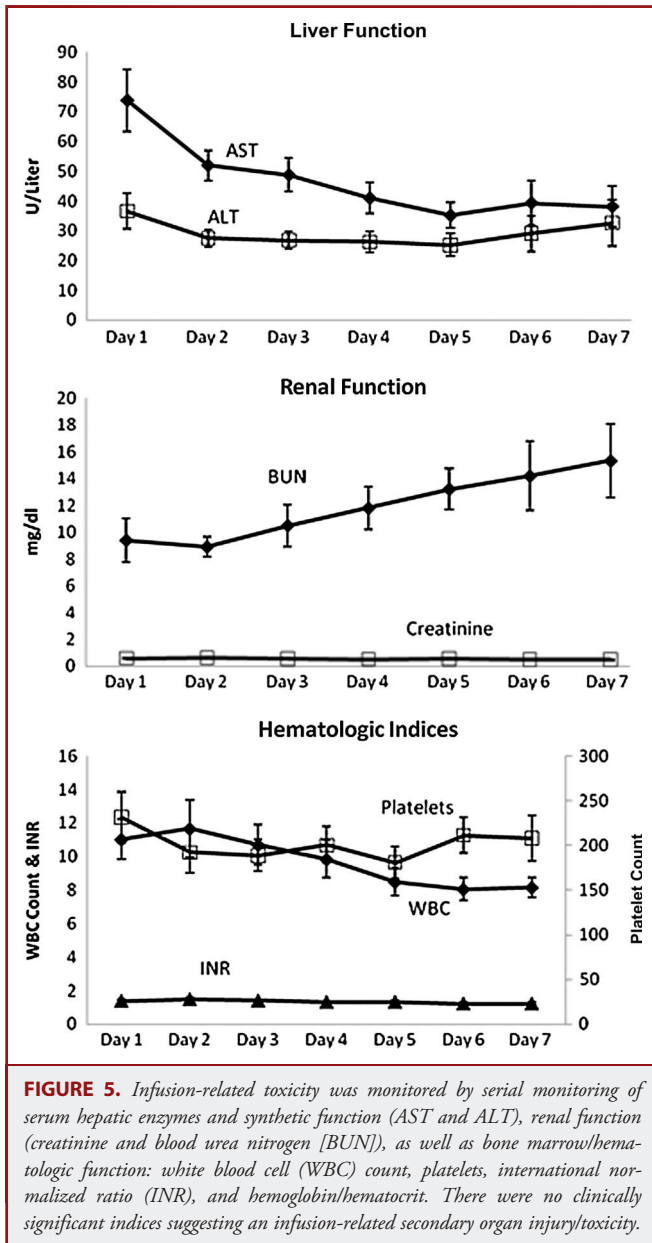
Bone marrow was transported in an anticoagulated blood collection bag to the Center for Cell and Gene Therapy for isolation of the mononuclear fraction. Before mononuclear cell enrichment, a nucleated cell count, differential count, and flow cytometric analysis were performed using the procedures described below.

The mononuclear cell (MNC) fraction was enriched from the bone marrow using Ficoll-Paque Plus density gradient separation. The bone marrow was filtered (170-µm blood filter) to remove spicules. The bone marrow was then layered onto the Ficoll-Paque Plus, centrifuged (400 × g, 30 minutes, ambient temperature), and the MNC layer was isolated. The MNCs were then washed 2 times with 5% human serum albumin in normal saline and adjusted to the appropriate concentration for administration.

Release criteria for the cell product were: Endotoxin < 5.0 EU/mL (measured by Endosafe, Charles River, Wilmington, Massachusetts); Gram stain negative; >70% cell viability by Trypan Blue exclusion.

Additional quality control included bacterial/fungal sterility with extended culture times of 14 and 28 days, respectively (Bactec blood culture system, Becton Dickinson Bioscience, Franklin Lakes, NJ), mycoplasma by polymerase chain reaction (PanVera, Madison,





Wisconsin), progenitor cell colony formation, and flow cytometric analysis for surface markers, differential, and cell viability.

Colony Formation

Assays for colony forming unit (CFU) potential were performed to determine initial cell proliferation properties. Six 35-mm-diameter gridded culture dishes were used for granulocyte, macrophage CFU (CFU-GM), granulocyte, erythrocyte, macrophage, megakaryocyte CFU (CFU-GEMM), and erythroid burst-forming units (BFU-E). Three 35-mm gridded culture dishes were plated at 2.5×10^4 cells and the remaining three at 1×10^4 ; CFU-GM, CFU-GEMM, and BFU-E were cultured using the Methocult CFU-kit (Stemcell Technologies,

Vancouver, British Columbia). Fibroblast CFU (CFU-F) were grown from 2×10^6 , 1×10^6 , and 0.5×10^6 cells plated with Mesencult basal medium (Stemcell Technologies, Vancouver, British Columbia) supplemented with mesenchymal stem cell stimulatory factor and fetal bovine serum. All CFUs were counted after 14 days of culture.

