

An Overview of Domestic and International Clinical Trials for Delivery of Cellular Therapies to the Spinal Cord

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Although varied, pathological afflictions to the spinal cord have limited therapeutic options and often carry a poor prognosis. This range includes traumatic (eg, spinal cord injury), inflammatory (eg, multiple sclerosis), and neurodegenerative (eg, amyotrophic lateral sclerosis [ALS], spinal muscular atrophy [SMA]) conditions. Numerous therapies have been tested for the treatment of spinal cord injury in a combination of domestic and international preclinical and clinical studies. None has demonstrated clinical efficacy and been approved for use. The current standard of clinical practice for traumatic spinal injury remains surgical decompression and spinal stabilization. Multiple sclerosis, an autoimmune condition in which the myelin protein is thought to provoke an immune response, is modified by both nonspecific immunomodulators such as corticosteroids and more targeted agents such as Tysabri (Natalizumab, Biogen Idec). This occurs at the risk of sometimes severe off-target effects and fails to restore lost myelination in disease-affected plaques. Finally, degenerative pathologies such as ALS and SMA portend a course of progressively worsening neurological function and a fatal prognosis. ALS is affected only minimally by currently available therapeutics, with Rilutek (Riluzole, Sanofi-Aventis) improving life expectancy by a few months. Therapies for SMA are focused solely on symptom alleviation.

It is with this background that cellular therapies have been considered for the treatment of spinal cord afflictions. Table 1 itemizes the completed international trials (2001-2009) that have assessed the safety and efficacy of cellular therapeutics for traumatic and degenerative indications. These studies have set the precedent for the current domestic and international trials assessing the delivery of cellular therapeutics that are underway. Contemporary trials assessing cellular therapies for neurodegenerative, inflammatory, and traumatic spinal conditions are introduced. Sections are arranged according to therapy delivery route. Table 2 reviews ongoing cellular delivery trials that employ either an intravenous or intrathecal therapy delivery route. Table 3 explores ongoing or near-term trials that use direct intraspinal microinjection to deliver a cellular graft. Citation sources have included searches of the MedLine (www.pubmed.gov) and National Institutes of Health (www.clinicaltrials.gov) databases, as well as personal communications of the senior author.

INTRAVENOUS DELIVERY

Only 1 trial¹⁶ exploring the intravenous delivery of a cellular therapy for treatment of spinal pathology is currently underway, as shown in Table 2. Inclusion criteria include a pediatric population of 1 to 15 years of age, an incomplete or complete spinal cord injury, and an injury that occurred between 6 months and 4 years before treatment. Autologous bone marrow progenitor cells are harvested to a quantity of up to 5 mL/kg, are processed, and are given through a single intravenous infusion. The primary outcome measure is the American Spinal Injury Association (ASIA) classification assessment that is completed at 1, 30, and 180 days after transplantation. The same group recently completed a phase I trial assessing the safety of intravenous autologous bone marrow progenitor cell delivery for acute traumatic brain injury in a pediatric population followed up for 6 months.²¹ While noting the safety of the approach in this trial, the authors indicated that their choice of cellular graft was guided in part by (1) small bone marrow progenitor cell size preventing a pulmonary “first-pass” effect, (2) ease of availability, (3) lack of ethical concerns, (4) data supporting lack of bone marrow progenitor cell tumorigenicity, and (5) lack of a need for cell banking and scaling for autologous application. The authors cite numerous possible mechanisms of effect, which include an immunomodulatory and local anti-inflammatory actions, as well as the paracrine secretion of growth factors and cytokines that may support an “at-risk” cellular population.

