4-1-2022

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JNS SPINE

CLINICAL ARTICLE

J Neurosurg Spine 37:321–330, 2022

Ten-year safety of pluripotent stem cell transplantation in acute thoracic spinal cord injury

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OBJECTIVE The purpose of this study was to evaluate the safety of oligodendrocyte progenitor cells (LCTOPC1) derived from human pluripotent stem cells administered between 7 and 14 days postinjury to patients with T3 to T11 neurologically complete spinal cord injury (SCI). The rationale for this first-in-human trial was based on evidence that administration of LCTOPC1 supports survival and potential repair of key cellular components and architecture at the SCI site.

METHODS This study was a multisite, open-label, single-arm interventional clinical trial. Participants (n = 5) received a single intraparenchymal injection of 2×10^6 LCTOPC1 caudal to the epicenter of injury using a syringe positioning device. Immunosuppression with tacrolimus was administered for a total of 60 days. Participants were followed with annual in-person examinations and MRI for 5 years at the time of this report and will be followed with annual telephone questionnaires for 6 to 15 years postinjection. The primary endpoint was safety, as measured by the frequency and severity of adverse events related to the LCTOPC1 injection, the injection procedure, and/or the concomitant immunosuppression administered. The secondary endpoint was neurological function as measured by sensory scores and lower-extremity motor scores as measured by the International Standards for Neurological Classification of Spinal Cord Injury examinations.

RESULTS No unanticipated serious adverse events related to LCTOPC1 have been reported with 98% follow-up of participants (49 of 50 annual visits) through the first 10 years of the clinical trial. There was no evidence of neurological decline, enlarging masses, further spinal cord damage, or syrinx formation. MRI results during the long-term follow-up period in patients administered LCTOPC1 cells showed that 80% of patients demonstrated T2 signal changes consistent with the formation of a tissue matrix at the injury site.

CONCLUSIONS This study provides crucial first-in-human safety data supporting the pursuit of future human embryonic stem cell–derived therapies. While we cannot exclude the possibility of future adverse events, the experience in this trial provides evidence that this cell type can be well tolerated by patients, with an event-free period of up to 10 years. Based on the safety profile of LCTOPC1 obtained in this study, a cervical dose escalation trial was initiated (NCT02302157).

Clinical trial registration no.: NCT01217008 (clinicaltrials.gov)

https://thejns.org/doi/abs/10.3171/2021.12.SPINE21622

KEYWORDS spinal cord injury; GRNOPC1; LCTOPC1; AST-OPC1; human embryonic stem cells; clinical trials; central nervous system; trauma; thoracic

ABBREVIATIONS AE = adverse event; AIS = American Spinal Injury Association Impairment Scale; DSMB = Data and Safety Monitoring Board; hESC = human embryonic stem cell; HLA = human leukocyte antigen; IND = Investigational New Drug; ISNCSCI = International Standards for Neurological Classification of Spinal Cord Injury; LEMS = lower-extremity motor score; NLI = neurological level of injury; SAE = serious AE; SCI = spinal cord injury; UEMS = upper-extremity motor score; UTI = urinary tract infection; ZPP = zone of partial preservation.

ACCOMPANYING EDITORIAL See pp 317–320. DOI: 10.3171/2022.1.SPINE211573.

SUBMITTED April 30, 2021. **ACCEPTED** December 20, 2021.

INCLUDE WHEN CITING Published online April 1, 2022; DOI: 10.3171/2021.12.SPINE21622.

* S.L.M. and R.E. share first authorship of this work, and E.W. and R.G.F. share senior authorship of this work.

To date, there are no treatments approved by the FDA to induce neurological recovery following spinal cord injury (SCI). Several interventions, including glucocorticoids,[1] modulation of voltage-gated channels,[1–3] tetracycline antibiotics,[4] and cell-based therapies,[5–7] have been studied in clinical trials; however, none to date have met critical registration endpoints. In this report we describe what is to our knowledge the first-in-human phase 1 safety clinical trial of oligodendrocyte progenitor cells (LCTOPC1) derived from human pluripotent stem cells, which have mechanistic properties to support survival and potential repair of key cellular components and architecture at the SCI site.

The initial characterization of the LCTOPC1 population was reported by Nistor et al. in 2005,[8] who showed that these cells could differentiate into oligodendroglial progenitors. Subsequent studies in an acute incomplete rat contusion injury model demonstrated that the oligodendroglial progenitor cells survived after delivery to the SCI site[9] and led to remyelination of denuded axons. When delivered in the acute injury period, the cells led to improvement in locomotor function as measured in standardized behavioral testing.[9] Based on proof-of-principle study data, preclinical studies were conducted to support translation into safety clinical trials. Preclinical studies demonstrated that the intended clinical, cryopreserved, human equivalent dose formulation of LCTOPC1 could survive and migrate after injection in the SCI site, produce neurotrophic factors to support cell survival, and provide remyelination potential to support denuded axons at the SCI contusion site.[10] Moreover, studies demonstrated that the cells did not produce teratomas and did not lead to increased pain in injured animals.[10]

The data from these studies formed the foundation for an Investigational New Drug (IND) application to initiate this phase 1 clinical trial, which was reviewed by the FDA, the Data and Safety Monitoring Board (DSMB), the SCI clinical community, surgical and outcomes steering committees, internal and external ethics committees, internal and clinical trial site stem cell research oversight committees, and the IRB for each participating clinical trial site. There was consensus that the data supported clinical testing in patients with acute, complete, thoracic spinal cord lesions. As a first-in-human study, the trial design accounted for the need to minimize the risk to participants, and hence individuals with complete SCI localized between the thoracic neurological levels T3 and T11 were chosen for intervention. The trial was an open-label, unblinded, nonrandomized, non–placebo-controlled study performed to establish the safety of intraparenchymal injection of LCTOPC1 and to monitor changes in neurological function.

