

Autologous Bone Marrow Derived Mononuclear Cell Therapy for Spinal Cord Injury: A Phase I/II Clinical Safety and Primary Efficacy Data

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Abstract

Objective: We sought to assess the safety and therapeutic efficacy of autologous human bone marrow derived mononuclear cell transplantation on spinal cord injury in a phase I/II, nonrandomized, open-label study, conducted on 297 patients.

Materials and Methods: We transplanted unmanipulated bone marrow mononuclear cells through a lumbar puncture, and assessed the outcome using standard neurologic investigations and American Spinal Injury Association (ASIA) protocol, and with respect to safety, therapeutic time window, CD34+ cell count, and influence on sex and age.

Results: No serious complications or adverse events were reported, except for minor reversible complaints. Sensory and motor improvements occurred in 32.6% of patients, and the time elapsed between the injury and the treatment considerably influenced the outcome of the therapy. The CD34+ cell count determined the state of improvement, or no improvement, but not the degree of improvement. No correlation was found between level of injury and improvement, and age and sex had no role in the outcome of the cellular therapy.

Conclusion: Transplant of autologous human bone marrow derived mononuclear cells through a lumbar

puncture is safe, and one-third of spinal cord injury patients show perceptible improvements in the neurologic status. The time elapsed between injury and therapy and the number of CD34+ cells injected influenced the outcome of the therapy.

Key words: Spinal cord injury, Regenerative therapy, Phase I/II clinical trial, Stem cells, Hematopoietic stem cells

Repair and replacement of the defective or damaged neuronal tissue, and restoration of normal functioning, is the objective of stem cell therapy to spinal cord injury. Stem cell transplant is used currently as a novel strategy to overcome physical discontinuity and support axonal growth in experimental models of spinal cord injury (1, 2). The multifarious potential of cellular therapy support restoration of axonal connections, limits tissue damage and scarring, facilitates remyelination repair, and replaces and re-establishes lost neural tissue and its circuitry (3).

The adult mammalian spinal cord contains neural stem and/or progenitor cells that slowly multiply throughout life and differentiate exclusively into glia (4). Regeneration of human spinal cord following injury is under the provision of growth permissive environment, neutralization of inhibitory influences, and the neuronal resident stem cells demonstrate that extensive cell proliferation and gliogenesis follow spinal cord trauma (1, 5).

Acute or chronic paraplegia resulting from severe spinal cord injury, is therefore, considered to be an irreversible condition (6). Whereas the postinjury inflammation of the damaged area of a spinal cord induces functional recovery in lower vertebrates, the secondary damage elicited by inflammatory reaction

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aggravates the injury in higher animals including humans (7).

The use of adult progenitors to the repair of spinal cord injury has been attempted with much disparity. Embryonic stem cells have high regenerative potential, but were marred with allogenic complications, uncontrolled multiplication, and differentiation, besides being unpopular owing to ethical considerations (8). Human umbilical cord mesenchymal stem cells though shares most of the characteristics with mesenchymal stem cells derived from bone marrow, the possibility of allogenic rejection, and restricted accessibility, hamper their use in clinical practice (9).

Bone marrow contains hematopoietic stem cells and nonhematopoietic—side population mesenchymal stem/progenitor cells—that exhibit a broad degree of plasticity, commensurate with other adult stem cell populations (including the ability to differentiate in vitro and in vivo into nonmesodermal cell types such as neurons and astrocytes) (10). Bone marrow derived mononuclear cells also have been reported to promote repair and regeneration of nervous tissue within the central and peripheral nervous system, although the mechanism by which this occurs remains unknown (11). It appears that bone marrow derived mononuclear cells may facilitate recovery from spinal cord injury by remyelinating spared white matter tracts, and/or by enhancing axonal growth. In addition, low immunogenicity of hMSCs was confirmed by survival of donor cells without immunosuppressive treatment.