INTRATHECAL

Four trials are currently exploring an intrathecal route for delivery of a cellular graft. Each trial consists of a 1-time delivery of a predefined cellular dose after percutaneous access through a lumbar puncture. Each of the listed trials uses autologous cells, and none uses an immunosuppression regimen. Two are being sponsored by the same company (TCA Cellular Therapy, Covington, Louisiana) and are assessing ALS¹² and spinal cord injury,¹³ respectively. In both trials, adult mesenchymal stem cells are obtained from iliac crest bone marrow aspiration followed by purification and passaging. After 2 to 3 weeks, a 1-time injection is performed. In the ALS trial, patients are confirmed to have moderate to severe disease by the El Escorial criteria. Planned enrollment is 6 patients. Primary safety outcome measures assess

TABLE 1. Completed/Terminated Intraspinal Cellular Transplantation Trials^a

Year	Country/ Sponsor	Cells	Indication	Delivery	Inclusion Criteria	Status	Immunosuppression	Reference or NCT
2000	Israel and Belgium (Proneuron Biotech)	Adult autologous macrophages	SCI	Syringe with a 30-gauge fixed needle	Acute complete SCI (ASIA A) between C5 and T11	Phase I completed	No	Knoller et al ¹
2001	China	Cultured fetal OECs	SCI	Handheld injections at the caudal border of the lesion Freehand injections	Chronic SCI n = 8	Phase I completed	Unknown	Huang et al ²
2001	Italy	Adult autologous MSCs	ALS	18-Gauge cannula pump injector supported by a table-fixed arm	Definite ALS n = 16	Complete	No	Mazzini et al ^{3,4}
2003	China	Cultured fetal OECs	SCI	Freehand injections	Chronic SCI n = 9 n = 171	Complete	Unknown	Huang et al ⁵
2003	Israel and US (Proneuron Biotech)	Adult autologous macrophages	SCI	Syringe with a 30-gauge fixed needle Freehand injections at the caudal border of the lesion	Acute complete SCI (ASIA A) between C5 and T11 n = 61	Phase II suspended	No	http://www.proneuron.com, NCT00073853
2003	China	Cultured fetal OECs	ALS	Freehand injections	Probable or definite ALS n = 327	Complete	Unknown	Chen et al ⁶
2004	Australia	Cultured autologous OECs	SCI	Hamilton syringe with a 28-gauge beveled needle Stabilized system mounted to the operating table	Chronic complete SCI (ASIA A) between T4 and T10 n = 3	Phase I complete	No	Feron et al ⁷
2006	Turkey	Autologous BMCs	ALS	Intraspinal, freehand Intrathecal Intravenous Intraspinal	ALS n = 13	Phase II	No	Deda et al ⁸
2007	Korea	Autologous BMCs	SCI	Not described Intraspinal	Acute complete SCI (ASIA A)	Phase I/II	No	Yoon et al ⁹
2008	Poland	OEC	SCI	Not described Intraspinal	Complete SCI (ASIA A)	Complete Phase I ongoing	Unknown	NCT01231893
2009	Italy	Autologous BMCs	ALS	Not described Table-mounted arm for positioning syringe	Definite or probable spontaneous ALS n = 10	Phase I complete	No	Mazzini et al ^{3,10}

Continues

TABLE 1. (Continued)

Year	Country/ Sponsor	Cells	Indication	Delivery	Inclusion Criteria	Status	Immunosuppression	Reference or NCT
2009	Spain	Autologous BMCs	ALS	22-Gauge needle, table-mounted arm for positioning syringe	Definite ALS n = 10	Phase I/II complete	Unknown	NCT00855400, Blanquer et al ¹¹
2010	US (Geron Corp)	Human ESC-derived oligodendrocytes	SCI	Syringe positioning device that attaches to the frame of the operating room table	Subacute complete thoracic SCI (ASIA Grade A) n = 11	Withdrawn	Yes	www.geron.com, NCT01217008

^aALS, amyotrophic lateral sclerosis; ASIA, American Spinal Injury Association; BMC, bone marrow cell; ESC, embryonic stem cell; MSC, mesenchymal stem cell; SCI, spinal cord injury.

