Administration of Autologous Bone Marrow Stem Cells Into Spinal Cord Injury Patients Via Multiple Routes Is Safe and Improves Their Quality of Life: Comprehensive Case Studies

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Presently, there is no cure or effective treatment for spinal cord injury (SCI). Studies in SCI patients have shown that for a treatment to be effective it must primarily improve their quality of life. Numerous studies have shown that stem cells represent an alternative treatment for various disorders and have shown promise in several disease/trauma states. For instance, the use of autologous CD34+ stem cells has been shown to ameliorate symptoms of several disorders such as leukemia, cardiomyopathy, diabetes, and several autoimmune diseases, including multiple sclerosis. For the first time, we report eight case studies of SCI (four acute, four chronic) with approximately 2 years of follow-up that were administered bone marrow stem cells (BMSCs) via multiple routes: directly into the spinal cord, directly into the spinal canal, and intravenously. Magnetic resonance imaging illustrated morphological changes in the spinal cord of some of the patients following BMSCs administration. Comprehensive evaluations demonstrate improvements in ASIA, Barthel (quality of life), Frankel, and Ashworth scoring. Moreover, in order to assess bladder function, we designed a simple numerical clinical scoring system that demonstrates significant changes in bladder function following BMSCs administration. To date, we have administration BMSCs into 52 patients with SCI and have had no tumor formations, no cases of infection or increased pain, and few instances of minor adverse events. These studies demonstrate that BMSCs administration via multiple routes is feasible, safe, and may improve the quality of life for patients living with SCI.

Key words: Spinal cord injury; Bone marrow stem cells (BMSCs); Quality of life

INTRODUCTION

Spinal cord injury (SCI) is a devastating disorder afflicting millions across the world (18). Presently, there is no cure or effective standard of care for SCI. Many treatments have been tested in clinical trials, including the use of methylprednisolone (9,10), GM1 ganglioside (24), decompression (51), and 4-aminopyridine (14), all of which have only produced marginal benefits with adverse side effects. The present standard of care for SCI is methylprednisolone and/or decompression. However, neither of these treatments has prevented the pathological cascade triggered by SCI and the efficacy of methylprednisolone is questionable (29).

Tissue loss from primary trauma to the spinal cord and the complexity of cell types required for functional recovery exemplifies a need for cellular replacement strategies (23,35,46). Scientists are compelled to find a cure or effective treatment for SCI; however, the heterogeneity of human SCI represents an enormous challenge for finding a standard of care. With our present technology, realistically the SCI community would greatly benefit with a treatment that promotes partial functional recovery leading to an improved quality of life (2,3).

Stem cells have been identified in various adult organs where they are thought to contribute to tissue repair. The expanding field of adult stem cell research has demonstrated numerous results concerning the broad differentiation potential of adult stem cells. Compared to embryonic stem cells, adult tissue-specific stem cells have a limited self-renewal ability and plasticity, but yet they have been proven to be multipotent. For example, neural stem cells were found to repopulate the hematopoietic system (5), as well as differentiate into all three...
germ layers (16). Furthermore, bone marrow cells (MAPCs) were shown to generate neuronal phenotypes as well as endoderm (32), while muscle-derived stem cells have been shown to express neural markers (1). These results demonstrated the feasibility of using different adult stem cell populations for autologous therapeutic applications in animal models. Many cell-based strategies have been successful in SCI animal models, but when clinically translated to the human condition they have all produced little or no effect (49).

Numerous studies have shown that CD34+ stem cells (HSCs) represent an alternative treatment for various disorders in humans. For instance, the use of autologous CD34+ stem cells has been shown to ameliorate symptoms of several disorders, such as leukemia (4), cardiomyopathy (37), diabetes (45,52), and several autoimmune diseases, including multiple sclerosis (20). This demonstrates the heterogeneous potential that CD34+ stem cells have for clinical applications.

Based on the inadequate standard of care for SCI, we sought to develop a rigorous clinical transplantation program. The objective of this study was to demonstrate that multiple route administration of bone marrow stem cells (BMSCs) for SCI is safe and feasible. In addition, administration with BMSCs via multiple routes: directly into the spinal cord, directly into the spinal canal, and intravenously improves the quality of life for SCI patients. This novel multiroute technique of BMSCs administration may be an ideal method to assure that the cells reach their necessary target in order to promote repair. Using this technique, we have had no cases of tumor formation, infection, or increased pain, and few instances of minor adverse events.

**MATERIALS AND METHODS**

**Patients Guidelines**

All studies were approved in accordance with the ethical committee of Luis Vernaza Hospital, Guayaquil, Ecuador. Acute and chronic patients with spinal cord injuries were enrolled in this study and an informed consent was obtained from each patient. All patients were evaluated prior to enrollment in this study. Some of the inclusion criteria were: have a spinal cord injury with paraplegia or paraparesia; not have any impediments to the responsible adult if under 18; have a desire and be motivated to participate in the study; and have an absolute understanding of the informed consent. Some of the exclusion criteria were: doubting that you can follow the specific study outlined; depression, psychosis, or any other mental disorders; no alcohol or drug abuse; other diseases, especially those with blood-related disorders; no active infections; no patients who have taken immunosuppressants within the last month; no multiple acute injuries; no active pressure ulcers of the skin, especially in the iliac crest region; no patients that cannot follow a strict physical therapy regimen; no obesity; and no patients with a life expectancy of less than 2 years. Following admittance into the study patients underwent an extensive medical evaluation, including magnetic resonance imaging, psychological examination, and neurological examination by physicians trained with the Frankel scale and American Spinal Injury Association (ASIA) impairment scale. Patients were also evaluated using the Ashworth scale (spasticity), Barthel Index (quality of life), and a newly developed bladder function scale designated the Geffner, Gonzalez, Santacruz, and Flor (GGSF) Bladder Function Scale. All patients underwent standard physical therapy prior to and after transplantation. Patients were classified into acute or chronic injury according to the International Campaign for Cures of spinal cord injury Paralysis (ICCP) guidelines (chronic injuries are defined as patients who have been injured longer than 1 year where the preceding 6 months there were no changes in functional capacity) (21). All acute patients were not treated with any other medications prior to BMSCs transplantation.