Determining the long-term safety of stem cell therapeutic agents is a critical step in enabling future trials to investigate the use of novel stem cell or combination therapies. Ten years postimplantation, there have been no medical or neurological complications to indicate that LCTOPC1 implantation is unsafe. Specifically, there have been no serious adverse events (SAEs) related to the procedure, cell implant, or immunosuppression. In addition, there have been no significant changes in neurological function. Safety data from this first-in-human study support progression to a clinical trial for individuals with cervical SCIs.

Methods

Clinical Study Design

This study was a phase 1, multicenter, nonrandomized, single-group assignment, interventional clinical trial. Participants were enrolled from one of seven centers in the United States. The study was registered on clinicaltrials.gov (NCT01217008).

The primary endpoint was safety, as measured by the frequency and severity of adverse events (AEs) related to LCTOPC1, the injection procedure used to administer LCTOPC1, and/or the concomitant immunosuppression administration. The secondary endpoint was neurological function as measured by sensory scores and lower-extremity motor scores (LEMSs) on the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) examinations. The eligibility criteria are summarized in Supplemental Table 1. At the time of this report, participants had been followed by protocol for a total of 5 years of in-person visits and will continue to be followed for an additional 10 years of annual telephone visits. Figure 1 provides an overall study schema for the clinical trial.

Study Oversight and Monitoring

In addition to FDA review, the protocol and study design were reviewed by a steering committee. Due to the nature of the study product, the protocol was also reviewed by an overall study Embryonic Stem Cell Research Oversight (ESCRO) committee as well as individual site ESCRO committees where required. As noted above, safety monitoring occurred via an External Medical Monitor, Sponsor Medical Monitor, and DSMB.

Informed Consent

The study was conducted in compliance with the protocols of the Declaration of Helsinki, and according to the International Conference on Harmonization (ICH) Guidance for Industry, Good Clinical Practice (GCP): Consolidated Guidance (ICH E6), and all other regulatory and institutional requirements, including those for subject privacy, informed consent, IRB or Independent Ethics Committee approval, and record retention.

Due to the potential for long-term risks of human embryonic stem cell (hESC) administration, two protocols and thus two informed consent forms were required: one for the administration of LCTOPC1 and 1-year follow-up (CP35A007) and the other for follow-up from years 2 to 15 following product administration (CP35A008). Written informed consent for both protocols was obtained for all individuals prior to study enrollment.

Study Participants

Male or female participants from 18 to 65 years of age with acute traumatic SCI were eligible for study participation. As this was a first-in-human study, with a risk of neurological deterioration, inclusion was limited to neu-

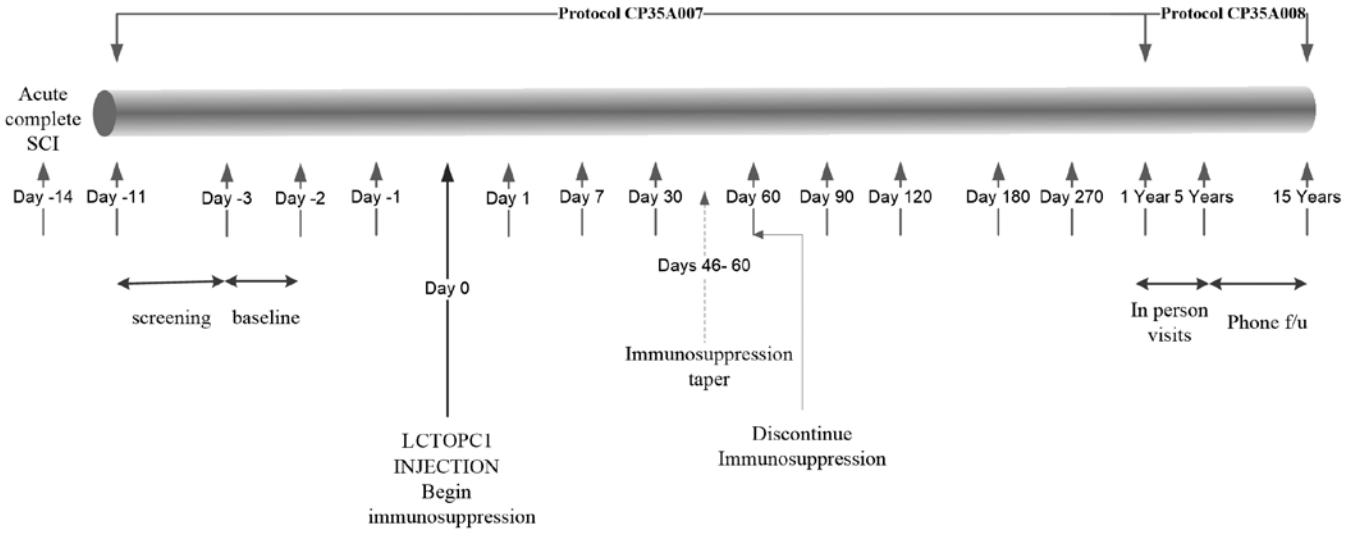

FIG. 1. Phase 1 clinical trial study schema. f/u = follow-up.

rologically complete injuries (American Spinal Injury Association Impairment Scale [AIS] grade A), with a single neurological level of injury (NLI) from levels T3 to T10, with no spared motor function in < 5 levels (i.e., zone of partial preservation [ZPP]) below the single neurological level. These inclusion criteria were chosen to minimize loss of function if neurological deterioration were to occur.

