ONLINE ONLY

Supplemental material

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

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

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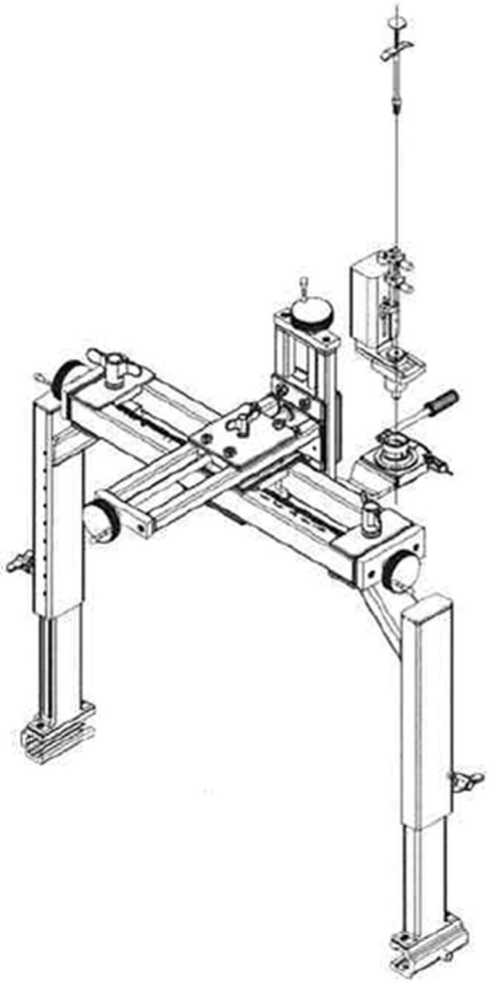

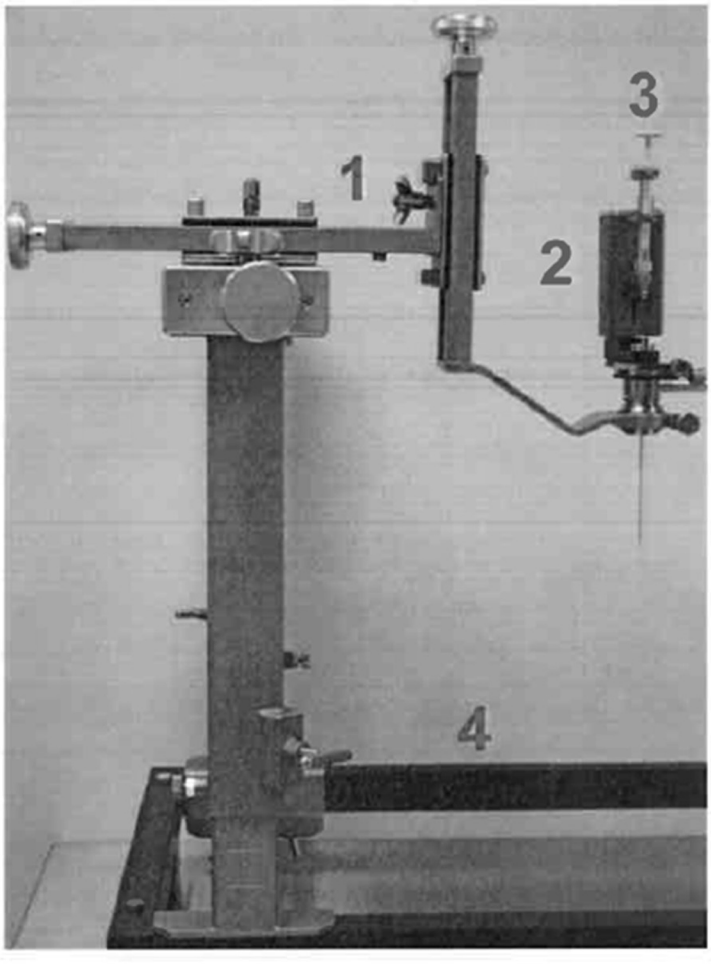

Supplemental Figure 1. The Syringe Positioning Device is composed of three main subassemblies: (1) Support Frame Assembly; (2) Microdrive Assembly; (3) Syringe/Needle Assembly. The Spinal Surgery Top (also referred to as Jackson Table) (4) is not considered to be part of the Syringe Positioning Device. The Syringe Positioning Device (SPD) is composed of an integrated set of components indicated for use in assisting the surgeon with accurate positioning of the needle at a predetermined location within the spinal cord. It is also designed to hold the needle at that location during and up to several minutes after injection of prepared doses of LCTOPC1.