**Isolation of Autologous Bone Marrow Cells for Administration**

Bone marrow was harvested by aspiration at a minimal number of sites under intrathecal or no anesthesia depending on the individual case. Bone marrow (100 ml) was harvested using only one skin puncture site on the right and left sides. A multiholed needle was introduced into the iliac bone between both posterior iliac spines; 5-ml aspirations were collected at a time for a total of 10 aspirations on the left and 10 aspirations on the right. The bone marrow was placed in a blood collecting bag with 15,000 units of sodium heparin and kept on ice. Using a satellite bag system and centrifugation at 1500 rpm for 20 min, we obtained the buffy coat. The buffy coat was transferred into a bag containing 75 ml of ficol-hypaque with 5000 units of heparin and centrifuged at 1000 rpm for 30 min. The supernatant, which contains the mononuclear cells, was then washed with sterile saline solution and placed into a blood collecting bag and a sample was processed for FACS analysis to obtain CD34+/CD45− cell counts. The mononuclear cells were resuspended in saline and autologous plasma for a total volume of 80 ml. The average total of mononuclear cells obtained for transplantation was $4 \times 10^8$ cells. Within that administration population there was an average of $90 \times 10^8$ CD34+ cells. The administration quantity was based upon preclinical work on SCI animal models (35) and human autologous stem cell transplantation into cardiac myopathies (6,41,42).
Identification of Cells by Fluorescence Activated Cell Sorting (FACS)

FACS analysis was performed using the FACS Calibur from Becton, Dickinson (BD) (Franklin Lakes, NJ). The ISHAGE method was used for obtaining CD34+ cell counts as previously described (25). Briefly, samples were incubated with CD34 and CD45 monoclonal antibodies followed by Pharm Lyse Lysing solution, TruCount Tubes, and Via Probe (7-AAD) (all from BD). The acquisition and analysis of data was composed using Cell Quest software (BD). Prior to data acquisition, a gate was established to exclude CD34−, CD45− events in order to eliminate any debris that may contaminate the sample.

Administration of Bone Marrow Stem Cells

All patients were administered with BMSCs using the same paradigm. Under general anesthesia we perform a radioscopic assessment of the vertebral injury area. After careful evaluation of the injury site, a laminectomy(s) was performed in order to expose the spinal cord. Following clear visibility of the spinal cord, we carefully removed the scar tissue and detethered the cord. Using a 21-gauge needle attached to a syringe, multiple micro-punctures were then performed and 1 ml of cell suspension was injected into multiple locations in and around the injury epicenter and into any intraspinal cavities for a total of 20 ml. The dura was then sutured shut and another 30 ml of the cell suspension was administered into the spinal canal. The remaining 30 ml was administered intravenously for a total of 80 ml of cell suspension. We used a multiple route administration delivery system in order to assure that the BMSCs reach their appropriate target. It has been previously shown that intravenous administration of BMSCs had little to no effect on SCI patients (48).

Neurological Evaluation

Following acceptance into the study all patients underwent an initial evaluation that consisted of the use of the ASIA, Frankel, and Ashworth scales (Tables 1 and 2). Follow-up testing was done at approximately 6 months, 1 and 2 years after administration except in a few cases indicated so (Table 3). All neurologists involved did not participate in any recruitment for the study. The ASIA scale was used to evaluate motor and sensory function as previously described (48). The Frankel score was also used to classify each SCI patient with definitions as follows: A = complete paralysis, B = sensory function only below the injury level, C = incomplete motor function below injury level, D = fair to good motor function below injury level, and E = normal function. The widely used and accepted modified Ashworth score (34) was used in order to measure spasticity changes following transplantation with definitions as follows: 0 = no increase in tone, 1 = slight increase in muscle tone, manifested by a catch and release or minimal resistance at the end of the ROM when the affected part(s) is moved in flexion or extension, 1+ = slight increase in muscle tone, manifested by a catch, followed by minimal resistance throughout the remainder (less than half) of the ROM, 2 = more marked increase in muscle tone through most of the ROM, but affected part(s) easily moved, 3 = considerable increase in muscle tone, passive movement difficult, and 4 = affected part(s) rigid in flexion or extension. We evaluated these three clinical parameters because they are widely accepted assessment methods used in SCI and we are not equipped to perform somatosensory evoked potentials or motor evoked potentials.

Quality of Life Evaluation and Bladder Function Evaluation

The Barthel Index (www.strokecenter.org) was used in order to document changes in quality of life following administration of BMSCs. Briefly, there are 10 categories [feeding (0, 5, 10), bathing (0, 5), dressing (0, 5, 10), bowel (0, 5, 10), bladder (0, 5, 10), toilet use (0, 5, 10), transfers—bed to chair and back (0, 5, 10, 15), mobility—on level surfaces (0, 5, 10, 15), and stairs (0, 5, 10)] for a maximum score of 100. After carefully evaluating bladder function with urodynamic studies we noticed a need for a simplified in-depth scoring system that takes into account the method of voiding following SCI. We designed the Geffner, Gonzalez, Santacruz, and Flor (GGSF) scale, which is a bladder function scoring system from 0 to 6. A score of 0 = no urinary bladder sensation or function, 1 = patients with cystostomies that when are closed may involuntarily void through the urethra, 2 = bladder sensation or autonomic symptoms and inability to void, 3 = bladder sensation or autonomic symptoms and passive voiding (spontaneous release of urine), 3.5 = patients with open cystostomies that have bladder sensation or autonomic symptoms and passively void through the urethra (spontaneous release of urine), 4 = bladder sensation with incomplete voiding (needs catheterization to complete voiding), 5 = bladder sensation with active ability to void; however no control while voiding, 6 = complete bladder control. Definitions are listed in Table 5, and the scoring sheet is listed in Table 4.