Poststabilization MRI was used to confirm the presence of a single spinal cord lesion with sufficient visualization of the spinal cord for 30 mm above and below the injury epicenter to enable postinjection safety monitoring. Participants had to be eligible for an elective surgical procedure to inject LCTOPC1 between 7 and 14 days following SCI (Supplemental Table 1).

Investigational Product and Mode of Administration

LCTOPC1 is a cell population containing a mixture of oligodendrocyte progenitor cells and other characterized cell types that are obtained following differentiation of undifferentiated hESCs from the H1 stem cell line, produced at the University of Wisconsin in 1998.[11]

LCTOPC1 is a cryopreserved cell therapy product. At the time of cryopreservation, each vial contained 7.5 × 10^6 viable cells in 1.2 ml of cryoprotectant solution. LCTOPC1 was supplied in 2.0-ml cryovials and shipped to the clinical sites in the vapor phase of liquid nitrogen and stored under the same conditions at the site. Prior to administration, LCTOPC1 was thawed and prepared by study personnel who were trained and qualified in the preparation of the study drug.

Participants received a single administration of 2 × 10^6 viable LCTOPC1 cells suspended in Hanks' Balanced Salt Solution (HBSS), with a total volume per dose of 50 µl. The rationale for selection of this dose was based on preclinical studies involving rats and mice and on dose extrapolation to humans using two methods: 1) comparing the relative sizes of the human and rat spinal cords and 2) evaluating tumorigenicity risks with respect to the absolute number of injected cells. During the development of LCTOPC1, a dose of 2 × 10^6 cells was the highest dose that was feasible to administer in the injured rat spinal cord and the rat was the largest animal that could be utilized to satisfy the animal number required for the IND-enabling studies for this novel product. Hence, to be conservative, 2 × 10^6 cells, the highest dose tested in rats, was used as the dose for the phase 1 trial.

The intended route of administration for LCTOPC1 was intraparenchymal at a depth of 6 mm in the midline and 5 mm caudal to the epicenter of injury as determined by MRI, as modeled in preclinical studies.[10] A caudal injection was selected out of an abundance of caution to avoid any potential direct tissue damage above the injury level. Based on preclinical studies, it was anticipated that the injected cells would migrate rostrally throughout the injury site.[10] The 7- to 14-day time frame was chosen based on results of animal studies suggesting poor graft survival for implantation within the first 7 days of injury[9] while attempting to maximize the potential neuroprotective and remyelinating effect. A custom-designed syringe positioning device (Supplemental Fig. 1) was utilized to assist neurosurgeons with the controlled delivery of the cells.[12]

Immunosuppression

Participants who received LCTOPC1 also received tacrolimus to prevent rejection of this allogeneic, cell-based product. Immunosuppression with tacrolimus was initiated between 6 and 12 hours after injection of LCTOPC1. If the participant was unable to take oral medication, tacrolimus was administered intravenously at a starting dose of 0.01 mg/kg/day, given as a continuous intravenous infusion. Participants were switched to oral tacrolimus as soon as possible. The starting dose for oral tacrolimus was 0.03 mg/kg/day, divided into 2 daily doses. The tacrolimus dose was adjusted to achieve a target whole-blood trough level of 3 to 7 ng/ml.

On day 46, the tacrolimus dose was decreased by 50% (rounded to the nearest 0.5 mg, as this was the smallest capsule size available). On day 53, the tacrolimus dose

was decreased by another 50% (rounded to the nearest 0.5 mg). If the rounded total daily dose was 0.5 mg or lower, the participant received a 0.5-mg dose once per day until tacrolimus was discontinued. Tacrolimus was discontinued at day 60. The dose of tacrolimus was lowered if the trough blood level exceeded 7 ng/ml. In addition, an expert reviewed all ISNCSCI examination forms to assess whether there were any changes in neurological function that may have been associated with tacrolimus tapering and/or discontinuation.

Follow-Up and Assessments

An overview of study visits for the 1-year protocol follow-up (CP35A007) and 2- to 15-year protocol follow-up (CP35A008) is provided in the study schema (Fig. 1). As this was a first-in-human clinical trial of cells derived from hESCs, a high number of study visits and long-term follow-up were required. In the 1-year protocol, three study visits were required prior to product administration, with 13 evaluations in the first year following study administration (Supplemental Tables 2 and 3). For the long-term protocol, annual visits were required in years 2–5. Subsequent to the year 5 annual visit, follow-up was by annual telephone questionnaires (Supplemental Document 1) and in-person evaluations, as necessary. Telephone assessments included documentation of all new medications taken for longer than 30 days, admissions to the hospital, and documentation of SAEs and AEs.

