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Phase I-II Clinical Trial Assessing Safety and Efficacy of Umbilical Cord Blood Mononuclear Cell Transplant Therapy of Chronic Complete Spinal Cord Injury

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Running Header: Umbilical Cord Blood Mononuclear Therapy of SCI

ABSTRACT

Umbilical cord blood (UCB) mononuclear cells (UCBMNC) transplants improve recovery in animal spinal cord injury (SCI) models. We transplanted UCBMNC into 28 people with chronic complete SCI in Hong Kong (HK) and Kunming (KM). Stemcyte Inc. donated UCBMNC isolated from human leukocyte antigen (HLA≥4:6) matched UCB units. In HK, four participants received four 4-µL (1.6 million cells) injections into dorsal entry zones above and below the injury site and another four received 8-µL (3.2 million cells) injections. The 8 participants averaged 13 years after C5-T10 SCI. Magnetic resonance diffusion tensor imaging of 5 participants showed white matter gaps at the injury site before treatment. Two participants had fiber bundles growing across the injury site by 12 months and the rest had narrower white matter gaps. Motor, walking index of SCI (WISCI) and spinal cord independence measure (SCIM) scores did not change. In KM, five groups of four participants received four 4-µL (1.6 million cells), 8-µL (3.2 million cells), 16-µL (6.4 million cells), 6.4 million cells plus 30mg/kg methylprednisolone (MP), or 6.4 million cells plus MP and a 6-week course of oral lithium carbonate (750 mg/day). KM participants averaged 7 years after C3-T11 SCI and received 3-6 months of intensive locomotor training. Before surgery, only 2 participants walked 10 meters with assistance and did not need assistance for bladder or bowel care before surgery. The rest could not walk or do their bladder and bowel care without assistance. At a year (41-87 weeks), WISCI and SCIM scores improved, i.e. 15/20 participants walked 10 meters (p=0.001); 12/20 did not need assistance for bladder care (p=0.001) or bowel care (p=0.002). Five participants converted from complete to incomplete (2 sensory, 3 motor; p=0.038) SCI. We conclude that UCBMNC transplants and locomotor training improved WISCI and SCIM. Additional clinical trials are proposed.

Keywords: umbilical cord blood, spinal cord injury, mononuclear cells, lithium, central pattern generator

INTRODUCTION

Umbilical cord blood (UCB) mononuclear cell (UCBMNC) transplants improve walking recovery in rat [1-18] and dog [19-24] spinal cord injury (SCI) models. Investigators gave the cells by intravenous infusion [1,25,26], intrathecal injection [27,28], or transplantation into the spinal cord [3,4,6,15,21]. A few investigators used allogeneic canine [19,23,29] or fetal rat UCB [13], some directly infused human UCB intravenously [1,8,15] while others used human UCBMNC enriched for CD34+ cells [3,5-7,10,16,21,25,30-35] or CD45+ cells [28], mesenchymal cells cultured from human [4,11,12,19,22,24,27,36-46] or canine UCB [19,23,29,47], human UCB cells selected for neural characteristics [48-53], somatic stem cells [18], human UCBMNC transfected to express growth factors [54,55], human UCBMNC combined with olfactory ensheathing glia [56] or lithium chloride [12]. In addition, many groups studied human umbilical cord tissue-derived mesenchymal cells [14,19,24,29,36-39,45,46,57-79]

UCBMNC may improve recovery through multiple mechanisms, including secretion of anti-inflammatory cytokines [9,30-32], release of growth factors [8,10,11,46], upregulation of matrix metalloproteinases [31], downregulation of tissue plasminogen activator [32], prevention of apoptosis [30], facilitation of myelination [7,22,49], reduced gliosis [24,47], and increased angiogenesis [35]. Although several groups have claimed that UCB differentiate into neural precursors [80,81] or neural stem cells [82-85], none have provided convincing evidence of neuronal or astroglial production by UCB stem cells transplanted into animal spinal cords.

Lithium stimulates stem cell proliferation [86], neurogenesis [87], and regeneration of long spinal tracts.[88-90] Systemic lithium treatment increases neurotrophin expression in contused rat spinal cords after transplants of neonatal rat mononuclear cells, including nerve growth factor (NGF), neutrophin-3 (NT-3), and glial derived neurotrophic factor (GDNF) known to stimulate spinal axonal growth. [91] Deng, et al. [12] reported that lithium combined with human UCBMNC improves locomotor recovery in rats after SCI. We therefore proposed to do clinical trials to assess safety and effects of lithium, UCBMNC, and UCBMNC plus lithium therapy of SCI.

Several groups have transplanted UCBMNC and UCB mesenchymal cells into people with SCI. In 2005, Kang, et al.[92] reported hip and thigh movement recovery after transplanting human leukocyte antigen (HLA) matched UCB "multipotent stem cells" into the spinal cord of a 37-year old woman with chronic SCI. In 2010, Ichim, et al.[93] transplanted UCBMNC into the spinal cord of a patient with chronic SCI. In 2011, Cordes, et al.[94] transplanted human CD34+ UCB cells into spinal cord of a patient with amyotrophic lateral sclerosis.[94] In 2013, Yao, et al.[96] reported improved autonomic function and somatosensory evoked potentials 12 months after intrathecal and intravenous injection of UCBMNC into 25 patients with chronic SCI (>6 months). Several groups have transplanted umbilical cord mesenchymal cells[97-100] intrathecally into patients with SCI. Except for the two case reports, none of the trials transplanted UCBMNC directly into the spinal cord.

We did Phase I and II clinical trials in Hong Kong (HK) and Kunming (KM) to assess the safety and efficacy of transplanting escalating doses of HLA-matched (≥4:6) UCBMNC into spinal cords of people with chronic (1-19 years after) complete C5-T11 SCI. The phase I trial in HK transplanted 1.6 or 3.2 million UCBMNC into spinal cord above and below the injury site. The patients (average 13 years after injury) did not receive any walking training and we did magnetic resonance diffusion tensor imaging (MR-DTI) to visualize long spinal tracts. The phase II trial in KM randomized 20 patients with chronic (average 7 years after) complete C5-T11 SCI to five treatment groups receiving 1.6, 3.2, or 6.4 million UCBMNC, 6.4 million UCBMNC with a 30-mg/kg bolus dose of methylprednisolone (MP), or 6.4 million UCBMNC with MP and a 6-week course of oral lithium carbonate. In KM, the patients started 3-6 months of intensive locomotor training and were assessed at 6 weeks, 3 months, 6 months, and one year after surgery for changes of American Spinal Injury Association and International Spinal Cord Society (ASIA/ISCOS) impairment scale (AIS) classification, motor and sensory scores, the walking index of spinal cord injury (WISCI), the spinal cord independence measures (SCIM), modified Ashworth scale (MAS) for spasticity, visual analog scale (VAS) for pain, and severe adverse events (SAE).

MATERIALS AND METHODS

Inclusion and exclusion criteria. The trials included male and female adults (18-60 years old) with chronic (≥1 year), neurologically stable (≥6 months), C5-T11 neurological levels, and complete (ASIA/ISCOS Impairment Scale or AIS A) SCI. We excluded people who were in another trial within 4 weeks, who had surgical or medical risks, or who were pregnant or lactating.

Treatments. Participants were assigned sequentially to five treatment groups: Group A received four 4-μL injections of UCBMNC (100,000 cells/μL), Group B received four 8-μL injections, Group C received four 16-μL injections, Group D received 16-μL injections plus a 30mg/kg intravenous bolus of methylprednisolone sodium succinate (MP), and Group E received four 16-μL injections plus MP and a 6-week course of oral lithium carbonate (750 mg/day). In HK, 8 participants were assigned to only groups A and B (n=4/group). In KM, 20 participants were assigned to all 5 groups (n=4/group) and received intensive locomotor training for 6 hours/day, 6 days/week, and for 3-6 months.

The primary outcome measure was ASIA/ISCOS motor and sensory scores [101,102]. Secondary outcomes include ASIA/ISCOS Impairment Scale (AIS), Walking Index of Spinal Cord Injury or WISCI [103], Spinal Cord Independence Measure or SCIM [104], Modified Ashworth Scale or MAS for spasticity [105], Visual Analog Score or VAS for pain [106]. We categorized adverse events by severity, relevance, significance, and outcomes.

Adverse Events, Neurological, and SCIM Assessment. The surgical teams reported adverse events, judging severity and relevance of the events. At 6 weeks, 6 months, and one year after surgery in HK, a rehabilitation team consisting of physical therapist and an occupational therapist assessed the patients under the supervision of an orthopedic surgeon and head of the spinal rehabilitation team. In KM, a team of doctors and nurses evaluated the patients. All examinations were videotaped. The China Spinal Cord Injury Network staff monitored the trials and data collection of both trials.