Supplemental Table 1. Inclusion and Exclusion Criteria

Inclusion Criteria
Participants were eligible for the study if all of the following inclusion criteria were met prior to dosing of LCTOPC1: 1. Neurologically complete, traumatic SCI (American Spinal Injury Association Impairment Scale A); spared motor or sensory function < 5 levels below the relevant sensory or motor (right or left) level. 2. Single neurological level from T-3 through T-11. 3. From 18 through 65 years of age at time of injury. 4. Single spinal cord lesion on a post-stabilization MRI scan, with sufficient visualization of the spinal cord for 30 mm above and below the injury epicenter to enable post-injection safety monitoring. 5. Informed consent for this protocol and the long-term follow-up protocol provided and documented (i.e., signed informed consent forms) no later than 11 days following injury. 6. Able to participate in elective surgical procedure to inject LCTOPC1 7 to 14 days following SCI.
Exclusion Criteria
Participants were not eligible for the study if any of the following exclusion criteria were met prior to dosing of LCTOPC1: 1. Spinal cord injury due to penetrating trauma. 2. Traumatic anatomical transection or laceration of the spinal cord based on prior surgery or MRI. 3. Spinal cord lesion with anteroposterior diameter of the spinal cord < 2 mm at point of maximal compression on a midline sagittal image from a post-stabilization MRI. 4. Any concomitant injury that could interfere with the performance, interpretation, or validity of neurological examinations, such as multiple spinal cord lesions, lumbar plexus injury, cauda equina injury, or traumatic brain injury. 5. Any treatment or pre-existing condition that could interfere with the performance, interpretation, or validity of neurological examinations, such as polyneuropathy, focal or multi-focal neuropathy, myelopathy, or radiculopathy. 6. Inability to communicate effectively with neurological examiner such that the validity of patient data could have been compromised. 7. Significant organ damage or systemic disease that would have created an unacceptable risk for surgery or immunosuppression. 8. Concomitant use at baseline of other immunosuppressive agents, such as corticosteroids, that would have created an unacceptable risk for additional immunosuppression with tacrolimus. 9. Need for mechanical support of ventilation (ventilator, continuous positive airway pressure, bi-level positive airway pressure), excluding supplemental oxygen, at baseline. 10. History of any malignancy. 11. Pregnant or nursing women. Female participants of childbearing potential agreed to prevent pregnancy by the use of contraception for 365 days following LCTOPC1 injection; male participants agreed to use contraception to prevent pregnancy in any female partners of childbearing potential for 365 days following LCTOPC1 injection. 12. Positive blood test for antibodies to human immunodeficiency virus types 1 or 2, antibodies to hepatitis B virus core antigen, or antibodies to hepatitis C virus. 13. Panel reactive antibodies (PRA) ≥ 20%; if a site lab reported PRA only for human leukocyte antigen (HLA) Class I or separately for HLA Class I and II, exclusion was based solely on the PRA for HLA Class I. 14. Serum creatinine above the established limit for the normal range at individual study center laboratories at baseline. 15. Liver function tests > 2× the established upper limit for the normal range at individual study center laboratories at baseline. 16. Hematocrit ≤ 27 % at baseline. 17. Positive blood cultures (48-hour culture results at Day -1). 18. Active untreated viral, fungal, or bacterial infection at baseline. 19. Evidence of surgical site infection at intended LCTOPC1 injection site at baseline. 20. Temperature ≥ 38.6° C at 2 time points from Day -1 through Day 0 prior to surgery for LCTOPC1 injection. 21. Body mass index > 35 kg/m2 or weight > 300 pounds. 22. Active participation in another experimental procedure/intervention. 23. Psychoactive substance use disorder (as defined by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) at any time during the 3 months preceding study entry. 24. History of major depression, schizophrenia, paranoia, or other psychotic disorder as defined by Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. 25. Participant who, in the opinion of the investigator, was unlikely to return for all follow-up visits as specified in the protocol. 26. Any condition that, in the judgment of the investigator, would have precluded successful participation in the study.