Safety Assessments

The primary endpoint of the phase 1 clinical trial was safety, as measured by the frequency and severity of AEs within 1 year of LCTOPC1 injection that were related to LCTOPC1, the injection procedure used to administer LCTOPC1, and/or the concomitant immunosuppression administration. Safety assessments included physical examination, vital signs, ISNCSCI neurological examination, pain questionnaire, electrocardiogram, MRI, laboratory tests for hematology and blood chemistry, laboratory tests for immunosuppression safety monitoring (whole-blood trough levels of tacrolimus and serum levels of creatinine, potassium, magnesium, phosphate, ionized calcium, aspartate aminotransferase, alanine aminotransferase, and total bilirubin), concomitant medications, and AEs. The severity of AEs and the characterization of SAEs were evaluated using standard FDA criteria.[13]

Neurological Assessments

The secondary endpoint was neurological function, including measurement of sensory scores and LEMSs. Neurological function was evaluated using the ISNCSCI examination for motor and sensory testing and for designation of the AIS grade.[14]

Exploratory Endpoints

Pain assessment was performed using the International Spinal Cord Injury Pain Basic Data Set.[15,16] A set of three questions was added to assess allodynia. These questions covered the presence and severity of pain provoked or increased by brushing, pressure, or contact with cold. Information on pain medication was collected as part of the assessment of concomitant medications.

Magnetic Resonance Imaging

Screening/baseline MRI was obtained between 3 and 5 days prior to injection (day –3 and day –5) of LCTOPC1 but no earlier than 4 days after SCI. The screening/baseline MRI included the brain, cerebellum, and entire spinal cord, with and without contrast (gadolinium-diethylenetriamine pentaacetic acid [Gd-DTPA]). If surgery for LCTOPC1 injection was subsequently delayed for more than 3 days, then a repeat MRI of the thoracic spine, without contrast, was obtained. Follow-up MRI scans of the spinal cord and cerebellum, with and without contrast (Gd-DTPA), were obtained on days 7, 60, 120, and 270 postinjection. A full central nervous system MRI, with and without contrast (Gd-DTPA), was obtained on days 30, 90, 180, and 365, as well as yearly between years 2 and 5. Image acquisition protocols were standardized. Image review was centralized and standardized by an independent radiologist at Radiology Imaging Associates Denver.

Human Leukocyte Antigen Typing and Immunological Monitoring

LCTOPC1 cells do not express human leukocyte antigen (HLA) class II and are resistant to NK-cell lysis.[17] However, one concern with the safety and potential efficacy of LCTOPC1 was the possibility of allogeneic rejection by the host's immune system. Immunosuppression was minimized in terms of duration to 60 days and dosed below the typical long-term maintenance therapy levels used for solid organ transplantation.

Peripheral blood and CSF samples from LCTOPC1-injected participants were collected according to protocol. A lumbar puncture to obtain 10 ml of CSF was conducted after receiving general anesthesia but prior to LCTOPC1 injection as well as at day 60 postinjection to assess for rejection of allogeneic cells as well as for immunological monitoring. The following CSF assessments occurred at the hospital laboratory: white blood cell count, glucose, total protein, oligoclonal banding, myelin basic protein, and immunoglobulin G index. In addition, CSF was evaluated by the sponsor to further assess immune response to LCTOPC1 and for the presence of LCTOPC1 (day 60) using a polymerase chain reaction–based assay. Peripheral blood was examined for the presence of antibodies specific for the donor-specific HLA molecules on LCTOPC1 and to detect T-cell–mediated responses to LCTOPC1.

Statistical Methods

Descriptive analysis was used due to the small sample size and the open-label and nonrandomized study design. The primary and secondary endpoints of this study are presented descriptively in table, figure, and text form.

Results

Study Participants

The first participant received implantation during the winter of 2010, and the last participant was enrolled in the

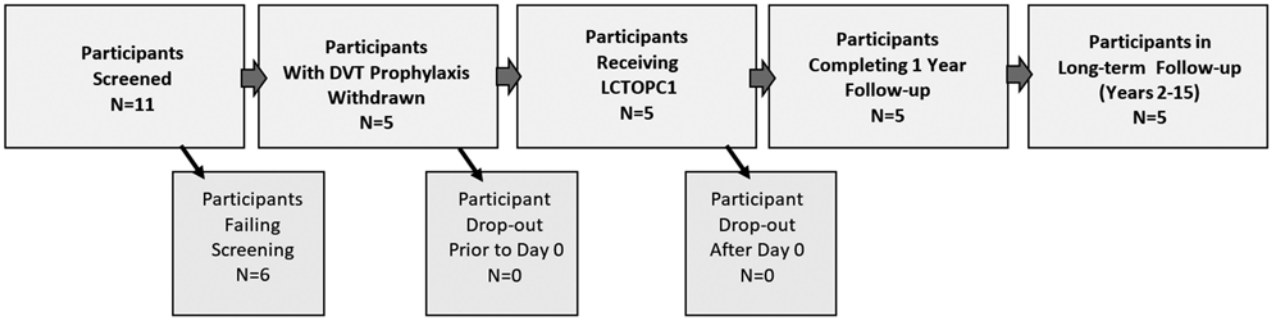

FIG. 2. Participant screening, treatment, and follow-up through the phase 1 clinical trial. DVT = deep vein thrombosis.

winter of 2011. Eleven patients with SCI were screened for enrollment, 6 of whom failed screening: 4 due to MRI artifacts which prohibited adequate spinal cord visualization, 1 based on the ISNCSCI examination (NLI T1), and 1 due to elevated liver enzymes. A total of 5 patients with SCI received LCTOPC1 at three study sites. Figure 2 provides a Consolidated Standard of Reporting Trials (CONSORT) flow diagram.