Materials and Methods

Selection of patients

Autologous bone marrow derived mononuclear stem cell phase I/II basic safety and preliminary efficacy study was approved by the Institutional Committee for Stem Cell Research and Therapy as per the Indian Council of Medical Research (ICMR) Stem Cell Guidelines 2006 and 2007, and registered provisionally under Clinical Trial Registry – India (CTRI, ICMR) No. REFCTRI-000448. We also followed the provisions of the ethical guidelines of the 1975 Helsinki Declaration. Before regular investigations and therapy a written consent was obtained from the patients, a periodic, 3-month follow-up was done to assess safety and improvement. All the patients selected for this trial

were already undergone spinal cord decompression, and stabilization before therapy with no apparent improvement in their motor and sensory activities below the level of injury. Because no patient had been reported within a week of injury, a control study was not possible, and the lack of improvement, or the degree of impairment at the time of presentation, was taken as basal level for assessment of safety and efficacy. Patients were classified according to American Spinal Injury Association (ASIA) scale of impairment, level of injury, degree of impairment, and assessed based on urodynamic study, somatosensory evoked potential, and magnetic resonance scan. Exclusion criteria were acute traumatic complications due to cord injury, myocardial infarction and coronary vascular diseases, chronic infections and liver, and renal insufficiency. The patients are classified as traumatic paraplegia, traumatic quadriplegia, and nontraumatic myelopathy, and also at the level of injury (supplementary data S1). The study is a nonrandom, open-labeled, interventional cohort study, wherein historical controls are used for safety and efficacy assessment.

Isolation and processing of human-bone-marrow-derived mononuclear cells

One hundred to 120 mL of bone marrow was aspirated from the iliac crest in a heparinized (1 mL/5000 U) bottle and diluted in Dulbecco's phosphate buffered saline (without calcium and magnesium) at a ratio of 1:2. The aspiration was layered on Ficoll (Ficoll-Paque PLUS, 1.077 g/L, Stem Cell technologies, Vancouver, BC, Canada V5Z 1B3), and centrifuged at 450g for 30 minutes. The mononuclear cell interface was carefully removed, and washed twice in Dulbecco's phosphate buffered saline at 400g for 10 minutes. The resultant pellet was added with RBC lysing solution (0.7% ammonium chloride) and incubated for 2 minutes. The lysing was arrested by adding 0.9% ice cold sodium chloride, and the cells were washed. The bone marrow derived cells were washed in Dulbecco's phosphate buffered saline until the lysing factors were removed, and finally, resuspended in required volume.

FACS characterization of bone marrow derived cells

One hundred microliter processed samples (1×10^6 cells/mL), 20 μ L of CD34, and 20 μ L of CD45 antibodies conjugated with PE and FITC respectively (BD Biosciences, San Jose, CA, USA) were added and

incubated for 15 minutes at room temperature in the dark. After incubation, 900 μ L of phosphate buffered saline was added to the stained cells and mixed well. To this mixture, 5 μ L of the 7-Amino Actinomycin D (7-AAD) dye was added, and again incubated in dark for 10 minutes at room temperature. The cells were vortexed and aliquoted for characterization in FACS area (BD). One lakh event was acquired for analysis of the cellular composition of the sample. The total viability was assessed by 7-AAD method.

Pyrogen testing by limulus amebocyte lysate method

To a 100- μ L processed sample, 100 μ L limulus amebocyte lysate reagent (Endotoxin Test Kit, Cambrex Walkersville, MD, USA) was added, and incubated for 1 hour at 37°C. Clotting of the sample was considered as positive indication for pyrogens at 0.06 EU/mL. All samples negative for this test were used for infusion to the patients.

Statistical Analyses

Statistical analyses involved the analyses of variance for nonparametric variables (age, time elapsed between injury and therapy, number of mononuclear cells injected, and CD34+ cells injected); chi-square or Fisher exact test were performed to analyze the nominal or ordinal variables (sex, injury level, and improvement). All tests were considered significant at *P* values that were less than .05 and 0.1. Statistical analyses were performed with SPSS software for Windows (Statistical Product and Service Solutions, version 12.0, SSPS Inc, Chicago, IL, USA).

Follow-up study

A 3-month periodic follow-up study was designed to elucidate neurologic and motor improvements, and assessed by 2 independent neurologists, using ASIA protocol and standard neurologic investigations. Somatosensory evoked potentials were used to evaluate sensory conduction from peripheral neurons along the spinal cord to the cortex. Urodynamic study was done to assess bladder control. Patients were classified as improved, if they showed improvement in any of these studies. Outpatients, or those patients who could not afford to come to the hospital, or referral centers, were assessed, based on the response of the follow-up along with the certification provided by qualified medical and paramedical personal.

