Introduction

Advances from experimental animal research are translating into clinical trials using stem cells and other cells/tissue for severe spinal cord injury (SCI), a condition previously thought to be hopeless. The olfactory mucosa contains neural stem cells (NSCs) and olfactory ensheathing cells (OECs).1–4 Rats with complete or partial spinal cord transections demonstrate functional improvement after transplant of olfactory mucosa,5 olfactory mucosa–derived NSCs,6,7 or olfactory mucosa–derived OECs.8–10 Because olfactory mucosa contains SCI repair–promoting cells (NSCs and OECs) and is readily accessible with minimally invasive techniques,11,12 a human pilot clinical study was done to determine the safety and feasibility of olfactory mucosa autografts (OMAs) in severe, chronic traumatic SCI.12 We
found that the OMA procedure was safe and feasible. Two of 7 subjects improved from ASIA Impairment Scale (AIS) grade A to C. Interpretation of results was limited by the small number of patients and the lack of a control group. The potential improvement of these 7 OMA patients was constrained by the lack of an intense rehabilitation program and uncertainty about the optimal type of rehabilitation. We hypothesized that 3 components are critical for functional improvement after complete SCI: (a) OMA containing NSCs, (b) graft site management, and (c) an intense rehabilitation program.

First, olfactory mucosa is believed to be an ideal graft for SCI because it can be acquired autologously with minimally invasive techniques, and pieces large enough to fill a 3- to 4-cm cavity can be obtained. Using a person’s own olfactory tissue may allow NSCs to integrate in a controlled manner without rejection or tumor formation that limits embryonic stem cell use. Unlike mesenchymal cells, Schwann cells, and OECs used for chronic SCI, the normal course of the olfactory mucosa NSCs includes neurons. In fact, the olfactory system has the fastest rate of neurogenesis in the adult nervous system.

Second, the graft site must be modified before the graft is implanted. Preparing the injury site for grafting is in some cases technically challenging and may require several hours of surgery. The scar varies in composition (astrocytic, fibrotic, or mixed) and thickness. Large cysts, multiple small cysts, or no cysts may be present. It is necessary to partially remove the scar superiorly and inferiorly to allow for transplant integration and bridging. In some cases only scar tissue remains at the injury site and must be removed.

Third, extended intense rehabilitation may be essential for recovery to strengthen physical components such as the skeletal, muscular, and vascular systems and to remodel the neural circuitry. It was unclear at the start of this study as to what would be the most effective rehabilitation program to promote recovery after NSC therapy. Repetition of unskilled movements, strength training, or exercise training alone is not sufficient to induce motor map reorganization that is crucial to functional improvement after injury. In fact, SCI results in a disconnection syndrome that not only severs brain–spinal cord connections but also circuitry all over the neuraxis (eg, cortex–pons). The rehabilitation that seemed to be the most effective in combination with OMA was overground ambulation, called BIONT (brain-initiated overground nonrobotic/nonweight supported training), which allows compensatory or novel walking patterns. Body weight–supported treadmill training (BWSTT) has also been considered as a potential therapy.

The main objective of the study is to determine the safety and efficacy of OMA in an additional 20 patients with chronic, severe SCI in humans enrolled at 1 of 3 different rehabilitation programs, which allowed us to compare BIONT with robotic BWSTT for intensive training.

Materials and Methods

Patient Selection and Inclusion Criteria

This phase I/II nonrandomized, noncontrolled prospective open-label study was approved by the Ethics Committee of the Hospital Egas Moniz, Centro Hospitalar de Lisboa Ocidental, Lisbon, Portugal, and meets the requirements of national agencies and ethical standards. All procedures were performed after obtaining written informed consent. Written consent included permission to culture and analyze a biopsy from the tissue to be grafted. Patients were fully aware of the experimental nature of the treatment, unclear outcomes, and possible side effects such as pain, spasticity, autonomic dysreflexia, worsening of motor or sensory function, infection, and unforeseen adverse events.