In this trial, the most common mechanism of injury was motor vehicle related for 4 of 5 patients, with a fall being the cause of injury in 1 patient. Four of 5 participants enrolled were male. The cohort age ranged from 21 to 32 years.

Participant Follow-Up

At the time of this report, all participants had completed their 10th year of follow-up. In agreement with the FDA, the trial was structured to begin with 5 years of in-person evaluation followed in years 6 through 15 with telephone interviews. During the first 5 years of the study, 24 of 25 in-person annual visits were completed. One participant did not participate in the year 5 in-person visit but has participated in scheduled telephone follow-up. From year 6 to the current time, 25 of 25 annual telephone interviews have been completed. All 5 participants have completed 10 years of follow-up interviews.

TABLE 1. AEs and SAEs

AE & SAE System Organ Class Preferred Term	No. of Events	No. (%) of Pts (n = 5)
All events	174	
Nervous system disorders	19	4 (80.0)
Eye disorders	2	2 (40.0)
Gastrointestinal disorders	16	5 (100)
General disorders & administration site conditions	8	3 (60.0)
Immune system disorders	2	2 (40.0)
Infections & infestations	42	5 (100)
Injury, poisoning, & procedural complications	10	5 (100)
Investigations	5	3 (60.0)
Metabolism & nutrition disorders	3	2 (40.0)
Musculoskeletal & connective tissue disorders	33	5 (100)
Psychiatric disorders	8	2 (40.0)
Renal & urinary disorders	7	4 (80.0)
Reproductive system & breast disorders	1	1 (20.0)
Respiratory, thoracic, & mediastinal disorders	2	2 (40.0)
Skin & subcutaneous tissue disorders	11	3 (60.0)
Surgical & medical procedures	2	2 (40.0)
Vascular disorders	3	2 (40.0)

Pt = patient.

Primary Outcome Measure: Evaluation of Safety

All SAEs and AEs (related and unrelated to procedure, cell implant, or immunosuppression) are summarized in Table 1 and described below.

SAEs Related to Procedure, Cell Implant, or Immunosuppression

There were no SAEs related to the procedure, cell implant, or immunosuppression. There were no findings of clinical concern on MRI scans of the full central nervous system through 5 years postinjection in any participant. During long-term telephone follow-up, participants denied having any fever of unknown cause or any changes in sensation in chest, arms, or legs (other than described below), and no participants have been diagnosed with any type of cancer. No participants died during the study. Safety events were monitored by the DSMB and no suspension rules were triggered.

SAEs Unrelated to Procedure, Cell Implant, or Immunosuppression

Three participants have reported 4 SAEs unrelated to the procedure, cell implant, or immunosuppression. These SAEs included urinary tract infection (UTI) and subsequent transitory autonomic dysreflexia in 1 patient, pyelonephritis in 1 patient, and a mood disorder in 1 patient.

AEs Related to Procedure, Cell Implant, or Immunosuppression

AEs Categorized by Grade

Over the course of the trial, 25 AEs were judged by the investigators to be possibly related to LCTOPC1 (grade 1/mild [n = 9], grade 2/moderate [n = 15], and grade 3/severe [n = 1]). The grade 3 AE was described as a burning sensation in the trunk and lower extremities first reported

on day 57 postinjection with grade 1 severity, increasing to grade 3 severity on day 90 postinjection. This neuropathic pain resulted in 3 additional grade 2 severity AEs and was ongoing through year 9 follow-up. Grade 2 AEs included surgical site pain, hypomagnesemia, UTI, vaginal yeast infection, emesis, upper-back pain, shoulder pain, pain with range of motion, and autonomic discomfort during catheterization relieved after treatment with lidocaine. Grade 1 AEs included hypomagnesemia, UTI, fever, headache, nausea, and spasticity.

AEs Categorized by Relation to Procedure, Cell Implant, or Immunosuppression

Nine of the 25 related AEs were deemed possibly related specifically to the injection procedure. Eight of the 9 were grade 1 or 2 in severity and 1 was grade 3. The AEs were predominantly transient postoperative pain, 1 fever, and 1 UTI. There were no AEs attributed to the cell implant. Moreover, the immunosuppression regiment was well tolerated, and all 5 participants completed the regimen per protocol. Sixteen of the 25 AEs were deemed possibly related specifically to the immunosuppression. Seven grade 1 AEs and 9 grade 2 AEs were judged to be possibly related specifically to tacrolimus. These AEs were primarily known common adverse reactions to tacrolimus (nausea/emesis, low magnesium level, infections). Among reported infections, 1 of 7 was a vaginal yeast infection and 6 of 7 infections were in the urinary tract, which is a common complication of SCI.