Magnetic Resonance Imaging (MRI)

For all MRI studies a General Electric 1.0 Tesla closed system was used. In order to determine the site of injury, we analyzed several T1 and T2-weighted images as previously described (22). Prior to acceptance into the study, patients underwent MRI and only patients...
Table 1. Neurological Evaluations: Asia Impairment Grade/Frankel Grade/Ashworth Score

<table>
<thead>
<tr>
<th>Case Study</th>
<th>Prior to Administration</th>
<th>6 Months After Administration</th>
<th>1 Year After Administration</th>
<th>2 Years After Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 1</td>
<td>A/B/0</td>
<td>C/C/2</td>
<td>C/C/3</td>
<td>C/C/1</td>
</tr>
<tr>
<td>Case 2</td>
<td>A/A/3</td>
<td>A/C/1</td>
<td>A/C/1</td>
<td>C/C/2*</td>
</tr>
<tr>
<td>Case 3</td>
<td>A/A/0</td>
<td>ND</td>
<td>A/C/1</td>
<td>A/C/1</td>
</tr>
<tr>
<td>Case 4</td>
<td>A/A/0</td>
<td>C/C/1</td>
<td>C/C/1</td>
<td>C/C/1</td>
</tr>
<tr>
<td>Chronic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 5</td>
<td>B/C/1</td>
<td>B/C/0</td>
<td>C/D/1</td>
<td>C/D/1</td>
</tr>
<tr>
<td>Case 6</td>
<td>C/D/3.5</td>
<td>C/D/3</td>
<td>D/D/3.5</td>
<td>D/D/ND</td>
</tr>
<tr>
<td>Case 7</td>
<td>A/A/0</td>
<td>C/C/1</td>
<td>C/C/1</td>
<td>C/C/1†</td>
</tr>
<tr>
<td>Case 8</td>
<td>C/C/2</td>
<td>C/C/2</td>
<td>C/D/1</td>
<td>C/D/0</td>
</tr>
</tbody>
</table>

A summary of neurological evaluations for eight case studies. ND indicates not done. Case 4, 5, 6, 7, and 8 can walk with the aid of a walker or other medical device. There were few changes in spasticity (Ashworth score) for all cases described. The criteria of scoring systems are described below.

*1 year 6 months.
†1 year 3 months.

ASIA Impairment Scale
A complete: no preservation of function below level of injury, and no sacral sparing (S4–S5)
B incomplete: sensory but no motor function is preserved below the neurological level and includes the sacral segments S4–S5
C incomplete: motor function is preserved below the neurological level, and more than half of key muscles below the neurological level have a muscle grade less than 3
D incomplete: motor function is preserved below the neurological level, and at least half of key muscles below the neurological level have a grade of 3 or more
E normal: motor and sensory function are normal

Frankel Scale
A complete paralysis
B sensory function only below the injury level
C incomplete motor function below injury level
D fair to good motor function below injury level
E normal function

Modified Ashworth
0 no increase in tone
1 slight increase in muscle tone, manifested by a catch and release or minimal resistance at the end of the ROM when the affected part(s) is moved in flexion or extension
1+ slight increase in muscle tone, manifested by a catch, followed by minimal resistance throughout the remainder (less than half) of the ROM
2 more marked increase in muscle tone through most of the ROM, but affected part(s) easily moved
3 considerable increase in muscle tone, passive movement difficult
4 affected part(s) rigid in flexion or extension

with clear midsagittal T2-weighted images of the lesion site were allow into the study. The lesion site was quantified according to the midsagittal T2-weighted image and the vertebral segments were identified in order to isolate the area of interest for administration of BMSCs.

RESULTS

Table 3 illustrates the demographics of each case (Cases 1–4 acute; Cases 5–8 chronic). Bone marrow isolated from each patient was evaluated by FACS analysis for the presence of CD34+ stem cells (Fig. 1, Table 3). The patients were administered with an average of 1.2 × 10^6 CD34+ cells per kilogram of body weight for an average total of 90.0 × 10^6 CD34+ cells per administration (Table 3). Prior to BMSCs administration each patient underwent an MRI and neurological examination. At approximately 6 months, 1 year, and 2 years following BMSCs administration the patients all underwent follow-up MRIs and neurological exams. Following administration of BMSCs there are noticeable morphological changes within the spinal cord as illustrated by sequential MRIs of an acute patient (Fig. 2a–d) and chronic patient (Fig. 2e–h) taken prior to administration, 6 months after BMSCs administration, 1 year after BMSCs administration, and approximately 2 years after BMSCs administration (Fig. 2). These studies illustrate that administration of BMSCs directly into the spinal
Table 2. ASIA Motor Score/Sensory Light Touch Score/Sensory Pin Prick Score

<table>
<thead>
<tr>
<th>Case Study</th>
<th>Prior to Administration</th>
<th>6 Months After Administration</th>
<th>1 Year After Administration</th>
<th>2 Years After Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 1</td>
<td>50/64/64</td>
<td>54/72/72</td>
<td>54/83/80</td>
<td>56/90/90</td>
</tr>
<tr>
<td>Case 2</td>
<td>50/49/49</td>
<td>52/50/50</td>
<td>52/54/54</td>
<td>52/54/57*</td>
</tr>
<tr>
<td>Case 3</td>
<td>50/42/42</td>
<td>ND</td>
<td>51/58/58</td>
<td>51/60/64</td>
</tr>
<tr>
<td>Case 4</td>
<td>50/76/76</td>
<td>52/78/78</td>
<td>56/80/82</td>
<td>58/80/84</td>
</tr>
<tr>
<td>Chronic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 5</td>
<td>52/88/88</td>
<td>54/88/88</td>
<td>64/100/101</td>
<td>68/100/101</td>
</tr>
<tr>
<td>Case 7</td>
<td>50/70/70</td>
<td>54/70/70</td>
<td>54/70/73</td>
<td>61/72/73†</td>
</tr>
<tr>
<td>Case 8</td>
<td>58/86/86</td>
<td>58/88/88</td>
<td>66/98/98</td>
<td>70/98/98</td>
</tr>
</tbody>
</table>

Detailed ASIA motor and sensory scores for all eight cases. Motor scoring: 0 = total paralysis, 1 = palpable or visible contraction, 2 = active movement, gravity eliminated, 3 = active movement against gravity, 4 = active movement against some resistance, and 5 = active movement against full resistance. Scores are accumulated from right and left sides and are based upon evaluating a total of five arm and five leg muscle groups (total of 100 points maximum). The ASIA sensory scoring is for light touch and pin prick: 0 = absent, 1 = impaired, and 2 = normal. There are 28 dermatomes assessed for a total of 112 possible points. ND indicated not done.