Patients were selected among individuals who had an SCI more than 1 year previously, were chronically paraplegic or tetraplegic, and were referred by Portuguese and Italian rehabilitation centers that specialize in the treatment of people with SCIs between April 2003 and December 2006 (Table 1). Costs for surgery and rehabilitation were paid by their respective governments. The rationale for selecting chronic (more than 12 months) SCI patients was to circumvent spontaneous recovery bias.

The inclusion criteria were the following: grade A or B on the AIS, age ≥18 and ≤40 years, presence of a cervical spinal cord lesion ≤3 cm or thoracic spinal cord lesion ≤4 cm, absence of significant nasal and paranasal sinus pathology, and absence of additional serious medical problems, brain disease, or psychological disturbance.

Twenty patients were enrolled in the study (17 men and 3 women). The mean age of the patients was 30.2 ± 5.7 years. Demographic data and clinical and imaging/radiological characteristics of the patients are presented in Table 1. Lesions resulted from road traffic accidents in 14 patients, sports accidents in 4, and work-related accidents in 2 patients. Lesions varied between 1.3 and 4 cm in the maximum vertical axis as measured on both the T1 and T2 weighted magnetic resonance imaging (MRI). Fifteen patients were AIS grade A, and 5 patients were AIS grade B. One patient (patient 12) was accepted with low SCI because a recent publication showed reversal of severely denervated muscle after electrical stimulation. Transplants were done from 18 to 189 months after injury (mean = 49 months). Sham operations were not considered because of the difficulty of ethical justification as this would entail an increased risk for the placebo group.
Table 1. Demographic, Clinical, and Neurological Features of the Patients

<table>
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<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (Years)</th>
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<th>P/T</th>
<th>Length of Lesion</th>
<th>AIS Grade</th>
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</tbody>
</table>

Abbreviations: SCI, spinal cord injury; P, paraplegic; T, tetraplegic; C, cervical; T, thoracic; AIS, ASIA Impairment Scale.

Operation

Briefly, pieces of olfactory mucosa were removed, cut in small pieces, and grafted into the spinal cord lesion site after performing a laminectomy. The detailed protocol for transplantation and surgical procedure has been reported previously. Microbiological examinations of the nasal cavities were performed routinely before and at the beginning of the operation. Specimens of the olfactory mucosa graft and scar tissue removed from the spinal cord were examined for histopathological and immunocytological purposes. In 6 cases, the olfactory mucosa graft specimens were cultured to derive NSCs.

Preoperative and Postoperative Rehabilitation

All patients had preoperative rehabilitation (31.8 ± 6.8 h/wk with a mean duration of 34.7 ± 30 weeks) and postoperative rehabilitation (32.7 ± 5.2 h/wk with a mean duration of 92 ± 37.6 weeks). The preoperative rehabilitation was carried out up to the time immediately prior to the operation (maximum 7-day delay). Baseline measures were determined after the preoperative rehabilitation, which was intended to ensure a stabilized neurological status. The rehabilitation included physical therapy strategies for encouraging motor function at and below the lesion, enabling walking training as soon as possible. The rehabilitation program at all 3 centers consisted of the following: 2 hours of passive, assisted range of motion and strengthening exercises; 2 to 3 hours of functional training for balance, posture, standing, and transfers; and 2 to 3 hours of pregait and gait activities. With regard to the walking training strategies, the Centro de Medicina de Reabilitação Rovisco Pais (RP), Tucha, Portugal, rehabilitation center focused on robotic-assisted bodyweight-support treadmill training (BWSTT, Lokomat) for subjects 2 to 6, 8, and 11; and the Serviço de Medicina Física e de Reabilitação do Hospital S. Sebastião (SS), Feira, Portugal (subjects 1, 7, 9, 10, and 12) and Centro Giusti (CG), Florence, Italy (subjects 13-20) rehabilitation centers performed BIONT. BIONT is an assisted overground walking training, with loading on the hips, knees, and feet to promote somatomotor biofeedback. There is freedom of movement (unrestricted by braces and other rigid bodyweight suspension systems) to allow the development of new, atypical motor patterns that may induce functional connections with supraspinal centers. BIONT focuses on the affected and nonaffected parts of the body.