Unit Selection. Frozen plasma-depleted UCB units were donated by Stemcyte Inc. (Covina, CA) and processed for mononuclear cells by Vista Biologics (Carlsbad, CA). We selected units from by matching human leukocyte antigens (HLA≥4:6), initially low-resolution A, B, and DR and confirmed by medium (HLA-A and B) to high-resolution (HLA-DR) typing after transplantation. All units fulfilled National Marrow Donor Program (NMDP) standards for UCB transplantation.[107] Units from donors who may have had hepatitis B (e.g. positive maternal antibody) were excluded.

Cell Preparation. Vista Biologics (Carlsbad, California) prepared the cells for transplantation. Each frozen cord blood unit was thawed at 4°C, washed to reduce dimethylsulfoxide (DMSO) concentration from 10% to <1%, treated with human DNAase (Pulmozyme, Genentech), and centrifuged in a Ficoll Hypaque (GE Healthcare Life Science, 1.077 specific gravity gradient) to isolate the UCBMNC. The cells were suspended (1 million cells/ml) in animal product-free CO_2-independent media (Invitrogen, CA) and shipped at 12-28°C. We shipped 38 test units from Carlsbad to Hong Kong and Kunming. About 10% of UCBMNC were lost per day of shipment. Shipping at room temperature enriched the UCBMNC for monocytes (35-45%), CD34+ or CD133+ cells (3-4%), nucleated red cells (1-2%), and mesenchymal (CD105 ~1%) cells.

Transplantation. Upon arrival at hospital, the cells were washed in saline, a small aliquot was removed to count cells that excluded trypan blue dye, and the remaining cells were suspended in normal saline containing 1% human albumin (CSL, Australia) so that each μL has ~100,000 trypan-blue excluding mononuclear cells. The cells were loaded into a 27-gauge (27G x ¾" or 0.4 x 19 mm, EXEL INT Sterile Scalp Vein Set) needle attached to a 100-μL Hamilton syringe with flexible tubing. After laminectomy, durotomy, and removal of adhesions between spinal cord and surrounding tissues, the surgeons manually inserted the needle 3 mm deep at a 45° angle (bevel up) into left and right dorsal root entry zones (DREZ) at 5 mm above and 5 mm below the injury site, and slowly (1 μL/minute) injected 4, 8, or 16 μL of cell suspension into each of the 4 sites. The dura was sutured to prevent cerebrospinal fluid leak. Postoperative care included analgesia and antibiotics as necessary.

Magnetic Resonance Imaging and Diffusion Tensor Imaging. In HK, we used a 3-Tesla magnetic resonance imaging (MRI) scanner (Achieva X-series, Phillips Healthcare, the Netherlands) to obtain diffusion tensor images (DTI) of the spinal cord. Conventional MRIs were first obtained (3D T2W and T2W) to locate the injury site. Fiber tracts were selected for tractography by manually identifying regions of interest (ROI) above the injury site for descending fibers and below the injury site for ascending fibers. Using software purchased from the manufacturer (Phillips Healthcare), we first quantified the fractional anisotropy of the selected ROI, yielding ratios that indicated the degrees to which diffusion of water is anisotropic, calculated with eigenvaleus (λ_1, λ_2, λ_3...) of the diffusion tensor.

Initial scans were obtained from six volunteers without spinal cord injury (six of the investigators volunteered). The FA values of normal spinal cords were quite reproducible, ranging from 0.65 to 0.55 from C1 through T12, declining in the more distal segments. Standard errors of average FA values were usually ±0.05, suggesting that the measurements are reproducible. In addition, we did MR-DTI scans of seven people with complete spinal cord injury (SCI) who did not receive surgery or cell transplantation. Every one had a gap at the injury site. One person was recruited to be part of the trial but we unfortunately could not find a suitable 4:6 HLA match. The participant agreed to have followup MR-DTI and the gap was unchanged 2 years later.

Clear and distortion free DTI images were obtainable from only 5 of the 8 participants in the HK trial. In three participants, distortion from fixation instruments was too great to allow the tractography. Once a set of MR-DTI parameters were determined for a satisfactory DTI in a participant, further scans on the participant were done with the same parameters, so that the images could be compared over time. Three participants agreed to have followup scans at 1.5 years after surgery and one participant was rescanned at 2 years. We compared the white matter gap in DTI images obtained before treatment and at one year or later in three participants. Two participants showed evidence of long bundles crossing the injury site and growing progressively into proximal and distal spinal cord over time.

Locomotor Training. In HK, the clinical trial participants did not receive locomotor training. In KM, at 14 days after surgery, the participants started locomotor training according to the Kunming locomotor scale (KLS) described earlier [108]. The participants initially stood with help (KLS II), then without help (KLS III), walked in a rolling walker with minimal (KLS IV) or no assistance (KLS V), or walked with a 4-point walker (KLS VI) without assistance. Participants walked as much as 6 hours a day and 6 days a week for 3-6 months. They typically walked 3 hours in the morning and 3 hours in the afternoon. The participants left hospital at 3 or 6 months, the latter if they were still improving at 3 months. One-year followup ranged from 41-87 weeks (41-87w).

Statistical Analyses. We used IBM SPSS Statistics (version 22) to do repeated measures analysis of variance (ANOVA) to assess ASIA, SCIM and VAS score changes over time (Time) and treatments groups (Group), the Kruskal-Wallis test to compare AIS, MAS, WISCI, and KLS among treatment groups, and Wilcoxon signed-rank test to compare AIS, MAS, WISCI and KLS before and 41-87w after treatment, and Spearman correlation to relate KLS and WISCI. Missing data were assumed to equal the last observation, i.e. last observation carried forward (LOCF). To assess WISCI score changes (ΔWISCI) and HLA-matching, we used ANOVA and Scheffés *post hoc* test (Statview) and the Chi-Square contingency test (Prism 6, GraphPad Software). We used the Chi-Square test to assess HLA-matching among treatment groups. All ±values indicate standard errors, P-values of <0.05 were considered significant, and analyses were based on intention-to-treat.

Consent, Approvals, and Registrations. Each participant gave informed consent. Institution review boards of the Chinese University of Hong Kong and University of Hong Kong approved the trial and the Hong Kong Department of Health approved the trial. The Ethics Committee of Kunming People's Liberation Army Hospital of Chengdu Military Command approved the trial. Western IRB (Seattle, WA) gave an "approvable" rating for both trials. The Military Medical Ministry and Yunnan Department of Science and Technology awarded grants for the KM trial. The two trials were registered on https://www.clinicaltrials.gov as NCT01046786 and NCT01354483.

RESULTS

Participants. Eight people (1 female, 7 males) participated in HK and 20 people (4 females, 16 males) participated in KM. At enrollment, the ages of participants averaged 42.6±2.7 (range 29-53) in HK and 36.9±2.4 (18-53) years old in KM, respectively 12.8±2.6 (2-20) and 7.2±1.2 (2-20) years after injury. All 8 participants in HK were "complete" (AIS A) with C5-T10 neurological levels and 19/20 participants in KM were AIS A with C5-T11 neurological levels and one was sensory incomplete (AIS C) with C3 neurological level. Figures 1 and 2 summarize the HK and KM data. All had significant spinal fractures and most had metallic implants to stabilize the spinal fractures. In HK, all participants had WISCI scores of 0. In KM, 2 participants had WISCI scores of 2 before treatment.

Treatments. Cord blood units were selected based on low-resolution HLA matching (HLA≥4:6) for HLA-A, -B, and –DR and then checked for medium (HLA-A and –B) and high resolution (-DRB1) matching after transplantation. In HK, 2 participants matched 6:6 and six matched 5:6 at low resolution but subsequent medium-high resolution matching showed one participant matching at 6:6, one at 5:6, and six at 4:6. In KM, 6 participants matched 6:6, ten 5:6, and four 4:6 at low resolution and subsequent medium-high resolution matching showed 9 participants matching at 5:6, seven at 4:6, three at 3:6, and one at 1:6. Low-resolution HLA matches differed among KM treatment groups (X^2=15.67, df=8, p=0.0474) but medium-high resolution matches did not (X^2=13.17, df=12, p=0.3565). All participants received planned doses of cells. One Group E participant (#18) received placebo instead of lithium. Three KM participants did not complete locomotor training, two due to possible tibial fractures (#2, #8) and one due to knee swelling (#20).

Diffusion Tensor Imaging. All MR-DTI showed gaps at the injury site (Figure 3). At 6-18 months, two participants had progressive fiber growth crossing the gap. Figure 4 shows pre-treatment, 6-month, and 1-year MR-DTI of a participant with T4 SCI. Figure 5 shows MR-DTI's of an uninjured spinal cord, spinal cords with narrow and wide gaps, fibers crossing the gap, and 2 spinal cords with narrower gaps at 12 months after transplantation.