*1 year 6 months.
†1 year 3 months.

Table 3. Case Studies Demographics

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Gender</th>
<th>Age</th>
<th>Weight (kg)</th>
<th>Injury Level</th>
<th>Injury Type</th>
<th>CD34/kg Cell/10e6</th>
<th>Viability CD34 (%)</th>
<th>Time of BMSCs Administration After SCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>28</td>
<td>80</td>
<td>T9</td>
<td>gunshot</td>
<td>1.43</td>
<td>89.62</td>
<td>1.5 months</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>33</td>
<td>75</td>
<td>T4</td>
<td>gunshot</td>
<td>1.1</td>
<td>82.22</td>
<td>7 months</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>28</td>
<td>79</td>
<td>T5–6</td>
<td>fall</td>
<td>1.5</td>
<td>77.62</td>
<td>13 days</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>31</td>
<td>67</td>
<td>T12–L1</td>
<td>fall</td>
<td>0.94</td>
<td>96.27</td>
<td>5 days</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>37</td>
<td>86</td>
<td>T12</td>
<td>car accident</td>
<td>1.2</td>
<td>91.22</td>
<td>6 years 3 months</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>42</td>
<td>72</td>
<td>T4</td>
<td>gunshot</td>
<td>1.3</td>
<td>91.93</td>
<td>21 years 10 months</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>27</td>
<td>80</td>
<td>T11</td>
<td>gunshot</td>
<td>0.88</td>
<td>91.15</td>
<td>5 years 10 months</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>44</td>
<td>68</td>
<td>T12</td>
<td>fall</td>
<td>1.43</td>
<td>89.62</td>
<td>6 years 9 months</td>
</tr>
</tbody>
</table>

Demographics of each patient are described including the type of injury, number, and viability of CD34+ cells that were administered in the cell suspension, and time of administration following SCI. Each patient had an average of 1.2 million CD34+ cells/kg of body weight administered (average of 90 million CD34+ cells total transplanted). Cases 1–4 are acute injuries while cases 4–8 are chronic.
same MRI intensity of normal tissue and dorsal recuperation of normal spinal cord signal below the injury level. Approximately 2 years post-BMSCs administration the patient has progressed significantly with an ASIA impairment grade of C, motor score 56, and Frankel grade of C. In addition, there was sensation through the S4–5 dermatome (light touch or pinprick; score of 90, 90, respectively) (Tables 1 and 2, Fig. 3a–c). Quality of life (Barthel score) increased from a score of 20 to 90 (Fig. 4a) and bladder function improved from no function to complete bladder control (Fig. 5a). The patient can stand on parallel bars and takes small strides with the use of a walker or crutches. Interestingly, the patient now comes to physical therapy riding a quad motor bike.

**Case 2**

A 33-year-old female sustained a gunshot wound to the thoracic area resulting in an injury to the spinal cord at the T4 vertebral level. Initial evaluation of the patient’s MRI illustrated a transection at T5.2–T6.1 and a severe contusion injury with edema from T4.3 to T5.1. The patient’s evaluation prior to BMSCs administration demonstrated that she sustained a complete injury (ASIA impairment grade A, motor score 50, Frankel grade A) with no motor functions preserved below the level of injury. In addition, there was no sensation (light touch or pinprick; score of 47, 47, respectively) below the T7 dermatome (Tables 1 and 2, Fig. 3a–c). Eleven days following her SCI, the patient underwent a first surgery in order to decompress the spinal cord and remove the bullet fragment from the spinal canal. Following decompression, the patient had no functional changes even though she underwent a strict rehabilitation regimen; therefore, administration of BMSCs was performed 7 months after spinal cord injury. At 6 months following BMSCs administration the patient progressively started to improve (Tables 1 and 2, Figs. 3a–c, 4a, 5a). At 6 months postadministration her ASIA motor and sensory scoring had improved slightly (52, 50, and 50 for motor, sensory light touch, and sensory pin prick, respectively) (Table 2, Fig. 3a–c) while her quality of life (Barthel Score) improved 35 points (Fig. 4a). Her most recent MRI (18 months) illustrated the persistence of a complete transection at T5.2–T6.1 with scar tissue at T5.2–T6.1. In addition, there was retraction of supraposterior cord fibers. There was no evidence of edema. At 18 months following BMSCs administration, her bladder function has improved from none to having sensation (Fig. 5a). Also, she improved from an ASIA impairment grade A to C (52, 54, and 57 for motor, sensory light touch, and sensory pinprick, respectively), Frankel A to C, and her spasticity had decreased from a 3 to 2 (Tables 1 and 2, Fig. 3a–c). There was no sensation below the T9 dermatome. Overall, we have noticed a qualitative increase in her life. She can now stand on the parallels with braces on.