Outcome Measures

Safety and efficacy measures are presented in Table 2. Any improvement in the AIS grade scale or in the lower extremity motor scores is considered as evidence for true gains because motor scores were 0 in the legs of patients after preoperative rehabilitation.

Preoperative and postoperative assessments included the AIS neurological exam as described in International Standards for Neurological and Functional Classification of Spinal Cord Injury Patients; standard electromyography (EMG) after asking the subject to move particular musclesrieness.12 Microbiological examinations of the nasal cavities were performed routinely before and at the beginning of the operation. Specimens of the olfactory mucosa graft and scar tissue removed from the spinal cord were examined for histopathological and immunocytological purposes. In 6 cases, the olfactory mucosa graft specimens were cultured to derive NSCs.

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and somatosensory evoked potentials (SSEP, cortically recorded after tibial nerve stimulation); urodynamic studies; full spinal cord MRI scan; otolaryngological evaluation including a general ear, nose, and throat examination, nasal endoscopy, olfactory evaluation, and computed tomography (CT) scan of the nose and paranasal sinuses; and psychological assessment. Psychological testing aimed to detect conditions such as active psychosis, major depression, anxiety disorder, severe mood disorder, suicidal behavior, alcohol addiction, drug addiction, low cognitive resources, and unrealistic expectations about treatment results.

All preoperative and postoperative neurological assessments were done unblinded in each rehabilitation center by the same trained SCI clinician and scheduled prior to OMA. The assessors were trained specifically for AIS (ASIA) assessments. To reduce bias, the transplanted patients and the other SCI patients treated in each center were assessed in the same sessions. Eurotrials Scientific Consultants (Lisbon, Portugal) collected and analyzed data from each center.

The patients from rehabilitation centers SS and CG were also assessed for Functional Independence Measure (FIM) and Walking Index for Spinal Cord Injury (WISCI) scales. Pain was assessed via interviews asking the patients to identify painful areas, to describe the pain using standard descriptors, and to identify temporal aspects of pain. Spasticity was evaluated clinically. Neurological status of the patients was evaluated every 6 months after OMA. The mean duration for follow-up was 27.7 months (range = 12-45 months) postoperatively.

Statistical Analysis

The statistical power consideration given the sample size limitation (N = 20) required implementation of nonparametric exact tests rather than asymptotic parametric tests. Nonparametric Wilcoxon signed rank test was employed to test the existence of any statistically significant difference between premeasures and the last nonmissing postmeasurement values. The decision on the statistical significance of the findings was made using an α level of .05.

Results

Safety Issues

There was no mortality in our series. All the patients recovered olfaction during the follow-up, 95% of them within 2 months. Five patients experienced adverse events resulting from the treatment. Three of these patients had minor complications that resolved spontaneously (subcutaneous collection of cerebrospinal fluid along the incision) or with simple treatments. One patient developed a late (1 year) irritable bowel syndrome that required dietary changes and medication.

One patient (patient 8) had a more severe complication requiring hospital readmission. He developed aseptic meningitis 2 weeks after surgery, associated with sensory and motor neurological deterioration, changing the AIS grade from B to A. MRI imaging showed evidence of spinal cord edema. The acute manifestations subsided in 3 weeks with vancomycin and dexamethasone. The patient recovered to AIS grade B in 2 months, but sensory status only partially recovered. This was the only patient who had a reduction in AIS grade.

Efficacy

AIS assessments. The data obtained using the AIS and ASIA scores are summarized in Tables 3, 5, 6, 7, and 8. The estimated mean change was statistically significant (p < .01, Table 4) in all the neurological measures (motor arms, motor legs, light touch, and pin prick scores). The changes were assessed between the baseline and the last evaluation (28 ± 11 months). Eleven (55%) patients improved their AIS grades: 6 patients from grade A to grade C, 3 patients from grade B to grade C, and 2 patients from grade A to grade B (Table 3). Nine (45%) of the patients who scored 0 at baseline for lower extremities improved from 4 to 22 at the last evaluation, including distal segmental muscles exceeding 3 motor segments (Table 6).