Flow Cytometry

A 3-color direct immunofluorescent labeling method was used in the evaluation of progenitor cells and lymphocyte subsets. To ensure standardization between products, antibody cocktails were prepared for each panel: (1) 7AAD to assess overall sample viability; (2) CD45 + CD14 to identify lymphocytes, monocytes, and granulocytes; (3) CD45 + CD19 + CD3 to identify T and B subsets; (4) CD45 + CD16 + CD56 + CD3 to identify T and natural killer (NK) subsets; and (5) Lin1 + CD34 + CD45 and Lin1 + CD133-2 + CD34 to identify hematopoietic stem cells and other progenitors cells. The following antibodies were used at saturated concentrations in the preparation of the cocktails: Lineage 1 cocktail (CD3, CD14, CD16, CD19, CD20 and CD56), CD45 (clone 2D1) conjugated to fluorescein isothiocyanate; CD34 (clone 8G12), CD19 (clone 4G7), CD16 (clone B73.1) and CD56 (clone MY31) conjugated to phycoerythrin; and CD3 (clone SK7) and CD34 (clone 8G12) conjugated to peridinin chlorophyll protein. All antibodies were obtained from BD Biosciences (San Jose, CA). The cells were acquired within 30 minutes using a FACScan cytometer and CellQuest Pro software for both acquisition and analysis (BD Biosciences, San Jose, CA). For lymphocyte subsets, 20|000 total events were acquired and 100|000 events were acquired for progenitor cells. The lymphocytes were analyzed using CD45 and lymphocyte light scatter gates. The progenitor cells were evaluated using ISHAGE gating strategy, with additional gating to identify the Lineage[neg]CD34+ subset and CD34 vs CD133 subsets. The immunophenotype characterization data are shown in Table 3 and Figure 3, and the CFU potency data are shown in Figure 4.

Monitoring for Infusion-Related Toxicity

PELOD Scoring

The pediatric logistic organ dysfunction (PELOD) score is an outcomes measure of the degree of multiple organ dysfunction in pediatric patients that has been prospectively validated.¹² The PELOD score was prospectively calculated daily from 12 variables derived from 6 organ system categories: neurological, cardiovascular, renal, respiratory, hematological, and hepatic. For each variable, the most abnormal daily variable was used. If the patient was iatrogenically sedated with concomitant neuromuscular blockade, the premedation GCS was used to calculate the PELOD score.

Pulmonary

Pulmonary function was measured using the Murray score, which is an accepted composite of the following variables: PaO₂:FiO₂ ratio (P:F ratio), positive end-expiratory pressure, respiratory compliance, degree of chest radiograph infiltrate.¹³ The final score is an average of the aggregate summed components. For perspective, a Murray score of 3 indicates profound respiratory failure and has been used as the primary entry criteria for extracorporeal life support.

Renal

Creatinine and blood urea nitrogen were monitored daily as an index of renal function, along with hourly urine output.

TABLE 2. Description of Functional and Neuropsychological Outcome Measures^a

Functional Outcome	
Glasgow Outcome Scale (GOS)	Rating scale assessing outcome from 1-death to 5-good recovery; dichotomized into good outcome/moderate disability and severe disability/persistent vegetative state/death categories.
Glasgow Outcome Scale-Expanded for Children (GOS EC)	Expansion of GOS to include disability rating scale assessing need for medical, rehabilitation, psychiatric, and educational/vocational support services to better discriminate factors contributing to mild, moderate, and severe disability. <i>Total score</i>
Pediatric Injury Functional Outcome Scale	Disability rating scale examining post-traumatic changes in motor, daily living skill, communication, social, cognitive, physical, and academic status. <i>Total score</i> .
Adaptive Behavior Assessment System II	A norm-based assessment of adaptive skills in communication, school/home living, self-care, social, motor, and leisure domains. <i>Composite standard score</i> .
Neuropsychological Outcome	
Wechsler Abbreviated Scale of Intelligence	General cognitive functioning: 2-subtest version assesses vocabulary and visual analytical reasoning; <i>IQ score</i> .
Coding	Processing speed: Paper-pencil test assesses speed of transcribing symbols. <i>Scaled score</i> .
Grooved Pegboard	Fine motor speed and coordination: Manipulative dexterity task assessing dominant and nondominant hand performance. <i>Time to completion-dominant hand</i> .
Listening Recall	Working memory: Holding and manipulating verbal information held in working memory. <i>Standard score</i> .
Verbal Learning	Declarative memory: Verbal memory task assessing initial learning of a word list and retention following a 30-min delay. <i>Scaled score for immediate and delayed recall</i> .

^aDependent variables of interest are in italics. Functional outcome measures are based on caregiver report. Neuropsychological outcomes assessing general cognitive functioning, fine motor coordination, working and declarative memory, and processing speed are based on direct assessment of performance.

Neurological

A complete neurological exam and GCS was performed daily. Any episode of posttraumatic seizures was documented. ICP, CPP (mean arterial pressure-ICP), was recorded as the values at 0700 hours and the minimum and maximum values each day. These are graphically represented in Figures 5a and 5b as related to the osmotherapeutic serum sodium concentrations.

