CLINICAL STUDY PROTOCOL AST-OPC1-01

A Phase 1/2a Dose Escalation Study of AST-OPC1 in Subjects with Subacute Cervical Spinal Cord Injury

Investigational Product:	AST-OPC1 (oligodendrocyte progenitor cells)
Protocol Number:	AST-OPC1-01
Development Phase:	Phase 1/2a
IND No:	BB-IND 13673
Sponsor:	Asterias Biotherapeutics, Inc. 6300 Dumbarton Circle Fremont, CA 94555
Medical Monitor	Susy Chen, MD Senior Medical Director Phone: 510-456-3851 or 650-400-3924 Email: schen@asteriasbio.com
Original Protocol Date:	21 August 2014
Amendment 1:	20 February 2015
Amendment 2:	03 May 2016
Amendment 3:	07 July 2017

SIGNATURE PAGE

STUDY TITLE: A Phase 1/2a Dose Escalation Study of AST-OPC1 in Subjects with Subacute Cervical Spinal Cord Injury

By signing this protocol acceptance page, I confirm I have read, understand, and agree to conduct the study in accordance with the current protocol.

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Principal Investigator:

_____
Print Name

_____ _____
Signature Date

PROTOCOL SYNOPSIS

Name of Sponsor/Company:	**Asterias Biotherapeutics, Inc.**
Title of Study:	**A Phase 1/2a Dose Escalation Study of AST-OPC1 in Subjects with Subacute Cervical Spinal Cord Injury**
Study center(s):	Multi-Center
Study duration (years):	Approximately 4 years for this protocol with an additional 14 years under a separate long term follow-up companion protocol
Objectives	**Primary:** • The primary objective of this study is to evaluate the safety of 3 sequential escalating doses of AST-OPC1 administered at a single time-point between 21 and 42 days post-injury, inclusive, to subjects with subacute cervical spinal cord injuries (SCI). **Secondary:** • The secondary objective is to evaluate changes in neurological function following administration of AST-OPC1.
Endpoints	**Primary:** • The primary endpoint is safety, as measured by the frequency and severity of adverse events within 1 year (365 days) of AST-OPC1 injection that are related to AST-OPC1, the injection procedure used to administer AST-OPC1, and/or the concomitant immunosuppression administered. **Secondary:** • The secondary endpoint is neurological function as measured by upper extremity motor scores and motor level on International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) examinations at 30, 60, 90, 180, 270, and 365 days after injection of AST-OPC1. **Exploratory**: Potential improvements in arm/hand function, self-care ability, and overall volitional ability will be evaluated by changes from baseline on the following endpoints: • Graded and Redefined Assessment of Strength, Sensibility, and Prehension (GRASSP) is a quantitative measure of upper limb function in cervical SCI • Spinal Cord Independence Measure (SCIM), particularly the SCIM self-care subscale, assesses the recovery of the ability of individuals with SCI to perform basic everyday tasks • Spinal Cord Ability Ruler, which is a quantitative linear measure of volitional performance inclusive of all subjects in the study population. The Spinal Cord Ability Ruler is derived from the ISNCSCI and SCIM assessments.

<table>
<tr><td>Name of Sponsor/Company:</td><td colspan="5">Asterias Biotherapeutics, Inc.</td></tr>
<tr><td>Title of Study:</td><td colspan="5">A Phase 1/2a Dose Escalation Study of AST-OPC1 in Subjects with Subacute Cervical Spinal Cord Injury</td></tr>
<tr><td>Study Design:</td><td colspan="5">An open label, dose escalation, cross-sequential, multi-center study</td></tr>
<tr><td>Investigational product, dosage and mode of administration:</td><td colspan="5">• AST-OPC1 - Cryopreserved human embryonic stem cell-derived oligodendrocyte progenitor cells (OPCs), supplied to clinical sites in sterile, single-dose, single-use, 2.0 mL Corning cryovials. The cryopreserved drug product will be thawed, washed, re-suspended in Isolyte®S and 1% human serum albumin (HSA) and loaded into the injection syringe at the clinical site.
• Doses to be studied: one administration of 2 x 10^6, 1 x 10^7, or 2 x 10^7 AST-OPC1 cells suspended in the injection medium as shown in the dose table below.
• AST-OPC1 must be injected directly into the spinal cord using the Syringe Positioning Device (SPD), which includes the syringe and needle.
• Subjects who receive AST-OPC1 will also receive a short course of low-dose tacrolimus to prevent immunological rejection. Tacrolimus will be initiated 6-12 hours after AST-OPC1 injection, continued for 46 days, tapered from Day 46 to Day 60, and discontinued at Day 60.</td></tr>
<tr><td rowspan="4">Doses to be Studied</td><td>Dose (# of cells)</td><td>Concentration (cells/µL)</td><td>Volume per injection</td><td>Number of Injections</td><td>Total Volume Administered</td></tr>
<tr><td>2 x 10^6</td><td>4 x 10^4</td><td>50 µL</td><td>1</td><td>50 µL</td></tr>
<tr><td>1 x 10^7</td><td>2 x 10^5</td><td>50 µL</td><td>1</td><td>50 µL</td></tr>
<tr><td>2 x 10^7</td><td>2 x 10^5</td><td>50 µL</td><td>2</td><td>100 µL</td></tr>
<tr><td>Study Population:</td><td colspan="5">Number of Subjects Planned: Up to 35 subjects across five cohorts will be enrolled and receive AST-OPC1 as shown in the table below. Enrollment within each cohort will be staggered, and advancement to Cohorts 2-5 will follow the rules described in the section immediately below the table.

<table>
<tr><td>Cohort</td><td>ASIA Impairment Scale (AIS) Grade</td><td>AST-OPC1 Dose (# of cells)</td><td>Number of Subjects</td></tr>
<tr><td>1</td><td>A</td><td>2 x 10^6</td><td>3</td></tr>
<tr><td>2</td><td>A</td><td>1 x 10^7</td><td>5-8</td></tr>
<tr><td>3</td><td>A</td><td>2 x 10^7</td><td>5-8</td></tr>
<tr><td>4</td><td>B</td><td>1 x 10^7</td><td>5-8</td></tr>
<tr><td>5</td><td>B</td><td>2 x 10^7</td><td>5-8</td></tr>
</table>
Staggered Enrollment within each cohort: Enrollment in Cohort 1 will be staggered such that there are at least 10 days between administration of</td></tr>
</table>

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	AST-OPC1 to each subject. Enrollment within Cohorts 2-5 will be staggered after the initial subject in each cohort is dosed, with at least 8 days between administration of AST-OPC1 to the first and second subjects. If the first two subjects are successfully dosed with no serious adverse events (SAEs) that would trigger study suspension (per the study Suspension Rules), then the remaining subjects in that cohort may be dosed without further staggering. An additional staggering rule for Cohorts 2-5 is that a minimum of 14 days must elapse between the dosing of any two consecutive subjects who both have a C4 neurological level of injury (NLI). This 14-day stagger for consecutive C4 NLI subjects applies throughout each of these cohorts and also would supersede the 8-day stagger noted above if the first two subjects in any cohort both have a C4 NLI. **Rules for Advancement to Cohorts 2-5:** • Enrollment in Cohort 2 may commence after all three subjects in Cohort 1 have completed 30 days of follow up after AST-OPC1 administration AND the safety data for Cohort 1 have been reviewed by the Data Monitoring Committee (DMC). • Enrollment in Cohort 3 may commence after at least five subjects in Cohort 2 have completed 30 days of follow up after AST-OPC1 administration AND the safety data from Cohorts 1 and 2 have been reviewed by the DMC. • Enrollment in Cohort 4 may commence after at least two subjects in Cohort 2 have completed 30 days of follow up after AST-OPC1 administration AND if there are no SAEs that would trigger study suspension. • Enrollment in Cohort 5 may commence after at least two subjects in Cohort 3 AND at least five subjects in Cohort 4 have completed 30 days of follow up after AST-OPC1 administration AND if there are no SAEs that would trigger study suspension. For the planned DMC reviews noted above, the DMC will recommend whether enrollment of the next dose cohort can commence or whether additional follow up of the current cohort is needed prior to commencing enrollment in the next cohort. **Maximum Number of Subjects**: A maximum of 35 subjects will receive AST-OPC1.
Inclusion Criteria:	• Sensorimotor complete, traumatic SCI (ASIA Impairment Scale A) for Cohorts 1, 2, and 3 • Sensorimotor incomplete, traumatic SCI (ASIA Impairment Scale B)

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	for Cohorts 4 and 5 • ISNCSCI Neurological Level of Injury (NLI) from C-5 to C-7 or a C4 NLI with an upper extremity motor score (UEMS) ≥ 1 • From 18 through 69 years of age at time of consent • Single spinal cord lesion on a post-stabilization magnetic resonance imaging (MRI) scan, with sufficient visualization of the spinal cord injury epicenter and lesion margins to enable post-injection safety monitoring • Informed consent for this protocol and the companion long term follow-up protocol must be provided and documented (i.e., signed informed consent forms) no later than 37 days following injury • Able to participate in an elective surgical procedure to inject AST-OPC1 21-42 days following SCI
Exclusion Criteria:	• SCI due to penetrating trauma • Traumatic anatomical transection or laceration of the spinal cord based on prior surgery or MRI • Spinal cord lesion with anteroposterior (AP) diameter of the spinal cord < 2 mm at point of maximal compression on a midline sagittal image from a post-stabilization MRI • Any concomitant injury that interferes with the performance, interpretation or validity of neurological examinations, such as multiple spinal cord lesions, brachial/lumbar plexus injury, cauda equina injury or traumatic brain injury • Any treatment or pre-existing condition that interferes with the performance, interpretation or validity of neurological examinations, such as polyneuropathy, focal or multi-focal neuropathy, myelopathy or radiculopathy • Inability to communicate effectively with neurological examiner such that the validity of patient data could be compromised • Significant organ damage or systemic disease that would create an unacceptable risk for surgery or immunosuppression • Acute, symptomatic pulmonary embolism that would create an unacceptable risk for surgery • Concomitant use at baseline of other immunosuppressive agents, such as corticosteroids, that would create an unacceptable risk for additional immunosuppression with tacrolimus • Need for mechanical support of ventilation (ventilator, continuous positive airway pressure [CPAP], bi-level positive airway pressure [BiPAP]), excluding supplemental oxygen, at baseline • History of any malignancy (except non-melanoma skin cancers) • Pregnant or nursing women. Female subjects of child bearing potential must agree to prevent pregnancy by the use of contraception for 1 year following AST-OPC1 injection; male subjects must agree to use contraception to prevent pregnancy in

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	any female partners of child bearing potential for 1 year following AST-OPC1 injection. • Positive blood test for antibodies to human immunodeficiency virus (HIV) types 1 or 2, antibodies to hepatitis B virus (HBV) core antigen or antibodies to hepatitis C virus (HCV) • Panel reactive antibodies (PRA) ≥ 20%; if a site lab reports PRA only for HLA Class I or separately for HLA Class I and II, exclusion will be based solely on the PRA for HLA Class I • Serum creatinine >1.5X above the established limit for the normal range at individual study center laboratories at baseline • Blood levels of aspartate aminotransferase (AST) or alanine aminotransferase (ALT) > 3X the established upper limit for the normal range at individual study center laboratories at baseline • Hematocrit below the study center lower limit for elective surgery at baseline • Positive blood cultures (48 hour culture results at Day -1) • Active untreated viral, fungal or bacterial infection at baseline • Evidence of surgical site infection at intended AST-OPC1 injection site at baseline • Temperature ≥ 38.6°C at two time points from Day -1 through Injection Day prior to surgery for AST-OPC1 injection • Body mass index (BMI) > 35 or weight > 300 lbs. • Active participation in another experimental procedure/intervention • Psychoactive substance use disorder (as defined by DSM-5) at any time during the 3 months preceding study entry • History of untreated or poorly controlled major depression, schizophrenia, paranoia, or other psychotic disorder as defined by DSM-5 • A subject who, in the opinion of the investigator, is unlikely to return for all follow-up visits as specified in the protocol • Any condition that, in the judgment of the investigator, would preclude successful participation in the study
Duration of treatment:	Subjects will receive a single administration of AST-OPC1, which must be given 21-42 days following SCI. Subjects will be followed for 1 year post-injection of AST-OPC1 under this protocol and for an additional 14 years under a companion long term follow-up protocol. Subjects must agree to and sign the informed consent for long-term follow-up in order to participate in this study. Subjects who have pharmacological deep vein thrombosis (DVT) prophylaxis withdrawn in preparation for surgery, but do not receive AST-OPC1, will be followed for safety for 30 days.

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Study assessments:	**Safety:** Measurements to assess safety will include: physical exam, vital signs, electrocardiogram (ECG), neurological exam, ISNCSCI exam, MRI, pain questionnaire, concomitant medications, adverse events, laboratory tests for hematology, blood chemistry, and immunosuppression safety monitoring. **Efficacy:** Measurements to assess efficacy will include: ISNCSCI examinations at baseline and at 30, 60, 90, 180, 270, and 365 days post-injection of AST-OPC1. **Exploratory:** Exploratory assessments will be performed to further evaluate upper and lower extremity motor function (e.g, GRASSP), bowel and bladder function, functional and volitional ability (e.g. SCIM), pain, and donor-specific immune responsiveness to AST-OPC1.
Sample size:	The sample size is based on the study design considerations for a Phase 1/2a study. Power calculations have not been performed, as the primary objective is to collect safety and tolerability information on a limited number of subjects per dose level and AIS Grade, and an effect size cannot yet be estimated.
Statistical methods	**Populations:** **All Subjects Receiving AST-OPC1:** This population is defined as all subjects who receive AST-OPC1. Primary safety and efficacy analysis will be on this population. **All Subjects:** This population is defined as all subjects who receive AST-OPC1 plus those who have pharmacological DVT prophylaxis withdrawn but do not receive AST-OPC1. Safety analyses will be presented for this population through study Day 30. **Analyses:** The analyses for safety will be done using descriptive statistics focused on the incidence, severity and relatedness of adverse events from AST-OPC1, the injection procedure to administer the product and concomitant immunosuppression. Efficacy will be analyzed by change in motor level and motor scores based on ISNCSCI examinations, as well as by other exploratory assessments of arm/hand function, self-care ability, and overall volitional performance.

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SUMMARY OF AMENDMENTS

Amendment 3: 07 July 2017

Rationale for changes: The primary changes in this amendment are: 1) expansion of the inclusion criteria to patients with a C4 neurological level of injury (NLI) if their upper extremity motor score (UEMS) is ≥ 1, and 2) changing the dosing window to 21-42 days post-SCI. The rationale for each of these protocol changes is described below. This amendment also includes several minor revisions that are summarized in the table immediately following this rationale section.