Rehabilitation centers that focused on BIONT therapy (SS and CG) had patients with better motor recovery compared with the rehabilitation center that focused on BWSTT (RP; Figure 1).

Of the 15 patients without anal sensation at the baseline evaluation, 9 had recovered at the last follow-up: 5 patients in the first 12 months and the others later. Additionally, 6 ASIA grade A patients (patients 1, 7, 9, 10, 12, and 19)
Table 3. Summary of AIS Grades

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<th>12 Months</th>
<th>18 Months</th>
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<th>36-48 Months</th>
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Abbreviations: AIS, ASIA Impairment Scale; LOCF, last observation carried forward; —, no evaluation done.

Table 4. Summary of Outcome Measures

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<tr>
<td>FIM</td>
<td>71.8 ± 20.9</td>
<td>86.8 ± 25.9</td>
<td>15.0</td>
<td>13*</td>
<td>−3.18</td>
</tr>
<tr>
<td>Paraplegics</td>
<td>90 ± 12.2</td>
<td>110 ± 9.0</td>
<td>20.0</td>
<td>5</td>
<td></td>
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<tr>
<td>Tetraplegics</td>
<td>60.5 ± 16.9</td>
<td>72.3 ± 21.8</td>
<td>11.8</td>
<td>8</td>
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Abbreviations: SD, standard deviation; WISCI, Walking Index for Spinal Cord Injury; FIM, Functional Independence Measure.
*P ≤ .001.
*P ≤ .01.

Recovered superficial sensation at S4-S5 segments. Five of the 20 patients without voluntary anal contraction recovered: 1 patient in the first 12 months and the others later. Of the 15 patients without bladder sensation at the baseline evaluation, 5 recovered the sensation of bladder fullness: 1 patient in the first 12 months and the others later. Only 1 of the 20 patients without bladder control recovered, by the 22nd month after surgery.

Functional and walking assessment. Thirteen patients from 2 rehabilitation centers (SS and CG) were assessed for functional studies. All the patients had improvements in FIM and WISCI scores (Figure 1). The mean FIM scores changed from 71 ± 23 at the baseline to 85 ± 28 at the last follow-up (P < .01). The WISCI scores changed from 0.2 ± 0.4 at the baseline to 7.4 ± 2.6 at the last follow-up (P < .01). WISCI scores were higher and achieved earlier in
paraplegic than in tetraplegic patients ($P < .01$). There was no difference between paraplegic and tetraplegic patients in the gain in FIM scores ($P > .05$).

Hip flexor muscle recovery was observed initially at 13 ± 11 months (patients 1, 7, 9, 10, 12, 13, 14, 15, and 19). Knee and ankle muscle recovery was observed initially at 22 ± 7 months (patients 1, 7, 9, 10, 12, 13). This suggests a proximal-distal pattern of recovery.

Of the 13 patients assessed by functional studies, 1 paraplegic patient (patient 9) can ambulate with 2 crutches and knee braces with no physical assistance and 10 other patients can ambulate with walkers with or without braces with physical assistance. One tetraplegic patient (patient 13) ambulates with a walker, without knee braces or physical assistance.
Electrophysiological assessments. New voluntary activity in response to voluntary effort was documented by EMG in 15 (75%) of the patients. In 6 tetraplegic patients (patients 1, 6, 8, 13, 16, and 20) and in 5 paraplegic patients (patients 7, 9, 10, 12, and 19) voluntary motor potentials were found in lower limb muscles.

New SSEP findings by tibial nerve stimulation were recorded at a cortical level after tibial nerve stimulation in 4 patients (patients 2, 4, 14, and 16). An example of this activity is shown for RP patient 2 (see Figure 2).