Hematological

A complete blood count with differential and platelet count was obtained daily.

Hepatic

The hepatic transaminases (AST and ALT) were measured daily as an index of hepatic injury/toxicity.

Functional and Neuropsychological Outcome Testing

Functional and neuropsychological outcome measures were used to evaluate the child’s global outcome at 1 and 6 months postinjury. Table 2 details the functional and neuropsychological outcome measures used; dependent variables of interest are in italics. Functional outcome measures are based on caregiver report. Neuropsychological outcomes assessing general cognitive functioning, fine motor coordination, working and declarative memory, and processing speed are based on direct assessment of performance. All neuropsychological testing was completed by a licensed, board-certified neuropsychologist.

Imaging Protocols

MRI volumetric determinations were as follows. Data were acquired using a 3-Tesla GE scanner with an 8-channel parallel imaging head coil

(NovaRad, American Fork, Utah). We acquired 2D spin echo T2-weighted, fluid-attenuated inversion recovery, DTI, gradient echo and 3D anatomic T1-weighted volumes. Gray matter, white matter, and CSF volumes were estimated from the T2-weighted data acquired with spatial resolution ~ 1 mm × 1 mm × 5 mm. The contrast between CSF and normal-appearing white and gray matter was adequate for tissue segmentation. The tissue segmentation analysis was conducted using the statistical parametric mapping toolbox in Matlab as detailed in previously published work.^{14,15} All images were reviewed in a blinded fashion by the director of the DTI laboratory in the Department of Diagnostic and Interventional Imaging at the University of Texas Medical School at Houston.

TABLE 3. Patient Demographics^{a,b}

Age at presentation/injury	8.8 ± 0.9 ^c
Sex	
Male	7
Female	3
GCS at presentation	6 ± 0 ^c
Hypoxia (first 24 h)	0
Hypotensive (first 24 h)	0

^aNo patient experienced hypotension or hypoxia during the initial 24 h postinjury, removing this potential confounder on outcomes measures.

^bGCS, Glasgow Coma Scale.

^cRepresents mean ± SEM.

TABLE 4. Flow Cytometric Analysis of Bone Marrow and Cell Product Administered^a

Marker	Initial Bone Marrow Mean % ± SE	Mononuclear Cell Product Mean % ± SE
Cell viability (by 7-AAD staining)	96.65 ± 0.61	98.24 ± 0.18
Total CD34+	2.13 ± 0.30	4.12 ± 0.59
Lin ⁻ CD34+	0.74 ± 0.08	1.36 ± 0.10
Lin ⁻ CD34+ CD133 ⁻		0.63 ± 0.09
Lin ⁻ CD34+ CD133+		0.87 ± 0.09
Lin ⁻ CD34[neg] CD133+		0.02 ± 0.01
T cells [CD3+]	8.87 ± 0.76	15.44 ± 1.29
B cells [CD19[pos]]	4.38 ± 0.45	7.89 ± 1.12
NK cells [CD56+ CD16+ CD3 ⁻]	1.50 ± 0.24	2.71 ± 0.46
3-part differential		
Lymphocytes	16.10 ± 1.50	27.13 ± 2.38
Monocytes	3.68 ± 0.60	7.12 ± 1.30
Granulocytes	67.10 ± 2.21	45.76 ± 4.25

^aCell viability and the differential immunophenotyping data are shown for the final infused product.

RESULTS

Demographics

The clinical demographic description of patients enrolled into the study is described in Table 3. Patients were enrolled beginning in 2005 and 6-month follow-up was completed in 2008. Patients were enrolled longitudinally and not concurrently, allowing for a complete safety review of each patient before enrolling the next patient.

Bone Marrow Harvest Hemodynamics

There were no significant hemodynamic changes during the bone marrow harvest, nor was there a significant drop in the hemoglobin/hematocrit after the procedure (Figure 2).

Cell Dose/Characterization

Each patient received 6×10^6 mononuclear cells/kg body weight, and preinfusion, there was over 98% viability. The profile of the cells is shown in Table 4 and Figure 3. Per kilogram body weight, there were approximately 1×10^6 CD34+, Lin⁻, CD133+ cells infused in each patient, and approximately 1×10^4 of CD34⁻, Lin⁻, CD133+ cells. These are more primitive cells with a greater degree of pluripotency.

Infusion-Related Toxicity/Organ Function

PELOD Score

The PELOD data predicted a mortality of 11.7% at day 2 (the worst score of the ICU stay). No deaths were noted. More importantly, the PELOD trend after day 2 was improvement, with resolution of organ dysfunction (Table 5).

Pulmonary

No patient developed adult respiratory distress syndrome or significant impairment in oxygenation/ventilation as evidenced by the stable Murray scores and P:F ratios (Table 5).

Hepatic

There was no evidence of clinically significant hepatocyte injury, because the liver enzymes did not significantly increase, although there was a slight increase, which could be attributed to the use of fosphenytoin and/or phenytoin. There was no evidence of hepatic synthetic dysfunction as evidenced by a stable international normalized ratio (Figure 6).