**Case 3**

A 28-year-old male fell from a tree approximately 8 m and sustained an injury to his spinal cord at the T5–6 vertebral spinal level. Initial MRI illustrates an oblique hemisection on the left side at T5.1–T6.1 and contusion with edema cranial at T4.3–T3.1 and caudal at T6.2–T7.3. There is dilation of the ependyma below the injury

**Figure 1.** Fluorescence activated cell sorting (FACS) results. Following mononuclear cell isolation each case had an aliquot of cells sent for identification of CD34+ cells. A representative plot of all cases demonstrates the identification of CD34+ cells in the bone marrow of patients with SCI.
and posterior vertebral displacement narrowing the canal. Initial neurological evaluation demonstrates that the patient sustained a complete injury (ASIA impairment grade A, Frankel A) with no motor function preserved below the injury area (motor score 50) (Tables 1 and 2, Fig. 3a–c). In addition, there was no sensation (light touch or pinprick; score of 42, 42, respectively) below the T5 dermatome (Table 2, Fig. 3b, c). Patient was ad-

Figure 2. MRI evaluations were performed for each case at several intervals. MRI images of an acute patient (Case 1) prior to administration (a), at 6 months (b), at 1 year (c), and approximately 2 years (d) after administration of BMSCs demonstrates structural changes of the spinal cord as time progresses following administration of BMSCs. The images illustrate a lesion of the spinal cord at T9 from a bullet (a). As time progresses there is the formation of a syringomyelic cavity with spinal cord thickening and the recuperation of normal signal below the injury site (d). MRI images of a chronic patient (Case 5) prior to administration (e), at 6 months (f), at 1 year (g), and at approximately 2 years (h) after administration demonstrates structural changes of the spinal cord as time progresses following administration of BMSCs. The images illustrate a lateral hemisection of the spinal cord at T11 with residual cavities at T12.1–T12.2 (e). At approximately 2 years following BMSCs administration the MRI illustrates a decrease in the residual cavity at T12.1 (h). Arrows denote injury area. (a–d) Acute injury; (e–h) chronic injury.
Table 4. Geffner, Gonzalez, Santacruz, and Flor (GGSF) Bladder Function Scale

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No urinary bladder sensation or function*†‡§</td>
</tr>
<tr>
<td>1</td>
<td>Patients with cystostomies that when are closed may involuntarily void through the urethra*</td>
</tr>
<tr>
<td>2</td>
<td>Bladder sensation or autonomic symptoms and inability to void*†‡§</td>
</tr>
<tr>
<td>3</td>
<td>Bladder sensation or autonomic symptoms and passive voiding (spontaneous release of urine)*†‡</td>
</tr>
<tr>
<td>3.5</td>
<td>Patients with open cystostomies that have bladder sensation or autonomic symptoms and passively void through the urethra (spontaneous release of urine)*</td>
</tr>
<tr>
<td>4</td>
<td>Bladder sensation with incomplete voiding (needs catheterization to complete voiding)†‡§</td>
</tr>
<tr>
<td>5</td>
<td>Bladder sensation with active ability to void; however no control while voiding</td>
</tr>
<tr>
<td>6</td>
<td>Complete bladder control</td>
</tr>
</tbody>
</table>

*Patients use suprapubic cystostomies in order to void their urinary bladder.  
†Patients use a catheter in order to void their urinary bladder.  
‡Patients with a urine collector.  
§Patients that manually compress (massage) the hypogastric region in order to void their urinary bladder.

ministered BMSCs 13 days following SCI and metal rods were placed in order to stabilize his vertebral column. The patient never showed up for his rehabilitation regimen and was given inadequate care at home immobile for 32 days. The patient acquired a giant bed sore that had to be surgically removed. The bed sore caused an infection, leading to a definitive colostomy and removal of the left femoral head. Due to the severe bed sore the patient had many autologous skin graft surgeries in order to repair the damaged area. The patient had an initial Barthel score of 5 (Fig. 4a), bladder function score of 0 (Fig. 5a), and Ashworth (spasticity) score of 0 (Table 1). At approximately 2 years post-BMSCs administration the patient has an improved Frankel grade of C, an ASIA impairment grade of A with some improvements in his sensory score (60, 64 for light touch and pin prick, respectively), and no sensation below the T11 dermatome (Tables 1 and 2, Fig. 3b, c). His quality of life score (Barthel Index) has increased from 5 to 40 (Fig. 4a) and bladder function increased from no bladder function to bladder sensation or autonomic symptoms and passive voiding (spontaneous release of urine) (Fig. 5a). We were unable to perform additional MRIs after placement of the metal rods. Considering the challenges presented with his bed sore and the fact that he has not performed any rehabilitation regimen, this patient has improved his quality of life following administration of BMSCs.

Case 4

A 31-year-old male fell from a ladder approximately 3 m and sustained an injury to his spinal cord at the T12–L1 vertebral level. The patient was evaluated immediately after injury. MRI of the vertebral column illustrated posterior vertebral displacement of L1 over T12 grade II and severe narrowing of the spinal canal. There was a contusion with edema at T12.2–L1.3 with a hematoma. The initial neurological evaluation demonstrates that the patient sustained a complete injury (ASIA impairment grade A, Frankel A) with no motor function preserved below the injury level (motor score 50) (Tables 1 and 2, Fig. 3a). There was no sensation

Table 5. Definitions and Terms

<table>
<thead>
<tr>
<th>Term</th>
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<tr>
<td>Bladder sensation</td>
<td>A feeling or awareness of conditions within the urinary bladder resulting from the stimulation of sensory receptors</td>
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<tr>
<td>Bladder function</td>
<td>The action performed by the urinary bladder (i.e., the ability to void urine)</td>
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<tr>
<td>Suprapubic cystostomies</td>
<td>A connection between the urinary bladder and the skin to drain urine from the bladder</td>
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<tr>
<td>Void</td>
<td>The ability to evacuate (empty) the urinary bladder</td>
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<tr>
<td>Involuntary</td>
<td>Independent of or contrary to volition</td>
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<tr>
<td>Dysesthesia</td>
<td>Abnormal sensations on the skin, such as a feeling of numbness, tingling, burning, itching, electric shock, pins and needles</td>
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<tr>
<td>Autonomic symptoms</td>
<td>Dysesthesia, pyloric erection, pain, burning, sudden hypertension, sweating, and bradycardia normally triggered by distention of the urinary bladder</td>
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<tr>
<td>Passive voiding</td>
<td>Evacuation or partial evacuation of the urinary bladder without any control</td>
</tr>
<tr>
<td>Manual bladder (hypogastric) compression for voiding</td>
<td>Through active compression (massage) of the hypogastric region, urine is released through the urethra</td>
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**Figure 3.** Graphical representations of the ASIA motor, ASIA sensory light touch, and ASIA sensory pin prick. There are motor improvements in all cases but most notable are acute cases 1 and 4, and chronic cases 5, 6, 7, and 8 (a). ASIA sensory light touch and pin prick scores demonstrate that there are improvements in all cases but most notable for acute cases 1 and 3 and chronic cases 5, 6, and 8 (b, c). *The last follow-up for Case 2 was at 1 year 6 months. **The last follow-up for Case 7 was at 1 year 3 months. ND, not done.
Figure 4. Quality of life evaluation performed using the Barthel Index Score for eight cases. Barthel scoring indicates an improvement in quality of life for all four acute spinal cord injury cases (a) and all four chronic spinal cord injury cases (b) following administration of BMSCs. The greatest improvements occurred from prior to administration to 6 months after administration of BMSCs (a, b). ND, not done. **The last follow-up for Case 2 was at 1 year 6 months. *The last follow-up for Case 7 was at 1 year 3 months.