Urodynamic studies. In 5 postoperative urodynamic studies, patients were now able to detect a full bladder (patients 1, 7, 9, 10, and 12). Particularly important are the result obtained in patient 9, with EMG evidence of sphincter voluntary contraction.

Magnetic resonance imaging. As in our previous study, MRI showed a complete or almost complete filling of the lesion site in all patients, suggesting long-term graft viability. The grafted area has a “salt and pepper,” “popcorn,” or mixed appearance. The “popcorn” appearance, a more heterogeneous and multiloculated pattern, is usually observed in the more chronic stage after the surgical treatment (Figure 3). There was no evidence of neoplastic tissue overgrowth or syringomyelia.

Pain and spasticity. Most of the pain reported in the study was musculoskeletal or surgical pain that resolved normally. One patient developed a late (1 year) irritable bowel syndrome, which could be also interpreted as a visceral neuropathic pain that subsided 5 years after surgery. No significant changes were observed in spasticity in the first weeks after surgery, as found in other studies.

Spinal Cord Scar and Olfactory Mucosa Analysis

The most common observation was a scar of mixed composition containing astrocytic processes, fibroblasts, collagen, and laminin, with peripheral type axons interspersed, and single or multiple cystic cavities in different proportions.
Very rarely neurons were found, and in many cases we observed macrophagic infiltration predominantly within the glial rather than the fibrotic component of the scar. In thoracic lesions, areas of collagen and peripheral type axons were marginally involved in astrocytic glial fibers, whereas in cervical lesions, a single cyst cavity lined by astrocytic glial scar with variable collagen content from the walls of surrounding vessels was found. In the most severe SCI cases, there was laceration of the dura mater, and the spinal canal was filled with bone fragments and large amounts of collagen, particularly in some thoracic patients.

Six of the olfactory mucosa biopsy samples were selectively cultured, and neurons were generated from the stem cells. Graft culture after dissociation of the olfactory mucosa biopsies revealed about 100,000 stem cells in each cubic millimeter of olfactory mucosa, which is the typical size of each piece of olfactory mucosa tissue implanted.33 The number of pieces required to fill a cavity (generally more than 30) was dependent on the size of the cavity in the spinal cord after removing some of the scar tissue. Methicillin-resistant Staphylococcus aureus (MRSA) contamination was present in the olfactory mucosa graft biopsy from patient 8 even though the preoperative testing was negative.

**Discussion**

In this study, the safety and feasibility of OMA was supported. There was only 1 serious adverse event of aseptic meningitis. Close monitoring of patients using neurological, functional, and electrophysiological testing demonstrated indications of efficacy in a number of patients that seemed to relate to OMA combined with the BIONT rehabilitation program as opposed to OMA and BWSTT as the rehabilitation intervention.

**AIS and ASIA Changes**

After preoperative rehabilitation, 15 patients were AIS grade A and 5 were AIS grade B. Subsequent to OMA and postoperative rehabilitation, 11 (8 tetraplegics and 3 paraplegics) improved in AIS grade. Six improved by 2 grades and 5 by 1 grade. All the patients having rehabilitation at SS improved in AIS grade along with most of those who had rehabilitation at GC (primarily tetraplegic patients). No improvement was observed in AIS grade in the predominantly tetraplegic patients at RP receiving BWSTT.
were similar differences in the changes in ASIA scores. All patients had motor scores of 0 for the legs after preoperative rehabilitation, and there was no change in primarily tetraplegic OMA patients at RP, an average increase of 2.75 in the primarily tetraplegic OMA patients receiving therapy at GC, and a striking mean increase of 15.4 in the primarily paraplegic OMA patients at SS. No change was observed in motor arms in RP tetraplegic OMA patients; there was a mean increase of 8 in tetraplegic OMA patients at GC. Similar results depending on the rehabilitation center were found in sensory scores for light touch (LT) and pin prick (PP): RP: (LT = −.53; PP = −4.9), GC (LT = 22.9; PP = 16.1), and SS (LT = 42.8; PP = 44). The mean overall changes observed in ASIA scores are beyond the minimal detectable changes (motor = 0.29; sensory pinprick = 7.8; sensory light touch = 12.95) as recently reported.33 Although results are striking at 2 of the centers using BIONT rehabilitation, functional changes are most important to the patients.