Adverse Events. Nine adverse events occurred in 3 participants in HK. One participant developed neuropathic pain (probably related), hyperthyroidism (probably unrelated), and hypertension (probably unrelated). A second participant developed a thin subdural hematoma and pneumocephalus due to cerebrospinal fluid (CSF) loss during surgery. He also developed back sore. Both resolved spontaneously. A third participant developed subarachnoid hemorrhage (definitely related), neuropathic pain (probably related), and colon cancer (not related); the first two resolved spontaneously. In KM, 68 adverse events occurred in 19 participants: 43 were unrelated, 17 definitely, 1 probably, and 7 possibly related to treatment. The most common event was post-operative wound swelling and pain in 9 participants. All adverse events resolved with routine therapies. No patient had neurological loss. In the 28 participants in the two trials, 5 had serious adverse events (SAE). One participant (Group A K1) had slow wound healing and low serum protein; both resolved on a high protein diet. Another (Group A K2) developed a CSF leak and wound dehiscence that required re-operation. He was later found to have an old tibial fracture and stopped locomotor training. A third (Group A H2-2) had blood pressure increase requiring hospitalization. A fourth (Group C, K12) had left leg swelling and thrombosis of vena iliaca externa treated by vena cava filter (unrelated). A fifth participant (Group B H1-3) had colon adenocarcinoma discovered at 21 months (probably unrelated).

Spasticity and Pain. In HK, the 8 participants had no or mild spasticity before and after treatment. Three had severe neuropathic pain (VAS>50) before treatment that decreased (88 to 76, 51 to 28, 67 to 0) after treatment and two developed neuropathic pain after treatment (0 to 62, 12 to 24). In KM, 5 participants had mild spasticity (MAS=1) and 2 had moderate spasticity (MAS=2) before treatment; at 41-87w, 7 participants had 1-point increases of MAS scores while 2 participants had 1-point decreases, not significant (X^2=2.977, df=4, p=0.562). Before treatment, 5 participants had VAS scores of 12-50 out of 100. Between w0 and 41-87w, VAS score increased in 3 participants (range: +15 to +69) but decreased in 4 participants (range -5 to -43), not statistically significant (Time: F=0.015, df=1, p=0.905; Group: F=0.0470, df=4, p=0.757; Group•Time: F=1.232, df=4, p=0.339). Two participants in Group E had high VAS scores; lithium reduced VAS scores in both.

ASIA Grade, Levels, and Scores. In HK, 1 of 8 participants (7%) converted from AIS A to B, 4 gained 2-5 points in touch scores, and 2 had 2-3 point motor score increases. In KM, 2 participants switched from AIS A to B (10%) and three from AIS A to C (15%). Neurological levels descended one segment in 6 participants (30%). Ten participants (50%) gained 1-10 touch points and 9 (45%) gained 1-8 pinprick points between w0 and w41-87. Mean sensory scores increased over time, i.e. touch scores increased 1.7 points (Time, F=9.869, df=1, p=0.007; Group, F=0.346, df=4, p=0.299; Group•Time, F=0.535, df=4, p=0.712) and pinprick scores increased 2.6 points (Time, F=8.984, df=1, p=0.009; Group, F=8.984, df=4, p=0.284; Group•Time, F=0.455, df=4, p=0.768). Motor scores did not change significantly (Time: F=1.800, df=1, p=0.200; Group: F=0.145, df=4, p=0.962; Group•Time: F=0.800, df=4, p=0.544), one participant gained 2 and another gained 4 points.

Locomotor Training. Participants in HK did not receive locomotor training. In KM, 17 of 20 participants received intensive locomotor training. Before treatment, 4 participants could not stand (KLS I), fifteen needed help to stand (KLS II), and one walked in a rolling walker with minimal assistance (KLS IV), i.e. an assistant pulled on ropes to stabilize knees during walking. By w14-24, 17 of 20 participants (85%) were training at KLS IV (figure 6). Nine participants went home at 14 weeks because they reached a plateau (n=6) or stopped training (n=3) due to tibial fractures in two cases or swollen knee in one case. After going home, four participants did not continue walking and regressed. At 41-87w, only thirteen (65%) walked at KLS IV and two (10%) walked unassisted with 4-point walkers (KLS VI). KLS at 41-87w differed significantly from w0 (Z=3.532, p<0.0005).

Walking Recovery. In HK, no participants walked before or after treatment. In KM before treatment, 16 participants (80%) could not walk (WISCI 0), one walked <10m (WISCI 1), one walked 10m (WISCI 2) in parallel bars with braces and 2 assistants, and two walked 10m in a walker with braces and no assistants (WISCI 9). At 41-87w, 5 participants had WISCI 0, two were WISCI 2, six (30%) walked 10m with one assistant (WISCI 6), and seven (35%) walked without assistance (WISCI ≥7). WISCI scores at 41-87w differed from W0 (Z=3.315, p=0.001). WISCI and KLS correlated highly (r=0.925, p<0.0005).

SCIM Scores. The SCIM has 19 subscores covering self-care, respiration and sphincters, and mobility. In HK, SCIM scores did not change significantly. In KM, most participants increased their SCIM scores. Repeated measures ANOVA confirmed that mean SCIM scores increased over time (F=51.194, p<0.0005) by 19.6±2.67 points between w0 and 41-87w. Fourteen of 19 SCIM subscores, i.e. except feeding, grooming, respiration, outdoors mobility (>100m) and stair management, improved significantly between w0 and 41-87w (Figure 7-8). The self-care subtotal score accounted for 3.6 points while the respiration and sphincter subtotal accounted for 9.7 points, and the mobility subtotal accounted for 6.3 points. The bladder, bowel, and toilet subscores accounted for almost half of the SCIM score improvement. At w0, 2 (10%) participants were independent for bladder and bowel care but twelve (60%) became independent by 41-87w. Two (10%) participants were catheter-free at w0 but ten (50%) were catheter-free at 41-87w. Bowel care subscores indicate that 12 (60%) participants did not require assistance for bowel procedures at 41-87w, compared to 2 participants before treatment. Likewise, 65-70% of participants could transfer from bed to wheelchair and from wheelchair to toilet and tub at 41-87w, compared to 5-30% at w0. At 41-87w, 35% of participants could walk without assistance or supervision indoors and for moderate distances up to 100m. For distances >100m, only 2 participants walked and the rest used wheelchairs. Table 1 shows means and standard errors of total SCIM and subscores, as well as t-values, Wilcoxon Z values, and p-values.

Treatment Effects. Comparison of walking scores among the treatment groups revealed that all 8 participants in group B and C showed improvement of WISCI scores by 6 points or greater. Only 2 participants showed improved walking in groups A and D, and only 1 participant in group E. Although this was not statistically significant, the data does not support beneficial effects of MP or lithium. Almost every participant except for one in Group B showed improved SCIM scores. Severe adverse events (SAE) did not differ amongst the groups, occurring in four of the five treatment groups, with two in Group A, one in Group B, one in Group C, and one in Group D. Figure 9 shows the ANOVA of change of WISCI scores in the five treatment groups. Mean change of WISCI scores increased from 3.5 to 6.1 to 9.1 in Groups A, B, and C, fell to 2.9 in Group D, and fell to 0.4 in Group E.

Discussion

The HK trial showed that 4-8 µL of UCBMNC can be safely injected into spinal cord above and below the injury site. MR-DTI suggest that white matter gaps decreased at the injury site and two participants showed bundles of fibers growing across the injury site into surrounding spinal cord at 6-18 months. However, the participants did not recover motor function. The KM trial showed that 4, 8, and 16 µL of UCBMNC can be safely injected into the spinal cord. Over half of the participants recovered walking with minimal or no assistance by 6-12 months after UCBMNC transplants and locomotor training, as well as increased independence in activities of daily living, including self-care, bowel and bladder function, and mobility.   This is an unprecedented recovery for complete chronic SCI.

Walking recovery is rare in patients with chronic complete SCI [109-111]. The finding that 15:20 participants (75%) with chronic complete SCI could walk 10m and seven (35%) walked 10m without manual assistance a year after treatment is unprecedented. SCIM indoor mobility subscores confirmed that 7 participants (35%) walked indoors without assistance at one year after treatment. Likewise, mobility subscores for moderate distances showed the same 7 participants (35%) walking 10-100m without assistance, 2 (10%) walking with supervision, and the rest using wheelchairs. For distances of >100m, only 2 participants (10%) walked while the rest used wheelchairs. Thus, 35% of participants used walking for indoors and for moderate distances <100m but most participants preferred wheelchairs for longer distances >100 m.