Rationale for including patients with a C4 neurological level of injury (NLI)

The expansion of the inclusion criteria to patients with a C4 NLI is based upon three primary factors: i) C4 is the second most common NLI following traumatic SCI, ii) patients with a C4 NLI have an extremely high unmet medical need due to the complete loss of functional movement in all four limbs, and iii) the safety of both AST-OPC1 and the injection procedure to date have been very favorable in subjects with a C5-C7 NLI. In addition, two subjects with a C4 NLI have already been dosed in this study under pre-approved protocol deviations after concurrence was obtained from both FDA and the clinical site IRB. There have been no intraoperative complications or SAEs related to the injection procedure for any subject in the study, including these two C4 subjects. The first of these two subjects received 10 million AST-OPC1 cells in Cohort 2 (AIS-A) and has completed nine months of follow up. This subject has converted to AIS-C, has improved two motor levels on the left side and one motor level on the right side, and has gained 11 points on the upper extremity motor score (UEMS) relative to baseline. The second subject with a C4 NLI was dosed very recently.

A key safety consideration for patients with a C4 NLI is that an ascending neurological level (i.e., to C3 or higher) could result in a loss of one's ability to breathe without the assistance of a ventilator. To date, a total of 17 subjects have received AST-OPC1 in this study, and no subject has had an ascending NLI following AST-OPC1 administration. In addition, of the 12 subjects who have reached the six-month follow up visit, 11 have improved at least one motor level on at least one side relative to baseline and 1 has a stable motor level relative to baseline. In order to provide an additional margin of safety, patients with a C4 NLI must have an UEMS ≥ 1 in order to be eligible for this study and a staggering rule has been added for Cohorts 2-5 that requires a 14-day stagger between the dosing of any two consecutive subjects who have a C4 NLI.

Rationale for changing the dosing window to 21-42 days post-SCI

The current dosing window of 14-30 days was selected to avoid the early hemorrhage and inflammation that occurs following SCI, as well as the scar tissue formation that occurs in the chronic phase of SCI. This window was also based on the preclinical data available prior to the initiation of this clinical study. However, Asterias recently completed a new preclinical study to further evaluate the appropriate dosing window for AST-OPC1, and the findings from this study suggest that the optimal dosing window in human subjects may extend to at least 60 days post-SCI (see Investigator's Brochure for details).

Asterias reviewed these new preclinical data with the current study investigators and several SCI experts to determine whether the dosing window should be adjusted. Consideration was also given to the planned inclusion of patients with a C4 NLI. Based on these reviews, it was determined that a dosing window of 21-42 days post-SCI would still be squarely within the subacute period but would also allow more time for patients to be medically stable prior to undergoing elective surgery for AST-OPC1 administration.

Amendment 2: 03 May 2016

The primary changes in this amendment were: 1) reduction of the staggering within each cohort to 8 days only between the first two subjects, 2) addition of two cohorts of subjects who are AIS-B, and 3) expansion of the acceptable age range to 18-69 years old at the time of SCI. This amendment also included several administrative changes and minor revisions to the study endpoints, inclusion/exclusion criteria, and schedule of assessments.

Amendment 1: 17 February 2015

This amendment contained a few minor administrative updates, corrected several minor typographical errors in the original protocol, and eliminated the UAB-IMR assessments. The UAB-IMR was eliminated because the clinical investigators and several expert SCI consultants noted that any clinically meaningful return of motor function in the lower extremities would be observed on both the ISNCSCI exam and the UAB-IMR exam. Thus, the latter exam was determined to be redundant for this clinical study.

Summary of changes in Amendment 3:

No.	Change	Section(s)
1	**Study Objectives:** Updated to reflect that the dosing window is being changed from 14-30 days post-SCI to 21-42 days post-SCI	Synopsis, Sections 2, 3.1
2	**Study Population:** Added an additional staggering rule for Cohorts 2-5 that requires a 14-day stagger between the dosing of any two consecutive subjects who have a C4 neurological level of injury (NLI)	Synopsis, Sections 3.1.1, 3.1.2
3	**Inclusion Criteria:** Expanded to include patients with a C4 neurological level of injury (NLI) if their upper extremity motor score (UEMS) is ≥ 1	Synopsis, Section 4.1
4	**Inclusion Criteria:** Changed the age requirement to apply at the time of consent rather than at the time of injury	Synopsis, Section 4.1
5	**Inclusion Criteria:** Changed the requirement for obtaining informed consent within 25 days post-SCI to within 37 days post-SCI to reflect the new dosing window	Synopsis, Section 4.1
6	**Inclusion Criteria:** Changed the requirement for being able to participate in an elective surgical procedure to inject AST-OPC1 to 21-42 days post-SCI to reflect the new dosing window	Synopsis, Section 4.1
7	**Exclusion Criteria:** Added an exclusion criterion for acute, symptomatic pulmonary embolism that would create an unacceptable risk for surgery based on a recommendation from the Data Monitoring Committee and after consultation with the lead neurosurgeon for the study	Synopsis, Section 4.2
8	**Duration of Treatment:** Updated to reflect the new dosing window	Synopsis
9	**Rationale for AST-OPC1 Administration in Subacute SCI:** Updated to reflect the new dosing window, which is supported by new preclinical data that is provided in the accompanying revision to the Investigator's Brochure	Section 1.3.3
10	**Complications of Spinal Cord Injury:** Added a section that lists these complications to clarify which adverse events would be expected due to the fact	Section 1.4.1

	that all subjects suffered an SCI prior to study entry	
11	**Surgical Injection Procedures:** Updated to note that one of the risks is hemorrhage and/or fluid collection in tissues between the incision site and the spinal cord	Section 1.4.2
12	**AST-OPC1 Administration:** Added recommendations for placement of an arterial line in all subjects and for intraoperative monitoring of somatosensory evoked potentials in AIS-B subjects based upon consultation with several study investigators and surgeons	Section 6.4
13	**Tests and Observations:** Deleted testing for ionized calcium based on the recommendation of a clinical expert and because several site labs were unable to perform this test, which resulted in several protocol deviations. In addition, there have been no clinically significant abnormalities of ionized calcium in any subject to date.	Section 7 (all subsections in which blood chemistry testing is performed)
14	**Tests and Observations:** Deleted the GRASSP test from post-injection Day 30 and added the GRASSP test to post-injection Day 90 based on the advice of our expert consultant for GRASSP	Sections 7.7, 7.10; Table 7-1
15	**Tests and Observations:** Deleted the fasting blood glucose test at post-injection Day 60 because subjects receive extremely low doses of tacrolimus during the Day 46-60 taper period and because there have been no clinically significant abnormalities for this test in any subject to date	Section 7.9; Table 7-1
16	**Efficacy Analyses:** Corrected two typographical errors	Section 9.3.2

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or term	Definition
AIS	ASIA Impairment Scale
ALT	alanine aminotransferase
ASIA	American Spinal Injury Association
AST	aspartate aminotransferase
AST-OPC1	investigational product (formerly GRNOPC1)
BBB	Basso, Beattie, and Bresnahan behavior locomotor score
BUN	blood urea nitrogen
CFR	Code of Federal Regulations
CNS	central nervous system
CSF	cerebral spinal fluid
DLT	dose-limiting toxicity
DMC	Data Monitoring Committee
DTPA	diethylenetriamine pentaacetic acid
ECG	electrocardiogram
eCRF	electronic case report form
EDTA	ethylenediaminetetraacetic acid
ESC	embryonic stem cells
FDA	Food and Drug Administration
GCP	Good Clinical Practices
GRASSP	Graded Redefined Assessment of Strength, Sensibility and Prehension
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HSA	human serum albumin
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISNCSCI	International Standards for Neurologic Classification of Spinal Cord Injury
MCB	master cell bank
MedDRA	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
NLI	Neurological Level of Injury
OPC	oligodendrocyte progenitor cell(s)

Abbreviation or term	Definition
PPCM	posttraumatic cystic myelopathy
SCI	spinal cord injury
SCIM	Spinal Cord Independence Measure
SCOPE	Spinal Cord Outcomes Partnership Endeavor
uhESC	undifferentiated human ESC

1 INTRODUCTION

1.1 Spinal Cord Injury

Spinal cord injury (SCI) is a devastating and currently untreatable condition, aside from symptomatic treatments for some of the resulting complications. SCI results in complete or partial loss of motor, sensory and autonomic function. The morbidities associated with SCI are both profound and life-changing and may include pain, bowel and urogenital dysfunction, urinary tract infections, hemorrhoids, pressure ulcers, deep vein thrombosis, pulmonary embolism, respiratory dysfunction and infections, autonomic dysreflexia, spasticity, heterotopic ossification, syringomyelia, anemia, and hypotension. To date there are no FDA-approved treatments to induce neurological recovery following acute SCI. Thus, there remains a significant unmet medical need in SCI, particularly in patients with more severe injuries who are initially classified as ASIA Impairment Scale Grade A or B (Curt 2008).

Worldwide, the annual incidence of SCI is estimated to be 15 to 40 cases per million of population (Sekhon 2001). In the United States there are approximately 12,000 new cases annually (National Spinal Cord Injury Database 2013). The most common causes of SCI are motor vehicle crashes, falls, violence (such as gunshot wounds), and sports injuries. SCI predominantly affects men (80.7%), and the average age at time of injury is 42.6 years. Since 2010, the most frequent categories of injury are as follows: incomplete tetraplegia (40.6%), incomplete paraplegia (18.7%), complete paraplegia (18.0%), and complete tetraplegia (11.6%). Currently, the leading causes of death for persons with SCI are pneumonia and septicemia.

The pattern of tissue damage after SCI in humans is complex and highly variable (Kakulas 1999a, Kakulas 1999b), but generally can be grouped into 4 categories: contusion cyst, cord maceration (due to severe compression), cord laceration, and solid cord injury (Bunge 1993). Roughly 80% of all human SCI is caused by contusion/compression type injuries as opposed to about 20% that are due to laceration (e.g., knife or gunshot wound).

Even though the patterns of gross pathology are variable, many of the molecular and cellular changes after SCI are consistently observed in the acute, subacute, and late phases following injury (Kakulas 1999a). These changes are induced by the initial mechanical insult (primary injury) and are collectively referred to as the secondary injury. The exact pathophysiology of secondary injury in human SCI is not known and is thus inferred from animal models of SCI that produce similar patterns of histopathological tissue damage.

Since the majority of human SCI cases are caused by contusion/compression, the majority of animal studies on the mechanisms of secondary injury have focused on this type of injury. It is also for this reason that the majority of the nonclinical efficacy and safety studies of AST-OPC1 were conducted in a rodent contusion SCI model.

1.2 AST-OPC1

1.2.1 General Description

AST-OPC1 (formerly known as GRNOPC1) is a cell population that contains a mixture of oligodendrocyte progenitor cells (OPCs) and other characterized cell types that are obtained following differentiation of undifferentiated human embryonic stem cells (uhESCs). The starting material for the production of AST-OPC1 is an H1 master cell bank produced from the H1 uhESC line derived at the University of Wisconsin in 1998. The H1 line was approved on January 29, 2010 for inclusion in the National Institutes of Health Human Embryonic Stem Cell Registry and is authorized for research using federal funding.

AST-OPC1 is manufactured using a controlled and highly specialized differentiation protocol. After the differentiation of uhESC is complete, the cells are harvested and cryopreserved, generating the AST-OPC1 investigational product**.** AST-OPC1 has been characterized by the expression of several molecules associated with oligodendrocyte precursors and is further characterized for the expression of markers known to be present in other cell types, such as neurons, epithelial cells, astrocytes, endodermal cells, mesodermal cells, or uhESCs. The presence of these cell types is controlled through the manufacturing process and through specified limits for their levels.

AST-OPC1 has been shown to have three potentially reparative functions which address the complex pathologies observed at the SCI injury site. These activities of AST-OPC1 include production of neurotrophic factors, stimulation of vascularization, and induction of remyelination of denuded axons, all of which are critical for survival, regrowth and conduction of nerve impulses through axons at the injury site. Additional information is provided in the Investigator's Brochure.

1.2.2 Nonclinical Testing

Rigorous nonclinical testing was conducted on AST-OPC1, with an emphasis on evaluating the safety and efficacy of the product. In vitro and in vivo models were utilized to assess:

- phenotype and cellular function

- survival, migration, function and potential therapeutic utility in SCI
- distribution within and outside the CNS after injection directly into a spinal cord contusion
- recognition by the allogeneic human immune system
- potential toxicity to multiple organ systems including the induction of allodynia
- toxicity to neurological function should immunological rejection occur
- potential to form human teratomas or ectopic tissue (defined as tissue types not found in the CNS nor intended to be produced by AST-OPC1)

Results of these studies are provided below and in the Investigator's Brochure.

1.2.3 Pharmacology

AST-OPC1 was found to have properties that promote repair of the pathology in SCI. AST-OPC1 produced neurotrophic factors which, upon implantation in the subacute period after injury, could potentially promote survival of damage spinal cord tissue as well as promote regeneration of injured axons (Zhang 2005). AST-OPC1 was also demonstrated to induce remyelination of demyelinated axons in rats with either a cervical or thoracic contusion SCI and to form compact myelin around dysmyelinated axons in the mutant Shiverer mouse, which lacks compact myelin in the CNS. In nonclinical studies with long term follow up, AST-OPC1 was also found to induce the formation of new blood vessels within the injury/injection sites.

When injected into the spinal cords of athymic nude rats 7 days following a cervical or thoracic SCI, AST-OPC1 survived in virtually all animals as measured 9 months after injection when an anti-asialoGM1 antibody was used to inactivate xenograft reactive, host natural killer cells. Similarly, AST-OPC1 survived for at least 9 months as a xenograft in immunocompetent Sprague Dawley rats when appropriate immunosuppression was administered.

AST-OPC1, which contains oligodendrocyte progenitor cells, matured when injected into the spinal cord in rats with thoracic SCI. At 180 days postinjection, human cells that expressed markers of differentiated oligodendrocytes were found at the lesion site. At this point, few human cells expressed markers for proliferation, indicating that the proliferative capacity of AST-OPC1 diminished as the cells matured.

Recovery of locomotor activity was improved in rats with moderately severe cervical or thoracic contusion injuries that were injected with AST-OPC1 at the lesion site 7 days after injury. Improvement in locomotor activity was measured using a well-established behavioral

rating scale (Basso 1996) and by unbiased kinematic gait analysis using the TreadScan system (CleverSys Inc.). In these nonclinical efficacy studies, animals were followed for 2-4 months and significantly improved locomotor function relative to controls was observed at both cell doses tested: 2.4×10^5 cells and 1.5×10^6 cells. There was no obvious dose response in these studies. When AST-OPC1 was administered 2 or 10 months postinjury, no improvements in locomotor activity were observed. This lack of functional benefit in chronic SCI may have been due to extensive glial scar formation at the injury site.