**Functional Improvement**

Functional changes were measured with urodynamic studies and WISCI and FIM scores. Bladder sensation or control can dramatically modify the quality of life of these patients. One patient recovered bladder control at almost 2 years after OMA, and 25% of the patients could now accurately detect when the bladder was full during urodynamic studies.

All the 13 patients in whom WISCI was measured demonstrated improvement. Slightly greater improvement was observed in the paraplegic patients, with the greatest improvement in patient 9, who went from no mobility to being able to ambulate 10 m with braces and crutches without assistance. The results are clinically relevant because they include recovery of motor voluntary activity in lower limbs, both proximal and distal, which are reflected by the gait improvements in a significant proportion of the patients. Although independent walking of a previous motor complete SCI patient might be an achievable goal, any recovery even with modest changes can have profound effects on the quality of life in the patients. This is particularly true in cervical SCI where individuals who can crawl by themselves provide some degree of locomotion independence in case of an emergency. There was a correlation between motor leg scores and WISCI (r = .69; n = 8). Because the patients were aware of their treatment, their motivation to improve their function, prevent immobility related side effects, and improve transfers may have been high.

The 13 who had FIM scores measured also improved, and the improvement correlated with their WISCI scores (r = .75; n = 13), motor arms (r = .83; n = 8), and motor legs (r = .75; n = 13). Again greater absolute improvement was observed in the paraplegic patients. Curt et al34 found that some functional improvement as measured by Spinal Cord Independence Measure can occur in the first year after injury in complete SCI patients without concomitant changes in neurological condition or conductivity, so it was important to determine if there were any electrophysiological measures that point to neural repair.

**Electrophysiological Evidence of Recovery**

In 15 of the 20 patients, we demonstrated EMG activity in muscles on command below the level of injury, where previously no signal was present. This new voluntary control of muscles suggests that OMA had mediated a change across the injury site. This is further supported by the finding of SSEPs in some patients, objectively validating conductivity SCI repair.34

**Safety Concerns and Adverse Events**

A particular area of concern was the possibility of the introduction of pathogens in using a mucosa that is normally exposed to the air. We routinely perform preoperative and intraoperative microbiological examination of the nasal cavities and tissue graft. One of the 20 patients (patient 8) developed, 2 weeks after surgery, an aseptic meningitis syndrome (cerebrospinal fluid profile showed a high protein content and low glucose level, <100 cell count, predominant mononuclear cells, lymphocytes, and monocytes, with negative microbiological assays) and negative microbiological assay of blood, associated with MRI evidence of spinal cord edema and sensory and motor neurological deterioration. This patient had an intraoperative MRSA-positive nasal swab and graft examination (culturing required several days) following a negative preoperative microbiological evaluation. Secondary contamination due to surgical instrumentation when the graft passes through nasal pathways was suspected.

New visceral pain was present in 1 tetraplegic patient (patient 1) at 1 year postsurgery and interfered with daily activities. It was relieved with diet and medication (antidepressants and spasmylics). It was strikingly postural dependent subsiding with recumbent position and was present during the current follow-up but subsided sharply 5 years after surgery. Because this patient was AIS grade A before OMA we do not know if this was associated with sensory and autonomic recovery or was due to maladaptive plasticity mechanisms after tissue transplantation.35 The prevalence of pain in chronic SCI is high but the visceral one is low (5%).36 Temporary musculoskeletal pain and spasm associated with physiotherapy efforts was present in several patients but neuropathic pain was not reported in any other patient.