(light touch or pinprick; score of 76 and 76, respectively) below the T12 dermatome (Table 2, Fig. 3b, c) and he had an initial Ashworth score of 0 (Table 1). Quality of life scoring demonstrates an initial Barthel score of 30 (Fig. 4a) and no bladder function (Fig. 5a). Five days following initial trauma posterior rods were placed in order to stabilize the vertebral column and administration of BMSCs was done. At 2 years following BMSCs administration the patient has improved significantly (ASIA impairment grade C, motor score 58, Frankel score C) with active movement against gravity at the hip flexors and sensation through the L1 dermatome and at the S2–S3 dermatome (light touch and pin prick; 84 and 80, respectively) (Tables 1 and 2, Fig. 3a–c). Barthel scoring demonstrates an improvement from an initial score of 30 to 90 (Fig. 4a) while bladder function went from absolutely no function to bladder sensation with active ability to void; however, no control while voiding (Fig. 5a). We were unable to do any more MRI due to the placement of metal rods. This patient can
walk a couple of blocks with the use of a walker and ankle braces.

**Case 5**

A 37-year-old male sustained an injury to the spinal cord at the T12 vertebral spinal level from a car accident. The patient was evaluated 6 years 2 months following injury. MRI prior to BMSCs administration illustrated an anterior disc herniation at T11–12 and a lateral hemisection of the spinal cord at T11.3. In addition, there was a residual cavity from T12.1 to T12.2 (Fig. 2e). The initial neurological evaluation demonstrated that the patient sustained an incomplete injury (ASIA impairment grade B, Frankel C) with only palpable or

**Figure 5.** Evaluation of bladder function using a newly designed bladder function assessment scoring system entitled Geffner, Gonzalez, Santacruz, and Flor (GGSF) Bladder Function Score. There were significant improvements in bladder function for acute SCI Cases 1 and 4 (a) and chronic SCI Case 7 (b) after administration of BMSCs. In addition, chronic SCI Cases 5 and 6 improved to complete bladder control. Overall, all SCI cases evaluated had an improvement in bladder function as assessed using the GGSF scale. Full scale descriptions are attached as an appendix. ND, not done. **The last follow-up for Case 2 was at 1 year 6 months. The last follow-up for Case 7 was at 1 year 3 months.**
visible contractions at the L2 level (ASIA motor score 52, 1 point on each side at the L2 level) (Tables 1 and 2, Fig. 3a). In addition, there was impaired sensation (light touch or pinprick; score of 88 and 88, respectively) through the S4–5 dermatome (Table 2, Fig. 3b, c) and an Ashworth score of 1 (Table 1). Initial Barthel score was a 65 (Fig. 4b) and he had bladder sensation with active ability to void; however, no control while voiding (GGSF score of 5) (Fig. 5b). Six years and 3 months following SCI the patient underwent a partial disectomy and administration of BMSCs. At 2 years following administration the patient has improved significantly with the MRI illustrating a disc herniation at T11–12, a lesion at T11.3, and a small cavity at T12.1 (Fig. 2h). His neurological evaluation demonstrates an ASIA impairment grade of C (improved motor score of 68, scores of 1–3 from the L2–S1 level), and Frankel D (Tables 1 and 2, Fig. 3a). His ASIA sensory score has increased from 88 light touch and 88 pin prick to 100 light touch and 101 pin prick (Table 2, Fig. 3c, b). Barthel score is now 100 (maximum value) and he has full control of his bladder (Figs. 4b and 5b, respectively). This patient can now walk with braces and crutches for more than 1 h.

Case 6
A 42-year-old male sustained a gunshot wound to the chest in 1984 that penetrated his vertebral column and was lodged in his dura at the T4 vertebral spinal level, causing an injury to his spinal cord. The patient immediately underwent a laminectomy at T3–4 and surgical removal of the bullet. MRI prior to BMSCs administration illustrated that he has an oblique lesion at T3.2–T4.1 and hypoplasia. There is a residual cavity at T3.2–T4.2. His neurological evaluation prior to administration (21 years 10 months after initial trauma) demonstrated that he is categorized as an incomplete injury (ASIA impairment grade C, Frankel D) with active movement, gravity eliminated through the L4 level on the left/L3 level on the right and palpable or visible contractions at the L5–S1 level on the left side only (ASIA motor score 62) (Tables 1 and 2, Fig. 3a). In addition, there was no sensation below the T7 dermatome on the right and impaired through the S4–5 dermatome from T6 (light touch or pinprick; score of 66 and 66, respectively) (Table 2, Fig. 3b, c). Initial Barthel score was 55 (Fig. 4b) and he had bladder sensation with active ability to void; however, no control while voiding (score of 5) (Fig. 5b). His 2-year follow-up MRI illustrated minor changes with an oblique lesion at T3.3–T4.3 and hypoplasia. There remains the residual cavity at T3.2–T4.2. Neurological evaluation 2 years after BMSCs administration demonstrated a significant improvement of motor function with an ASIA impairment grade of D, motor score 94, and Frankel D grade (Tables 1 and 2, Fig. 3a). Moreover, his sensory scoring has improved from a 66 light touch/66 pin prick to a 94 light touch/94 pin prick (Table 2, Fig. 3b, c). Barthel score has improved dramatically to a maximum score of 100 and he has complete bladder function (Figs. 4b and 5b, respectively). He now has the ability to walk using forearm crutches and is able to walk up and down stairs.