Several histological parameters were measured in the spinal cords of injured animals that received AST-OPC1. At 2-4 months postinjection, increased survival of corticospinal tract axons immediately adjacent to the lesion epicenter was observed, as well as regeneration of rubrospinal and reticulospinal axons through the AST-OPC1 grafts. Animals that received AST-OPC1 7 days after SCI showed significantly less injury-related cavity formation at 9 months postinjection than was observed in control animals. In addition, the lesion sites into which AST-OPC1 had been injected contained numerous human cells that were traversed by multiple fascicles of myelinated axons, and increased oligodendrocyte-mediated remyelination was seen in the adjacent white matter.

1.2.4 Biodistribution Studies

Studies were performed to determine the distribution of AST-OPC1 throughout the CNS and the rest of the body upon injection into the SCI site using the product as formulated for clinical trials. AST-OPC1 was injected at doses of 2.4×10^5 and 2.4×10^6 cells into a moderately severe cervical or thoracic contusion injury in athymic nude rats 7 days after injury. At time points ranging from 2 days to 6 months posttransplantation, both CNS and peripheral tissues were harvested and tested for the presence of human cells as detected by quantitative polymerase chain reaction (PCR). A parallel study examined the distribution of AST-OPC1 in the CNS using a histological endpoint. The major findings from these studies were:

- AST-OPC1 was retained primarily within 2 cm rostral and caudal to the injection sites in the cervical or thoracic spinal cord
- AST-OPC1 was detected in the spinal cord white matter up to 5 cm rostral and caudal to the injection sites, and the number of human cells progressively decreased as the distance from the injection site increased
- At supraspinal levels, AST-OPC1 was restricted to white matter tracts in the brainstem and pons/medulla

- In a few cases, detectable but not quantifiable amounts of human DNA were found in the cerebellum, hippocampus, or peripheral tissues, ranging from approximately 0.1 to 1 human cell per 1,000,000 rat cells
- Human cells were detectable in the meninges directly over the injection site
- Human cells were detectable in cerebral spinal fluid (CSF) 2 days after injection, but not at 14 or 180 days
- Dose did not appear to influence the distribution of the cells within or outside the CNS

Acute biodistribution of AST-OPC1 was also assessed following injection of 1 x 10^7 cells at C6 in the in the uninjured spinal cord of the Gottingen pig using the clinical syringe positioning device. Survival of AST-OPC1 was observed at 2 and 14 days post-implantation. AST-OPC1 was not observed in the brain by histological examination. Administration of AST-OPC1 did not affect mortality rates and was well tolerated.

1.2.5 Toxicology

Multiple nonclinical studies were conducted to evaluate the potential toxicity and tumorigenicity associated with the administration of AST-OPC1 to the spinal cord. The results of these studies are summarized in the Investigator's Brochure. The only significant finding was the formation of ectopic tissue (atypical glandular structures or cartilaginous tissue) at the spinal cord lesion site in some animals administered AST-OPC1. Pooled analysis of the studies that used 9-month follow up and AST-OPC1 that passed all current release testing for clinical use showed a 2-3% incidence of ectopic tissue with the injection sites. These ectopic tissue foci were all very small and there was no evidence of adverse effects in the adjacent spinal cord tissue. There was also no evidence of any clinical adverse effects related to the ectopic tissue. In addition, very few proliferative cells were present in these foci of ectopic tissue, which suggests that the potential for continued enlargement via cellular proliferation is very low.

1.2.6 Clinical Experience to Date

Five subjects with thoracic SCI have received AST-OPC1 (formerly GRNOPC1) injections in a previous open-label Phase 1 safety study (Geron Protocol CP35A007). All subjects had a last fully preserved neurological level from T3 – T11 and received a single injection of 2 x 10^6 cells between 7 and 14 days after the injury. Subjects received temporary immunosuppression with tacrolimus, which was tapered at Day 46 of follow up and discontinued at Day 60 per protocol. There were no serious or unexpected adverse events related to AST-OPC1, tacrolimus, or the injection procedure. There were 5 AEs judged to be possibly related to AST-OPC1. One of these AEs was a brief mild elevation of body

temperature. The remaining four AEs all occurred in 1 subject and were primarily neuropathic pain reported as a burning sensation in the trunk and lower extremities. Pain of this type and distribution is also a common complication of SCI.

There were no reports of cyst formation or enlarging masses at the injection sites on MRI scans. All 5 subjects completed 1 year of follow up under protocol CP35A007 and are continuing long-term follow up under a companion protocol, CP35A008. Additional information is provided in the Investigator's Brochure.

1.3 Rationale for Current Study Design

1.3.1 Cervical Spinal Cord Injuries

As the initial safety profile for AST-OPC1 at a dose of 2 x 10^6 cells is favorable in thoracic SCI, the next target patient population in which to clinically test AST-OPC1 are subjects with severe cervical spinal cord injuries who are initially classified as ASIA Impairment Scale (AIS) Grade A or B. There is a strong medical and scientific rationale for the transition to subjects with cervical SCI. Individuals with severe cervical SCI have an enormous unmet medical need due to the loss of function in all four limbs as well as multiple additional sequelae such as impaired bowel and bladder function, reduced sensation, spasticity, autonomic dysreflexia, deep vein thromboses, sexual dysfunction, increased infections, decubitus ulcers and chronic pain. These individuals frequently require significant assistance for self-care and activities of daily living. They also have a significantly decreased lifespan - the life expectancy of an individual suffering a C5-C8 cervical spinal cord injury at age 20 is 20 years less than that of a similarly aged individual with no SCI (Spinal Cord Injury Facts and Figures at a Glance, February 2013, https://www.nscisc.uab.edu/).

Scientifically, the injured cervical spinal cord is a much better location than the upper or middle thoracic spinal cord to test the safety and potential activity of AST-OPC1. This is partly due to the fact that denervated neuronal pools in the lumbar enlargement can be many levels below a thoracic injury whereas lower levels of the cervical enlargement are only a short distance from the epicenter of a cervical injury (Sharp 2010). Therefore, regeneration and/or repair of damaged axons mediated by AST-OPC1 could theoretically result in substantial reinnervation of lower cervical segments and have a significant impact on upper extremity motor function.

The advantages of conducting clinical trials in patients with severe cervical SCI were recently reported by Steeves et al. (2011, 2012) together with the Spinal Cord Outcomes Partnership Endeavor (SCOPE). These studies analyzed several large SCI databases and found that only 21% and 26% of people living with C4-C7 cervical AIS-A spinal cord injury recovered ≥2 upper extremity motor levels at 24 and 48 weeks after injury, respectively. These studies further showed that a ≥2 level improvement in motor score led to a statistically significant increase in the Spinal Cord Independence Measure (SCIM) self-care subscore, suggestive of a measurable association between improvement in neurological function and a clinically meaningful functional outcome. The SCIM will also be used as an exploratory endpoint on its own since recent studies have shown that AIS-A and AIS-B patients exhibit a similar degree of recovery on the SCIM with current standard of care (Curt 2008).

Additional new outcome measures and endpoints will be explored that can further elucidate recovery of function across both complete and incomplete cervical SCI patients. One of these measures is the Graded Redefined Assessment of Strength, Sensibility, and Prehension (GRASSP). It has been shown that a baseline GRASSP exam conducted at 1 month post-SCI can predict upper limb function and self-care ability at 6 and 12 months post-SCI (Velstra 2014). Another new exploratory endpoint that will be utilized is the Spinal Cord Ability Ruler, which is a quantitative linear measure of volitional performance inclusive of all subjects in the study population (Reed, submitted). The Spinal Cord Ability Ruler is derived from the ISNCSCI and SCIM assessments.

1.3.2 AST-OPC1 Dose Selection

1.3.2.1 Dose Selection with Respect to Potential Efficacy

Rats with cervical contusion injuries that received a low dose of 2.4×10^5 AST-OPC1 cells in a pivotal nonclinical cervical efficacy study exhibited significantly greater recovery of locomotor function as assessed by the TreadScan system than vehicle control animals ($p = 0.045$) after 1 month of follow up. Since each segment of the human spinal cord has roughly a 50-fold larger volume than the corresponding rat spinal cord segment, it is estimated the human equivalent dose (HED) for efficacy is approximately 1.2×10^7 AST-OPC1 cells ($= 50 \times [2.4 \times 10^5]$). However, it is anticipated that the optimal therapeutic dose in humans could be lower or higher than the estimated HED because AST-OPC1 has several potential mechanisms of action and it is unclear how these scale separately and collectively from rodents to humans. Therefore, three doses of 2×10^6, 1×10^7, and 2×10^7

cells were selected for the proposed trial in order to bracket the estimated HED with a sufficient range to maximize the probability of detecting potential efficacy while maintaining an acceptable safety profile based on the nonclinical and clinical data acquired to date.

1.3.2.2 Dose Selection with Respect to Safety

The first proposed dose of 2×10^6 cells has already been evaluated for safety in the previous thoracic SCI trial. No complications due to the injection procedure were noted in the thoracic trial, and the adverse events deemed possibly related were all Grade 1 or 2, and were all postoperative symptoms commonly seen after spine surgery (e.g., transient pain or fever).

The increase from the first dose (2×10^6 cells in 50 µL) to the second dose (1×10^7 cells in 50 µL) only entails increasing the concentration of AST-OPC1 such that both of these doses will be delivered via a single injection with a 50 µL volume. Therefore, the neurosurgeons consulted for this protocol view this initial dose escalation as a very small step with respect to the potential risks associated with the injection procedure. In addition, the safety of administering the second dose (1×10^7 cells in 50 µL) was demonstrated in the uninjured pig cervical spinal cord at C6. This study confirmed the minimal expected tissue damage associated with injections into the uninjured spinal cord, and no evidence of efflux or cellular dissemination via the CSF was observed.

The third dose represents an additional injection of 1×10^7 cells in 50 µL at a second site within the lesion in a manner similar to that used for the rodent safety studies. This dose still represents a 6-12X safety margin relative to the highest dose tested in the rat safety studies, particularly with respect to the total volume injected. Also, as noted in Section 3.1.2 of this protocol, dose escalation will occur in accordance with the rules for cohort advancement.

1.3.3 Rationale for AST-OPC1 Administration in Subacute SCI

It is hypothesized that the subacute phase of SCI is the optimal time window in which to administer AST-OPC1. This phase avoids the early damage that leads to apoptosis of endogenous oligodendrocytes and occurs soon enough to allow OPCs to migrate to denuded axons before extensive glial scarring has occurred. This hypothesis is supported by studies in rodent models of SCI that have shown functional benefits when AST-OPC1 or similar cells are injected 7 days after SCI, but no benefit if the interval between injury and injection is greater than 8 weeks (Keirstead 2005, Karimi-Abdolrezaee 2006). Since spontaneous functional recovery in rats with contusion SCI begins to plateau at about 6

weeks post-injury, injection of AST-OPC1 at 7 days corresponds to about 1/6 (17%) of the time elapsed between injury and the onset of recovery plateau.

The subacute phase of SCI is thought to be much longer in humans given that the rate of spontaneous recovery typically begins to plateau at about 6 months after SCI (Fawcett 2006). Extrapolating from the nonclinical efficacy data in rodents, injection of AST-OPC1 when 1/6 of the time to onset of recovery plateau has elapsed in humans would correspond to about 30 days post-injury.

The original dosing window of 14-30 days was selected to avoid the early hemorrhage and inflammation that occurs following SCI, as well as the scar tissue formation that occurs in the chronic phase of SCI. This window was also based on the preclinical data available prior to the initiation of this clinical study. However, Asterias recently completed a new preclinical study to further evaluate the appropriate dosing window for AST-OPC1, and the findings from this study suggest that the optimal dosing window in human subjects may extend to at least 60 days post-SCI (see Investigator's Brochure for details).

Asterias reviewed these new preclinical data with the current study investigators and several SCI experts to determine whether the dosing window should be adjusted. Consideration was also given to the planned inclusion of patients with a C4 NLI. Based on these reviews, it was determined that a dosing window of 21-42 days post-SCI would still be squarely within the subacute period but would also allow more time for patients to be medically stable prior to undergoing elective surgery for AST-OPC1 administration. Therefore, AST-OPC1 will be administered to subjects in this study at 21-42 days after SCI – a timeframe during which the patient should have stabilized from the acute injury but still within a window when clinical benefit might be anticipated based on extrapolation from nonclinical studies.

1.3.4 Immunosuppression

In vitro studies were also performed to assess the immunogenicity of AST-OPC1 with respect to the allogeneic human immune system (Okamura 2007). These experiments demonstrated that AST-OPC1 was capable of only weakly stimulating allogeneic T-cell proliferation in a mixed lymphocyte reaction assay. Although AST-OPC1 was found to express Class I human leukocyte antigens, Class II expression was not detected. Further experiments in this study indicated that AST-OPC1 is resistant to lysis by human natural killer cells as well as antibodies contained in normal human serum. These in vitro studies

suggest that AST-OPC1 may be a poor target for immune-mediated responses in an allogeneic setting.

Based upon these results, it was hypothesized that if an immune response against AST-OPC1 were to occur in a human subject, it would likely consist of a weak T-cell attack. Therefore, the previous trial of AST-OPC1 utilized a low dose of tacrolimus for immunosuppression. In addition, the tacrolimus dose was tapered beginning at Day 46, and tacrolimus was withdrawn completely at Day 60. Since it was not known whether this immunosuppression regimen was sufficient to prevent donor-specific immune responses to AST-OPC1, extensive immune monitoring was performed.

The immune monitoring of subjects who received AST-OPC1 focused on identifying responses from the humoral and cellular components of the immune system. Blood and CSF were collected from subjects at baseline and at either multiple time points after AST-OPC1 injection (for blood) or at Day 60 after injection (for CSF). Serum and CSF samples were used to assay for the presence of donor specific antibodies. Peripheral blood mononuclear cells were used to detect reactive T cells against AST-OPC1. The results of the immune monitoring assays showed no detectable humoral or cellular immune responses against AST-OPC1 that were sustained at successive time points through 1 year of follow up for any of the five subjects in that study.

Therefore, tacrolimus administration and tapering will follow the procedure used in the previous clinical study (see Section 6.5 for details). This study will also include an immunological monitoring plan similar to the one used in the previous clinical study to evaluate whether an immune response to AST-OPC1 will occur following immunosuppression withdrawal.