Results

A phase I/II clinical trial study involving a BMDC transplant through a lumbar puncture was done on patients with traumatic paraplegia (n=215), traumatic quadriplegia (n=49), and nontraumatic spinal cord myelopathy (n=33)—the latter included transverse myelitis, postinfective secondary demyelination, and hemiplegic disorders. The samples were processed in a class 1000 room, and were pyrogen-free (limulus amebocyte lysate test) before injection. A follow-up was done at 3, 6, 12, 15, 18, and 21 months after the cellular therapy, and the follow-up varied from 18.4 to 20.5 months.

Safety

No patient had experienced any serious adverse event, such as inflammation in spinal cord, systemic infections, and gastrointestinal bleeding upon BMDC reinfusion. Table 1 outlines the adverse events that occurred during the therapy. Except for fever, headache, and a tingling sensation, no complications or wound infections were observed in the patients. Teratoma was not reported in any of the patients receiving the therapy. Because bone marrow harvesting and reinfusion through a lumbar puncture is an invasive clinical procedures, the patients had been subjected to local anesthesia and maintained on anti-inflammatory drugs during the hospital stay. Mild-to-moderate neuropathic pain was observed in few patients after 15 to 30 days of therapy, and was resolved using medications.

Traumatic paraplegia

On an average, 32.6% out of 215 traumatic paraplegic patients showed improvements in sensory and motor functions (Table 2). Whereas a thoracic level injury had 37% chance of improvement, 30% $\pm$ 2% patients showed improvement in other levels of

Table 1. Posttherapeutic adverse events.

Adverse event	Treatment group			Total percentage (n=297)
	Traumatic paraplegia (%) (n=215)	Traumatic quadriplegia (%) (n=49)	Nontraumatic spinal injury (%) (n=33)	
Fever	31.6	34.6	30.3	31.9
Headache	17.2	36.7	36.4	22.5
Tingling sensation	23.2	20.4	24.2	22.8
Spasm	0.46	0.00	0.00	0.33
Neuropathic sensory symptoms (burning, pain sensations)	5.1	8.16	6.06	5.7

injury: 66% of the patients within this study had a T-level injury—the statistical improvement was equally applicable to injuries at other sites, notwithstanding a percent improvement or nonimprovements documented in D and S level of injuries, respectively, owing to a low sample number. Thus, there was no association between level of injury and improvement, despite C-level injuries that responded poorly to the treatment. Similarly, there existed no statistical significance in the improvements between the sexes that average between $30\% \pm 2\%$. Nevertheless, at 0.1 level of confidence (and not in 0.05 level), there existed a statistical significance that favored better results with the male patients, partly reflecting that there were more males in the sample (80.5%) (supplementary data S2).

The number of bone-marrow derived mononuclear cells, and the CD34+ hematopoietic stem and progenitor cell count, did not vary considerably between improved and nonimproved cases. Nevertheless, the CD34+ cell count was marginally higher in improved cases and might reflect on improvement and efficacy of the treatment. Interestingly, a statistically significant correlation was observed between the times elapsed between the injury and improvement. Patients reported to the

stem cell trial therapy, with a mean time elapsed of 2.6 ± 2 years, showed more response to the therapy than the patients reported with a mean of 3.8 ± 4 years (Table 3). Therefore, the fresher the injury, the better is the response to the stem cell regenerative therapy. Age was not a factor influencing the outcome of the therapy, although younger patients responded better than the older patients.

Traumatic quadriplegia

A total of 32.7% of patients showed improvement in sensory and motor scales (Table 2). Traumatic quadriplegia patients had a skewed ratio in the level of injury, with a cervical injury (c) constituting 89.8% of the patients. At 0.1% confidence level, there found a correlation between the improvement and the level of injury with C level injury favoring the outcome of the therapy. No correlation had been found between the sexes, and improvement of, or between, sex and level of injury (supplementary data S3).

Time elapsed between injury and therapy had no influence on the outcome of the therapy, as the mean difference of time elapsed between the improved and unimproved cases ranged as close as 0.15 years, which was significant at an 0.15% confidence level. Similarly, the outcome of the therapy was not influenced by age or by the total number of mononuclear cells injected. Nevertheless, 0.1% significance was found between the CD34+ cell count and the improvement, suggesting a positive role play by the hematopoietic stem cells in the therapeutic outcome (Table 4).

Nontraumatic spinal cord myelopathy

The statistical inference of the use of cellular therapy to diverse spinal disorders of nontraumatic nature, including postinfective demyelination, or cord contusion rested on the common safety, efficacy, and regenerative ability of the BMDC. Diversity in cause

Table 2. Efficacy of BMDC therapy to spinal cord injury.