Supplemental Table 2 Schedule of Events From Screening to Day 90

Visit:	Screen	Day -3	Baseline	Day -1[1]	Day 0	Follow-up[2]								
Procedures Day (window):	**-11 to -3**	**-3**	**-2**	**-1**	**0**	**1**	**1 to 7**	**7 (±1)**	**7 to 30**	**30 (±3)**	**30 to 60**	**60 (±7)**	**60 to 90**	**90 (±7)**
Informed consent[3]	X													
Demographics and medical history	X													
Physical exam	X[4]		X[1,5]			X[5]		X[5]		X[5]		X[5]		X[5]
Vital signs	X		X[1]	X	X	X		X		X		X		X
Neurological exam			X					X		X		X		X
ISNCSCI exam	X		X[1]	X[6]				X		X	X[7]	X	X[7]	X
UAB-IMR			X					X		X		X		X
Pain questionnaire										X				X
Bowel and bladder questionnaire and SCIM										X[8]				
Electrocardiogram	X							X						
MRI	X[9]							X[10]		X[11]		X[10]		X[11]
Pregnancy test, if applicable	X													
HIV, Hep B, Hep C, and panel reactive antibodies	X													
Hematology	X		X[1]			X		X		X		X		X
Blood chemistry panel 1[12]	X		X[1]			X		X		X		X		X
Blood chemistry panel 2[13]							X[13,15]		X[13,15]		X[13,15]	X[16]		
Blood chemistry panel 3[14]									X[14,15]		X[14,15]	X[16]		
48-hour blood cultures		X												
HLA typing			X											
Fasting blood glucose			X							X		X		
Tacrolimus level							X[17]		X[17]		X[18]	X[19]		
Blood for immune response (shipped)			X			X		X		X	X[20]	X	X[20]	X
Blood for xenotransplantation (shipped)			X							X				
Withhold pharmacological DVT prophylaxis				X[21]										
Restart pharmacological DVT prophylaxis						X[22]								
Begin tacrolimus					X[23]									
Taper tacrolimus dose											X[24]			
Discontinue tacrolimus												X		
CSF via LP (10 mL)					X[25,26]							X[26]		
AST-OPCI injection					X									
Concomitant medications	X		X[1]	X	X	X		X		X		X		X
Adverse events	X		X[1]	X	X	X		X		X		X		X

ALT = alanine aminotransferase; AST = aspartate aminotransferase; CSF = cerebrospinal fluid; DVT = deep vein thrombosis; Gd-DTPA = gadolinium diethylenetriamine pentaacetic acid; HIV = human immunodeficiency virus; Hep = hepatitis; HLA = human leukocyte antigen; ISNCSCI = International Standards for Neurological Classification of Spinal Cord Injury; LP = lumbar puncture; MRI = magnetic resonance imaging; SCI = spinal cord injury; SCIM = Spinal Cord Independence Measure; UAB-IMR = Alabama-Birmingham Index of Motor Recovery.