1.4 Potential Risks

1.4.1 Complications of Spinal Cord Injury

There are many medical complications that affect individuals who have suffered a traumatic spinal cord injury. These risks include, but are not limited to:

- allodynia
- anemia
- autonomic dysreflexia
- bowel dysfunction
- deep vein thrombosis (DVT) with or without pulmonary embolism

- hemorrhoids
- heterotopic ossification
- hyperthermia
- hypotension
- hypothermia
- pain
- pressure ulcers
- respiratory depression/dysfunction
- respiratory infections
- spasticity
- syringomyelia
- tachycardia
- urinary tract infections

Development of any one or more of the complications noted above will be considered an anticipated (expected) adverse event (AE) in this study. Risks that are specific to AST-OPC1 are described below, and they fall into three categories: 1) risks related to the surgical procedure for AST-OPC1 injection, 2) risks secondary to immunosuppression, and 3) potential risks of the AST-OPC1 cells.

1.4.2 Surgical Injection Procedures

AST-OPC1 is injected directly into the spinal cord during an open surgical procedure under general anesthesia. The procedure requires a posterior approach, laminectomy, and opening of the dura. These steps are common in intradural spinal cord operations, and have several known risks, including:

- anesthesia-associated risks
- compression of nerve roots
- CSF leak
- deep vein thrombosis and pulmonary embolus
- further tissue damage to the spinal cord
- hemorrhage and/or fluid collection in tissues between the incision site and the spinal cord
- infection (incision site, intraspinal/CNS, pneumonia, urinary tract)

- intraspinal hemorrhage
- pain
- spinal cord tethering

These risks will be minimized as much as possible by selecting participating sites with highly qualified spine surgeons and ensuring good perioperative care. In the previous thoracic study of AST-OPC1 (Geron Protocol CP35A007), there were no serious or unexpected adverse events related to the injection procedure. There were 9 AEs in that study that were judged to be possibly related to the injection procedure. These were all Grade 1 or Grade 2 in severity and were predominantly transient postoperative pain, one brief, mild, elevated temperature, and one urinary tract infection.

1.4.3 AST-OPC1

As outlined in Section 1.2.6, clinical experience has been obtained in 5 subjects with thoracic SCI in Geron Protocol CP35A007. There were no serious or unexpected adverse events related to AST-OPC1, nor was there any evidence of cyst formation or enlarging masses at the injection sites on MRI scans. At this stage, all potential risks discussed below are theoretical.

Based on the results of nonclinical studies, the potential risks of AST-OPC1 include the formation of ectopic tissue (atypical glandular structures and/or cartilaginous tissue) at the injection site and/or teratomas. Since no teratomas were observed in any animals injected with AST-OPC1 from lots that are intended for clinical use, the estimated likelihood of this risk in human subjects is very low. In addition, the foci of ectopic tissue that were observed were all contained within AST-OPC1 grafts and were much smaller (< 1 mm diameter) than the lesion cavities in control animals.

If an area of ectopic tissue develops, it is possible that this ectopic tissue may not reach a size that is detectable on magnetic resonance imaging (MRI) (about 1–2 mm in length) until after 1 year following AST-OPC1 injection. In contrast, many individuals with SCI have lesion cavities in the spinal cord that are at least several millimeters in length and/or damaged spinal tissue that may exhibit areas of heterogeneous signal on MRI. In addition, approximately 21–28% of all individuals with SCI will develop progressive posttraumatic cystic myelopathy (PPCM), also known as posttraumatic syringomyelia (Brodbelt 2003) in which the initial lesion cavity expands and causes further destruction of spinal tissue above and/or below the original injury level. Development of PPCM is usually slow, with a median time of onset at 20 months after injury (Edgar 1994). Only about 1–9% of individuals with

SCI will develop clinical symptoms due to PPCM (Brodbelt 2003). These symptoms might include asymmetrical motor loss, increased pain or temperature sensitivity above the level of the lesion, increased spasticity, and autonomic dysreflexia.

In over 95% of PPCM cases, the cystic cavity attains a length of at least 4 cm before clinical symptoms become evident (Edgar 1994). No treatments are recommended for patients who have evidence of PPCM on MRI scans but do not have clinical symptoms.

1.4.3.1 Xenotransplantation Related Risks

Another theoretical risk of AST-OPC1 is that it may contain heretofore unknown and currently undetectable murine viral or retroviral adventitious agents. This theoretical risk is a consequence of the original culturing of the H1 uhESC line on a layer of irradiated murine (mouse) embryonic feeder cells. However, the H1 line was adapted to feeder-free culture conditions prior to generating the H1 Master Cell Bank (MCB) from which AST-OPC1 is produced. This MCB has been demonstrated to be free from known murine viral and retroviral adventitious agents. Precautions with regard to xenotransplantation that will be taken in this study are described in Appendix A.

Additional warnings and precautions are outlined in the Investigator's Brochure.

1.4.3.2 Reproductive Risks

Nonclinical studies of AST-OPC1 biodistribution following injection into the spinal cord have not shown evidence of quantifiable AST-OPC1 cells outside of the CNS. Thus, the potential reproductive risks of AST-OPC1 are unknown, but would theoretically decrease as the cells mature into adult oligodendrocytes, which are not found outside the CNS. In addition, very few proliferative cells are present in the AST-OPC1 grafts beyond six months post-injection.

The effects of AST-OPC1 on a fetus or nursing child are unknown. Pregnant or nursing women are excluded from this study because participation requires immunosuppression with tacrolimus and multiple MRI scans with gadolinium-DTPA contrast, both of which present potential additional risk to a pregnant subject.

1.4.4 Tacrolimus

Common adverse reactions to tacrolimus may be found in the package insert for tacrolimus and can include infection, malignancy, nephrotoxicity, hypertension, diabetes, neurotoxicity, and potassium imbalance (http://www.astellas.us/docs/prograf.pdf). Tacrolimus will be given for a finite duration (up through Day 60) and dose will be tapered after Day 46. Blood levels

of drug will be monitored throughout and doses adjusted accordingly to maintain levels between 3 and 7 ng/mL. Concomitant medications and food products will be monitored to avoid untoward interactions.

The tacrolimus regimen in this study is identical to that used in the previous thoracic SCI trial of AST-OPC1 (Geron Protocol CP35A007). There were no serious or unexpected adverse events related to tacrolimus in that study and all five subjects completed the course of tacrolimus per protocol. There were 16 adverse events reported as possibly related to immunosuppression with tacrolimus. These were all Grade 1 or Grade 2 in severity and were consistent with the common adverse reactions to tacrolimus including: nausea, urinary tract infection, and decreased magnesium blood level.

2 STUDY OBJECTIVES

The primary objective of this study is to evaluate the safety of 3 sequential escalating doses of AST-OPC1 administered at a single time-point between 21 and 42 days post-injury, inclusive, to subjects with severe, subacute cervical spinal cord injuries (SCI).

The secondary objective is to evaluate changes in neurologic function following administration of AST-OPC1.

3 INVESTIGATIONAL PLAN

3.1 Overall Study Design

This clinical development program includes 2 proof-of-concept companion trials that are designed to explore the safety and potential efficacy of AST-OPC1 in subjects with recent cervical spinal cord injuries, i.e., occurring between 21 and 42 days prior to AST-OPC1 administration. Study AST-OPC1-01 is a Phase 1/2a dose-escalation protocol encompassing injection (2×10^6, 1×10^7, or 2×10^7 AST-OPC1 cells) and 1 year of follow-up as described herein (See **Figure 3-1**). Study AST-OPC1-02 is a long-term follow-up protocol presented separately.

AST-OPC1-01 is an open-label, dose escalation, cross-sequential, multicenter Phase 1/2a study. Subjects with cervical AIS-A SCI will receive 1 of 3 sequential dose levels of AST-OPC1 injected directly into the spinal lesion within 21 and 42 days after the original injury. Subjects with cervical AIS-B SCI will receive 1×10^7, or 2×10^7 AST-OPC1 cells. All subjects will also receive tacrolimus for 60 days following injection to prevent immune rejection. Subjects will be followed for safety and neurologic function for 1 year under this protocol and will then be required to participate in a separate long-term safety follow-up protocol for 14 years. In addition, eligible subjects who were enrolled and had DVT prophylaxis withdrawn prior to Day 1 but who never received the AST-OPC1 injection will be followed for safety for 30 days and assessed separately. All adverse events will be monitored in an ongoing fashion by the Sponsor's medical monitor and by an independent Data Monitoring Committee (see Section 7.14.1).

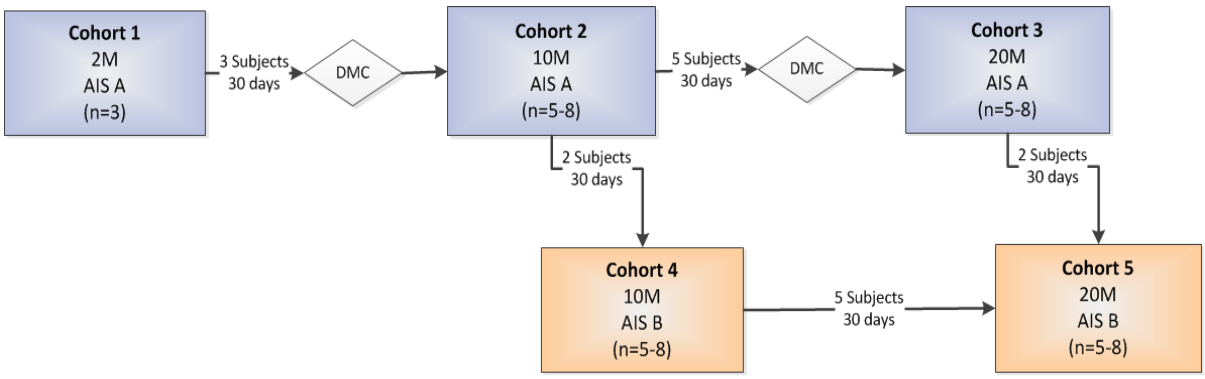
3.1.1 Staggered Enrollment within each Cohort

Enrollment in Cohort 1 will be staggered such that there are at least 10 days between administration of AST-OPC1 to each subject. Enrollment within Cohorts 2-5 will be staggered after the initial subject in each cohort is dosed, with at least 8 days between administration of AST-OPC1 to the first and second subjects. If the first two subjects are successfully dosed with no serious adverse events (SAEs) that would trigger study suspension (per the study Suspension Rules), then the remaining subjects in that cohort may be dosed without further staggering.

An additional staggering rule for Cohorts 2-5 is that a minimum of 14 days must elapse between the dosing of any two consecutive subjects who both have a C4 neurological level of injury (NLI). This 14-day stagger for consecutive C4 NLI subjects applies throughout

each of these cohorts and also would supersede the 8-day stagger noted above if the first two subjects in any cohort both have a C4 NLI.

Figure 3-1: Study Schematic:



3.1.2 Rules for Advancement to Cohorts 2-5

Enrollment in Cohort 2 may commence after all three subjects in Cohort 1 have completed 30 days of follow up after AST-OPC1 administration AND the safety data for Cohort 1 have been reviewed by the Data Monitoring Committee (DMC).

Enrollment in Cohort 3 may commence after at least five subjects in Cohort 2 have completed 30 days of follow up after AST-OPC1 administration AND the safety data from Cohorts 1 and 2 have been reviewed by the DMC.

Enrollment in Cohort 4 may commence after at least two subjects in Cohort 2 have completed 30 days of follow up after AST-OPC1 administration AND if there are no SAEs that would trigger study suspension.

Enrollment in Cohort 5 may commence after at least two subjects in Cohort 3 AND at least five subjects in Cohort 4 have completed 30 days of follow up after AST-OPC1 administration AND if there are no SAEs that would trigger study suspension.

For the planned DMC reviews noted above, the DMC will recommend whether enrollment of the next dose cohort can commence or whether additional follow up of the current cohort is needed prior to commencing enrollment in the next cohort.

The rationale for the 8-day stagger (or the 14-day stagger between consecutive subjects with a C4 NLI) is that nearly all of the potential surgical complications, if they occur, will become clinically evident within the first few days after surgery. Thus, any serious complications (i.e., SAEs) would be evident within the first few days after surgery and would be reported within 24 hours of their occurrence (as required for all SAEs).

In addition, subjects in this study will receive a complete neurological exam and MRI scan for safety monitoring at 7 days after AST-OPC1 injection. The study suspension rules (See Section 7.14.2) include several rules that could be triggered by potential complications of the AST-OPC1 injection procedure, such as: death or unexpected neurological deterioration, evidence of direct spinal cord damage related to the volume of injection, or any CSF infection or inflammation that is more than a transient reaction to AST-OPC1 injection.

3.2 Number of Subjects

A total of 23-35 subjects are planned to receive AST-OPC1. A maximum of 35 of subjects will receive AST-OPC1.

3.3 Investigational Sites

This is a multicenter study with approximately 8 institutions participating.

3.4 Study Duration

Enrollment is expected to take approximately 36 months. The treatment and observation period for individual subjects on this study (excluding long-term follow-up study) is approximately 12 months. The total duration of this study is about 4 years.

4 SELECTION OF STUDY POPULATION

Prior to completing any protocol-specific tests or procedures, the subject will sign informed consent both for the primary protocol AST-OPC1-01 and the long-term follow-up protocol AST-OPC1-02.

4.1 Inclusion Criteria

1. Sensorimotor complete, traumatic SCI (ASIA Impairment Scale A) for Cohorts 1, 2, and 3
2. Sensorimotor incomplete, traumatic SCI (ASIA Impairment Scale B) for Cohorts 4 and 5
3. ISNCSCI Neurological Level of Injury (NLI) from C-5 to C-7 or a C4 NLI with an upper extremity motor score (UEMS) ≥ 1
4. From 18 through 69 years of age at time of consent
5. Single spinal cord lesion on a post-stabilization magnetic resonance imaging (MRI) scan, with sufficient visualization of the spinal cord injury epicenter and lesion margins to enable post-injection safety monitoring
6. Informed consent for this protocol and the companion long term follow-up protocol must be provided and documented (i.e., signed informed consent forms) no later than 37 days following injury
7. Able to participate in an elective surgical procedure to inject AST-OPC1 21-42 days following SCI

4.2 Exclusion Criteria

1. SCI 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 (AP) 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 interferes with the performance, interpretation or validity of neurological examinations, such as multiple spinal cord lesions, brachial/lumbar plexus injury, cauda equina injury or traumatic brain injury
5. Any treatment or pre-existing condition that interferes 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 be compromised

7. Significant organ damage or systemic disease that would create an unacceptable risk for surgery or immunosuppression

8. Acute, symptomatic pulmonary embolism that would create an unacceptable risk for surgery

9. Concomitant use at baseline of other immunosuppressive agents, such as corticosteroids, that would create an unacceptable risk for additional immunosuppression with tacrolimus

10. Need for mechanical support of ventilation (ventilator, continuous positive airway pressure [CPAP], bi-level positive airway pressure [BiPAP]), excluding supplemental oxygen, at baseline

11. History of any malignancy (except non-melanoma skin cancers)

12. Pregnant or nursing women. Female subjects of child bearing potential must agree to prevent pregnancy by the use of contraception for 1 year following AST-OPC1 injection; male subjects must agree to use contraception to prevent pregnancy in any female partners of child bearing potential for 1 year following AST-OPC1 injection.