AEs Unrelated to Procedure, Cell Implant, or Immunosuppression

At year 6, 1 participant reported an increase in frequency and intensity of muscle spasms attributed to functional electrical stimulation cycling. This participant reported resolution of these symptoms during years 7 through 10 and is currently not using any medication for muscle spasms. In year 9, a different individual received outpatient treatment after developing a deep vein thrombosis. In the 10th year of follow-up, a third individual received a baclofen pump and began taking oral medications for migraines and type 2 diabetes.

Secondary Outcome Measure: Neurological Assessment

After discharge from acute inpatient rehabilitation and through the first 5 years postimplantation, participants continued to be evaluated in person according to the schedule shown in Fig. 1 and Supplemental Tables 2 and 3. Of note, between baseline and year 5, participants' annual in-person evaluations included at least 13 ISNCSCI examinations. All participants had an AIS grade of A on enrollment in the trial and all participants have maintained the same impairment grade. The highest and lowest single NLIs enrolled in the study were T3 and T8, respectively. Only the individual with T3 NLI improved to T4 with a sensory ZPP initially at T4 bilaterally noted to improve to T5 on the left and T6 on the right at 1-year follow-up. In total, 3 of 5 participants experienced at least one level of improvement in their ZPP. All participants began and ended the 5 years of in-person ISNCSCI examination with intact upper-extremity motor function with an upper-extremity motor score (UEMS) of 50 out of 50 and an LEMS of 0 out of 50 (Table 2). Over the course of 5 years of in-person follow-up, sensory examination results have not materially changed. Figure 3 provides a diagrammatic representation of the motor and/or sensory function of each patient at baseline and at 5 years post–LCTOPC1 administration.

MRI Findings

No participant exhibited evidence of an enlarging cyst, enlarging mass, spinal cord damage related to the injection procedure, intramedullary hemorrhage, CSF leak, epidural abscess or infection, inflammatory lesions in the spinal cord, CSF flow obstruction, or masses in the ventricular system. No evidence of any adverse neurological changes or adverse changes on MRI was reported during tacrolimus tapering or after tacrolimus discontinuation. MRI results during the long-term follow-up period in patients administered LCTOPC1 cells showed that 80% of patients demonstrated T2 signal changes consistent with the formation of a tissue matrix at the injury site.

Immune Monitoring

LCTOPC1 is an allogeneic cell therapy and is potentially sensitive to rejection by the recipient immune system. As a baseline assessment, HLA class I and class II molecular typing was performed for 10 alleles of the donor LCTOPC1 cells and peripheral blood cells of each of the 5 recipients. The potential development of a cellular immune response to LCTOPC1 was assessed and showed no evidence of T-cell–mediated responses to LCTOPC1 even after cessation of tacrolimus immunosuppression in any of the serum samples of the 5 recipients. In addition, CSF samples obtained through lumbar puncture did not show signs of antibody or T-cell responses to LCTOPC1.

Discussion

In January of 2009, the journal *Nature* reported that LCTOPC1 would enter "the world's first clinical trial of a therapy generated by human embryonic stem cells."[18] At the time, pharmaceutical research in acute SCI was considered a relatively recent development.[19] Although no clinical trial of hESC-derived cell lines had ever been assessed in any context, procedures for other intraparenchymal injections of cellular products (e.g., activated autologous macrophages) into the spinal cord had been evaluated,[6] providing a partial roadmap for LCTOPC1-based studies.

We present the primary and secondary outcome measures of 5 participants who received 2×10^6 allogeneic hESC-derived oligodendrocyte progenitor cells within 7–14 days postinjury. The primary results from the first 10 years of follow-up establish the safety and feasibility of intraparenchymal LCTOPC1 injection. The injection procedure and the low-dose immunosuppression regimen were well tolerated. At the time of this report, all 5 participants who received LCTOPC1 had demonstrated no evidence of neurological deterioration or adverse findings on MRI scans. No unanticipated SAEs related to LCTOPC1 have been reported with 98% follow-up of participants (49 of 50 annual visits) through the first 10 years of the clinical trial.

TABLE 2. ISNCSCI exam results at baseline and years 1 and 5

				Neurological Level					ZPP				
				Sensory		Motor			Sensory		Motor		
Study Visit	TSS	UEMS	LEMS	Rt	Lt	Rt	Lt	NLI	Rt	Lt	Rt	Lt	AIS Grade
Pt 1													
Baseline	ND	ND	0	T6	ND	T6	ND	ND	T7	T7	T6	ND	A
Yr 1	111	50	0	T6	T7	T6	T7	T6	T7	T7	T6	T7	A
Yr 5	111	50	0	T7	T6	T7	T6	T6	T7	T7	T7	T6	A
Pt 2													
Baseline	125	50	0	T8	T8	T8	T8	T8	T9	T9	T8	T8	A
Day 270*	129	50	0	T8	T8	T8	T8	T8	T10	T10	T8	T8	A
Yr 5	122	50	0	T7	T7	T7	T7	T7	T10	T10	T7	T7	A
Pt 3													
Baseline	112	50	0	T6	T6	T6	T6	T6	T8	T8	T6	T6	A
Yr 1	112	50	0	T6	T6	T6	T6	T6	T8	T8	T6	T6	A
Yr 4*	114	50	0	T6	T7	T6	T7	T6	T8	T8	T6	T7	A
Pt 4													
Baseline	121	50	0	T7	T8	T7	T8	T7	T8	T9	T7	T8	A
Yr 1	123	50	0	T7	T8	T7	T8	T7	T9	T10	T7	T8	A
Yr 5	123	50	0	T7	T8	T7	T8	T7	T9	T9	T7	T8	A
Pt 5													
Baseline	82	50	0	T3	T3	T3	T3	T3	T4	T4	T3	T3	A
Yr 1	95	50	0	T4	T4	T4	T4	T4	T6	T5	T4	T4	A
Yr 5	97	50	0	T4	T5	T4	T5	T4	T6	T6	T4	T5	A

ND = unable to determine; TSS = total sensory score.