Late conversions from AIS A to B or C are also rare. As Kirschblum, et al. [112] pointed out, among 987 patients who were neurologically complete (AIS A) at one year, only 3.5% improved to AIS B and 1.05% improved to AIS C and D by 5 years. In HK, 1 of 8 participants converted from AIS A to B (12.5%). In KM, 2 participants converted from AIS A to B (10%) and three from AIS A to C (15%), a conversion rate of 25%. These changes were statistically significant (Z=2.070, p=0.038) but did not vary among treatment groups.

SCIM scores indicated significant improvements in independence for bladder function. Before treatment, 18 participants (90%) required assistance for bladder care and 15 participants (75%) used catheters. At w14-24, 16 participants (80%) were still catheterizing. At 1 year after treatment, 12 (60%) participants did not need assistance for bladder care and 11 participants (55%) no longer used catheters. Three participants (15%) did not use catheters or drainage devices.

Bowel function also improved. Before treatment, 6 participants (30%) had irregular or low frequency bowel movements (<1/3 days) and 90% required assistance. By discharge from hospital at 14-24 weeks, all participants became regular but 75% still needed assistance and had occasional accidents. By 41-87w, however, 60% did not require assistance and rarely had accidents. Four participants (20%) had no accidents. We plan to bring the participants back for further evaluation.

MP and lithium may have reduced walking recovery. Animal studies suggest that MP improves survival of transplanted cells [113] and lithium should improve walking recovery [90] after acute SCI. Only 2 of 8 participants (25%) in Groups D (6.4 million cells plus MP) and E (6.4 million cells plus MP and lithium) recovered walking to 6 points on WISCI, compared to 8 of 8 participants (100%) in Groups B (3.2 million cells) and C (6.4 million cells). Lithium reduced neuropathic pain in two participants in Group E, consistent with our earlier report that lithium reduces neuropathic pain.[114]

Change in WISCI scores (ΔWISCI) increased with cell dose. As Figure 9 shows, ANOVA of ΔWISCI in the five treatment groups showed progressive increase in ΔWISCI in Groups A, B, and C but ΔWISCI decreased in Groups D and Group E. Post hoc tests (Scheffés) suggest that Group C and E differed significantly at $p < 0.0051$. However, one patient (#18) in Group E inadvertently received placebo rather than lithium tablets, one patient (#19) was already walking at WISCI 9 when the trial began, and one patient (#20) stopped walking training due to knee swelling. Thus, only one patient in Group E represented a valid comparison with Group C. Further clinical trials are needed to determine whether lithium is effective.

Two other findings are noteworthy in the KM trial. First, two participants had to discontinue locomotor training due to old bone fractures. We should screen participants in future trials for old bone fractures. Second, many participants did not show improved motor scores despite recovering walking and other programmed spinal cord functions, i.e. micturition and defecation. We hypothesize that UCBMNC transplants stimulated growth of axons that activate lumbosacral central pattern generators for walking, micturition, and defecation but only in participants who received intensive locomotor training. [115] This would explain why most patients could walk but could not voluntarily contract individual muscles or feel specific sensory signals in their legs at one year after treatment. It is possible that some patients will recover more voluntary motor and sensory function later.

Our trials left several critical questions unanswered. First, can intensive locomotor training alone improve locomotor function in people with chronic complete SCI? Several years ago, most doctors would have replied that locomotor training alone cannot restore locomotion to people with chronic complete SCI. Second, does untethering surgery improve the effects of intensive locomotor training? In our trials, all patients that received transplants also received untethering surgery. Many neurosurgeons [116-131] have reported beneficial effects of untethering surgery in patients with spina bifida or syringomyelic cysts. Third, does lithium improve locomotor recovery when combined with UCBMNC and intensive locomotor training? We [114] have previously observed that a 6-week course of lithium does not improve motor or sensory function in patients with chronic complete SCI but these patients did not receive any locomotor training. If lithium does not improve function when combined with UCBMNC and locomotor training, we should exclude it from future phase III trials.

We have proposed further phase II trials to answer these questions. The first trial will ascertain whether locomotor training alone or untethering surgery plus locomotor training restore walking in people with chronic complete SCI. This trial (NCT02663310, http://clinicaltrials.gov) is underway in Kunming, comparing walking outcomes of 30 people with chronic complete SCI, randomized to untethering surgery or no surgery, followed by 6 months of intensive locomotor training. We have applied for two phase II trials, one in India

and the other in the United States, to ascertain whether lithium improves locomotor recovery of participants with chronic SCI, randomized to UCBMNC transplants or UCBMNC plus a 6-week course of lithium, followed by 6 months of intensive locomotor training.

These trials will provide the following important information needed for design of pivotal phase III trials of UCBMNC treatment. First, if the trials show no significant benefits of adding lithium to UCBMNC transplants, lithium should be omitted from the phase III trials. Second, if locomotor training alone or untethering surgery plus locomotor training improves walking recovery in patients with chronic complete SCI, it would provide justification for a surgery control group involving untethering only. Finally, intensive locomotor training (6 hours a day, 6 days a week for 6 months) has not been practiced outside of Kunming. It is important to establish that such training is feasible elsewhere since our trials to date suggest that intensive locomotor training is essential for recovery of walking.

In summary, our data indicate that UCBMNC can be safely transplanted into the spinal cord of people with chronic SCI, intensive locomotor training is essential for motor recovery, and UCBMNC transplants combined with intensive locomotor recovery can lead to significant locomotor, bowel, and bladder recovery in people with chronic complete SCI. However, the patients did not recover much voluntary motor function. Some participants recovered sensory dermatomes close to the injury site and as many as a quarter of the patients recovered anal sensation and voluntary sphincter contraction, converting from AIS A to B and C. Further clinical trials are necessary to determine whether these improvements are due to UCBMNC, untethering surgery, or intensive locomotor training.

Acknowledgment

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The authors declare no potential conflicts of interest.

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Figure Legends

Figure 1. Neurological levels and scores in Hong Kong (HK). Each column represents a participant in the trial: dark green indicates segments with normal sensation and motor function, dark green with white letters indicates the neurological level before treatment, red with white letters indicates changed neurological level one year after treatment, light green indicates zone of partial preservation (ZPP) before treatment, and pink indicates ZPP after treatment. Individual participant data are listed, including age and years after injury, sex, medium-high resolution (medium for HLA-A and –B and high for HLA-Dr) HLA matches out of 6. AIS is ASIA:ISCOS Impairment Scale where A is complete, B is sensory incomplete, and C is motor incomplete that <50% of motor score in the legs. None of the participants received walking training or recovered walking; hence, no Kunming Locomotor Scores (KLS) or Walking Index of Spinal Cord Injury (WISCI) scores are listed. Motor score is the sum of muscle grades (0-5) for ten muscles on each side of the body, totaling 100 points. Touch and Pin refer to light touch and pinprick scores (0=no, 1=abnormal, 2=normal) for 28 dermatomes on each side of the body. MAS is modified Ashworth scale (0-4) for spasticity. VAS is visual analog scale (0-100) for pain. SCIM is spinal cord independence measure (0-100). Red indicates improvement. SAE (yellow) refers to severe adverse events.

Figure 2. Neurological levels and scores in Kunming (KM). Each column represents a participant in the trial. The color columns indicate the neurological level (green) at the time of treatment, improvements in neurological level (red), and improvements in ZPP or zones of partial preservation (pink), light green indicates ZPP before treatment. Two participants converted from complete to sensory incomplete, 3 participants were converted to motor incomplete (AIS C). Five participants had adverse events: WH = wound healing, TF = tibial fracture, KS = knee swelling. See legend for figure 1 for explanation.

Figure 3. A magnetic resonance diffusion tensor image (MR-DTI) of the spinal cord before treatment. White matter tracts were selected from regions of interest (ROI) above the below the injury site and tract-tracing software was then used to identify adjacent pixels with similar diffusion tensors. Descending tracts are colored purple and green while ascending tracts are colored blue. A clear gap was present in the spinal cord at C6 vertebral level.

Figure 4. Magnetic resonance diffusion tensor images (MR-DTI) of the spinal cord of a participant before operation (Pre-op), at 6 months (6m), and 12 months (12m) after treatment. Before operation, MR-DTI showed very atrophic descending fibers (blue), more ascending fibers (green), and a clear gap at the T4 injury site. At 6 months, the gap was still present. At 12 months, both ascending and descending fibers were crossing the gap (upper right). On the lower right image, ascending fibers (dark blue) were removed so that the descending fibers (light blue) could seen to extend into the lumbosacral spinal cord.