procedural safety and the development of neurological deficit beyond those expected for disease progression. Secondary outcome measures assess disease progression based on electrodiagnostic studies, pulmonary function testing, semiobjective assessments of motor function alteration, and disease-specific rating scale worsening followed over a 1-year period. In the spinal cord injury trial, inclusion criteria include an ASIA A injury below the C5 level, concordant with imaging findings, that occurred between 2 and 60 weeks before enrollment. Ten patients are planned for enrollment, with the primary outcome being listed as assessment of neurological and nonneurological factors associated with the procedure and graft. Using a variation of the approach described above, Windebank et al¹⁴ are attempting a target intrathecal infusion of 1 million cells in a single patient with ALS. As opposed to iliac harvest of bone marrow, a subcutaneous biopsy is used to obtain adipose tissue. This aspirate is subsequently purified and passaged to select for adult mesenchymal stem cells. Detailed information regarding primary and secondary outcome measures is not provided.

The only international cellular trial assessing intrathecal delivery currently reported is at the Hadassah Hebrew University Medical Center in Jerusalem, Israel. A total of 24 patients are being enrolled for either intramuscular or intrathecal autologous mesenchymal stem cell delivery. All patients meet the El Escorial criteria for definite or probable ALS. Twelve “early”-stage patients will receive multiple intramuscular injections into proximal upper arm musculature to a total of 24 injections with 1 million cells being delivered per injection. Twelve “late”-stage patients will receive a single intrathecal dose of 60 million cells. Determination of early vs late stage disease depends on duration of disease, ALS functional rating scale scores, pulmonary function testing, and muscle bulk assessments. Primary outcome measures explore the safety of intramuscular or intrathecal infusion. Secondary outcome measures assess ALS Functional Rating Scale-determined disease progression, pulmonary function, electrodiagnostic study results, time to tracheostomy, and overall survival. Patients are being followed up for 6 months.

INTRASPINAL DELIVERY

Six trials are planned or underway that use a direct intraspinal microinjection approach, as summarized in Table 3. Described delivery strategies include a table-mounted approach or a patient-mounted platform. Available documentation does not specify for 2 trials. Either 1 injection or serial injections may be attempted. Indications include SMA, ALS, and spinal cord injury. Immunosuppression is being explored in 1 trial, is not being used in 2 trials, and is unknown in 3 trials. Each of the trials is described in detail below.

The study currently being conducted by our group is a phase I clinical trial assessing serial microinjections into the ALS spinal cord.^{17,18} Novel elements of the trial design include the use of a risk-escalation paradigm, the use of a patient-stabilized microinjection approach, and the use of immunosuppressants with a nonautologous cell source. Because of the vulnerability of the ALS spinal cord, risk to

TABLE 2. Ongoing/Planned Intrathecal or Intravenous Cellular Transplantation Trials^a

Year	Country/ Sponsor	Cells	Indication	Delivery	Inclusion Criteria	Status	Immunosuppression	Reference or NCT
Intrathecal								
2010	Covington, LA (TCA Cellular Therapy, LLC)	Autologous human bone marrow– derived mesenchymal stem cells	ALS	Single intrathecal infusion	Moderate to severe ALS with El Escorial criteria	FDA phase I, ongoing n = 6 planned	No	NCT01082653 (http://www.tcaacellulartherapy.com , http://clinicaltrials.gov) ¹²
2010	Covington, LA (TCA Cellular Therapy, LLC)	Autologous human bone marrow– derived mesenchymal stem cells	SCI	Single intrathecal infusion	Subacute complete SCI below C-5 (ASIA Grade A)	FDA phase I, ongoing	No	NCT01162915 (http://www.tcaacellulartherapy.com , http://clinicaltrials.gov) ¹³
2010	Rochester, MN, (Mayo Clinic)	Autologous human adipose tissue– derived mesenchymal stem cells	ALS	Single Intrathecal infusion (1 million cells)	Adults with chronic onset of a progressive motor weakness	n = 10 planned FDA phase I, ongoing	No	NCT01142856 (http://clinicaltrials.gov) ¹⁴
2011	Jerusalem, Israel (BrainsStorm Cell Therapeutics, Ltd)	Autologous human mesenchymal bone marrow stromal cells secreting neurotrophic factors	ALS	Early stage: multiple intramuscular injections to triceps and biceps muscles (24 million cells); late stage: single intrathecal injection (60 million cells)	ALS disease duration < 2 y; and ALS-FRS- R scale > 30 (early stage) or ALS-FRS-R scale 15-30 (late stage)	n = 1 planned Israel Ministry of Health phase I/II, recruiting	No	NCT01051882 (http://www.brainstorm-cell.com , http://clinicaltrials.gov) ¹⁵
Intravenous								
2011	Houston, TX, (Memorial Hermann Healthcare System)	Autologous human bone marrow– derived progenitor cells	SCI	Single intravenous infusion	Children 1 to 15 y with chronic SCI	FDA phase I, recruiting	No	NCT01328860 (http://www.memorialhermann.org , http://clinicaltrials.gov) ¹⁶
						n = 10 planned		