**Improbability of Spontaneous Recovery for Chronic, Complete SCI**

Spontaneous late recovery is unlikely to be responsible for the gains reported because OMA was performed at least
18 months or more after SCI. It is reported that only 5.6% of AIS A (32/571 patients) and 20.2% (23/114 patients) of AIS B improved in grade from year 1 to 5 after SCI. In this study, 53.3% of AIS A and 60% of AIS B improved in grade. Because the 4 patients who improved the most in ASIA motor score for the legs (patients 1, 7, 9, and 12) had the OMA surgery performed more than 2 years after injury (3½, 10, 2½, and 5 years, respectively), spontaneous recovery is highly improbable. This result also suggests that the time after injury is not a critical factor in performing the OMA procedure.

**Rehabilitation Alone Insufficient for Recovery in Chronic, Complete SCI**

One of the limitations of this study is that there was no control group with rehabilitation alone to separate the effects of rehabilitation and OMA and rehabilitation. However, 8/20 had a year or more of intense rehabilitation before the OMA with no change in AIS grade, and their ASIA motor legs remained at 0. This strongly suggests that rehabilitation alone was not sufficient for these patients with complete SCI. Six of the 8 patients with a year or more of preoperative rehabilitation improved in AIS grade and had gains in ASIA motor leg scores (mean 13.5) subsequent to OMA and BIONT rehabilitation. Some recovery was present in 4 out of 6 of these patients at the 6-month evaluation after OMA. Again this suggests that the rehabilitation alone was not responsible for this recovery.

**OMA Alone Insufficient for Recovery in Chronic, Complete SCI**

OMA alone is not likely to be sufficient to improve function after a complete SCI. One of the important findings of this study is that rehabilitation, and possibly a particular type of rehabilitation program, has to be combined with OMA to get improvement. Although all rehabilitation programs probably greatly increase the health of the individual, functional gains as measured by the outcome measures used in this study and, most important, new voluntary muscle control were only observed in the BIONT groups at the SS and CG sites after OMA. No motor recovery was found in the robotic BWSTT program (RP), which primarily focused on the affected part of the body. For a OMA procedure performed in India, patients who were only given instructions to follow a rehabilitation program at home or at rehabilitation centers did not show any ASIA score improvement. Their compliance and the intensity of therapy is unknown. The amount of recovery of OMA patients who received BIONT rehabilitation at SS and CG was also greater than the first 7 OMA patients we previously reported. These findings stress the importance of not only combining OMA with rehabilitation but also that a BIONT-type rehabilitation program that focuses on the whole body may be necessary for improvement. The BIONT rehabilitation program is specifically goal directed at walking. Braces and support are minimized over time and load bearing is encouraged to achieve ambulation. The use of muscle groups not normally used in walking is encouraged, and the preinjury pattern of walking is not attempted. Other tissue/cell therapies, such as autologous bone marrow transplantation, fibroblast growth factor, and autologous Schwann cells, for chronic complete SCI with no or other types of rehabilitation programs have not reported any improvements.

Recent studies in patients with chronic paraplegia confirm structural changes in the cerebral cortex and descending motor pathways including corticopontine tracts that are not directly connected to the spinal cord proving that the SCI represents a disconnection syndrome. In SCI complete lesions, the creation of new neural pathways that mediate functional recovery require at least partial reconnection of anatomical circuits by scar removal and cellular transplantation strategies. Subsequent functional rewiring necessitates rehabilitation strategies to reorganize circuits in the brain and spinal cord for meaningful sensory–motor integration of new and/or remaining neural circuits that may require a brain-initiated rehabilitation program such as BIONT.

**Probable Mechanism of OMA Recovery**

Other studies demonstrate that it is unlikely that long tract regeneration (eg, corticospinal tracts) takes place in the mature mammalian nervous system after SCI. In hemisectioned spinal cord, new axonal sprouting connections with proprio spinal neurons proximal to the lesion site occur with formation of new intraspinal circuits relaying cortical input to distal locomotor centers. Accordingly, both modified and new neural pathways may mediate recovery in incomplete patients and presumably in complete patients after successful cell/tissue therapies. It is unrealistic to expect a restoration of neural tissue function to its original state. A reconnection by a repair/bridge mechanism provided by immature neurons with assistance from other cell types such as OECs and fibroblasts could provide a lesion site neuronal network relay station between both the rostral and caudal stumps of the spinal cord. Support for this idea is that neurons developed from the 6 olfactory mucosa biopsy samples cultured from our patients. The new surgical injury may also induce plasticity.