All 5 patients were AIS grade A at enrollment and there were no conversions to AIS B. The highest single and lowest NLIs enrolled in the study were T3 and T8, respectively. Only the patient with the T3 NLI improved to T4, with a sensory ZPP initially at T4 bilaterally noted to improve to T5 on the left and T6 on the right at 1-year follow-up. In total, 3 of 5 patients experienced at least 1 level of improvement in their ZPP.

* Patients were not able to follow up as directed.

Although this study did not demonstrate significant recovery, no participant exhibited evidence of neurological deterioration on ISNCSCI examinations through 5 years of in-person follow-up or 10 years of self-reported neurological function. It is important to note that potentially subtle changes in neurological function (better or worse) could occur that are not measurable by the ISNCSCI of the participants. In addition, there was a male sex predominance in this study cohort, which may potentially have implications for the generalizability of these results.

This study was not designed to assess efficacy; however, animal studies of LCTOPC1 produced improvements in motor function through mechanisms that appeared to represent remyelination as well as neuroprotection, suppression of inflammation, promotion of axonal regeneration, and/or homeostatic maintenance.[9,20] The proposed mechanism of locomotor function improvement included remyelination as well as expression of neurotrophic factors.[21] The limited signs of functional recovery in various human trials despite promising results in animals may be related to the relative severity of human injuries in comparison to preclinical studies with incomplete contusion, suggesting that subsequent studies with incomplete injuries may demonstrate recovery more similar to that seen in animal models.

Neuropathic pain in response to LCTOPC1 secondary to remyelination or neurotrophic factors was assessed using the International Spinal Cord Injury Pain Basic Data Set and a set of three questions to assess allodynia. Neuropathic at-level pain and below-level pain often have onset during the first several months after SCI, and by 1 year the prevalence of neuropathic pain approaches 60%.[22] The prevalence of pain in this study is consistent with the natural history of neuropathic pain. One participant experienced neuropathic pain reported as a burning sensation in the trunk and lower extremities that was considered possibly related to LCTOPC1, which persisted in long-term follow-up. The pain reported by this participant is consistent with two of the major categories of pain that are common following SCI: neuropathic pain at the level of injury (termed neuropathic at-level pain), and neuropathic pain that occurs diffusely below the level of injury (termed neuropathic below-level pain).[23] Unfortunately for affected individuals, both at-level and below-level neuropathic pain are often severe and persistent for at least 5 years after SCI, despite attempts at pain management.[24] In addition, 40% to 50% of individuals with these types of pain report their pain as severe or excruciating.[25] It is not possible to determine a cause-and-effect relationship between LCTOPC1 and a change in the incidence of long-term neuropathic pain in this small, open-label study.

Serial MRI studies did not demonstrate the formation of ectopic tissue and/or teratomas. In addition to the ab-