^aALS, amyotrophic lateral sclerosis; ALS-FRS, ALS Functional Rating Scale; ALS-FRS-R, ALS DEA, revised; ASIA, American Spinal Injury Association; FDA, Food and Drug Administration; SCI, spinal cord injury.

TABLE 3. Ongoing/Planned Intraspinal Cellular Transplantation Trials^a

Year	Country/Sponsor	Cells	Indication	Delivery	Inclusion Criteria	Status	Immunosuppression	Reference or NCT
2010	Atlanta, GA, (Neuralstem, Inc)	Allogenic human fetal spinal cord-derived spinal stem cells (NSI-566RSC)	ALS	Direct multiple injections to the ventral horn of the lumbar or cervical enlargement using a spine-mounted microinjection platform (0.5-1 million cells)	Adult probable or definite ALS defined according to El Escorial criteria	FDA phase I, recruiting (n = 18 planned)	Yes	NCT01348451 (http://www.neuralstem.com , http://clinicaltrials.gov) ¹⁷⁻¹⁹
2010	Spain	Autologous BMCs	ALS	Intraspinal 22-gauge needle; table-mounted arm for positioning syringe	Definite ALS	Phase I/II recruiting (n = 63 planned)	Unknown	NCT01254539
Intrathecal								
2010	Brazil	Autologous BMCs	SCI	Intraspinal Not described	SCI Frankel A classification	Phase I ongoing (n = 20)	No	NCT01325103
2011	California Stem Cells Inc, CA	Allogenic human ESC-derived motor neuron progenitor cells (MotorGraft)	SMA	Direct multiple injections to the ventral horn of the thoracic spinal cord	Infant (age 2-6 months) SMA type 1	FDA clinical hold; reviewing investigational new drug		http://www.californiastemcell.com ²⁰
2011	Zurich, Switzerland (Stem Cells, Inc)	Allogenic fetal brain-derived nervous system stem cells	SCI	Direct multiple injections to the inferior and superior border of spinal cord lesion (20 million cells)	Adult thoracic chronic SCI (ASIA A, B, or C)	Swissmedic phase I/II, recruiting	Yes	NCT01321333 http://www.stemcellsinc.com
2012	Italy	Autologous BMCs	ALS	Safety validated patient-mounted platform (same approach as in Neuralstem trial)	Definite ALS	Phase I/II ongoing	No	Personal communication

^aALS, amyotrophic lateral sclerosis; BMC, bone marrow cell; ESC, embryonic stem cell; FDA, Food and Drug Administration; SCI, spinal cord injury.