Renal

No patients developed renal failure or required dialysis. The serum creatinine did not rise significantly, although the blood urea nitrogen trended upward (staying within a physiological range) as a consequence of fluid management strategies used in the intensive care unit (Figure 5).

Neurological

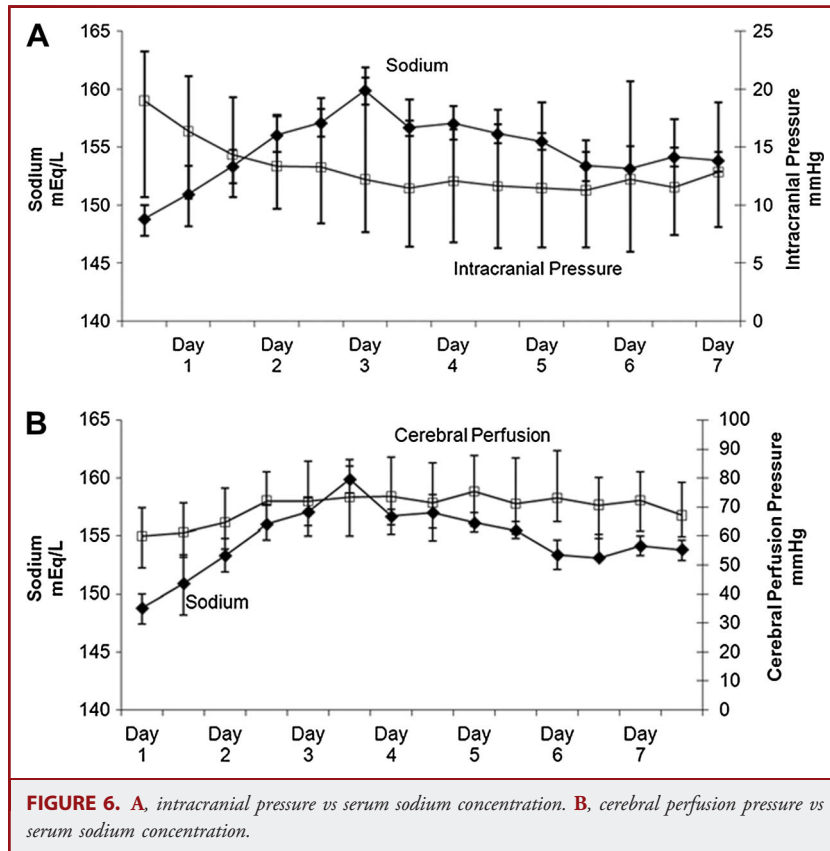
There were no postinjury seizures, episodes of refractory ICP elevations, or alterations in CPP (Figure 6), or new infarcts/ischemic events during the study.

TABLE 5. PELOD Score and Murray Scores are Shown as Indices of Secondary Organ Injury (n = 10/group)^a

	Pulmonary Toxicity and PELOD Scores ^b					
	Baseline	Day 2	Day 3	Day 7	Day 14	Day 21
Murray score	0.5 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.9 ± 0.2	0.3 ± 0.2	0.7 ± 0.7
P:F ratio	370 ± 42	346 ± 33	367 ± 42			
PELOD score	6 ± 2	8 ± 2	5 ± 1	5 ± 2	3 ± 1	1 ± 0*
GCS	6 ± 0	4 ± 1	5 ± 1	7 ± 2	11 ± 2**	13 ± 1***

^aPELOD, Pediatric Logistic Organ Dysfunction; P:F, PaO₂:FiO₂; GCS, Glasgow Coma Scale.

^bValues represent mean ± SEM. All statistical comparisons are by ANOVA with post hoc Tukey analysis; *represents statistical significance vs day 2; **represents statistical significance vs days 2 and 3; ***represents statistical significance vs day 2, 3, and 7. The GCS is a component of the PELOD score, and this accounts for the lower PELOD score in the first 7 days of the study. P:F ratios are shown for days 1 to 3 when all patients had arterial blood gases. Some were managed with pulse oximetry (Sao₂) and venous blood gases after day 3. There was no significant infusion-related lung injury or development of adult respiratory distress syndrome.



Longitudinal Functional and Neuropsychological Outcome Measures

Paired *t* tests were used to examine recovery of scores from the 1- to 6-month follow-up evaluations. Functional outcome scores were available for all children at each follow-up. Significant improvement was noted from the 1- to the 6-month evaluation. Figures 7a and 7b demonstrate change over time on the GOS-EC and dichotomized GOS. Every patient demonstrated improvement from 1 to 6 months, with 7 of 10 demonstrating normal or near-normal functioning on the GOS-EC. Similarly, 7 of 10 had a good outcome/mild disability on the standard dichotomized GOS; of the 3 of 10 without good outcomes, no patients died. At subsequent 24-month follow-up, 1 of the 3 has progressed to a good outcome. The adaptive behavior assessment scale data are shown in Figure 8, with each patient serving as his/her own internal control (preinjury questionnaire). Because this is a normalized score, each patient’s baseline should be near 100. Three of 10 had completely recovered; 3 of 10 had persistent, significant deficits (patients 3, 4, and 6), and 4 of 10 had mild deficits (patients 1, 2, and 10). These data mirror the GOS data.