1. If the day of injection was delayed, the following baseline tests and procedures were to be repeated on the new Day -1: brief physical examination, vital signs, ISNCSCI examination, hematology, blood chemistry panel 1, concomitant medications, and adverse events.
2. If a participant's participation terminated between Day 0 and Day 60, all tests and procedures scheduled for the Day 60 visit were collected at the final visit prior to termination from the study, if possible. If a participant's participation terminated between Day 60 and Day 365, all tests and procedures scheduled for the Day 365 visit were collected at the final visit prior to termination from the study, if possible.
3. Informed consent was collected for treatment and long-term follow-up.
4. Complete examination.
5. Brief examination.
6. Rectal examination only to confirm completeness of injury.
7. A single pre-tapering ISNCSCI examination was performed between Day 42 and Day 46. Additional ISNCSCI examinations were performed twice weekly from Days 46 to 60, and weekly from Days 60 to 90. For those participants who exhibited neurological improvement through Day 46 greater than what would have been expected for an individual with a neurologically complete, thoracic SCI, ISNCSCI examinations were performed 3 times per week from Days 46 to 60, and weekly from Days 60 to 90. In participants that remained neurologically complete, these ISNCSCI exams could have been brief, focusing on the zone of partial preservation and a rectal examination. If an examination overlapped with a scheduled ISNCSCI exam at Day 60 or 90, the same ISNCSCI could have been used for both days.
8. SCIM was collected as a questionnaire (versus observation of activities).
9. A screening/baseline MRI was obtained between Day -5 and Day -3, but no earlier than 4 days after SCI. This MRI included the brain, cerebellum, and entire spinal cord, with and without Gd-DTPA contrast. If surgery for AST-OPC1 injection was subsequently delayed for more than 3 days, then a repeat MRI of the T-spine, without contrast, was obtained.
10. MRI included the spinal cord and cerebellum, with and without Gd-DTPA contrast.
11. MRI included the brain in addition to the routine imaging of the spinal cord and cerebellum, with and without Gd-DTPA contrast.
12. Blood chemistry panel 1: serum albumin, alkaline phosphatase, blood urea nitrogen, total bilirubin, chloride, serum creatinine, glucose, potassium, total serum protein, sodium, AST, ALT.
13. Blood chemistry panel 2: serum creatinine, potassium, magnesium, phosphate, and ionized calcium were obtained daily for 1 week after initiation of tacrolimus, twice per week from Day 7 to Day 30, and once per week from Day 31 to Day 60.
14. Blood chemistry panel 3: serum AST, ALT, and total bilirubin were measured 1 week after initiation of tacrolimus and then once per week until Day 60.
15. When overlap with routine study visits occurred (Day 1, 7, 30, or 60), the same blood sample may have been used for both purposes. For example – panel 1 consisted of serum albumin, alkaline phosphatase, blood urea nitrogen, total bilirubin, chloride, serum creatinine, glucose, potassium, total serum protein, sodium, AST, ALT. Therefore, if panel 1 overlapped with panel 2, the following tests should have been added: serum magnesium, phosphate, and ionized calcium. If panel 1 overlapped with panel 3, no additional tests needed to be ordered.
16. Serum creatinine, potassium, magnesium, phosphate, ionized calcium, AST, ALT, and total bilirubin were obtained on Day 60, regardless of the time elapsed since the immediately preceding chemistry panel was obtained.
17. Whole blood levels (if given intravenously) or trough levels (if given orally) of tacrolimus were measured within 3 days after initiation of treatment and twice per week thereafter until Day 30. If tacrolimus administration was changed from intravenous to oral, or if the dosage was adjusted, a trough blood level was obtained within 3 days, after which blood level monitoring twice per week resumed through Day 30.

18. Whole blood trough levels of tacrolimus were measured once per week from Day 30 to Day 60. If the dosage was adjusted during this time (other than scheduled tapering from Days 46-60), a trough blood level was obtained within 3 days, after which blood level monitoring once per week resumed through Day 60.
19. Tacrolimus blood level and all serum samples were obtained on Day 60, regardless of the time elapsed since the previous blood collection.
20. Additional blood samples for immune response monitoring were obtained weekly from Days 46 to 60 and 60 to 90. When overlap with the Day 60 or 90 visit occurred, the same blood sample could have been used for both purposes.
21. Pharmacological DVT prophylaxis may have been stopped on Day -1 in preparation for surgery, depending on the prophylaxis used and standards of care at the clinical site.
22. Pharmacological DVT prophylaxis was re-started following the procedure to implant AST-OPC1, depending on the prophylaxis used and standards of care at the clinical site.
23. Began 6 to 12 hours post-injection of AST-OPC1.
24. At Day 46, the tacrolimus dose was decreased by 50% (rounded to the nearest 0.5 mg, since this was the smallest capsule size available). At 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 0.5 mg once per day until tacrolimus was discontinued.
25. CSF collection on Day 0 occurred via LP after anesthesia but prior to surgical incision.
26. The volume required at individual study sites for the following tests were sent to the hospital laboratory: white blood cell count, glucose, total protein, oligoclonal banding, myelin basic protein, and immunoglobulin G index. The remainder of the sample was processed and sent to an outside laboratory.