Figure 5. Magnetic resonance diffusion tensor images (MR-DTI) of a normal cervical spinal cord (A), an image of an injured spinal cord with a narrow gap before treatment (B), an image of an injured spinal cord with a wide gap before treatment (C). D, E, and F show MR-DTI from a participant before, 6 months after, and 1.5 years after treatment. Note the fibers crossing the gap. G, H, I, and J show MR-DTI from a participant before, at 6 months, 1 year, and 2 years after treatment. Note the narrowing of the white matter gap.

Figure 6. Kunming Locomotor Scale (KLS) and Walking Index of Spinal Cord Injury (WISCI). **KLS** represents locomotor training stages: I indicates inability to stand, II is standing with assistance, III is standing without assistance, IV is walking in rolling walker with minimal assistance, V is walking in rolling walker without assistance, VI

is walking in four-point walker without assistance. No participant trained with crutches (VII), cane (VIII), or without devices (IX, X). **WISCI** reflects ability to ambulate 10 meters (10m) with devices, braces, and assistants: 0 indicates inability to stand or to participate in assisted walking; 1 is ambulating <10m and 2 is ambulating 10m in parallel bars with braces and 2 assistants; 3 is ambulating 10m in parallel bars with braces and 1 assistant; 4 is ambulating 10m in parallel bars with no braces and 1 assistant; 5 is ambulating 10m in parallel bars without braces or assistant; 6 is ambulating 10m with a walker with braces & 1 assistant; 7 is ambulating 10m with 2 crutches, braces, & 1 assistant; 8 is ambulating 10m with walker, no braces, and 1 assistant; 9 is ambulating in a walker with braces and no assistant; 10 is ambulating with 1 cane or crutch, no braces, and 1 assistant; 11 is ambulating with 2 crutches, no braces, and 1 assistant; 12 is ambulating with 2 crutches, braces, and no assistant; 13 is ambulating in a walker without braces or assistants. No participant achieved WISCI scores higher than 13. Missing w24 data were assumed to equal w14 data and w48 refers to 41-87w.

Figure 7. Spinal Cord Independence Measure (SCIM) mobility scores. **Indoor** (indoor mobility on even surfaces), **Moderate Distances** (10-100m) and **Outdoors** (>100m): 0 = total assistance, 1 = electric wheelchair or manual assisted wheelchair, 2 = moves independently with manual wheelchair, 3 = walks with supervision, 4 = walks with walking frame or crutches by swinging, 5 = walks with crutches or two canes with reciprocal gait, 6 = walks with one cane, 7 = needs leg orthosis only, 8 = walks without walking aids. **StairMgt** (go up or down stairs): 0 = total assistance, 1 = ascends and descends ≥3 steps with support or partial assistance, 2 = ascends and descends at least 3 steps with rail, crutch, or cane, 3 = ascends and descends at least 3 steps without support or supervision. **Bedmobility** (turn in bed and actions to prevent pressure sores): 0 = total assistance to turn and to sit up in bed, to push up in wheelchair, with or without adaptive devices, 1 = performs one of the above actions without assistance, 2 = performed 2-3 of above without assistance, 3 = independent. **GroundWheelchair** (get into wheelchair from the ground): 0 = total

assistance, 1 = transfers independently with or without adaptive devices.

Figure 8. Spinal Cord Independence Measure Toilet and Transfer Functions. **Bladder**: 0 = indwelling catheter, 3 = residual urinary volume (RUV) >100ml with assisted catheterization, 6 = RUV <100ml with intermittent self-catheterization (ISC) and external drainage (ED) with assistance, 9 = ISC and ED without assistance, 11 = ISC without ED, 13 = RUV <100ml, only ED and no assistance; 15 = RUV <100ml, no ED. **Bowel**: 0 = irregular or very low frequency movements <1/3 days, 5 = regular, requires assistance for suppositories, rare accidents <2/month, 8 = regular without assistance, rare accidents, 10 = regular, no assistance or accidents. **Toilet**: 0 = total assistance, 1 = partial assistance, does not clean self, 2 = partial assistance, cleans self, 4 = independent but requires adaptive device, 5 = independent without adaptive devices. **WheelchairToilet** (transfers to and from toilet, locking wheelchair, lifting footrests, removing and adjusting armrests): 0 = total assistance, 1 = partial assistance or supervision, 2 = independent. **Bedwheechair** (transfers from bed to wheelchair): 0 = total assistance to lock wheelchair, lift footrests, remove and adjust arm rests, transferring, lifting feet, 1 = partial assistance or supervision and/or adaptive devices, 2 = independent or does not require wheelchair. **WheelchairCar** (approach a car, lock wheelchair, remove arm and footrests, transfers to and from car, bring wheelchair into and out of car): 0 = total assistance, 1 = partial assistance or supervision/adaptive devices, 2 = independent.

Figure 9. Analysis of Variance (ANOVA) of change of WISCI (Walking Index of Spinal Cord Injury) between week 0 and week 48. The ANOVA table indicated an F-value of 6.765 (p=0.0026) amongst treatment groups (Rx). The lower left graph shows means and standard errors of mean. Group A received the lowest dose of 1.6 million cells, B received a higher dose of 3.2 million, Group C-E received the highest dose of 6.4 million, Group D received the highest cell dose plus 30 mg/kg methylprednisolone (MP), and Group E received the highest cell dose plus MP and a 6-week course of oral lithium carbonate.

Rx	Group A				Group B			
Subject	H02-1	H01-1	H02-2	H01-5	H02-3	H02-4	H01-3	H02-6
Side	R L	R L	R L	R L	R L	R L	R L	R L
C5					C5			c5
C6	C6				C6			
C7		C7						
C8		C8			C8			
T1								T1
T2								
T3		T3						
T4			T4					T4
T5								
T6			T6			T6		
T7				T7				
T8						T8		
T9				T9			T9	
T10							T10	
T11							T11	
T12							T12	
L1								
Age	39	53	45	51	45	39	29	29
Years	20	5	5	16	17	18	19	2
Sex	M	M	M	M	M	F	M	M
HLA	4:6	6:6	4:6	5:6	4:6	4:6	5:6	5:6
AIS	A>A	A>A	A>A	A>B	A>A	A>A	A>A	A>A
Touch	20>23	34>36	49>50	59>62	22>20	56>56	69>74	32>32
Pin	20>20	26>27	49>50	57>53	22>20	56>55	70>71	18>18
Motor	26>29	40>42	50>50	50>50	22>24	50>50	50>50	32>32
MAS	1+>1+	1>0	0>0	1>1	1>1	0>0	1>1	1>1
VAS	0>0	88>76	0>0	51>28	0>62	67>0	12>24	0>5
SCIM	31>31	63>63	68>68	68>68	27>28	74>74	67>67	19>19
SAE			N,H,T		S,P,B		S,N,C	

Legend:
- Normal
- C6 Initial Level
- C8 48w level
- T3 Initial ZPP
- T11 48w ZPP
- N Neuropathic pain
- H hyperparathyroid
- T hypertension
- S subdural hematoma
- P pneumocephalus
- B back sore
- C colon cancer

Figure 1

Figure 2

Group	Subject	Age	Years	Sex	HLA	AIS	KLS	WISCI	Motor	Touch	Pin	MAS	VAS	SCIM	SAE
A	1	37	7.0	M	3:6	A=A	I>II	0=0	50=50	70>80	70>76	0=0	0=0	49>62	WH
A	2	47	10.0	M	3:6	A=A	I>II	0>2	24=24	51=51	51=51	0=0	0=0	23>27	TF
A	3	44	2.0	M	5:6	A=A	II>VI	9>13	50=50	64=64	64=64	0=0	12>7	49>76	
A	4	20	6.0	M	4:6	A>B†	I>IV	1>9	50=50	40=40	40=40	0=0	0=0	46>74	
B	5	53	3.0	M	4:6	A=A	I>IV	0>6	50=50	36=36	36=36	1=1	0>15	32>70	
B	6	35	12.0	M	5:6	A=A	II>IV	0>6	50=50	60=60	60=60	0=0	0=0	57>62	
B	7	24	7.0	F	4:6	C=C‡	II>IV	0>6	18>20	60>63	60>63	1>2	0=0	40=40	TF
B	8	35	3.0	M	4:6	A=A	II>IV	0>7	50=50	70>71	70>71	1>2	9>0	50>58	
C	9	50	20.0	M	4:6	A>C‡	I>IV	0>9	50=50	56>60	56=56	0=0	0=0	53>64	
C	10	47	14.7	M	2:6	A>C‡	II>VI	0>13	50=50	72>76	72>76	0=0	0=0	48>74	
C	11	27	7.4	M	5:6	A=A	II>IV	0>9	50=50	68>72	68>72	0>1	0>20	54>74	
C	12	45	3.2	F	5:6	A=A	II>IV	0>6	20=20	24=24	24=24	0=0	21>0	20>37	KS
D	13	18	1.6	M	5:6	A=A	II=II	0=0	50=50	46=46	46=46	2=2	0=0	28>55	
D	14	50	8.0	M	1:6	A=A	II>III	0=0	50=50	70=70	70=70	2>1	0=0	37>71	
D	15	41	2.8	M	5:6	A=A	II>IV	0>6	50=50	44>46	44>46	0>1	0>69	43>63	
D	16	34	13.8	M	5:6	A>C‡	II>IV	0>6	30>30	57>64	53>56	0>1.5	0=0	37>72	
E	17	42	13.2	M	5:6	A=A	II>I	0=0	50=50	44=44	44=44	0=0	22>0	70>73	
E	18	20	3.0	F	4:6	A=A	II=II	0=0	20>24	20>30	20>24	0>1	0=0	24>47	
E	19	29	4.0	M	4:6	A=A	IV>IV	9>9	50=50	48=48	48=48	1=1	0>6	45>84	
E	20	40	3.0	F	4:6	A>B†	II>III	0>2	30>30	24>32	24>32	1>0	50>7	26>30	KS