For neuropsychological assessment, 5 of the 10 patients were untestable on one or more measures at the 1-month follow-up and 1 patient remained untestable at the 6-month follow-up. For

statistical analysis, scores reflecting performance 4 standard deviations below the mean were assigned to these cases. This value was selected to be outside the range of typical performance and lower than scores obtained by participants who were able to comply with testing. As indicated in Table 6, significant increases in all functional and neuropsychological outcome scores were obtained from the 1- to the 6-month follow-up. Mean scores increased substantially from the deficient range (<2nd percentile) at the initial assessment for IQ, processing speed, and working memory to the low-average range. Significant improvement was also noted in the areas of fine motor speed and coordination and learning and retention of new information.

Longitudinal Structural Findings

The cMRI volumetric data for the MRI studies obtained at 1 and 6 months postinjury are shown in Figure 9. There was no significant change in grey matter, white matter, intracranial volume, or CSF volume over the time course of the study.

DISCUSSION

Our data demonstrate that the acute harvest of bone marrow and infusion of bone marrow mononuclear cells to acutely treat severe

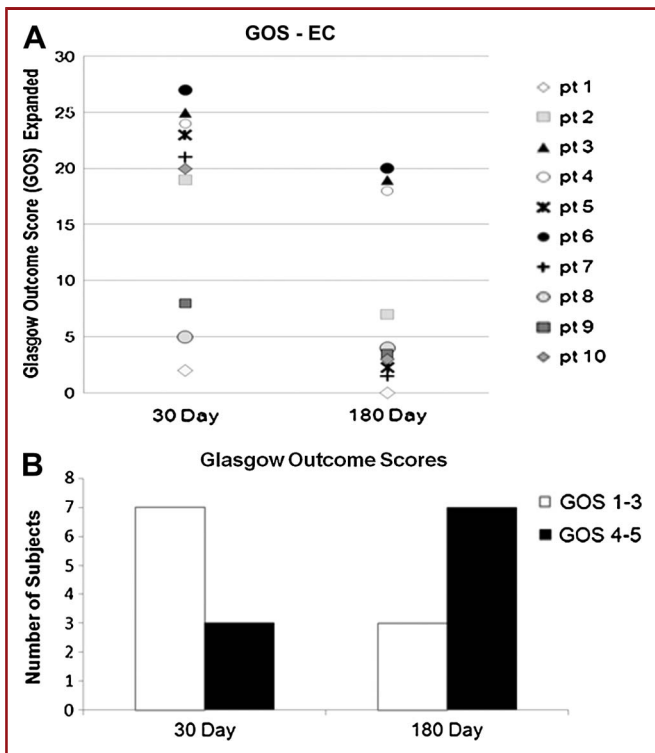


FIGURE 7. A, Glasgow Outcomes Score, expanded for children at 1 and 6 months postinjury (GOS-EC). B, dichotomized Glasgow Outcomes Score at 1 and 6 months postinjury. GOS, Glasgow Outcomes Score.

TBI in children is safe. There was no evidence of infusion-related toxicity of pulmonary, hepatic, renal, hematologic, or neurological organ systems. Further, the approach of using autologous bone marrow-derived cells in the setting of acute injury is logistically

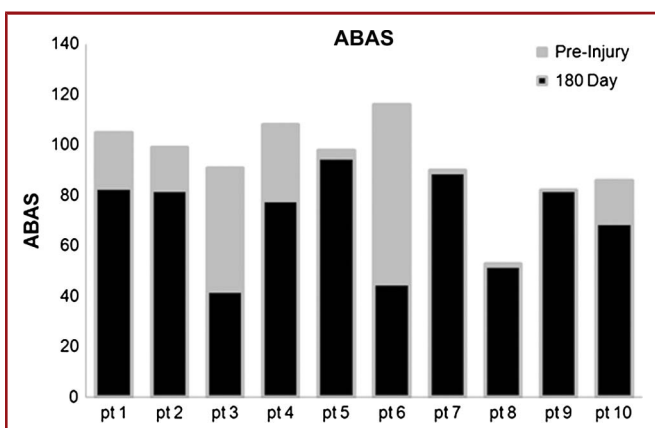


FIGURE 8. Adaptive behavior assessment scale (ABAS) graphic demonstrating preinjury functioning as determined by a parental-completed survey and a postinjury functioning by a neuropsychology evaluator. Mean age-normalized functioning is 100.