Supplemental Table 3. Schedule of Events from Day 120 to Day 365

Procedures	Follow-up			Final Visit[1]
Day (window):	120 (±7)	180 (±14)	270 (±14)	365 (±14)
Informed consent for companion long-term, follow-up study				X
Physical examination	X[2]	X[2]	X[2]	X[3]
Vital signs	X	X	X	X
Neurological examination	X	X	X	X
ISNCSCI examination	X	X	X	X
UAB-IMR	X	X	X	X
Pain questionnaire		X		X
Bowel and bladder questionnaire		X		X
SCIM		X[4]		X[4]
MRI	X[5]	X[6]	X[5]	X[6]
Hematology	X	X	X	X
Blood chemistry – panel 1[7]	X	X	X	X
Blood samples for immune response (shipped)	X	X	X	X
Blood samples for xenotransplantation (shipped)				X
Concomitant medications	X	X	X	X
Adverse events	X	X	X	X

ALT = alanine aminotransferase; AST = aspartate aminotransferase; Gd-DTPA = gadolinium diethylenetriamine penta-acetic acid; ISNCSCI = International Standards for Neurological Classification of Spinal Cord Injury; MRI = magnetic resonance imaging; SCIM = Spinal Cord Independence Measure; UAB-IMR = Alabama-Birmingham Index of Motor Recovery.

1. If a participant's participation terminated between Day 60 and Day 365, all tests and procedures scheduled for the Day 365 visit were collected at the final visit prior to termination from the study, if possible.
2. Brief examination.
3. Complete examination.
4. SCIM was collected as a questionnaire (versus observation of activities).
5. MRI included the spinal cord and cerebellum, with and without Gd-DTPA contrast.
6. MRI included the brain in addition to the routine imaging of the spinal cord and cerebellum, with and without Gd-DTPA contrast.
7. Serum albumin, alkaline phosphatase, blood urea nitrogen, total bilirubin, chloride, serum creatinine, glucose, potassium, total serum protein, sodium, AST, and ALT.

Supplemental Table 4. Description of SAEs

Participant ID	Event	LCTOPC1 Dose Date	Start Date / Resolution Date	AE Serious	AE Severity	Outcome	Relatedness to: LCTOPC1, surgical delivery of LCTOPC1, or immunosuppression
1101	Pyelonephritis	7-May-11	08DEC2011/ 20-Dec-11	Yes	Grade 2	Hospitalized, Resolved	Unrelated
1204	Urinary Tract Infection	16-Nov-11	06OCT2012/ 22-Oct-12	Yes	Grade 3	Hospitalized, Resolved	Unrelated
1002	Psychiatric disorder (Mood Disorder)	6-Oct-10	04MAR2015/ 6-Mar-15	Yes	Grade 3	Resolved with Sequelae	Unrelated
1204	Psychiatric disorder (Mood Disorder) (Increased autonomic dysreflexia)	16-Nov-11	05NOV2013/ 7-Nov-13	Yes	Grade 3	Resolved without Sequelae	Unrelated

Supplemental Document 1

Asterias GRNOPC1

CP35A008 Long Term Follow-up Study
Yearly Telephone Visit: ____

(± 30 Days)

Subject ID #: _____ Subject Initials: _____ Telephone Visit Date: _____ /_____ /_____
DD MMM YYYY

Study Investigator: Please ask the study subject the following questions and document their responses to each question - Yes or No. For Yes responses please ask additional follow up questions as needed to obtain the information necessary to make a decision whether to schedule the subject for an in person visit.