the patient is incrementally increased. The progression begins with unilateral lumbar microinjection series in nonambulatory patients and concludes with unilateral cervical plus bilateral lumbar microinjections in ambulatory patients over a series of 5 groups and 18 patients. This allows stepwise safety evaluation of injection series when completed in each of the following scenarios: nonambulatory vs ambulatory, unilateral vs bilateral, and thoracolumbar vs cervical. The use of a patient-stabilized microinjection platform and targeted delivery approach both recognizes the need for the standardization of the microinjection process to achieve reproducible accuracy and addresses the theoretical concern of patient movement during the microinjection process. Further information on the preclinical development of the stabilized microinjection platform and targeted microinjection delivery approach can be found elsewhere.²² Interim primary (safety) and secondary (functional) outcomes also can be found elsewhere.^{17,18} To date, 14 of 18 planned patients have received the microinjection approach without development of postoperative neurological worsening. Finally, the use of a cellular allograft with a multiagent immunosuppression regimen will begin to define the potential role for immunosuppression when delivering a nonautologous graft to the ALS spinal cord. Mazzini et al (L. Mazzini, personal communication) will be using the technology and approach described above for an upcoming intraspinal microinjection trial for ALS that uses the autologous bone marrow progenitors they have pioneered in previous work.³

Expanding on their prior work,¹¹ Moraleda and colleagues are currently recruiting for a phase I/II trial intended to enroll 63 patients to assess a combination of thoracic intraspinal microinjection and intrathecal infusion of autologous bone marrow-derived stem cells in patients with ALS. Provided documentation indicates the presence of a 3-arm study to include intraspinal microinjection, intrathecal cell delivery, and intrathecal placebo delivery. Available documentation does not specify whether patients are intended to receive concomitant intraspinal and intrathecal cellular delivery. The study primary outcome measure is the forced vital capacity with secondary outcome measures including quantification of neurological functional rating scale and respiratory functional outcomes. The trial proposed by California Stem Cells, Inc is the only planned trial to assess a direct cellular microinjection approach to treat SMA. Furthermore, it is planned for cellular transplantation into infants (age, 2-6 months). Investigational New Device documentation is currently in process with the Food and Drug Administration.

Two trials are currently recruiting for the treatment of spinal cord injury. Stem Cells, Inc is recruiting for evaluation of a fetal allograft delivered rostral and caudal to the level of a thoracic spinal cord injury. Limited documentation indicates that immunosuppression will be provided, although further information is not provided. With the use of a risk-escalation paradigm, cohorts are grouped by ASIA grade, with transplantation into ASIA A through C being assessed. Recent press releases indicate tolerance of delivery in the first 4 patients with a “complete” ASIA A injury.²⁰ Santos et al²³ are

attempting delivery of autologous bone marrow progenitors to the lesion area in “complete” thoracic spinal cord injury patients. An initial series of 20 patients is planned. The primary outcome measure, being followed up over a period of 6 months, is described as “safety” with secondary outcome measures including motor improvement as measured by the Frankel scale and improvements in sphincter control.

CONCLUSION

Early experiences in international cellular transplantation efforts have helped to inform the development of current and planned domestic and international trials. Our group has taken several lessons from these early efforts that we have attempted to incorporate into our trial design. First, published reports from early efforts underscored a role for a validated, stabilized delivery approach to optimize delivery safety and targeting reproducibility. Second, the vulnerability of these patient populations and the novelty of cellular delivery to the spinal cord have called attention to the role of a phase I trial design focused on risk escalation. Finally, the role of immunosuppression remains unclear. Many early translational efforts explored the use of autologous cellular grafts and did not use immunosuppression. More recent trial designs are exploring both autografts and allografts. As a result, the full range of immunosuppressive options is being tested, from none to multiagent regimens. Encouragingly, preliminary results from ongoing trials (Table 3) and reported outcomes for completed direct microinjection studies (Table 1) provide data supporting the safety of cellular delivery to the spinal cord even in the vulnerable patient populations under assessment. In this setting, data addressing the role of alternative cellular graft types, immunosuppression approaches, graft survival and migration potential, graft tumorigenicity, optimized injection parameters, and maximum tolerable doses should be forthcoming.

Disclosure

Neuralstem, Inc provided financial assistance for microinjection platform construction and is funding the phase I clinical trial that is described and is currently underway. Dr Boulis has received an inventor's fee for the microinjection platform and floating cannula. He is also eligible for royalties associated with future licensing of these technologies. The other authors have no personal financial or institutional interest in any of the drugs, materials, or devices described in this article.

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