Legend:
- Normal
- C7 Initial level
- C8 48w level
- T12 Initial ZPP
- L3 New ZPP
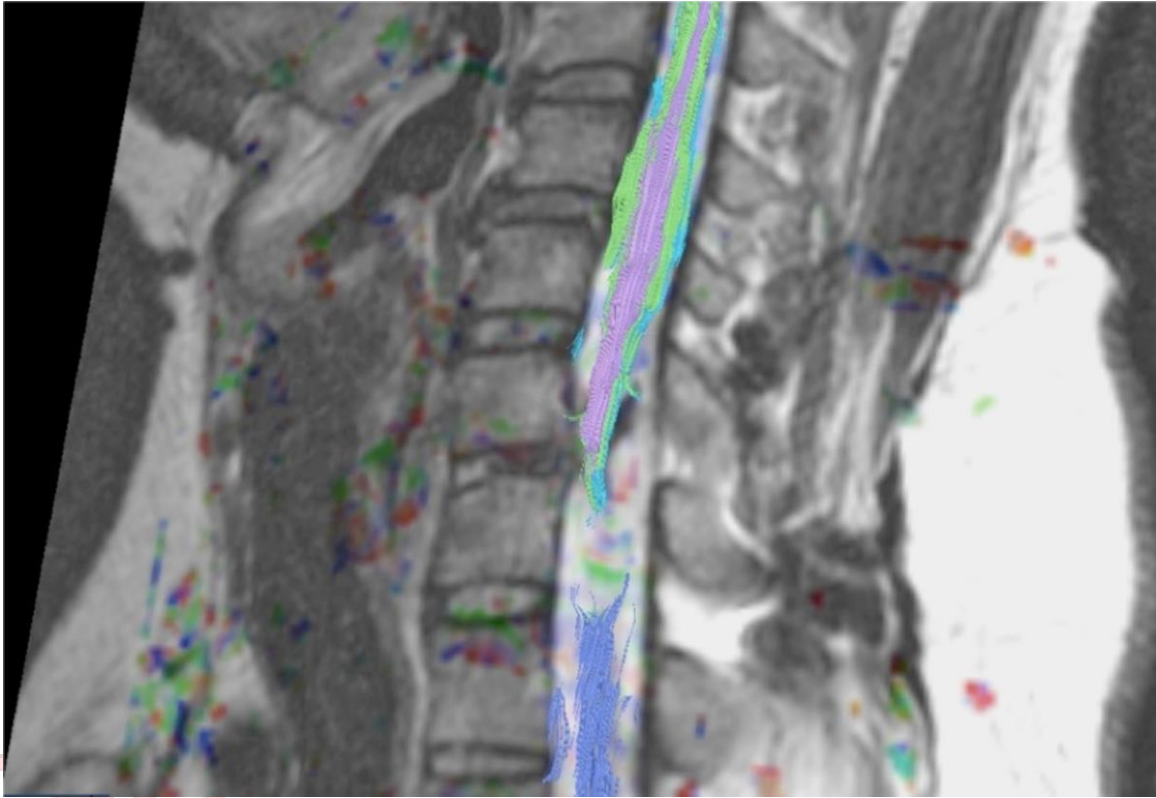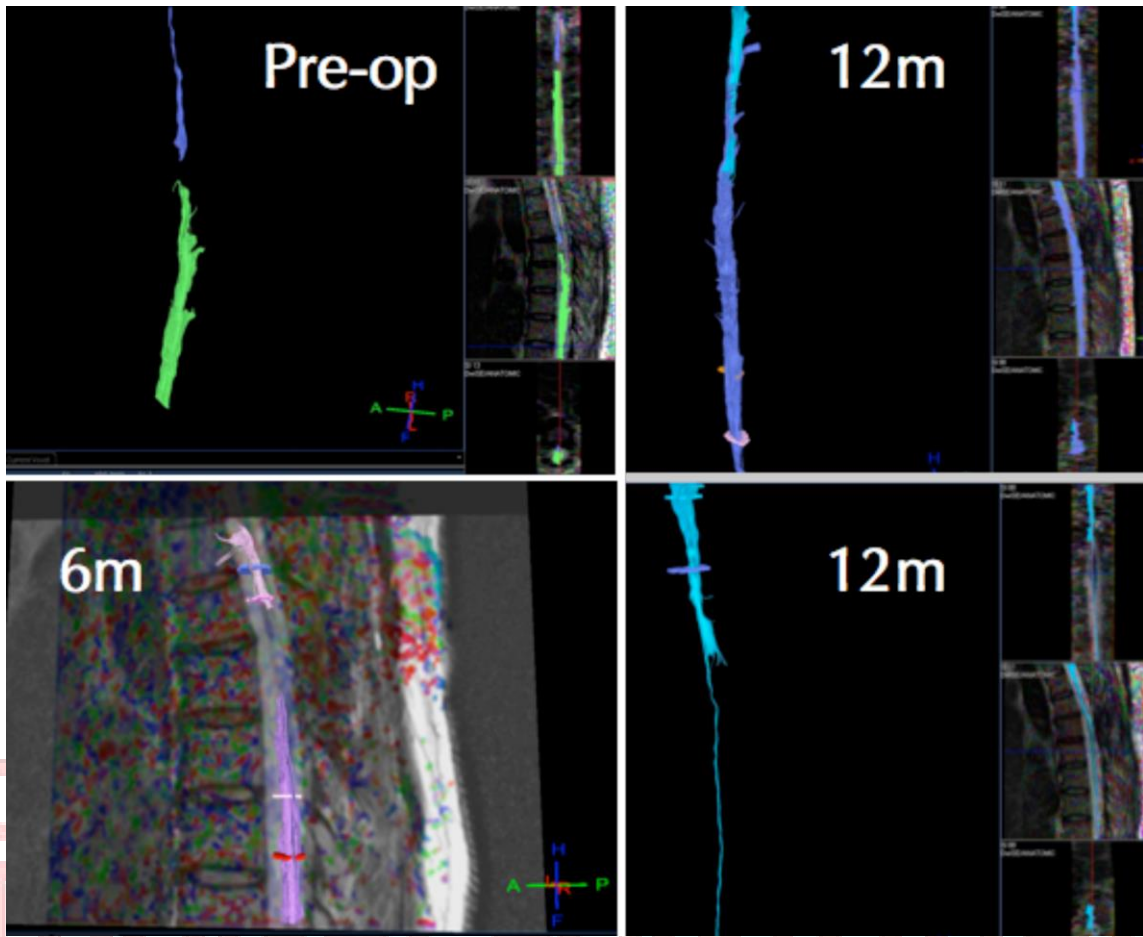- WH Wound Healing
- TF Tibial fracture
- KS Knee swelling