Improvement	Traumatic Cord Injury paraplegia (n=70)	Cord Injury quadriplegia (n=16)	Nontraumatic cord injury (n=11)	Total (n=97)
Sensory only	31 (44.3%)	9 (56.3%)	1 (9.1%)	41 (42.3%)
Bladder control	19 (27.1%)	3 (18.7%)	4 (36.4%)	26 (26.8%)
Predominantly motor power and reflexes	20 (42.3%)	4 (42.3%)	6 (54.5%)	30 (30.9%)
Sensory and motor power	19 (28.6%)	4 (25.0%)	4 (36.4%)	27 (27.8%)
Total improvement	70 (32.6%)	16 (32.7%)	11 (33.3%)	97 (32.66%)

Abbreviations: BMDC, Bone marrow derived mononuclear cell.

Table 3. Traumatic paraplegia – group assessment.

	Conclusions	n	Mean	SD	t	P
Age (y)	Improvement	70	32.81	11.68	-.715	.475
	No improvement	145	33.99	11.14		
Time elapsed between injury and therapy (y)	Improvement	70	2.61	2.87	-2.529	.012
	No improvement	144	3.83	4.06		
Number of cells injected	Improvement	70	3.77×10^8	2.23×10^8	.112	.911
	No improvement	145	3.72×10^8	3.02×10^8		
CD34+ cell counts (%)	Improvement	70	0.99	0.004	-1.482	.140
	No improvement	145	1.28	0.015		
CD34+ cells injected	Improvement	70	3.98×10^6	3.17×10^6	.218	.828
	No improvement	145	3.88×10^6	3.31×10^6		

and progress of the disease hampered the cohort approach, although improvement was documented in 33.3% of all cases (Table 2). Both sexes responded equally to the treatment with no apparent correlation between sex and improvement (supplementary data S4). Although there were variations in the number of mononuclear cells injected, the average number of CD34+ cells injected was higher in the responding or improved cases than those nonresponding (Table 5).

ASIA level classification and improvement

A total of 32.7% of the ASIA-classified patients showed improvement, in sensory and motor scale. All the spinal cord injury patients—both traumatic and nontraumatic patients—were classified

according to ASIA grade of impairment. A total of 97 out of 297 patients (32.7%) showed posttherapeutic improvements that could be translatable to an ASIA grade (Table 6). ASIA A level of impairment occurred in 83.8% of all patients. A 30.5% ASIA A patients progressed to ASIA B/C/D grades after the cellular therapy, and the recovery of sensation precedes the acquisition of motor power. Whereas the percentage of persons improved from ASIA B/C level of impairment to a higher level was greater than from ASIA A, it nevertheless constituted prominently of motor improvements (supplementary data S1). Gain in motor power and reflexes coincided with partial or good recovery of bladder control. In paraplegics, recovery of bladder control was dependent on motor

Table 4. Traumatic quadriplegia – group assessment.

	Conclusions	n	Mean	SD	t	P
Age (y)	Improvement	16	35.06	14.59	.926	.359
	No improvement	33	31.30	12.68		
Time elapsed between injury and therapy (y)	Improvement	16	3.91	3.88	.124	.902
	No improvement	33	3.77	3.82		
Number of cells injected	Improvement	16	3.88 x 10 ⁸	2.21 x 10 ⁸	-.540	.592
	No improvement	33	4.26 x 10 ⁸	2.38 x 10 ⁸		
CD34+ cell count (as %)	Improvement	16	1.07	0.629	.052	.958
	No improvement	33	1.06	0.597		
CD34+ cells injected	Improvement	16	4.70 x 10 ⁶	3.68 x 10 ⁶	.544	.589
	No improvement	33	4.18 x 10 ⁶	2.87 x 10 ⁶		

Table 5. Nontraumatic spinal cord lesions – group assessment.

	Conclusions	n	Mean	SD	t	P
Age (y)	Improvement	11	33.45	10.12	-1.292	.208
	No improvement	22	38.90	13.67		
Time elapsed between injury and therapy (y)	Improvement	11	6.66	7.40	.287	.779
	No improvement	22	5.97	3.96		
Number of cells injected	Improvement	11	4.20 x 10 ⁸	2.80 x 10 ⁸	.594	.563
	No improvement	22	3.66 x 10 ⁸	1.58 x 10 ⁸		
CD34+ cell count (as %)	Improvement	11	0.87	0.49	-.163	.871
	No improvement	22	0.91	0.58		
CD34+ cells injected	Improvement	11	4.53 x 10 ⁶	5.18 x 10 ⁶	.721	.484
	No improvement	22	3.34 x 10 ⁶	2.49 x 10 ⁶		

Table 6. ASIA Classification.