TABLE 6. Change over Time in Functional and Neuropsychological Outcome Scores^{a,b}

	1 Month	6 Month
Pediatric Injury Functional Outcome Scale (range, 26-104)	76.7 ± 25.6	50.3 ± 22.0*
GOS-EC (range, 0-27)	17.5 ± 9.1	7.9 ± 8.1*
WASI IQ (M = 100; SD = 15)	63.7 ± 28.1	83.3 ± 23.9*
Coding (M = 10; SD = 3)	3.1 ± 4.6	5.9 ± 5.2**
Grooved pegboard (M = 0; SD = 1)	-2.6 ± 2.2	-1.22 ± 2.3*
Listening Recall (M = 100; SD = 15)	59.2 ± 27.9	83.6 ± 30.5**
Verbal learning (M = 10; SD = 3)		
Immediate recall	3.9 ± 4.4	5.8 ± 4.4***
Delayed recall	3.6 ± 4.5	5.6 ± 3.8*

^aGOS-EC, Glasgow Outcome Scale-Expanded for Children; TBI, traumatic brain injury; WASI, Wechsler Abbreviated Scale of Intelligence IQ test.
^bValues represent mean ± standard deviation. Student paired t test was used to compare performance at 1 and 6 months after TBI: * P < .05, ** P < .01, *** P < .001.

feasible within the context of a well-developed pediatric trauma system and cellular therapeutics laboratory. Six-month follow-up data suggest structural preservation and continued improvement in functional outcomes. The dichotomized GOS at 6 months showed a 70% good to 30% bad outcome or death, which is similar to other recent major reports in pediatric severe TBI.^{2,3} However, there were no deaths in the current study, and this is probably due to the exclusion criteria that limited entry of patients with evidence of herniation on initial CT of the brain, GCS of 3 to 4, and initial ICP

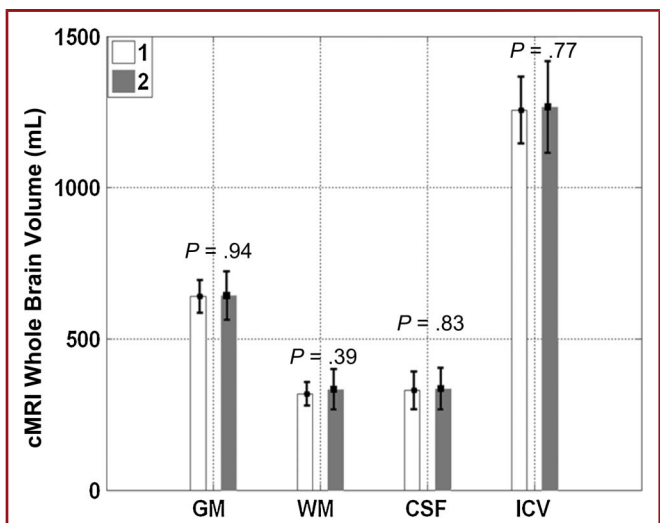


FIGURE 9. Conventional magnetic resonance imaging volumetry at 1 month (scan 1) and 6 months (scan 2) postinjury. There is no decrease in grey matter (GM), white matter (WM), or intracranial volume (ICV). There is no increase in CSF volume. Typically, after TBI, there is progressive loss of grey and white matter with an associated increase in CSF volumes. Over years there is a loss in total intracranial volume. TBI, traumatic brain injury.

of >40 mm Hg.^{4,5} Further, with long-term (24-month) follow-up, we may be able to estimate a treatment effect size on structural parameters (white and gray matter preservation) as well as functional/educational outcomes.

Our phase 1 study evaluating the potential toxicity of bone marrow harvest and reinfusion of the mononuclear fraction necessitated an uncontrolled design excluding the most severely injured patients. The inclusion of patients with severe TBI and the potential for herniation and death would not allow for the observation of toxicity and decrease study enrollment. Therefore, although structural preservation and improved functional outcomes were observed, our study is underpowered and not designed to conclude any difference with treatment. However, the observed safety of the protocol and promising preclinical research showing benefit from bone marrow-derived cell therapy for TBI warrants the implementation of controlled phase 2 trials.

Rationale for Cell Type, Dosing, and Route

Two major classes of progenitor cell-based therapies have emerged: autologous and heterologous or allogeneic. Within the heterologous class are subdivisions of embryonic, embryonic-like (cells-induced pluripotent stem cells), and adult; within the adult subdivision are fetal, specialized niche cells (subventricular zone derived neural stem cells, for example), and bone marrow-derived adherent (mesenchymal stromal cells [MSCs] and multipotent adult progenitor cells [MAPCs]) and nonadherent cells (BMMNCs) and umbilical cord blood cells (hUCBs), which are similar to the bone marrow mononuclear fraction. We chose to use autologous BMMNC for a number of reasons: (1) no immune barrier considerations; (2) the 5- to 8- μ m cell size (vs 13- to 19- μ m size for MSCs) precluded significant pulmonary sequestration, or the “pulmonary first pass effect” making intravenous delivery more practical¹⁶; (3) no in vitro culture/scaling issues for autologous application; (4) ready availability; (5) no issues with uncontrolled replication as with embryonic or fetal cells; and (6) no ethically objectionable issues with cell type. However, there are distinct advantages to an “off the shelf” product that is heterologous. Heterologous, expanded, and banked cells are available readily and can be dosed multiple times if necessary. No harvest or separation/enrichment/expansion is required in an acute timeframe. Some cell types can be used either in an autologous or heterologous application, but there are limitations on the treatment window because of the time needed to expand the cell population. MSCs or MAPCs could be derived from autologous bone marrow, plated, and expanded to yield a clinically relevant dose for treatment. However, this expansion process could take weeks to achieve the dose needed, effectively ruling out this approach for acute or even subacute applications. For these and other reasons, many groups have pursued the use of heterologous cells with the rationale that there is a low level of major histocompatibility complex (MHC) class II antigen expression, and rejection should be minimized.¹⁷ This remains an area of controversy, in that it is unclear if long-term cell engraftment is necessary for a beneficial neuroprotective effect, or if