Figure 3

Figure 4

A. Normal MR-DTI B. Pre-treatment C. Pre-treatment

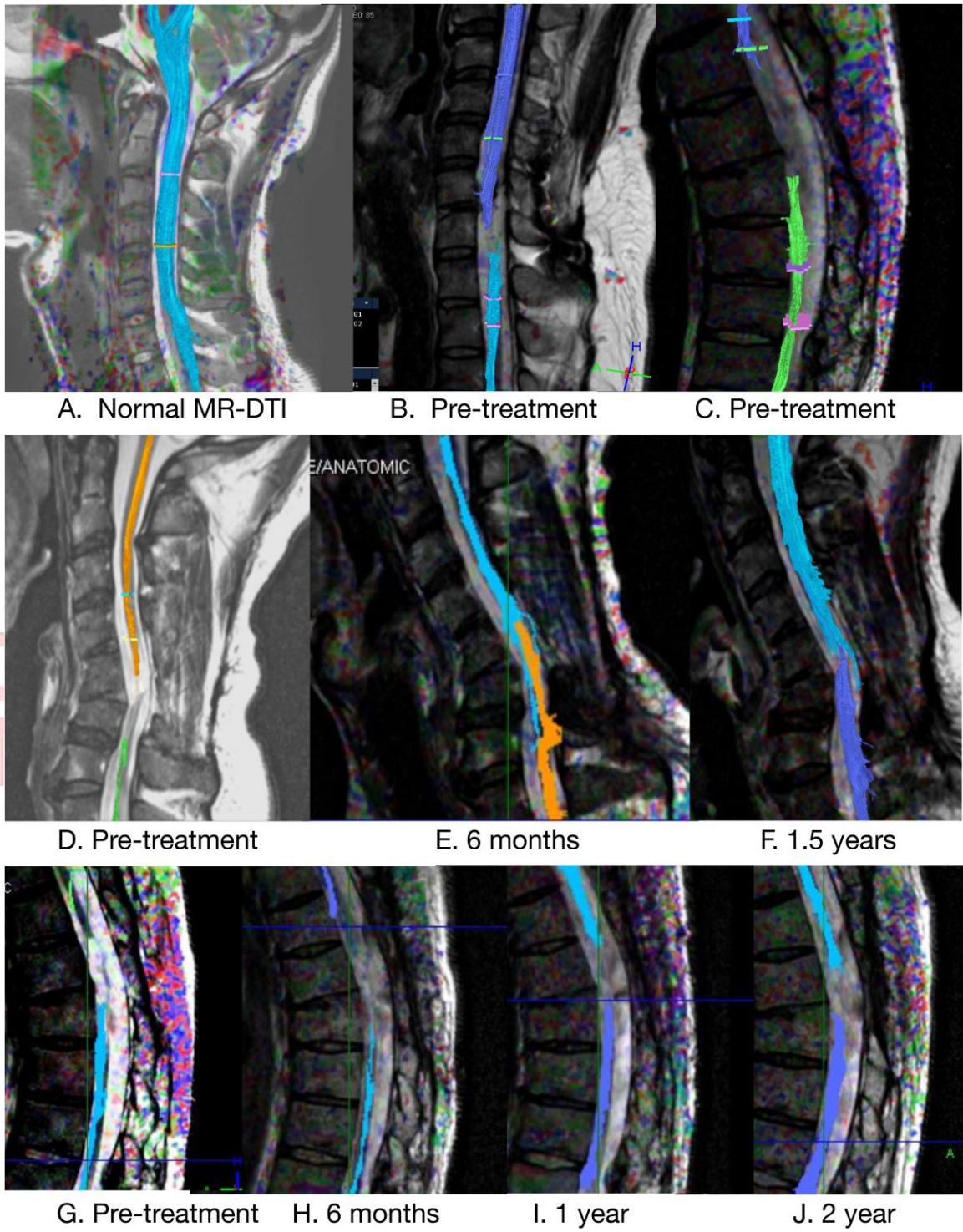D. Pre-treatment E. 6 months F. 1.5 years
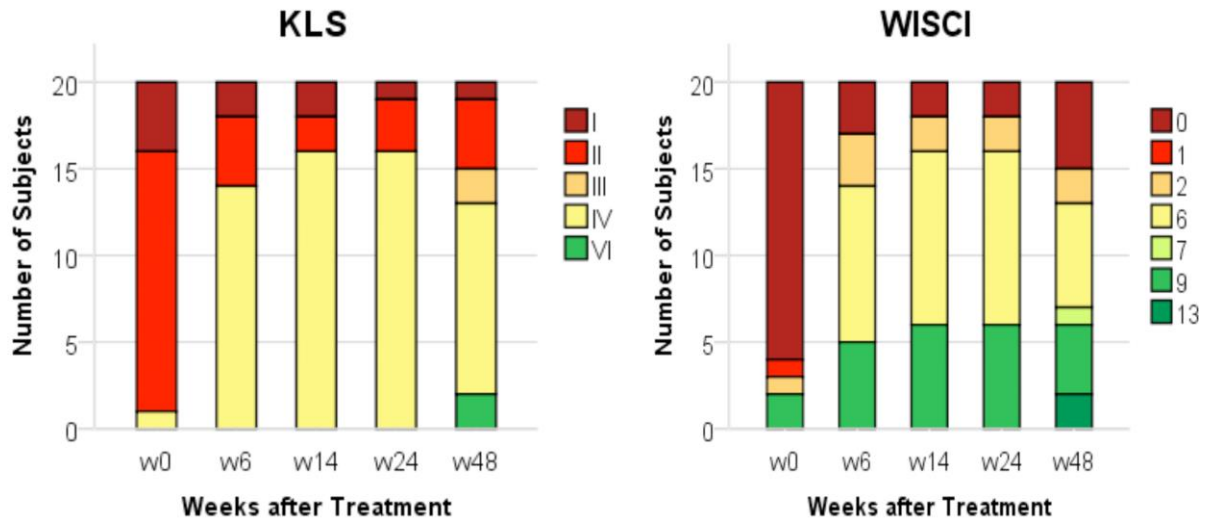
G. Pre-treatment H. 6 months I. 1 year J. 2 year
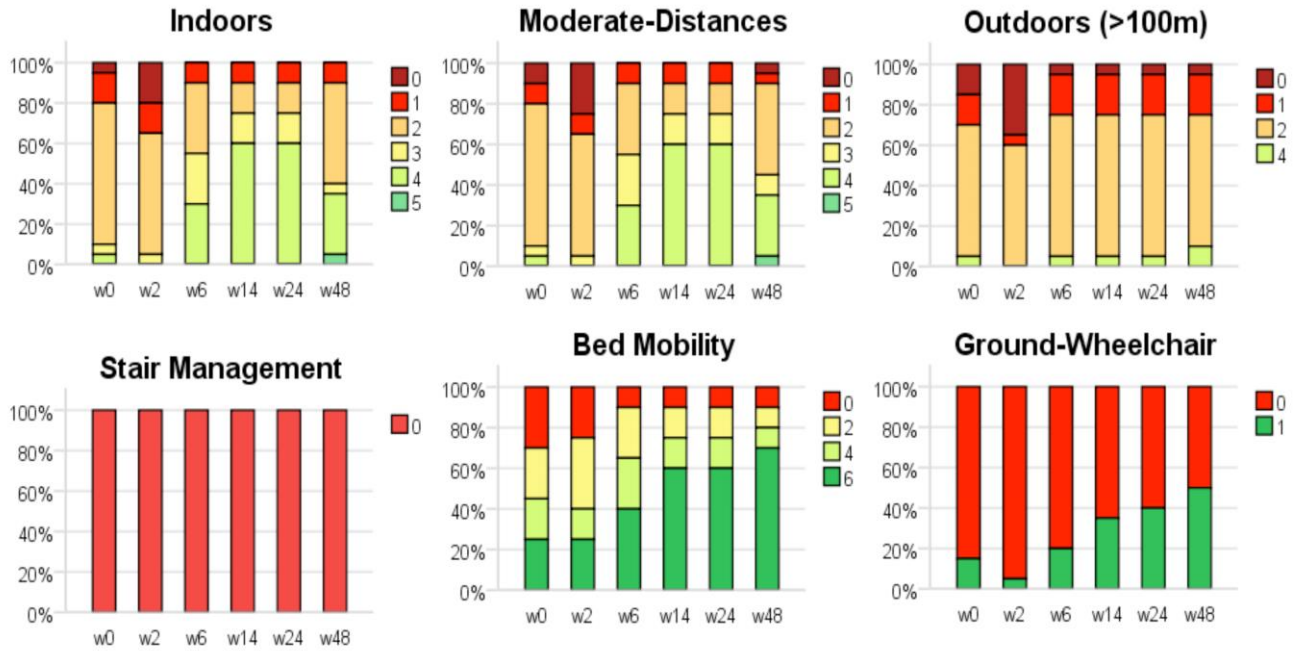
Figure 5