	Traumatic cord injury					Nontraumatic cord injury				
	Paraplegia					Quadriplegia				
ASIA Grade	A	B	C	D	Total	A	B	C	D	Total
Total cases	190	6	18	1	215	43	1	4	1	49
Number of improved cases	58	3	9	0	70	15	0	1	0	16
% of improvement	28.9	50.0	50.0	0.0	32.5	34.8	0.0	25.0	0.0	32.6

recovery, with or without sensory development, and sensory improvement alone is insufficient for bladder recovery. In quadriplegics and nontraumatic spinal disorders, all the patients who showed recovery in bladder control also exhibited motor improvements.

Discussion

Reparative strategies for neurodegenerative and spinal cord traumatic injuries involve cellular replacement, trophic, and a substrate support. Loss of cellular components, and myelination that occurs as a postinjury inflammatory process hampers the functional recovery, and adds to regenerative complexity of spinal cord (7). Reducing progressive tissue damage and scarring, facilitation of remyelination, and re-establishment of lost neural tissue and its circuitry, therefore, should be addressed for any successful cellular therapy (12-14).

Bone marrow derived mononuclear cellular therapy for acute and chronic spinal cord injury has proven as effective in experimental animal cases (15), and several preliminary attempts were made to use autologous BMDC in human clinical trials with much emphasis on safety (16). Clinical trials using autologous BMDC on spinal-cord-related ailments, or injuries such as amyotrophic lateral sclerosis, multiple sclerosis, or traumatic spinal cord injury were being conducted elsewhere, with limited sampling size (17-19). Except for minor tingling sensations in our study, no allergic or inflammatory reactions (20), or any major adverse reactions (21) so far have been reported with the use of autologous BMDC for spinal cord injuries. Fever and headache usually manifest in the first week after the therapy, and then become normal with medications. Neuropathic sensory symptoms developed a fortnight or 2 after the therapy, and also could be resolved with medications.

Further, it could be observed that the recovery of sensation was not universal; that it might or might not precede the motor control (including the bladder control) in a complete spinal cord injury (ASIA A), and the partial impairment (ASIA B/C) often leading to both sensory and motor improvement. This dichotomy in the *in vivo* development could not be explained, as it was observed that the neurons regenerated from adult BMDC was shown to exhibit the same morphologic and functional characteristics as neurons derived from adult brain stem/progenitor cells, and both had similar electrophysiologic response (10).

Comparative studies that estimated the effectiveness of hematopoietic stem cell and marrow stromal (mesenchymal) cell transplant for spinal cord injury also revealed no difference in the potential of these cells in restoring functional recovery of the injured spinal cord (22). Besides, our study suggested that although the mean CD34+ cell count in the mononuclear fraction was always higher in the improved cases, it did not influence the quality of improvement. Thus, it is apparent that functional recovery of spinal cord lesions was not only mediated by transdifferentiation of HSC/BMDC into functionally integrated neurons (23), but also by the milieu of the injury, the extent of trophic support to the injured neurons, and improvement in neuronal plasticity created by functional demands, rather than by overt tissue replacement by bone marrow-derived stem cells (5). Indeed the combination of bone marrow stem cell therapy, and functional demand created by exercise, resulted in significant, functional improvement in acute, spinal cord injury in Wistar rat models (24). A statistical significance in improvement at 0.1 level observed in our study between sexes would have partially reflected on the freedom that the male patients enjoy for regular physiotherapeutic exercises.

Because the cord tissue below and above the level of injury is vital for recovery of the neuronal functions, the therapeutic approach of intrathecal injection of undifferentiated BMDC for repair and remyelination of injured or demyelinated spinal cord is marred with contradicting results. In animal models, direct intralésional injection of undifferentiated MSCs does not lead to remyelination, and the transplanted MSCs migrate into areas of normal tissue, deposit collagen, and are associated with further axonal damage (25). Further, it was apparent that lumbar puncture delivery of MSC appears to be superior to intravenous delivery to the injured spinal cord. Cell engraftment and tissue sparing were significantly better after lumbar puncture delivery, and host immune response after lumbar puncture delivery was reduced compared with intravenous delivery (26). Besides being a minimally invasive method, MSCs delivered by lumbar puncture have been shown to reach the contused spinal cord tissues and exert a significant beneficial effect by reducing the injury size in the clinically relevant spinal cord contusion models (27).