ultimate cell MHC antigen expression occurs and tolerance is induced as a low-level microchimeric state. Many of the cytokines that these cells would be exposed to after TBI induce MHC antigen expression.¹⁸ Adding to the controversy is the growing recognition from biodistribution studies that significant long-term engraftment is not required to achieve therapeutic benefit in all cases. However, the attractive aspect of heterologous cell use is the “off the shelf” capacity with minimal logistical complexity to administration of the cells, including repeat and scalable dosing.

A fundamental question is how to administer the cells. The answer hinges, in part, on the purported mechanism of action and the biodistribution of injected cells in the setting of injury. Finally, the cell type affects the delivery route. We chose intravenous delivery because of the minimal pulmonary first-pass effect for BMMNCs and the lack of a focal lesion as with stroke, which would make stereotactic injection attractive.¹⁹ Further, the catheter delivery systems are standard and the risks of selective cerebral catheterization and injection was avoided. These considerations are more critical when the issue of using MSCs is explored, because the large size of MSCs has resulted in transient reductions in cerebral perfusion after intra-arterial delivery after stroke.²⁰

Structural Correlates to Functional Outcomes Data

Our study sought to examine whether there were any potential acute (and later) structural changes associated with BMMNC treatment of TBI. The rationale that this treatment may offer some structural preservation is based on the putative mechanism(s) of action described in preclinical studies.²¹ MRI volumetric studies of pediatric TBI patients (mean GCS of 6) have demonstrated reductions in whole brain volume (ICV), grey matter, white matter, and concomitant increases in CSF space compared with age-matched controls.²² Bendlin showed grey and white matter volume loss after TBI in adults over a similar time period as our study.²³ Similarly, we have shown a selective loss of corpus callosal volumes in pediatric TBI patients compared with age-matched control patients, correlating anatomic to their neuropsychological outcomes.²⁴ Data from our current study suggest that there is no early or 6-month postinjury volumetric loss, and longer-term imaging will assess whether these findings are durable. As the study by Wilde et al²² demonstrated an approximately 60 to 70% increase in CSF volume, and a 15 to 20% decrease in grey and white matter, we may be able to estimate a potential treatment effect for future study planning. This surrogate marker correlates strongly with functional outcomes.

Potential Mechanisms of Action for Adult Progenitor Cell Treatment of TBI: BMMNC, MSC, MAPC, hUCB, and Derivatives

The biological rationale for using any stem or progenitor cell therapy for the treatment of TBI falls into 1 of 2 major categories: neural or supporting element replacement or an anti-inflammatory/paracrine/immune biological response modifier. The issue of transdifferentiation was championed by many in the field starting in the late 1980s and early 1990s.^{7,25} Specifically, there were numerous studies in rodent TBI models using a variety of

bone marrow-derived cell populations, including bone marrow in a filtered, but unprocessed state as well as heterologous MSCs, using various cell tracking labels.²⁶ Chopp's team demonstrated similar effective approaches using various routes of delivery, including intravenous, intra-arterial, and direct intracranial implantation in rodent controlled cortical impact injury models. Initially, they promoted the idea that these cells migrated to the site of injury, and "transdifferentiated" into a neural phenotype.^{27,28} With use of BrdU labels, immunohistochemistry sections of the injured brains demonstrated labeled cells near the site of injury with neural phenotypes. Later, other investigators showed that this technique could yield erroneous results.¹⁷ The MSCs could migrate to the site of injury, die, and transfer the thymidine analog BrdU label to dividing cells that may express neural proteins and/or phenotypes (endogenous neural stem cells, for example, or microglial cells). Furthermore, there were potential issues with the size of MSCs and their ability to migrate across the pulmonary microvasculature in enough numbers to be therapeutic, especially if a reparative or reconstructive mechanism was being considered.²⁹ These and other data cast doubt on the idea of transdifferentiation as the putative therapeutic mechanism of action of these types of cells when administered after TBI. However, other potential mechanisms of benefit were explored. Numerous investigators began to study the inflammatory response to TBI, and how MSCs and/or other bone marrow-derived cells/hUCB could modify the response to injury. Similar approaches were pursued in the study of stroke. The inflammatory response to injury is characterized by a rapid increase in proinflammatory cytokines at the site of injury and the penumbra surrounding region.^{18,30} The cytokine response subsides by 72 hours, but the peak in cerebral edema (and ICP elevation) follows the early inflammation. Therefore, the timing of cell delivery, and the cellular microenvironment may significantly affect the potential therapeutic benefit of the treatment. We sought to deliver the cells within the acute injury time window with the rationale that the dosing must occur before/during the period of inflammation to down-regulate the inflammatory process (or more precisely, up-regulate the anti-inflammatory response). There are pros and cons to this approach. A potential flaw in this approach is that the regional microenvironment is too toxic for any cellular engraftment or even transient residence in the region of injury, with most of the infused cells dying upon reaching the region of interest. For this reason, many in the field have suggested that cell-based treatments should be given after 72 hours to minimize this potential problem. Two main subdivisions of thought have arisen in the anti-inflammatory mechanistic view: (1) stem cells of various types exhibit a paracrine secretion of growth factors and cytokines salvage "at-risk" cells in the penumbra around regions of injury^{31,32} and (2) infusion of stem cells systemically alters the cellular immune response to injury favorably such that tissue is preserved, and functional outcomes are improved.^{33,34} Others have produced some evidence of stem cell fusion with injured cells and possible exchange of cytoplasm to preserve the damaged neuron.