Case 7
A 27-year-old male sustained a gunshot wound to the scapula region that passed through his spine and exited his chest, causing a spinal cord injury at the T11 vertebral level. The patient was evaluated for BMSCs administration 5 years 9 months follow SCI. Initial MRI illustrated a fracture at T11.1 with a cavity from the projectile in the vertebral body and an oblique hemisection of the spinal cord at T11.1–T11.2. His neurological evaluation prior to BMSCs administration demonstrated that he has a complete injury (ASIA impairment grade A, Frankel A) with a motor score of 50, meaning the absence of all key muscles below the injury level (Tables 1 and 2, Fig. 3a). There was no sensation below the T11 dermatome (70 and 70 for light touch and pin prick, respectively) and his Ashworth score was 0 (Tables 1 and 2, Fig. 3b, c). His Barthel score was 35 and he had no bladder function (Figs. 4b and 5b, respectively). MRI evaluation 1 year 3 months following BMSCs administration illustrated the existence of the fracture at T11.1 and the oblique hemisection injury has expanded from T11.1–T12.1. There was a small residual cavity above the injury from T10.1–T10.3. Although his MRI demonstrates an expanded injury, his neurological evaluation indicated a significant functional improvement (ASIA impairment grade C, improved motor score of 61) (Tables 1 and 2, Fig. 3a). In addition, his sensory score has increased slightly with impaired sensation at the S2–S3 dermatome level (light touch 72, pin prick 73) (Table 2, Fig. 3b, c). There are little changes in his spasticity. Barthel scoring indicates a significantly improvement to a score of 85 (Fig. 4b) and his bladder function has improved from 0 to a 3 (sensation or autonomic symptoms and passive voiding) (Fig. 5b). One year 3 months following BMSCs administration with the aid of a walker and braces, this patient has gained the ability to walk.

Case 8
In 1999, a 44-year-old male fell 8 m and sustained an injury to the spinal cord at the T12 vertebral spinal level. Five days following SCI the patient had metal rods placed in order to stabilize his vertebral column, which were subsequently removed 4.5 years later. The patient was evaluated at the hospital for BMSCs administration in early 2006. His MRI prior to BMSCs administr-
istration illustrated a compression of the vertebral body at T12 with a 10% posterior displacement. There was compression of the spinal cord at T11.2–T12.1 with a residual cavity at T11.2–T12.3. His initial neurological examination with us indicated that he had an incomplete injury (ASIA impairment grade C, Frankel C) with active movement, gravity eliminated at L2 and L3 (motor score 58) (Tables 1 and 2, Fig. 3a). There was no sensation below the L3 dermatome (86 and 86, light touch and pin prick, respectively) and his Ashworth score was 2 (Tables 1 and 2, Fig. 3b, c). His Barthel score was a 55 (Fig. 4b) and he had bladder sensation with incomplete voiding (score of 4) (Fig. 5b). Two years following BMSCs administration his MRI demonstrated a lesion at T11.3–T12.1 with a residual cavity that has shrunk at T11.2–T11.3. There was a small edema below the lesion at T12.2–T12.3. Neurological evaluation indicated functional improvement (ASIA C, improved motor score of 70, and Frankel D) and a reduction in spasticity (Tables 1 and 2, Fig. 3a). There was impaired sensation through the S4–5 dermatome with an improved sensory score of 98 for light touch and pin prick, respectively (Table 2, Fig 3b, c). Barthel scoring indicates he has improved to the maximum score of 100 (Fig. 4b) and he has the ability to actively void his bladder (GGSF score 5) (Fig. 5b). Interestingly, even though he has the ability to walk with crutches and can go up and down stairs; because he has active movement against full resistance (score 5) in only 40% of his key muscles he continues to be considered ASIA impairment grade C.

**DISCUSSION**

The standard of care for acute SCI is methylprednisolone and/or decompression. Clinical studies using surgical intervention for acute SCI (decompression) have shown no difference in neurological recovery between operated and nonoperated patients (50,51). In three National Acute Spinal Cord Injury Studies (NASCIS I, II, and III), the use of methylprednisolone demonstrated minimal overall benefits (10–12). These benefits did not come without much skepticism of the post hoc analysis of the data (17,30,40). Indeed, the use of methylprednisolone or any steroid does not come without risk. In all three NASCIS studies the incidence of sepsis and pneumonia was higher in the methylprednisolone groups compared to other treatment groups. Moreover, gastrointestinal complications were also reported following high-dose methylprednisolone (10–12). According to a committee of Canadian physicians, the use of high-dose methylprednisolone is not an evidence-based standard of care for SCI (29).

It has been demonstrated that intravenous and intracranial transplantation of mononuclear cells into SCI is safe (48). Here we demonstrate that administration of BMSCs via multiple routes is feasible, safe, and most importantly improves the quality of life for both acute and chronic patients suffering from SCI. Moreover, there has been no incidence of infection or severe adverse reactions for any cases documented to date. This study clearly documents the feasibility of cell replacement strategies as an alternative therapy for SCI. In addition, we have a unique method of delivering the cells; all patients get cells directly into the spinal cord, directly into the spinal canal, and an intravenous injection of cells. Although multiple route administration limits the mechanistic understanding for cell-based applications, this assures us that the BMSCs will reach the necessary target in order to fulfill their niche and possibly promote host endogenous repair within the SCI area. Shi et al. demonstrated that bone marrow-derived CD34+ EPCs from canines migrated to the vascular injury site (44). In another study, autologous bone marrow CD34+ cells labeled with magnetic nanoparticles that were delivered into the spinal cord via lumbar puncture migrated into the injured site in patients with chronic SCI (13).