Although these results in mice might not reflect the human conditions, they nevertheless caution

clinicians to be precise while going for intralesional injection of the spinal cord injury. Besides, it highlights the importance of potential clinical consequences of heterogeneity of cells present in the mononuclear fractions of bone marrow in the repair of spinal cord. However, in a human clinical trial, with a 1-year follow-up, and involving direct intrathecal injection of HSCs, revealed that none of the patients had any clinical setback associated with the cellular therapy and showed marginal improvement compared with their preoperative status (19).

Similar effects, nevertheless, also were observed when bone-marrow-derived mononuclear cells were introduced through a lumbar puncture, wherein, the MSCs prevented astrogliosis and microglial activation, and spared and regenerated motoneurons (28). Further, autologous, bone-marrow-CD34+ cells, labeled with magnetic nanoparticles, delivered into the spinal cord via a lumbar puncture technique, migrated into the injured site in patients with chronic spinal cord injury (29). These studies suggest that effective cell transplant strategy might involve noninvasive delivery protocols for spinal cord injury.

The local microenvironment plays an important role in determining the fate of stem cells. Our results revealed an inverse relation between the time elapsed after the injury, with the developmental outcome (Table 3), revealing that the *in vivo* environment should be conducive to homing and cellular transformation (30). Bone marrow stromal cells (MSCs) can be differentiated into neuronal and glial-like cell types, under appropriate experimental conditions (31), and can replace damaged neurons and glia in injured spinal cord, and/or promote cell survival and axonal growth of the host tissue (3). In the milieu of the injured central nervous system (CNS), inflammation-activated astrocytes forms a unique environment that facilitates the proliferation and neural differentiation of bone-marrow-derived mononuclear cells and the activated astrocytes at different phase after CNS injuries might have distinct effects on bone-marrow-derived mononuclear cells (32). Further bone-marrow-derived mononuclear cells encountered to the neuronal inductive cytokines up-regulated the expression of early neural genes and differentiate into neuronal phenotype (33). Indeed, coculturing experiments have revealed that the differentiation ratio of bone-marrow-derived mononuclear cells in the presence of neural cells is

higher than that of BMDC grown only with differentiation factors (34). Besides the direct transformation, transplanted bone marrow stem/progenitor cells also promote neuronal regeneration of the injured cord by functioning as cellular scaffolds for growing axons (12), by producing neuroprotective immunomodulatory effect (35), and by providing instructive signals that directs the differentiation of neuronal stem cells and development of neuronal progeny (36).

Thus, it was apparent from our study that infusion of unfractionated and unmanipulated mononuclear bone marrow cells could repair the chronic spinal cord injury and bring about perceptible neurologic improvement. Differences in the quality of recovery of spinal functions were not related to the cellular therapy but to the spinal microenvironment. We presume that inflammatory and lymphoid fraction in the mononuclear fraction might have activated the glial cells and secrete trophic factors that favored healing of the scar, induced transdifferentiation of bone marrow stem cells and activation of resident neuronal stem cells (6, 32). Absence of any serious adverse events in our trial, therefore, might favor BM mononuclear transplant therapy over HSC therapy.

Indeed, human trials on spinal cord injury have been conducted with a combination of cells involving bone marrow mononuclear cells, effector T cells, and neuronal stem cells, for effective regeneration (21). However, further studies are required to distinguish the degree of effectiveness and improvement on administration with cultured HSC, bone marrow mesenchymal stem cells, or combination thereof.

In conclusion, our phase I/II clinical trial study suggests that

1. Autologous bone marrow derived mononuclear cellular therapy is safe, and devoid of any serious complications
2. Approximately one-third of the patients receiving the cellular therapy had shown some form of improvement—either sensory and motor or both
3. Lumbar puncture is a safe, efficient, and minimally invasive method of cellular delivery in chronic spinal cord injury
4. Recent spinal cord injury responds better than old injury does
5. Cervical and upper levels of injury showed poorer response to therapy than the lower levels of injury

6. The absolute number of CD34+ cells injected had a direct correlation to the outcome of the therapy
7. The outcome of BMDC therapy was not influenced by sex and age of the patients.

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