1. Have you experienced any changes in your neurological condition during the past 12 months?
   a. Voluntary muscle movement in your chest, arms, or legs? Yes ____ No ____
      i. If Yes, please describe the changes: _____

      _____

   b. Feeling (sensation) in your chest, arms, or legs? Yes ____ No ____
      i. If Yes, please describe the changes: _____

      _____

   c. Any other neurological changes? Yes ____ No ____
      i. If Yes, please describe the changes: _____

      _____

2. If Yes, to questions 1 a – c, was the change documented during a visit with your primary care or treating physician? Yes ____ No ____

   a. If Yes, what assessments/tests were done to confirm and document the above changes? (ISNCSCI Exam/Neurological assessment/MRI and/or CT scan of your level of injury)? _____

      _____

   b. If an MRI and/or CT were done, was there anything wrong with the MRI or CT? _____

      _____

   c. Can a copy of the report and images be requested from your physician and provided to your Asterias study physician for your study records and comparison? Yes ____ No ____

CP35A008 Long Term Follow-up Study
Yearly Telephone Visit: ____
(± 30 Days)

3. Have you experienced any fever of unknown cause over the past 12 months? Yes ____ No ____

   a. If Yes, what were the start and stop dates? ____ /____ /____ to ____ /____ /____

   b. What treatments/medications did you receive or take during that time? _____

   _____

4. Have you been diagnosed with any type(s) of cancer in the past 12 months? Yes ____ No ____

   a. If Yes, what type of cancer and where is it located? _____

   b. When were you diagnosed: ____ /____ /____

5. Have you been admitted to the hospital, for one day or more over the past 12 months?

   Yes ____ No ____ If Yes, what was the admission date? ____ /____ /____

   a. If YES, what was the reason or serious adverse event experienced? _____

   b. For the Investigator filling out this form; if Yes to #5 - is the SAE possibly related to the GRNOPC1 cells? Yes ____ No ____

   c. If Yes to 5b, an SAE report must be completed and this event reported to Asterias. Was the study SAE Form completed? Yes ____ No ____

6. Have you had any new medications prescribed to you and taken for longer than 30 days in the past 12 months? Yes ____ No ____

   If Yes, what medication(s) were prescribed and the reason for prescription (list below):

   _____

   _____

7. Have you or your female partner become pregnant or delivered a baby over the past 12 months?

   Yes ____ No ____ If Yes, which: ___ Pregnant ___ Delivered Baby ___ Miscarriage

   a. If pregnancy is ongoing when is the expected delivery date? ____ /____ /____

   b. If delivered baby, was the baby delivered healthy? Yes ____ No ____

      i. If No, are you willing to provide additional information on the baby? Yes ____ No ____

      If Yes to 7.b.i; Investigator please determine what additional information or medical records (if necessary) about the baby's health are needed.

CP35A008 Long Term Follow-up Study
Yearly Telephone Visit: ____
(± 30 Days)

8. Are you still agreeable to being contacted yearly for the study telephone follow-up calls?

   Yes ____ No ____

Based on the subject responses do any of the following apply?

9. I need to obtain additional information based on the response(s) to questions above:

   Yes ____ No ____ Follow up information is needed for question(s): _____

   a. I need to obtain this information from the subject: Yes ____ No ____

   b. Discuss the above responses with the subjects treating physician: Yes ____ No ____

   c. Request / Review the subject's recent medical records to further evaluate the above responses:

      Yes ____ No ____

10. Based on the subject responses to Questions 1 – 7 and the follow up information obtained from the subject; speaking with their treating physician or reviewing their recent medical records the subject should be scheduled for an in person clinic visit within 30 days: Yes ____ No ____

    a. If Yes, has the subject agreed to return to the clinic for an in person visit? Yes ____ No ____

       If Yes, what is the date of the Unscheduled visit? ____ /____ /____

11. If no "in person clinic visit" is needed the subject will be contacted in one year +/- 30 Days for their yearly telephone follow-up visit. Yes ____ No ____

12. Have you or your study coordinator confirmed the subjects contact information is current and accurate (home address / email / cell and/or home phone numbers)? Yes ____ No ____

_____
Investigator Printed Name

_____ _____ / _____ / _____
Investigator Signature DD MMM YYYY