13. Positive blood test for antibodies to human immunodeficiency virus (HIV) types 1 or 2, antibodies to hepatitis B virus (HBV) core antigen or antibodies to hepatitis C virus (HCV)

14. Panel reactive antibodies (PRA) ≥ 20%; if a site lab reports PRA only for HLA Class I or separately for HLA Class I and II, exclusion will be based solely on the PRA for HLA Class I

15. Serum creatinine >1.5X above the established limit for the normal range at individual study center laboratories at baseline

16. Blood levels of aspartate aminotransferase (AST) or alanine aminotransferase (ALT) > 3X the established upper limit for the normal range at individual study center laboratories at baseline

17. Hematocrit below the study center lower limit for elective surgery at baseline

18. Positive blood cultures (48 hour culture results at Day -1)

19. Active untreated viral, fungal or bacterial infection at baseline

20. Evidence of surgical site infection at intended AST-OPC1 injection site at baseline

21. Temperature ≥ 38.6°C at two time points from Day -1 through Injection Day prior to surgery for AST-OPC1 injection

22. Body mass index (BMI) > 35 or weight > 300 lbs.

23. Active participation in another experimental procedure/intervention

24. Psychoactive substance use disorder (as defined by DSM-5) at any time during the 3 months preceding study entry

25. History of untreated or poorly controlled major depression, schizophrenia, paranoia, or other psychotic disorder as defined by DSM-5

26. A subject who, in the opinion of the investigator, is unlikely to return for all follow-up visits as specified in the protocol

27. Any condition that, in the judgment of the investigator, would preclude successful participation in the study

5 ENROLLMENT

Site personnel should notify the Sponsor when screening initiates for an individual subject in order to ensure that the protocol specified rules for staggering within cohorts and for advancement to Cohorts 2-5 are followed correctly. This notification will also enable the Sponsor to ensure that the maximum number of subjects per each cohort is not exceeded.

After signing informed consent, potential subjects will be assigned a subject identification containing the site number and a sequential enrollment number. After eligibility assessment, subjects who are deemed eligible in accordance with the inclusion/exclusion criteria (Section 4) will be enrolled into the study.

6 STUDY TREATMENTS

6.1 Investigational Product

AST-OPC1 is a cryopreserved cell population containing a mixture of oligodendrocyte progenitor cells and other characterized cell types that are obtained following differentiation of uhESCs. At the time of cryopreservation, each vial contains 7.5 x 10^6 viable cells in 1.2 mL of cryoprotectant solution.

AST-OPC1 is supplied in 2.0 mL cryovials and is shipped to the clinical sites in the vapor phase of liquid nitrogen. The cryopreserved vials of AST-OPC1 should be stored in the vapor phase of liquid nitrogen in a validated storage tank.

Each clinical site will be supplied with AST-OPC1 starter kits and dose preparation kits by the Sponsor. These kits consist of reusable or disposable laboratory items required for the preparation of AST-OPC1 doses and are assembled by a contract manufacturing organization. Sites will also receive a reusable syringe positioning device that is required for AST-OPC1 injection. The starter kits, dose preparation kits, and syringe positioning devices should be stored at room temperature.

6.2 Dose Preparation

Prior to administration, AST-OPC1 will be thawed and prepared according to instructions for preparation by study personnel who have been trained in the AST-OPC1 dose preparation procedure. The dose preparation procedure is shown graphically in a process flow diagram provided in Appendix B and is described below. AST-OPC1 dose preparation may only be performed in a facility qualified for this purpose by the Sponsor and only by personnel who were previously trained and qualified by the Sponsor.

Dose preparation kits consist of presterilized, single-use disposable items required to prepare the AST-OPC1 dose. Dose preparation begins with the thawing of an appropriate number of vials of AST-OPC1. The thawed cells are washed and suspended in Isolyte®S and 1% HSA. A sufficient number of cells will be loaded into the 0.25 mL delivery syringe using an 18-gauge blunt loading needle in order to enable delivery of the assigned dose while taking into account the residual dead volume in the syringe and needle.

The loaded delivery syringe is sealed using a syringe Luer-Lok® cap, and labeled with the date prepared, drug name, dose concentration, and expiration time for the dose. The syringe is then placed in a sealable sterile pouch. The cap protects the prepared dose from unintentional ejection of cells. The pouch containing the secured syringe is then placed

inside a refrigerated (2-8°C) AcuTemp® PX1L container. The AcuTemp® container is secured by the use of a one-way plastic zip-tie mechanism with a tag label containing the subject information, date prepared, drug name, dose concentration, and expiration time for the dose.

Based upon data from stability studies of prepared doses, AST-OPC1 formulated in Isolyte®S and 1% HSA may be stored for up to 12 hours at refrigerated temperature (2-8°C) in the AcuTemp® PX1L container plus up to an additional 3 hours at room temperature prior to administration.

6.3 Prepared Dose Release Criteria and Sterility Testing

The AST-OPC1 dose preparation process at the clinical site is controlled by the verification of cell count and sterility. An initial cell count and viability test are performed and samples taken for both Gram stain testing and 14-day sterility testing. The dose is released from the preparation laboratory and administered to the subject based on a negative Gram stain result. *The 14-day sterility test result will not be available by the time of transplantation.* In the event of a positive result from the 14-day sterility test, the procedures to be followed are described below.

AST-OPC1 must be administered within 15 hours of preparation, which may include no more than 12 hours of storage at 2–8°C and an additional 3 hours at room temperature. Once the prepared AST-OPC1 dose is released from the dose preparation lab, the AcuTemp® PX1L container containing the dose is delivered to the operating room where the operating room staff ensures administration before this expiration time.

In the event of a positive 14-day sterility result, the testing laboratory will notify the dose preparation laboratory, the investigators, and the Sponsor within 24 hours. Organism identification and antibiotic sensitivity will be initiated immediately and the results will be communicated to the above personnel as soon as available. The subject will be notified of the results of the sterility test failure and will be monitored for evidence of infection. Additional testing may be performed and/or empiric antibiotic therapy may be initiated at the Investigator's discretion. The Investigator and the Sponsor's Medical Monitor will determine whether any additional action should be taken. The Sponsor will notify the FDA of the sterility failure, results of the investigation of cause, and any corrective actions within 30 calendar days after the Sponsor's initial receipt of the positive culture test result. If the subject experiences any serious or unexpected adverse event that could have resulted from

administration of contaminated product, the Sponsor will report this information to the FDA in an IND safety report no more than 15 calendar days (7 calendar days if fatal or life threatening) from the initial receipt of the sterility failure, in accordance with 21CFR312.32.

6.4 AST-OPC1 Administration

One day prior to the planned injection of AST-OPC1 (i.e., Day –1), subjects should have any ongoing pharmacological DVT prophylaxis suspended in preparation for surgery. These may be restarted on Day 1.

Surgery to inject AST-OPC1 must be delayed if the subject has a temperature ≥ 38.6°C at any 2 time points between Day –1 and Injection Day prior to surgery. Depending upon the duration of the delay of treatment, the Medical Monitor may request that certain baseline tests be repeated.

On Injection Day subjects will receive the dose of AST-OPC1 as determined by cohort assignment (Table 6-1). AST-OPC1 may only be administered by a licensed surgeon who has both training and experience in performing intradural surgical procedures on the spine. In addition, each surgeon must be trained and qualified by the Sponsor on the use of the SPD and the injection procedure prior to their first administration of AST-OPC1 (see Appendix C).

Table 6-1: AST-OPC1 Dose Cohorts and Injection Preparation

Cohort	Dose (# of cells)	Concentration (cells/µL)	Volume per Injection (µL)	Number of Injections	Total Volume Administered (µL)
1	2×10^6	4×10^4	50	1	50
2	1×10^7	2×10^5	50	1	50
3	2×10^7	2×10^5	50	2	100
4	1×10^7	2×10^5	50	1	50
5	2×10^7	2×10^5	50	2	100

The injection procedure requires an open or minimal-access surgical exposure from a posterior approach with the subject sedated under general endotracheal anesthesia. Neuromuscular paralyzing agents will also be administered to prevent subject movement during the injection procedure. This is necessary because if a subject moves while the needle is in the spinal cord, additional damage to the adjacent spared spinal cord tissue could occur.

It is recommended that the surgeon and anesthesiologist consider the placement of an arterial line for intraoperative blood pressure monitoring, since this could facilitate rapid detection and management of an episode of intraoperative autonomic dysreflexia. For subjects with incomplete SCI (i.e., AIS-B), intraoperative monitoring of somatosensory evoked potentials is recommended to determine whether these are adversely affected by the injection procedure.

The target injection site is the caudal portion of the spinal cord injury epicenter and within a region of damaged but nonhemorrhagic spinal tissue. The target level and site should be confirmed by review of the baseline MRI scan and other radiological studies, as needed. For dose Cohorts 3 and 5, the two injections should both be in the caudal half of the lesion epicenter, if possible, and should be spaced at least several millimeters apart in both the lateral and rostral-caudal directions. If it is not possible to perform both injections in the caudal half of the lesion epicenter, then the second injection may be performed in the rostral half of the lesion epicenter.

Due to the migratory ability of the cells in AST-OPC1, targeting of the injection site does not need to be performed in a stereotactic fashion. However, the surgeon should ensure that the target site is sufficiently deep (at least 6–7 mm) within the spinal cord to minimize the chance of AST-OPC1 efflux.

Once the target spinal level is exposed, direct visualization of the spinal cord surface at the target level should be obtained by laminotomy or laminectomy and opening of the dura. When the dura is opened, CSF will efflux and will not typically continue to flow over the posterior surface of the spinal cord. If needed, gentle aspiration may be used to prevent CSF from flowing directly over the injection site while the dura is open. The spinal cord target site should be confirmed by direct or endoscopic visualization and by intraoperative ultrasound (if available and of sufficient quality).

The syringe positioning device is then set up and a syringe/needle assembly containing AST-OPC1 is loaded. The syringe positioning device is then used to advance the needle tip to the target injection site within the spinal cord.

If the surgeon observes substantial deformation of the spinal cord at the onset of attempted needle penetration, the surgeon may elect to raise the needle, use a scalpel to make a small (~1 mm) longitudinal incision of the pia at the intended penetration site, and then reattempt to insert the needle using the syringe positioning device.

If spinal cord motion is deemed to be a problem, the surgeon will consult with the anesthesiologist, who may adjust the ventilator settings to decrease tidal volume and increase respiratory rate to minimize spine motion. If the surgeon deems that spinal motion will still interfere with delivery of AST-OPC1, ventilation can be suspended for several minutes while the needle is in the spinal cord.

Once the needle is at the target position, the surgeon will inject the AST-OPC1 dose over a period of 2 minutes. The surgeon should observe the needle entry site during injection using a surgical microscope or magnifying loupes to monitor for possible efflux of AST-OPC1, which would appear as an opaque liquid on the spinal cord surface. If efflux from the spinal cord is noted at the beginning of injection, the surgeon may consider advancing the needle deeper into the spinal cord. If efflux is still noted or if it occurs after a delay but prior to delivery of the specified volume, the surgeon should stop injection and record the amount delivered. The surgeon may then consider repositioning the needle a few millimeters caudal to the initial site and making an additional attempt to complete injection. However, the total volume of AST-OPC1 delivered to the spinal cord cannot be increased. In the previous clinical study of AST-OPC1 in thoracic SCI, efflux was not observed during the injection in any of the 5 subjects.

The injection needle is left in place for 1 additional minute following injection and then withdrawn using the syringe positioning device. If ventilator settings were altered during injection, normal ventilation should be resumed immediately after needle withdrawal. The surgical procedure is then completed according to the usual standard procedures.

If the surgeon determines that completion of the injection in any location may cause an unacceptable safety risk to the subject, the entire volume may not be delivered. The location(s) and volume(s) of AST-OPC1 actually delivered should be recorded in the eCRF.

6.5 Immunosuppression with Tacrolimus

Between 6 and 12 hours after the AST-OPC1 dose, subjects will commence a course of the immunosuppressant tacrolimus, given orally at a starting dose of 0.03 mg/kg/day (divided into 2 daily doses) Days 1 through 46, tapered by 50% between Days 47 and 52 (rounding to the nearest 0.5 mg), tapered by another 50% on Day 53, and discontinued after Day 60.

If a subject is unable to take oral medication, tacrolimus may be given intravenously at a dose of 0.01 mg/kg/day as a continuous infusion, switching over to oral medication as soon as practicable. During intravenous infusion of tacrolimus, standard monitoring for acute

anaphylaxis will be observed in accordance with approved labeling. Subjects will not be allowed to eat any grapefruit-containing food items through Day 60 as this may alter the pharmacokinetics of tacrolimus.

Blood samples will be taken for monitoring blood levels of tacrolimus (immediately prior to the first daily dose if given orally) twice per week starting on Day 3 (+/- 1 day) and continuing through Day 30, decreasing to once per week through Day 60 (or through termination of tacrolimus) (see Section 7). Dosing of tacrolimus will be adjusted in order to maintain blood levels between 3 and 7 ng/mL. Additional monitoring of blood chemistry for possible tacrolimus toxicity will be as outlined in Section 7.

Tacrolimus dosing may be decreased or discontinued for unacceptable toxicity at the investigator's discretion; this might include infection or uncontrolled fever, liver function test elevation, creatinine elevation, seizure, or thrombotic thrombocytopenic purpura.

6.6 Investigational Product Accountability

AST-OPC1 will only be dispensed to subjects enrolled in the study who have signed informed consent and will only be administered by investigators designated on the Form FDA 1572. The Investigator must ensure that subjects receive AST-OPC1 only from personnel who fully understand the procedures for dose preparation and for administering the investigational product.

Each shipment of investigational product will contain inventory documentation to assist the investigator and dose preparation laboratory in maintaining current and accurate study drug accountability records. The designated recipient will visually inspect the shipment, verify the quantity and condition of investigational product received, and provide written confirmation of receipt of the investigational product shipment as instructed by the Sponsor. Records at the institution will document shipment dates, lot numbers, and quantity of investigational product received.