Figure 6

Figure 7
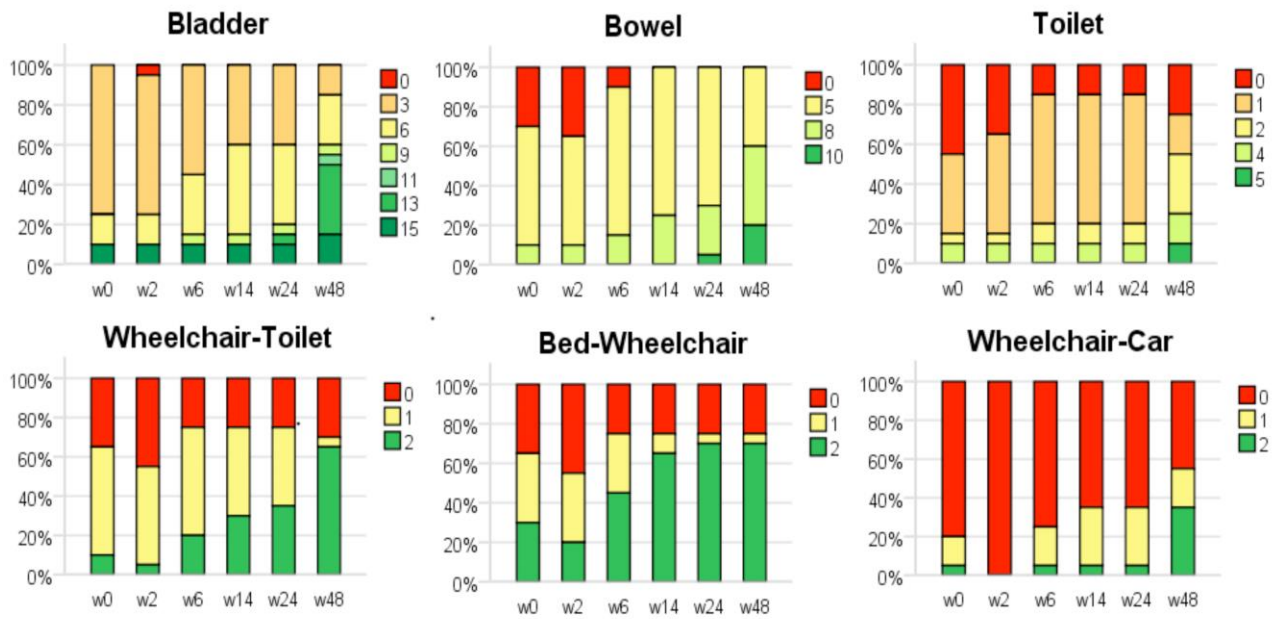
Figure 8

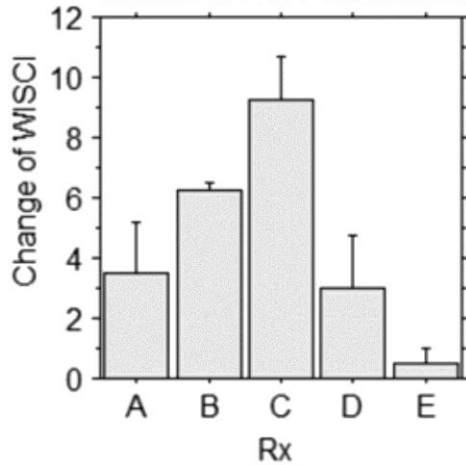**ANOVA Table for WISCI W48-W0**

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Rx	4	179.500	44.875	6.765	0.0026	27.060	0.965
Residual	15	99.500	6.633				

Interaction Bar Plot for WISCI W48-W0
Effect: Rx
Error Bars: ± 1 Standard Error(s)

Scheffe for WISCI W48-W0
Effect: Rx
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
A, B	-2.750	6.367	0.6884	
A, C	-5.750	6.367	0.0874	
A, D	0.500	6.367	0.9992	
A, E	3.000	6.367	0.6175	
B, C	-3.000	6.367	0.6175	
B, D	3.250	6.367	0.5460	
B, E	5.750	6.367	0.0874	
C, D	6.250	6.367	0.0557	
C, E	8.750	6.367	0.0051	S
D, E	2.500	6.367	0.7562	

Figure 9

Table 1. SCIM Scores and subscores

Dependent Variable	Max	W0±sem (CI 95%)	W48±sem (CI 95%)	Δ±sem	Paired Samples T-Test		Wilcoxon Signed Ranks Test	
					t	p	Z	p
SCIM Total	100	41·0±3·0 (34·7-47·3)	60·6±3·6 (52·9-68·2)	19·6±2·7	7.330	0.000	3.912	0.000
SCIM Self Care	20	11·9±1·4 (8·87-14·8)	15·4±1·4 (12·4-18·4)	3·6±0·8	4.440	0.000	3.305	0.001
1· Feeding	3	2·5±0·2 (2·14-2·86)	2·6±0·2 (2·34-2·96)	0·2±0·1	1.143	0.267	1.134	0.257
2. A. Bathing (upper)	3	1·8±0·2 (1·36-2·34)	2·4±0·2 (1·96-2·94)	0·6±0·2	3.040	0.007	2.521	0.012
B. Bathing (lower)	3	0·8±0·2 (0·28-1·32)	1·8±0·3 (1·20-2·40)	1·0±0·4	3.979	0.001	2.976	0.003
3. A. Dressing (upper)	4	2·8±0·4 (2·05-3·55)	3·3±0·3 (2·67-3·93)	0·5±0·2	2.236	0.036	2.041	0.041
B· Dressing (lower)	4	1·6±0·4 (0·78-2·85)	2·7±0·4 (1·81-3·49)	1·0±0·5	2.761	0.012	2.395	0.017
4. Grooming	3	2·3±0·3 (1·75-2·85)	2·6±0·2 (2·11-2·99)	0·3±0·1	2.032	0.056	1.890	0.059
SCIM R & S	40	18·8±1·2 (16·4-21·3)	28·5±1·3 (25·9-31·2)	9·7±1·5	6.570	0.000	3.728	0.000
5. Respiration	10	9·5±0·2 (9·08-9·92)	9·7±0·2 (9·36-10·04)	0·2±0·1	1.453	0.163	1.414	0.157
6. Bladder	15	4·6±0·8 (2·92-6·38)	9·8±1·0 (7·70-11·80)	5·1±1·0	4.972	0.000	3.218	0.001
7. Bowel	10	3·8±0·6 (2·53-5·07)	7·2±0·4 (6·27-8·13)	3·4±0·8	4.363	0.000	3.142	0.002
8. Toilet	5	0·9±0·3 (0·33-1·47)	1·9±0·4 (1·11-2·69)	1·0±0·3	2.874	0.010	2.553	0.011
SCIM Mobility R & T	10	4·5±0·8 (2·99-6·10)	7·6±0·8 (5·87-9·33)	3·1±0·6	5.431	0.000	3.755	0.000
9. Bed mobility	5	2·8±0·5 (1·69-3·91)	4·8±0·5 (3·82-5·78)	2·0±0·4	4.873	0.000	3.256	0.001
10. Bed Wheelchair	2	0·9±0·2 (0·56-1·34)	1·5±0·2 (1·03-1·87)	0·5±0·2	3.249	0.004	2.640	0.008
11. Wheelchair Toilet	2	0·8±0·1 (0·45-1·05)	1·4±0·2 (0·91-1·79)	0·6±0·1	4.485	0.000	3.207	0.001
SCIM Mobility I & O	30	5·8±0·7 (4·44-7·16)	9·0±0·9 (7·04-10·96)	3·2±0·7	4.913	0.000	3.398	0.001
12. Indoors	8	1·9±0·2 (1·53-2·27)	2·7±0·3 (2·15-3·25)	0·8±0·2	3.559	0.002	2.805	0.005
13. Moderate (≤100m)	8	1·9±0·2 (1·44-2·26)	2·7±0·3 (2·11-3·29)	0·9±0·2	3.489	0.002	2.853	0.004
14. Outdoors (>100m)	8	1·6±0·2 (1·21-2·09)	1·9±0·2 (1·47-2·33)	0·3±0·1	2.032	0.056	1.890	0.059
15. Stair Management	3	0	0	0			0.000	1.000
16. Wheelchair-car	2	0·3±0·1 (-0·01-0·51)	0·9±0·2 (0·47-1·33)	0·7±0·2	3.577	0.002	2.739	0.006
17. Ground-wheelchair	1	0·2±0·1 (-0·02-2·09)	0·8±0·3 (0·18-1·42)	0·7±0·3	2.156	0.044	2.714	0.007
SCIM Mobility All	40	10·3±1·3 (7·63-12·97)	16·6±1·6 (13·3-19·9)	6·3±0·9	6.779	0.000	3.789	0.000

Explanation. R & S refers to respiration and sphincters, R & T refers to room and toilet, I & O refers to indoor and outdoors. W0 is week 0 or baseline score while W48 represents 41-87w mean scores ± sem with 95% confidence interval (CI 95%). Δ is the difference (mean ± sem) between W0 and W48 scores. We used Paired Samples T-Test and Wilcoxon Signed Ranks Test to compare findings at W0 and W48.