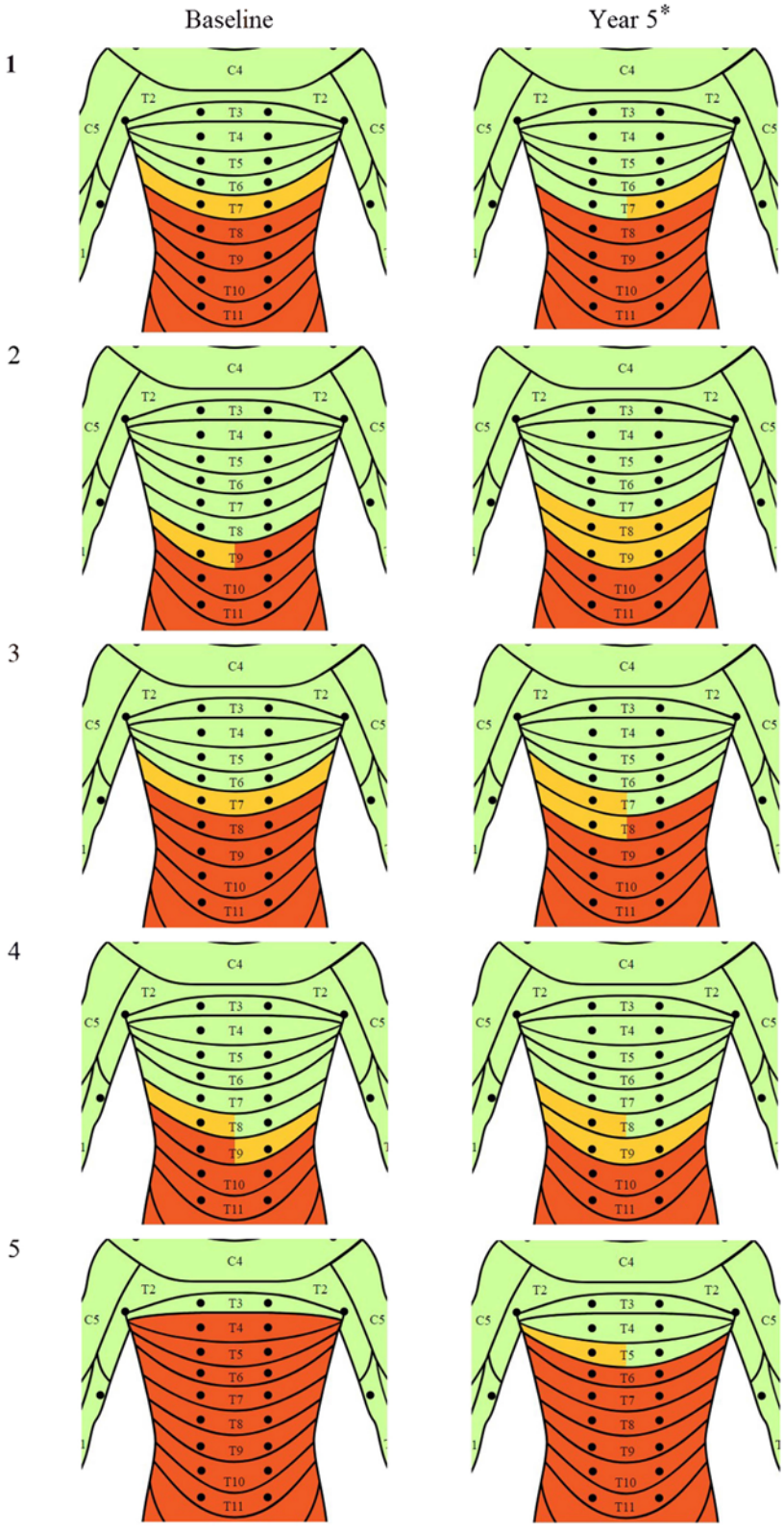

FIG. 3. ISNCSCI pretransplantation (baseline) and at year 5 for each of the 5 study patients. *Green* represents areas with normal motor and/or sensation, *red* represents areas with absent motor and/or sensation, *orange* areas represent sensation that is present but abnormal. *Participant 3 did not participate in year 5 follow-up; year 4 data are presented.

sence of space-occupying lesions, the natural history of chronic SCI MRI studies suggests that cavitary lesions will be identifiable in 58% of individuals who pursue thoracic-level cellular trials.[26] MRI results during the long-term follow-up period for LCTOPC1 were of particular significance because 80% of individuals showed T2 signal changes consistent with the formation of a tissue matrix at the injury site. Although the sample size is limited, these findings suggest that LCTOPC1 cells may have either durable engraftment and/or induced long-term changes which limited cavitation at the injury site.[27]

SCI is a relatively rare condition and the potential population of patients with T3–11 AIS grade A injuries represents less than 20% of acute SCI patients in the United States.[28] Despite the development of a nationwide network of seven treatment sites, the complexities of identification, consent, and implantation required more than 1 year to enroll 5 participants. In November 2011, the initial corporate sponsor, Geron Corporation, halted the trial before reaching the intended 8-participant cohort size, citing difficulty raising capital.[29] The stem cell program was ultimately acquired by Asterias Biotherapeutics, which initiated the Dose Escalation Study in Spinal Cord Injury clinical trial, enrolling individuals with cervical complete and sensory incomplete injuries (NCT02302157).

Conclusions

The LCTOPC1 thoracic SCI clinical trial is one of the longest-running clinical trials in the hESC field. The study provides crucial first-in-human safety data for future hESC-derived therapies. While we cannot exclude the possibility of future AEs, the experience in this trial provides evidence that these treatments can be well tolerated and event free for up to 10 years. In addition, this report supports the willingness of individuals to participate in long-term follow-up as well as setting a standard for corporate sponsors' commitment to data collection beyond their immediate financial interests. Based on the safety profile of LCTOPC1 obtained in this study, a cervical dose escalation trial was initiated (NCT02302157).

Acknowledgments

We thank the participants for enabling the success of the study. In addition, we gratefully acknowledge the infrastructure and support of all the participating institutions. We acknowledge all of the team members who contributed to the trial, especially Don Leslie, MD, and David Apple, MD, at Shepherd Center; James Crew, MD, and Marc Lee, MD, PhD, for serving as co-investigators at Santa Clara Valley Medical Center, as well as members of the team, including Chris Wilder, David Cutler, and Jake Kosarchuk, MD; Zachary Smith, MD, David Chen, MD, and Aruna Ganju, MD, for serving as principal investigators at Northwestern; Scott Anderson, MD, and David Dungan, MD, for providing radiological interpretation of participant MRI scans during the clinical trial; and Jerrod Denham for his leadership in developing the manufacturing and final formulation procedures for LCTOPC1.

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Disclosures

Drs. Jones, Lebkowski, and Wirth were employed by Geron, Inc., at the time of the study.

Author Contributions

Conception and design: all authors. Acquisition of data: all authors. Analysis and interpretation of data: all authors. Drafting the article: all authors. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Fessler. Administrative/technical/material support: Fessler. Study supervision: Fessler.

Supplemental Information

Online-Only Content

Supplemental material is available with the online version of the article.

Supplemental Materials. https://thejns.org/doi/suppl/10.3171/2021.12.SPINE21622.

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