Investigational product must be stored in a secure area at the institution. Only authorized personnel should have access to the investigational product. The investigator or dose preparation laboratory will maintain dispensation records including the identity of subjects to whom investigational product is dispensed, identity of authorized study personnel receiving, dispensing and disposing of investigational product, the dates of all activity, the lot number, and the quantity of investigational product. These records must be readily available for inspection by the Sponsor or regulatory authorities at any time.

Return of investigational product must be coordinated with the Sponsor prior to shipment. Accountability records should include the lot number(s) and quantity being returned.

Upon completion of the study, site personnel should ensure that all unused investigational product has either been returned or destroyed, as instructed by the Sponsor. Return and/or destruction of all investigational product must be documented in the accountability records. A copy of the institution's drug disposal or destruction policy will be provided to the Sponsor prior to disposal or destruction of investigational product. A copy of the accountability records must be returned to the Sponsor at the end of the study.

6.7 Prior and Concomitant Medications

There is no restriction on medications prescribed to the subject during participation in this trial, with the exception of additional immunosuppressive agents such as corticosteroids during the period of tacrolimus administration. In addition, pharmacological DVT prophylaxis must be discontinued from the day before until the day after surgery for AST-OPC1 injection. Subjects who received methylprednisolone immediately after the SCI, prior to study entry, will not be excluded. All concomitant medications will be recorded in the electronic case report form (eCRF).

7 TESTS AND OBSERVATIONS

All required tests and observations with their schedules are summarized in Table 7-1 and are described in more detail below. Prior to initiating screening tests, the Investigator or approved designee must obtain a signed informed consent for this protocol and the companion long term follow-up protocol. The day on which AST-OPC1 is injected is designated as Injection Day. The days prior to this are designated with negative numbers and the days following injection have positive numbers.

7.1 Screening (Days –11 to –3)

Tests and procedures to confirm eligibility (see Section 4) are summarized below. Tests may be conducted between 11 and 3 days prior to the planned day of surgery for AST-OPC1 injection unless otherwise noted.

Note: if the day of injection is subsequently delayed, all screening tests and procedures that are not repeated will still be deemed to have been collected within the appropriate pre-operative window.

- Obtain demographic data
- Past and current medical history
- Complete physical exam and vital signs
- ISNCSCI exam
- MRI (cervical spine and brain, with and without gadolinium-DTPA contrast) between Days –7 and –3
- ECG
- Hematology (CBC with differential, RBCs, hematocrit, platelets)
- Blood chemistry (albumin, alkaline phosphatase, ALT, AST, BUN, calcium, chloride, creatinine, glucose, magnesium, phosphorus, potassium, total protein, sodium, total bilirubin)
- Serology for HIV antibody, Antibody to Hepatitis B core antigen, and Hepatitis C virus antibody
- Panel reactive antibodies
- Serum pregnancy test for females of childbearing potential
- 48-hour blood cultures (to be drawn on Day -3)
- Concomitant medications
- Adverse events

7.2 Baseline (Days –2 to –1)

Note: As indicated below, some of the baseline assessments may be performed as early as Day -4 if required to accommodate clinical site staff availability.

- Brief physical exam and vital signs (check vital signs both days)
- Standard neurological exam (may be performed on Day -4 to -1)
- ISNCSCI exam (may be performed on Day -3 to -1, unless the screening ISNCSCI was performed on Day -3.)
- Partial Graded Redefined Assessment of Strength, Sensibility and Prehension (GRASSP; may be performed on Day -4 to -1)
- Spinal Cord Independence Measure (SCIM; may be performed on Day -4 to -1)
- Hematology (as before)
- Blood chemistry (as before)
- Fasting blood glucose (may be done pre-op on injection day)
- Blood for HLA typing (may be obtained on Day -4 to -1)
- Blood for immune response monitoring (may be obtained on Day -4 to -1)
- Blood for xenotransplantation archival (may be obtained on Day -4 to -1)
- Withhold pharmacological DVT prophylaxis on Day -1
- Concomitant medications
- Adverse events

If the day of injection is delayed, some or all of the following tests and procedures may need to be repeated prior to the new Injection Day. The determination of which tests and procedures to be repeated will be made by the Investigator after consultation with the Sponsor's Medical Monitor.

Note: baseline tests and procedures that are not repeated will still be deemed to have been collected within the appropriate pre-operative window.

- Brief physical exam and vital signs
- Brief ISNCSCI exam
- MRI (cervical spine, non-contrast only)
- Hematology (as before)
- Blood chemistry (as before)
- Concomitant medications
- Adverse events

7.3 Injection Day (Surgery for AST-OPC1 Administration)

- Vital signs

- CSF sample by lumbar puncture during pre-op or after induction of general anesthesia but prior to AST-OPC1 injection (~10 mL for white blood cell count, glucose, total protein, oligoclonal banding, myelin basic protein, and IgG index)
- AST-OPC1 Injection
- Begin tacrolimus administration 6 to 12 hours following AST-OPC1 injection
- Concomitant medications
- Adverse events

7.4 Post-Injection Days 1-6

- Brief physical exam on Day 1
- Vital signs daily
- Hematology (as before) on Day 1
- Restart DVT prophylaxis on Day 1 (if applicable)
- Blood chemistry (as before) daily
- Tacrolimus blood level on Day 3 (+/- 1 day)
- Concomitant medications
- Adverse events

7.5 Post-Injection Day 7 (+/- 1 day)

- Brief physical exam and vital signs
- Neurological exam
- ISNCSCI exam
- MRI (cervical spine, with and without gadolinium-DTPA contrast)
- ECG
- Hematology (as before)
- Blood chemistry (as before)
- Tacrolimus blood level
- Blood for immune response monitoring
- Concomitant medications
- Adverse events

7.6 Post-Injection Days 8-29

- Blood chemistry (as before) twice per week
- Tacrolimus blood level twice per week

7.7 Post-Injection Day 30 (+/- 3 days)

- Brief physical exam and vital signs

- Neurological exam
- ISNCSCI exam
- SCIM questionnaire
- Pain questionnaire
- Bowel and Bladder questionnaire
- MRI (cervical spine, with and without gadolinium-DTPA contrast)
- Hematology (as before)
- Blood chemistries (as before)
- Fasting blood glucose
- Tacrolimus blood level
- Blood for immune response monitoring
- Blood for xenotransplantation archival
- Concomitant medications
- Adverse events

7.8 Post-Injection Days 31-59

- Blood chemistry (as before) once per week
- Tacrolimus blood level once per week
- Decrease tacrolimus dose by 50% (rounded to the nearest 0.5 mg, since this is the smallest capsule size available) on Day 46
- Decrease tacrolimus dose by another 50% (rounded to the nearest 0.5 mg) on Day 53; if the rounded total daily dose is 0.5 mg or lower, the subject will receive 0.5 mg once per day until tacrolimus is discontinued
- Concomitant medications
- Adverse events

7.9 Post-Injection Day 60 (+/- 7 days)

- Brief physical exam and vital signs
- Neurological exam
- ISNCSCI exam
- SCIM questionnaire
- Hematology (as before)
- Blood chemistry (as before)
- Tacrolimus blood level
- Blood for immune response monitoring
- Discontinue tacrolimus

- CSF via lumbar puncture
- Concomitant medications
- Adverse events

7.10 Post-Injection Day 90 (+/- 7 days)

- Brief physical exam and vital signs
- Neurological exam
- ISNCSCI exam
- GRASSP
- SCIM questionnaire
- Pain questionnaire
- Hematology (as before)
- Blood chemistry (as before)
- Blood for immune response monitoring
- Concomitant medications
- Adverse events

7.11 Post-Injection Day 180 (+/- 14 days)

- Brief physical exam and vital signs
- Neurological exam
- ISNCSCI exam
- GRASSP
- SCIM questionnaire
- Pain questionnaire
- Bowel and Bladder questionnaire
- MRI (cervical spine, with and without gadolinium-DTPA contrast)
- Hematology (as before)
- Blood chemistries (as before)
- Blood for immune response monitoring
- Concomitant medications
- Adverse events

7.12 Post-Injection Day 270 (+/- 14 days)

- ISNCSCI exam
- GRASSP

- SCIM questionnaire
- Concomitant medications
- Adverse events

7.13 Post-Injection Day 365 (+/- 14 days)

- Complete physical exam and vital signs
- Neurological exam
- ISNCSCI exam
- GRASSP
- SCIM questionnaire
- Pain questionnaire
- Bowel and Bladder questionnaire
- MRI (cervical spine and brain, with and without gadolinium-DTPA contrast)
- Hematology (as before)
- Blood chemistries (as before)
- Blood for immune response monitoring
- Blood for xenotransplantation archival
- Concomitant medications
- Adverse events

Table 7-1: Summary of Tests and Observations

Part A

	Screen	Baseline	Surgery	Post-Injection		
	Days –11 to –3	Days –2 to –1	Injection Day	Days 1-6	Day 7	Days 8-29
Procedure					(+/- 1 day)	
Demographic data	**X**					
Past and current medical history	**X**					
Complete physical exam	**X**					
Brief physical exam		**X**		**Day 1**	**X**	
Vital signs	**X**	**X**[3]	**X**	**Daily**	**X**	
Neurological exam		**X**[5]			**X**	
ISNCSCI exam	**X**	**X**[1]			**X**	
GRASSP		**X**[5]				
SCIM		**X**[5]				
MRI[2]	**Day -7 to -3**				**X**	
ECG	**X**				**X**	
Hematology	**X**	**X**		**Day 1**	**X**	
Blood chemistry	**X**	**X**		**Daily**	**X**	**2/week**
Serology for HIV, HBV, HCV	**X**					
Panel reactive antibodies	**X**					
Pregnancy test, if applicable	**X**					
48-hour blood culture	**Day –3**					
Fasting blood glucose		**X**[4]				
Blood for HLA typing		**X**[5]				
Blood for immune response monitoring		**X**[5]			**X**	
Blood for xenotransplantation archival		**X**[5]				
Withhold DVT prophylaxis		**Day -1**				
CSF via lumbar puncture			**X**			
Begin tacrolimus			**X**			
Restart DVT prophylaxis				**Day 1**		
Tacrolimus blood levels				**Day 3**[6]	**X**	**2/week**
Concomitant medications	→					
Adverse events	→					

Table 7-1: Summary of Tests and Observations

Part B

	Day 30	Days 31-59	Day 60	Day 90	Day 180	Day 270	Day 365
Procedure	**(+/- 3 days)**		**(+/- 7 days)**	**(+/- 7 days)**	**(+/- 14 days)**	**(+/- 14 days)**	**(+/- 14 days)**
Complete physical exam							X
Brief physical exam	X		X	X	X		
Vital signs	X		X	X	X		X
Neurological exam	X		X	X	X		X
ISNCSCI exam	X		X	X	X	X	X
GRASSP				X	X	X	X
SCIM questionnaire	X		X	X	X	X	X
Pain questionnaire	X			X	X		X
Bowel/Bladder questionnaire	X				X		X
MRI[2]	X				X		X
Hematology	X		X	X	X		X
Blood chemistry	X	1/week	X	X	X		X
Fasting blood glucose	X						
Blood for immune response monitoring	X		X	X	X		X
Tacrolimus blood level	X	1/week	X				
CSF via lumbar puncture			X				
Blood for xenotransplantation archival	X						X
Concomitant medications	→	→	→	→	→	→	→
Adverse events	→	→	→	→	→	→	→

Footnotes for Table 7-1 Parts A and B

1 ISNCSCI exam (may be performed on Day -3 to -1, unless the screening ISNCSCI was performed on Day -3)

2 MRI of cervical spine and brain at Screen & Day 365; MRI of cervical spine only at Days 7, 30, 180

3 Vitals on day -2 and -1

4 May be done pre-op on Injection Day

5 May be performed as early as Day -4 if required to accommodate clinical site staff availability

6 Tacrolimus blood level on Day 3 may be obtained +/- 1 day

7.14 Procedural Details

7.14.1 Data Monitoring Committee

An independent DMC will be appointed at study initiation and will be comprised of physicians with extensive clinical trial experience and clinical expertise in either spinal neurosurgery or spinal cord injury rehabilitation. Members of the DMC will be independent of the investigators and the Sponsor and will have no financial, scientific, or other conflict of interest with the trial.

After all subjects in Cohort 1 have completed 30 days of follow up after AST-OPC1 administration, the safety data will be submitted to the DMC for review. The DMC will review these data and recommend whether enrollment of Cohort 2 can commence or whether additional follow up of the current dose cohort is needed prior to dose escalation. This process will be repeated after at least five subjects in Cohort 2 have completed 30 days of follow up, at which time the DMC will recommend whether enrollment in Cohort 3 can commence. Thereafter, the DMC will meet at least twice yearly for a trial overview. The DMC will also monitor the study for overall safety and for potential trends in efficacy.

Additionally, the DMC will also be notified of any fatal or life-threatening experiences associated with the use of AST-OPC1 within 7 calendar days of the Sponsor's initial receipt of the information. Serious, unexpected, and possibly related adverse events that are not fatal or life-threatening will be reported to the DMC within 15 days. The DMC will also receive all reports and images of a possible mass or ectopic tissue.

7.14.2 Suspension Rules

The DMC will receive rapid notification of all SAEs associated with the use of AST-OPC1 and of all reports of cyst/cavity formation. The DMC will operate under a high degree of flexibility and will tend to err on the side of protecting subjects' safety until satisfactory resolution of potential problems is obtained. Any event that triggers study suspension will be reported to the FDA and to the IRBs. If study suspension occurs, both enrollment of new subjects and administration of AST-OPC1 will be suspended.

The following events will be used as study suspension rules:

- Death or unexpected neurological deterioration, possibly related to AST-OPC1, the injection procedure used to administer AST-OPC1, and/or the concomitant immunosuppression administered

- Evidence of direct spinal cord damage related to the volume of injection
- Development of an enlarging mass or expanding ectopic tissue within the central nervous system, other than the expected filling of the lesion cavity by AST-OPC1
- Any CSF infection or inflammation that is more than a transient reaction to AST-OPC1 injection.

7.14.3 ISNCSCI Assessment Tool

The ISNCSCI was developed by the American Spinal Injury Association (ASIA) in 1982 in order to promote common definitions of neurological levels and the extent of complete injuries in patients with SCI and to achieve more consistent and reliable data among centers participating in the National Spinal Cord Injury Database. This tool is described in detail in Appendix D.

The ISNCSCI data forms will be reviewed periodically by an independent expert. The reviewer will look for inter- or intra-subject trends that may trigger a repeat examination or a DMC review.

7.14.4 MRI

General guidance on the objectives and procedures of the serial MRI scans is provided in Appendix E.