Translational Considerations for Future

There are numerous issues to address that will drive future translational considerations. Simple issues such as dosing (range, timing, number of doses), route of delivery (intravenous, intra-arterial, intracranial), cell fate/tracking, and cell modification/preconditioning are near-term factors to be considered as cell therapeutics are translated into practice. Few preclinical studies use multiple doses of cells, and fewer still use variable dosing/cell types over different times. It is easy to consider that various cell types/preparations would be more efficacious at different time points after injury. The putative mechanisms of action will determine, in large part, the necessity for specific routes of delivery, ie, if the cells do not need to get to the brain, then there is no need for more invasive routes of delivery. The ability to label cells with clinically available imaging (MRI-based) will help determine the ultimate mechanisms of action of these cells in patients. Specifically, if the overwhelming majority of the cells remain in the lungs and reticuloendothelial system with an improvement in outcomes via a down-regulation of the inflammatory response, then a greater focus can be placed on monitoring the mediators responsible for this effect. These types of data can drive the subsequent approaches to dosing and cell type.

Lessons Learned/Logistics

There are numerous logistical and practical requirements to pursue a cell-based, acute treatment protocol in children. Because this is a relatively new field, we believed that discussing the logistical lessons learned that are not routinely found in articles would be useful for others pursuing this type of protocol.

Regulatory Issues

Even autologous cell protocols used in a nonhomologous fashion must be performed under an investigational new drug application through the Food and Drug Administration/CBER branch.³⁵ Although there are many useful components to the investigational new drug process, this requires an extensive amount of documentation and oversight that is typically audited by an external Contract Research Organization. The auditing and verification of the data and adverse events, as well as the durable, legacy housing of research records must be considered in planning these trials. An external Data Safety and Monitoring Board must be established, convened, and meet after each patient enrollment with a full review of all adverse events, and attribution to the protocol must be determined. This is extraordinarily laborious for studies in critical care. After a safety review, a GO/NO GO decision must be made to move to the next enrollment. These board members must also be available for consultation for potential protocol deviations that could impact the patient.

Informed Consent

Pediatric patients are a vulnerable population, especially after TBI. Numerous safeguards must be in place to obtain informed consent, and we used a research consent intermediary to avoid the issue of "therapeutic misconception" surrounding our study.

Language

Not all of the pediatric neuropsychiatric outcomes measures have been validated in Spanish or other languages. This necessitates that the child and caregiver both are fluent in English for enrollment in the study. This must be considered in planning sample sizes/enrollment potential for treatment centers.

Coordination

The acute time frame and brief treatment window required significant multi-institution/personnel coordination, often on holidays/week-ends/after-hours. The trauma team and research team (University of Texas Medical School and Children's Memorial Hermann Hospital) coordinated bone marrow harvest with the bone marrow transplant physicians (MD Anderson Cancer Center) and the cellular therapeutics laboratory (Cell and Gene Therapy Center/Baylor College of Medicine). Lack of availability of any component prevents execution of the protocol. Imaging and initial to long-term follow-up was initiated as inpatients and return visits coordinated through multiple offices. Pitfalls included the rapid changes of image acquisition protocols/software upgrades, and the need for sedation of younger children during follow-up imaging studies that necessitated multiple visits to accomplish the neuropsychiatric testing. Although there are numerous other minor issues that arose during the trial, we anticipate complex multicenter organizational issues as we pursue subsequent follow-up in phase II studies and other phase I trials.

Infrastructure Requirements

A multidisciplinary, functional clinical team must exist, preferably with external verification of excellence for the standard care of the types of patients to be enrolled in the study (American College of Surgeons Level I Pediatric Trauma Center for this study). Bone marrow transplant team members are invaluable and become critical if an immunosuppression/heterologous approach is proposed. Outcomes measures must be tested with a battery of accepted evaluations in a vigorous manner, and this requires an extensive team to evaluate these patients. Advanced imaging capabilities are needed to follow structural correlates to functional outcomes. Finally, a validated current good manufacturing practices laboratory is required for cell processing, preferably operational 24/7 for acute interventions. All of these considerations differ somewhat depending on the protocol proposed, but they should be examined in detail as teams move forward in applying these investigative treatments.

Disclosures

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