**OMA as Source of Neural Stem Cells**

Stem cell treatment has the potential to be a major medical advance for SCI, and several types of stem cells are available such as embryonic, fetal, umbilical cord blood cells, and adult stem cells (bone marrow mesenchymal cells, fat cells, brain subventricular zone, olfactory mucosa,
and many other tissue types). Adult stem cells allow autolo-
gous transplants that avoid the problems of rejection, neoplasia,51 disease transmission,52-54 graft-versus-host dis-
ease,55 and ethical issues. In contrast to other readily available sources of adult stem cells, the normal fate of olfactory mucosa stem cells is to become neurons and sup-
port cells. Olfactory mucosa NSCs maintain telomerase activity and low apoptotic activity after several years in cul-
ture, and unlike hematopoietic stem cells and bone marrow mesenchymal cells, the capacity to replicate does not change with a person’s age.56 The olfactory mucosa is the only part of the adult nervous system capable of lifelong neurogenesis and axogenesis57 that is readily accessible.11,12 Neurons in the olfactory mucosa are continually being replaced by newly formed neurons.58,59 In culture, the olfactory mucosa is a source of stem cells that can become neurons,1,3,4 including motoneurons60 and oligodendrocytes.61

Other cell types in the olfactory mucosa may assist stem cell derivatives in bridging the injury site. OECs that are also present in the olfactory mucosa may contribute to the repair process. OECs obtained from the olfactory mucosa8 promote axonal remyelination and regeneration in the dam-
aged spinal cord. Equally favorable results were obtained using pieces of the lamina propria of the olfactory mucosa or cultured nasal OECs.9,10 OECs derived from the olfactory mucosa express a unique combination of developmentally important proteins not reported in olfactory bulb OECs.62 It also seems that adding other cells to OEC cultures such as fibroblasts63 or meningeal cells might increase the efficacy in SCI regeneration.64

Theoretical Support for Scar Removal
The composition of the scar (astrocytic, fibrotic, or mixed) implies that it is a physical and molecular barrier to axon regeneration and neural circuitry repair. The thickness and sometimes huge expanse of scar tissue necessitates that before any cell bridging attempt, the scar should be surgically reduced to the point of not damaging normal tissue, which can be accomplished by careful microscopic dissection.

Future Directions
Our studies offer strong support for the safety and feasibility of the OMA procedure. There are clear indications of efficacy based on neurological, functional, and electrophys-
iological testing that justify moving forward to a larger, controlled clinical trial. However, there are 3 important points to consider:

1. The technical challenges of OMA mean that expan-
sion to other sites with limited resources should be
approached conservatively. Our team prepared for
several years for this clinical study and proceeded slowly. Surgical techniques were perfected on cadavers. The nasal mucosa was examined in numerous cadavers to define the region where only olfactory mucosa is present. Given the complexity and invasiveness of the surgical procedure, the possibility of sham surgeries must be cautiously considered because of the risks involved.
2. New tests need to be developed for rapid recog-
nition of olfactory tissue to prevent any respira-
atory tissue in the graft.
3. The OMA procedure must be combined with an
intense rehabilitation program to obtain improve-
ment. The design of a randomized controlled clinical trial will require 2 arms given our results. Prior to randomization, all subjects should receive an intensive and specific rehabilitation effort for up to several months. This phase will help reduce con-
founders of latent function that a rehabilitation effort may bring out.65 One group would be ran-
domized to continued rehabilitation and another group to the surgical procedure with OMA and rehabilitation. The specific rehabilitation intervention will require continued consideration, given the experience we describe with BIONT compared with robotic-assisted therapy. In addition, the effort at driving training-induced plasticity must be con-
tinued intensively for at least 2 to 3 years.

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