If a subject becomes pregnant, the decision to perform subsequent MRI scans will be made in consultation between the Investigator, the local radiologist, the Sponsor, and the subject. The decision about performing an MRI will be based on findings from previous MRIs, clinical findings, the risk/benefit ratio, and timing (e.g., whether the MRI can safely be postponed until the subject is no longer pregnant). If an MRI is performed on a pregnant subject, gadolinium-DTPA contrast will not be used.

7.14.5 Pain Scale

Pain assessment will be performed using the International Spinal Cord Injury Pain Basic Data Set (http://www.iscos.org.uk/international-sci-data-sets). The purpose of the Basic Data Sets is to promote uniform data collection in a standard format, with precise instructions for each question (Biering-Sørensen 2006). The Basic Data Sets are intended to be collected with information from the Core Data Set, which includes information such as:

date of birth and injury, gender, cause of spinal cord lesion and neurological status, whether surgery was performed, and whether associated injuries are present (DeVivo 2006). Three questions have been added to the Pain Basic Data Set to assess allodynia based on expert recommendation.

Baseline pain assessments will not be performed due to proximity with the original SCI and surgery. Posttreatment analyses of changes in pain over time will be exploratory in nature.

7.14.6 Spinal Cord Independence Measure

The SCIM was developed as a comprehensive rating scale specifically for persons with SCI. The instrument assesses activities of daily living, capturing the economic burden as well as the impact on a subject's overall medical condition and comfort. Reliability and validity of the SCIM Version III were recently established in a multicenter international study (Itzkovich 2007). As with pain assessment, analyses of changes in SCIM will be exploratory.

7.14.7 Assessment of Urinary Tract and Bowel Function

Assessment of lower urinary tract function and bowel function will be performed using the respective International Spinal Cord Injury Basic Data Sets (http://www.iscos.org.uk/international-sci-data-sets). Basic Data Set information for lower urinary tract function and bowel function will be collected at key posttreatment intervals to assess changes in function; analyses will be exploratory.

7.14.8 Immune Response Monitoring

As described in Section 1.3.4, no acute or chronic immune responses to AST-OPC1 have been observed to date in the previous trial. Therefore, this study will utilize a similar immunological monitoring plan, but with a slightly reduced frequency of blood sample collection at earlier time points. Donor-specific responsiveness to AST-OPC1 will be evaluated using the mixed lymphocyte reaction to monitor T cell responses (Okamura 2007) and panel reactive tests to measure AST-OPC1-specific alloantibodies. Additional assays may include cytokine and RNA changes analysis.

In addition, CSF will be collected via lumbar puncture immediately prior to AST-OPC1 injection and at study Day 60. The CSF will be tested for donor-specific responsiveness to AST-OPC1 and for evidence of inflammation, based upon total protein, IgG index, and oligoclonal bands.

8 TERMINATION

8.1 Study Completion

At the time of study completion, subjects will transition from this protocol to the long-term follow-up protocol AST-OPC1-02. Subjects will be reconsented at the time of transition.

8.2 Premature Discontinuation from Study

Subjects can withdraw from the trial on their own volition or be withdrawn by the Investigator at any time. If a subject who received AST-OPC1 is considering withdrawing from the study, the Investigator should discuss the implications of withdrawing and work with the subject to collect as much safety data as possible, including a final safety visit. If a subject who received AST-OPC1 voluntarily withdraws from this study, they will be nevertheless asked to participate in the long-term follow-up companion protocol AST-OPC1-02.

Specific instances that may precipitate discontinuation of individual subjects from the study are as follows:

- intercurrent illness precluding the continuation of protocol observations
- withdrawal of consent by the subject
- loss to follow-up
- death of subject

If the Investigator is unable to contact a subject for follow-up, the Investigator will attempt to contact the subject 3 times by phone. If unable to reach the subject by phone, a registered letter will be sent. If the Investigator is still unable to reach the subject, the subject will be considered lost to follow-up.

8.3 Study Discontinuation at Site

The study will be completed when all subjects who received AST-OPC1 have either completed the Day 365 visit, discontinued participation, or were lost to follow up.

The Sponsor retains the right to terminate the study at an individual institution and remove all the study materials from the study site for reasons including the following:

- completion of the study at the site
- unanticipated safety information indicating a potential and unacceptable health hazard caused by the investigational product

- significant protocol deviation or noncompliance on the part of the Investigator for reasons such as informed consent violations, other regulatory or ethical irregularities, unsatisfactory enrollment, or inaccurate data handling or reporting
- withdrawal of investigational product from research use
- termination of this study by the Sponsor

9 PLANNED ANALYSES

All analyses will be descriptive in nature. No inferential statistical tests will be conducted.

9.1 Sample Size Considerations

This trial employs a cross-sequential cohort design for Phase 1 dose escalation in cervical AIS-A and AIS-B subjects. Power calculations have not been performed, as the primary objective is to collect safety and tolerability information on a limited number of subjects per dose level and AIS grade.

9.2 Study Endpoints

The primary endpoint of this Phase 1/2a study is safety and includes observations related to AST-OPC1 tolerance, the injection procedures, and the protocol-specified immunosuppression regimen. Secondary and exploratory endpoints focus on preliminary evidence of efficacy.

9.2.1 Primary

- Incidence and severity of adverse events
- Changes in laboratory variables
- Changes at the injection site as monitored by MRI

9.2.2 Secondary

- Changes in neurologic function as assessed by the ISNCSCI

9.2.3 Exploratory

- Changes in posttreatment pain as assessed by the International Spinal Cord Injury Pain Basic Data Set
- Changes in aspects of daily living as assessed by the SCIM scale
- Changes in lower urinary tract and bowel function
- Changes in hand function on the GRASSP assessment
- Changes in volitional performance using the Spinal Cord Ability Ruler

9.3 Data Analysis Methods

For all safety and efficacy analyses, the All Treated population will be used, comprised of all subjects who received an injection of AST-OPC1. The 30-day safety data from subjects who had their DVT prophylaxis withdrawn but who never received AST-OPC1 will be assessed separately.

Conventional descriptive statistics will be used to summarize subject demography, baseline characteristics, disposition, and history. Discrete variables will be expressed with number and percentage and continuous variables with mean, standard deviation, median, and range as appropriate. Due to the small sample sizes per dose level and AIS grade planned for this study, data tabulations may be supplemented with subject narratives.

9.3.1 Safety Analyses

All reported adverse events will be coded to a preferred term and classified by System Organ Class using the current version of MedDRA. Adverse events will be listed by subject and will include preferred term, verbatim term, severity, seriousness, relationship, action taken, and outcome. Relationship will address attribution to the investigational product (AST-OPC1), the surgical procedure, and/or the immunosuppressive agent. Tabulations will be prepared for all adverse events, related events, Grade 3 and higher events, and serous events. Designated adverse events of special interest will be tabulated separately as appropriate.

Summary statistics of individual clinical laboratory parameters will be prepared by time period. Shift tables comparing baseline versus most abnormal value will be generated for selected analytes with standard toxicity grades. For selected laboratory analytes, the frequency of abnormal values and/or the incidence of Grades 3 and 4 values may be tabulated if appropriate.

Clinically significant changes in physical examination findings and ECGs will be presented descriptively. Vital signs will be summarized and listed by subject. Findings from immunological assessments will be presented appropriately.

Concomitant medications will be coded to generic term using the World Health Organization Drug Dictionary and will be tabulated across subjects and listed by subject.

9.3.2 Efficacy Analyses

Neurological function will be evaluated by characterizing upper extremity motor scores and motor level on the ISNCSCI exam (point estimate and 95% CI) by time point. The percentage of AIS-A subjects within Cohorts 2 and 3 who recover at least 2 motor levels at 180 days and at 365 days will be evaluated and compared to recent historical controls (Steeves, 2011 & 2012).

The results from the exploratory assessment tools (GRASSP, SCIM, Spinal Cord Ability Ruler, urinary tract and bowel function) will be tabulated and analyzed using statistical methods appropriate for each type of data set. Efficacy results will be presented by dose level and AIS grade, and across all subjects as appropriate.

10 ADVERSE EVENTS

The collection period for adverse events for the purposes of this protocol begins once the subject has signed the informed consent form and ends after 365 days of observation. Adverse events will be assessed at each clinic visit; all adverse events occurring between visits will be reported by the subject and recorded by the site staff. All unresolved events should be followed by the Investigator until resolved, deemed a chronic/irreversible condition, or the subject is lost to follow-up.

10.1 Adverse Event Reporting

The Investigator or designee is responsible for evaluating all adverse events, obtaining supporting documents, and determining that documentation of the event is adequate. All adverse events must be promptly entered into the eCRF. Details of the event must include description, severity, seriousness, relationship to investigational product, duration, action taken, and outcome.

10.1.1 Definitions

An adverse event is any untoward medical event that occurs to a study subject once the informed consent has been signed and until the subject's last study visit, whether or not the event is considered related to the investigational product. Examples include:

- Any sign, symptom, physical examination finding, or laboratory result that worsens in nature, severity or frequency compared with baseline
- All reactions from the investigational product including those occurring as a result of an overdose, abuse, withdrawal phenomena, sensitivity, or toxicity
- Concurrent illness
- Injury or accident

A preexisting condition is one that is present prior to or at the start of the study and is to be reported as part of the subject's medical history. Preexisting conditions should be reported as an adverse event only if the frequency, intensity, or the character of the condition worsens during the study.

An unexpected adverse event is one not identified in nature, severity, or frequency in the current protocol or Investigator's Brochure.

10.1.2 Grading of Adverse Event

Adverse event severity will be grade according to the following criteria:

- Grade 1, Mild: Awareness of symptom, but easily tolerated; usually transient requiring no special treatment; does not interfere with usual status or activities
- Grade 2, Moderate: May be ameliorated by simple therapeutic measures; may interfere with usual activities
- Grade 3, Severe: Incapacitating, inability to perform usual activities
- Grade 4, Life-threatening/Disabling: Subject at risk of death or significant disability at the time of the event
- Grade 5, Fatal

10.1.3 Relationship of Adverse Event

Relationship of the adverse event either to the investigational product, the surgical procedure, and the immunosuppressive agent will be determined by the Investigator, and will be categorized as:

- Unrelated: The occurrence of the event is not reasonably related in time OR the event is considered unlikely to be related to use of the investigational product, the surgical procedure, and/or the immunosuppressive agent. Other factors or evidence explain the occurrence of the event, such as the underlying condition, other medical conditions, concomitant medications, etc.
- Related: The investigational product, the surgical procedure, and/or the immunosuppressive agent are reasonably related in time to the adverse event.

10.2 Serious Adverse Events

10.2.1 Definitions

A serious adverse event is one that meets any of the following criteria:

- Results in death
- Is life threatening
- Requires inpatient hospitalization or prolongation of an existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect in the offspring of an exposed subject

An important medical event that may not result in death, be life-threatening, or require hospitalization, may be considered a serious adverse drug experience when, based upon appropriate medical judgment, it jeopardizes the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

A life-threatening adverse event is defined as any adverse experience that places the patient or subject, in the view of the Investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

10.2.2 Reporting

The Investigator must notify the Sponsor by telephone or fax of any serious adverse event within 24 hours of the Investigator's knowledge of the event. Telephone reports must be followed by a written report within 24 hours. Follow-up reports must be submitted in a timely fashion as additional information becomes available.

The Sponsor will notify the FDA and/or other regulatory authorities and all participating Investigators of any adverse event that is serious, unexpected and possibly related to the use of the investigational product, in accordance with 21CFR312.32 (c), IND Safety Reports. Regulatory authorities will be notified of any fatal and life-threatening experiences associated with the use of the investigational product as soon as possible but in no event later than 7 calendar days after the Sponsor's initial receipt of the information. Serious, unexpected and possibly related adverse events that are not fatal or life-threatening will be reported as soon as possible but in no event later than 15 calendar days after the Sponsor's initial receipt of the information.

The Investigator is responsible for notifying their IRB/IEC of all serious, related and unexpected adverse events.

10.2.3 Pregnancy

Pregnancy itself is not considered an adverse event. However, for any pregnancy that occurs during the study period, the Sponsor must be notified within 24 hours of the Investigator learning of the pregnancy. The site should maintain contact with the subject and obtain pregnancy outcome information. The Investigator must report follow-up information to the Sponsor regarding the course of the pregnancy, including perinatal and neonatal outcome.

10.3 Recording of Adverse Events

All adverse events, including observed problems, complaints, or symptoms, are to be recorded on the appropriate eCRF whether or not considered related to the investigational product. Documentation must be supported by an entry in the subject's source document.

Laboratory test abnormalities considered by the Investigator to be clinically relevant should be reported on the eCRF. Each event is to be evaluated for duration, severity, and causal relationship with the investigational product or other factors.

11 ADMINISTRATIVE AND ETHICAL PROCEDURES

11.1 Regulatory Authority Approval

The Investigator will ensure that the study is conducted in compliance with the protocol, the Declaration of Helsinki, and according to 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, Institutional Review Board (IRB) or Independent Ethics Committee (IEC) approval, and record retention

This study must have the approval of a properly constituted IRB/IEC. Before the investigational product is shipped to the study site under this protocol, the Investigator will provide the Sponsor with a copy of the IRB/IEC approval letter stating that the study protocol and informed consent form have been reviewed and approved for both this protocol and the companion long term follow up protocol.

11.2 Informed Consent

A properly written and executed informed consent form, in compliance with the Declaration of Helsinki, ICH E6 Section 4.8, 21 CFR Part 50.20, and other applicable local regulations, must be obtained for each subject prior to entering the subject into the study. According to 21 Code of Federal Regulations (CFR) Part 50.20, no Investigator may involve a human being as a subject in research covered by these regulations unless the Investigator has obtained the legally effective informed consent of the subject or the subject's legally authorized representative. An Investigator shall seek such consent only under circumstances that provide the prospective subject or the subject's legally authorized representative sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence. The information that is given to the subject or the representative shall be in language understandable to the subject or the representative.

The Sponsor will provide the Investigator with a study-specific template for the informed consent form. State and local laws and/or institutional requirements may require the disclosure of additional information in the consent form. The proposed consent form must be submitted to the Sponsor prior to submission to the IRB/IEC to ensure that it meets the Sponsor's standards for informed consent forms.

The IRB/IEC must approve the informed consent form. A copy of the approved consent form must be submitted to the Sponsor prior to subject screening.

Prior to the initiation of any procedures relating to the study, informed consent shall be documented by the use of a written consent form approved by the IRB/IEC and signed and dated by the subject or the subject's legally authorized representative at the time of consent. A copy of the signed informed consent form will be given to the person signing the form. The Investigator must keep each subject's signed consent form on file for inspection by authorized representatives of the Sponsor, the IRB/IEC, or a regulatory authority at any time.