Following primary trauma to the adult spinal cord there is evidence of hemorrhage and blood flow is attenuated within the spinal cord. This disruption in blood flow leads to spinal cord infarction, the disruption of the blood–spinal cord injury barrier, edema, the release of vasoactive molecules influencing spinal cord perfusion, and ischemia. This primary disruption ultimately leads to the death of millions of cells and the activation of secondary degeneration, which is a cascade of events that exacerbate primary trauma (18,36,38). Secondary degeneration is the present target for pharmaceutical intervention in acute SCI; however, any intervention would have to be applied rapidly as within 72 h the white matter in the spinal cord is thought to be irreversibly damaged (8) and cell death progresses for months due to the complicated cascade of secondary degeneration (19).

Autologous stem cells represent an alternative therapy when conventional methods have not worked for various devastating disorders. Specifically, CD34+ stem cells (HSCs) and/or endothelial progenitor cells (EPCs), which are easily attainable from bone marrow or peripheral blood. HSCs and EPCs are well known for their ability to promote angiogenesis (43,54). Angiogenesis is necessary for wound healing and likely plays a critical role in establishing a growth-permissive environment. Results have shown that EPCs play an essential role in repairing injured blood vessels and transplantation of EPCs into ischemic limbs of rats induced collateral vessel formation (31). Moreover, it was noted that transplanted EPCs secrete proangiogenic growth factors such as VEGF, HGF, and IGF-1, which lead to new vessel development (39,53). Studies indicate that progenitor
cells contribute not only to the restoration of injured tissues, but also to the pathological remodeling (26,33,53). Interestingly, in SCI revascularization occurs prior to nerve regeneration (55). Because BMSCs possess angiogenic properties, we hypothesize that improved blood flow and oxygen supply within the injury area may have contributed to the functional improvements seen in SCI patients transplanted with autologous BMSCs. Alternatively, it is well documented that BMSCs promote host endogenous repair (15,27,28). For instance, transplantation of marrow stromal cells into SCI rats promotes axonal regeneration (28), and transplantation of BMSCs into a mouse model of chemically induced pancreatic damage causes endogenous pancreatic regeneration (27).

Clearly, administration of autologous BMSCs is a promising cell replacement strategy for several human disorders.

One of the greatest challenges in finding a treatment or improving the quality of life for the SCI community is the vast heterogeneity of human SCI; no two injuries are alike. It is well understood that tremendous challenges exist in gathering data in order to validate a specific treatment type for SCI (47,49). Presently, we have performed administration of autologous BMSCs using our unique multiple route delivery system into 52 SCI patients. However, it has been challenging to gather comprehensive long-term data. Therefore, in this study we present the eight cases that we have with comprehensive long-term data. We presently have 25 cases of SCI with slightly greater than 3 months comprehensive follow-up. There have been no cases of tumor formation, increased pain, and/or deterioration of function following administration of BMSCs. There were very few adverse reactions observed following administration of BMSCs. We observed a transient lack of erection and/or ejaculation in four patients, sweating on one half of the body on two patients, and spinal cord canal fistula in one patient whose metal rods had to be withdrawn. Nonetheless, there is an overall improvement in the quality of life for patients with SCI following administration of BMSCs.

One of the challenges in this study was with patients that had clear improvements that cannot be reflected correctly by the ASIA impairment grade. There are several individuals that have large improvements in only one to two major muscle groups yielding a definite improvement in quality of life; however, ASIA impairment grade does not reflect these beneficial changes (7). For instance, Case 8 has gone from an initial ASIA motor score of 58 (motor score of 2 on each side at the L2 and L3 levels) to an ASIA motor score of 70 (motor score of 5 on each side at the L2 and L3 levels), meaning active movement against full resistance for the hip flexors and knee extensors. This individual can walk with crutches and can go up and down stairs, which is a vast improvement in his quality of life. Other challenges are whether to separate out the ASIA upper and lower extremity scores. In almost all of our cases lower extremity scores were nonexistent (total motor score of 50) and a 5–10 point increase may make a large difference if one separates the upper and lower extremity scores. In agreement with the ICCP panel, our personal observation is that separation of lower and upper extremities in the ASIA motor scale can better indicate functional improvement compared to combining the data, which may illustrate a nominal effect (47).

A survey of the SCI community illustrates that one of the most important aspects in improving their quality of life would be the ability to control bladder function (2). This study indicated that presently we would greatly benefit from the development of treatments that lead to partial functional recovery in order to improve the quality of life for the SCI community. Our data clearly demonstrate that administration of autologous BMSCs improves the quality of life for SCI patients (Figs. 4 and 5). Because bladder function is of great importance, we designed the GGSF bladder function score to facilitate bladder function assessment (Tables 4 and 5). It is more in depth compared to questionnaires in quality of life scoring systems, such as the Barthel index, which are a crude method of assessing bladder function (0 = incontinent, or catheterized and unable to manage alone; 5 = occasional accident; 10 = continent) yet measure overall quality of life changes. It is well understood that spontaneous recovery in SCI can occur for as long as 1 year following SCI (21). Indeed, this may have occurred within our acute SCI patients; however, it is important to note that all of our acute patients improved significantly with no signs of deterioration or impediment of presumed spontaneous recovery. Moreover, Case 2 had a laminectomy followed by physical therapy for 6 months with no improvements. Following administration of BMSCs, her quality of life improved.

In conclusion, these studies document an alternative cell-based method of treating SCI. Interestingly, it documents that BMSCs administration via multiple routes may be an effective treatment for patients that suffered a SCI from a gunshot wound. To the best of our knowledge this is the first documentation of SCI patients being administered BMSCs directly into the spinal cord following gunshot wounds. Presently, we are investigating long-term effects of BMSCs administration in quadriplegic injuries. However, the difficulties of tracking, monitoring, and assessing several parameters in patients that have been administered BMSCs remains our largest challenge. Nevertheless, we report here that administra-
tion of BMSCs via multiple routes is safe and feasible for several SCI types. Most importantly, it may improve the quality of life for many suffering from SCI.

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