11.3 Protocol Amendments

Any changes to this protocol will be initiated by the Sponsor in writing as a protocol amendment. The amendment must be submitted to the IRB/IEC, together with a revised informed consent form, if applicable. Written documentation of IRB/IEC approval must be received before the amendment may take effect.

11.4 Data Management and Study Monitoring

Clinical data will be recorded on eCRFs provided by the Sponsor. Entry of data onto eCRFs must be recorded accurately and complete. eCRFs must be completed on a timely basis and as requested by the Sponsor or its authorized representative in preparation for site monitoring. The investigator will retain an electronic copy of the eCRFs and the sponsor will have an electronic copy of the eCRFs. The Investigator will review all completed eCRFs and will verify the accuracy of the completed eCRFs.

The study will be monitored by employees or authorized representatives of the Sponsor. Each site will be monitored on a periodic basis to perform the following, and other activities as required:

- to ensure regulatory compliance
- to perform verification of source documentation for each subject to ensure accurate and complete collection of required data, and accurate and complete recording of data on eCRFs
- to ensure adherence to the requirements of the protocol including, but not limited to, required study procedures and safety reporting

The frequency of monitoring may vary depending on protocol compliance, enrollment rate and/or quality of the data. The Investigator and staff are expected to cooperate by preparing

and providing all requested study documentation at each site visit. The Investigator and staff are responsible for being present or available for consultation during routinely scheduled site visits. Between site visits, the site may also be routinely contacted by phone to assess enrollment, subject status and to address study-related issues.

Employees or authorized representatives of the Sponsor may conduct an audit of a clinical site at any time during or after completion of the study. The Investigator will be informed if an audit is to take place and advised of the audit scope. Representatives of the FDA or other regulatory agencies may also conduct an audit of the study. If informed of such an audit, the Investigator should notify the Sponsor immediately. The Investigator will ensure that auditors have access to the, study site facilities, original source documentation, eCRFs, and all other study files.

11.5 Study Termination

Upon completion of the study, the following activities, when applicable, will be conducted by the study monitor and the Investigator:

- return of all study data to the Sponsor
- data clarifications and/or resolutions
- accounting, reconciliation, and final disposition of used and unused investigational product; and
- review of site study records for completeness

In addition, the Sponsor reserves the right to temporarily suspend or prematurely terminate this study for any reason.

If the study is suspended or terminated for safety reasons, the Sponsor will promptly inform the Investigator, and will also inform the regulatory authorities of the suspension or termination of the study and the reasons for the action. The Investigator is responsible for promptly informing the IRB/IEC and providing the reasons for the suspension or termination of the study.

If the study is prematurely terminated, all study data must be returned to the Sponsor. In addition, the clinical site must conduct the final disposition of all unused investigational product in accordance with the Sponsor's procedures for the study.

11.6 Records Retention

The Investigator must retain all study records required by the Sponsor and by the applicable regulations in a secure and safe facility. The Investigator must consult an appropriate the Sponsor representative before disposal of any study records, and must notify the Sponsor of any change in the location, disposition or custody of the study files. The Investigator/institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (e.g., subject charts) as well as any original source documents that are electronic as required by applicable regulatory requirements. All study records must be retained for at least 2 years after the last approval of a marketing application in the US or an ICH region and until (1) there are no pending or contemplated marketing applications in the US or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. The Investigator/institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by the Sponsor agreement. The Sponsor must be notified and will assist with retention should Investigator/institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of the Sponsor to inform the Investigator/institution as to when these documents no longer need to be retained.

11.7 Confidentiality of Information

Subject medical information obtained for the purposes of this study is confidential; disclosure to third parties, other than those noted below, is prohibited. Upon the subject's request and written permission, medical information may be given to his/her personal physician or other appropriate medical personnel responsible for the subject's welfare.

Data generated for this study must be available for inspection on request to representatives of the FDA, other national or local health authorities, the Sponsor, and the associated IRB/IEC.

Release of research results or data that reveal subject names or other identifiers, such as photographs, audio or videotapes, must be carried out in accordance with Department of Health and Human Services Final Standards for Privacy of Individual Health Information,

45 CFR 164.508. Written authorization must be obtained from the subject and IRB/IEC prior to the release of such information. Identifiable subject data may not be used for purposes of promoting the investigational product.

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13 APPENDICES

Appendix A: Compliance with Xenotransplantation Guidelines

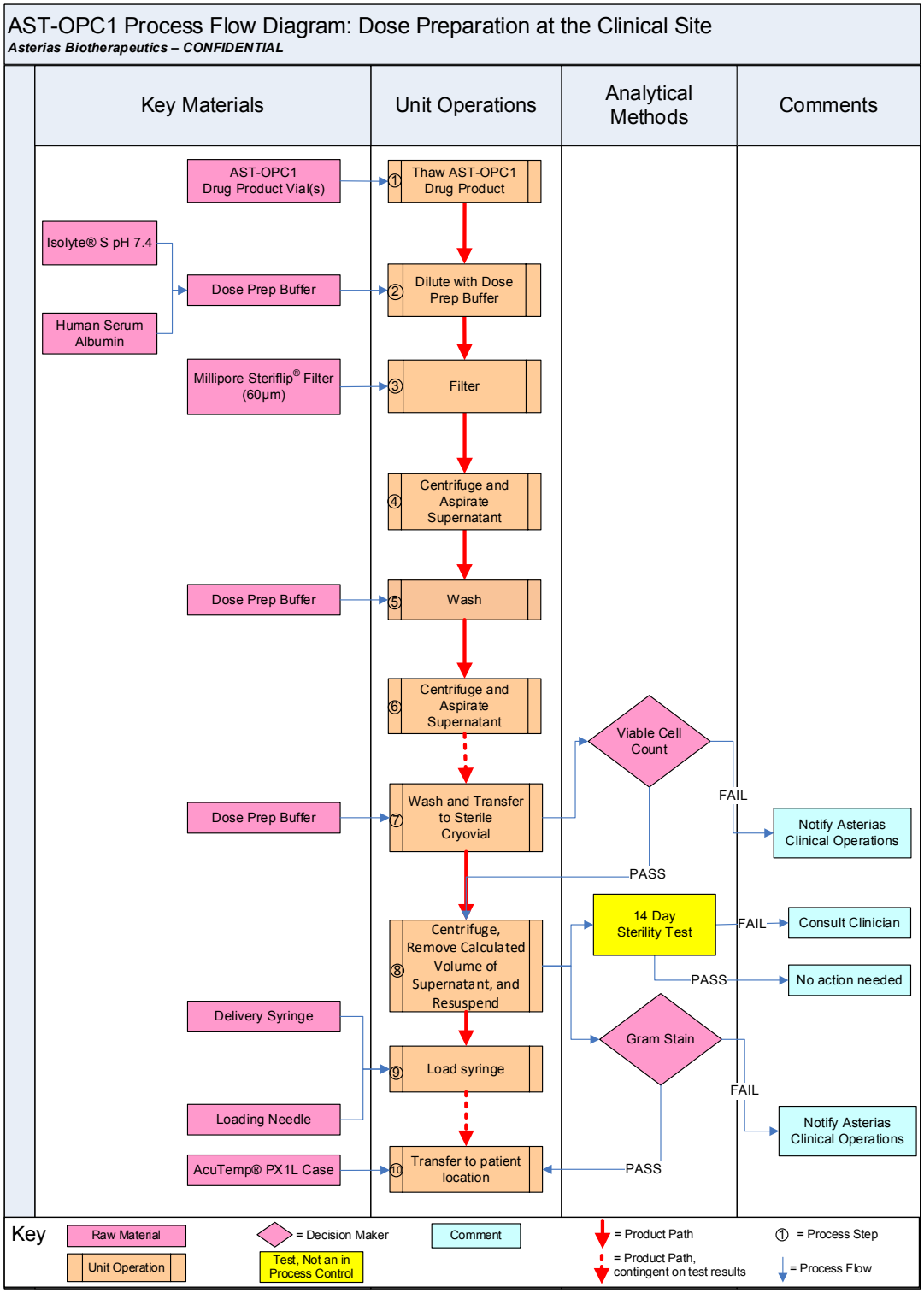
AST-OPC1 is produced from the H1 uhESC line, which was originally derived at the University of Wisconsin in 1998. The H1 line was established by isolating the inner cell mass from a donated excess embryo and culturing the cells from this mass on a layer of irradiated murine embryonic feeder cells.

As a consequence of this ex vivo contact between H1 undifferentiated cells and murine cells, AST-OPC1 meets the Public Health Service definition of a xenotransplantation product (Public Health Service 2001, CBER 2003). However, the Sponsor subsequently adapted the H1 uhESC line to feeder-free culture conditions prior to generating the H1 master cell bank (MCB) that is used to produce AST-OPC1. The Sponsor has also performed extensive testing of the H1 MCB and this testing has demonstrated that the MCB is free from known murine viral and retroviral adventitious agents. Because of the demonstrated freedom from known murine viral and retroviral adventitious agents, the FDA has recommended that a subset of the FDA and Public Health Services xenotransplantation guidance be followed. The plan below represents compliance with the FDA recommendations with respect to xenotransplantation issues:

- The Sponsor will archive vials from each manufactured lot of AST-OPC1 indefinitely for xenotransplantation safety monitoring. These samples may prove useful during retroactive investigation of clinically relevant events that might occur in subjects treated with AST-OPC1. The Sponsor will archive these vials at BioStorage Technologies, Inc. or an equivalent biorepository that is capable of storing samples in vapor phase liquid nitrogen and is fully compliant with applicable industry, regulatory, biosafety, and biomaterial handling regulations. In addition, the contract facility will have an inventory tracking system that is compliant with 21 CFR Part 11.
- The sample informed consent form includes language informing subjects that they will be receiving a xenotransplantation product.
- The sample informed consent requests that the subject inform their personal physician that the subject has received a xenotransplant product.
- At baseline (before AST-OPC1 injection), about 8 mL of blood will be obtained and divided into three (3) to five (5) 0.5-mL aliquots of citrated or EDTA-anticoagulated plasma and ≥ 2 aliquots of viable, cryopreserved leukocytes (1.0×10^7 cells/aliquot). These samples will be frozen on dry ice and sent to the same contract facility noted above. The samples will be archived indefinitely at -80°C.
- At postinjection Days 30 and 365, additional blood samples will be obtained, divided into aliquots, and archived indefinitely as noted above.

- In the event of a subject death, an autopsy will be requested and, if granted, a biopsy of the treatment site and any major organs related to the clinical circumstances resulting in death will be collected as: snap-frozen samples stored at -70°C; paraffin embedded tissue; and tissue that may be used for performing electron microscopy. Samples will be stored at BioStorage Technologies, Inc. or an equivalent biorepository. The sample informed consent form includes language stating that an autopsy will be requested in the event of a subject death.
- The Sponsor will maintain a database that captures the following information:
  - link between the lot number for the final product and the subject identifier
  - identification of dates and location for any archived final product and subject baseline samples
  - documentation of clinical events that could be related, potentially, to murine-derived infectious agents including fever of unknown etiology, neoplasias and neurological disorders
- All subjects who receive AST-OPC1 will receive long-term follow-up under a companion protocol, for which informed consent will be requested at the same time that consent for this protocol is obtained. The follow-up plan contains the following elements (see companion protocol AST-OPC1-02 for details):
  - request that the subject promptly notifies the Investigator of any change in address and/or telephone number
  - evaluation at 2 years after AST-OPC1 administration and annually thereafter through 5 years. Evaluation will include a physical exam, neurological exam, MRI scan of the spinal cord, and laboratory assessments as indicated.
  - blood sample at year 5 will be obtained and archived for xenotransplantation monitoring.
  - annual telephone contact at Years 6–15 for any adverse changes in neurological status or serious adverse health problems (if yes, subject will be asked to return to clinic for evaluation)

Appendix B: Flow Diagram of AST-OPC1 Dose Preparation



Appendix C: Site Personnel Training

Surgeon Training

Each surgeon must be trained and qualified on the use of the syringe positioning device prior to their first administration of AST-OPC1. Training will be provided by Sponsor personnel and an expert spine neurosurgeon who is familiar with the device. The training and qualification will be conducted using human cadavers at a facility equipped for surgical training on spine procedures.

The training will consist of: (1) an oral review of the Instructions for Use manual and the administration procedures, and (2) hands-on demonstration of the syringe positioning device setup and administration of AST-OPC1 into the cervical spinal cord in a human cadaver. Qualification will be performed by having the surgeon independently set up the syringe positioning device and administer AST-OPC1 at 2 spinal levels under the observation of Sponsor personnel and an expert spine neurosurgeon. If the injection(s) are not satisfactory in the expert's opinion, then the trainee must repeat the procedures, as appropriate.

Satisfactory or unsatisfactory completion of the injection qualification will be documented by the expert neurosurgeon.

Radiologist Training

MRIs will be read by a small, central neuroradiology group that has been trained and achieved consensus on how to interpret pre- and poststabilization images. Training will consist of discussion between radiologists and agreement on image interpretation for parameters such as length of contusion, length of edema, amount of spinal cord compression, and evidence of laceration or transection.

ISNCSCI Assessment Training

In order to perform the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) sensory/motor examination for this study, the assessors at each site must be therapists and/or physicians with at least 2 years of documented experience (e.g., in their curriculum vitae or other appropriate documentation) in frequently conducting the ISNCSCI exam. In addition, all assessors must submit a certificate confirming successful completion of the InSTeP training course on the American Spinal Injury Association website within the previous two years.

Appendix D: International Standards for Neurological Classification of Spinal Cord Injury

The ISNCSCI is the most commonly used neurological examination tool for classifying severity of SCI for both clinical and research purposes (ASIA 2002). A reference manual was developed to support the motor/sensory examination, as well as scoring, scaling and application (ASIA 2003).

The neurological examination consists of both motor and sensory examinations that have been standardized to promote consistency (Figure 13-1). The ISNCSCI examination consists of the following:

- Motor examination: 10 key muscles are tested in each half of the body. Each muscle is graded from 0 to 5 and then the total score is calculated, the maximum being 100.
- Sensory examination: 28 key regions are tested in each half of the body for pinprick and light touch sensations. Each region is given a score of 0 (absent), 1 (impaired), 2 (normal) or NT (not testable). The total score is then calculated, with a maximum of 112.

In addition, based on the data collected from the motor and sensory examinations, neurological level, completeness of neurological loss, the zone of partial preservation and the ASIA Impairment Scale can be determined.

Figure 13-1: ISNCSCI Scoring System

Front Side

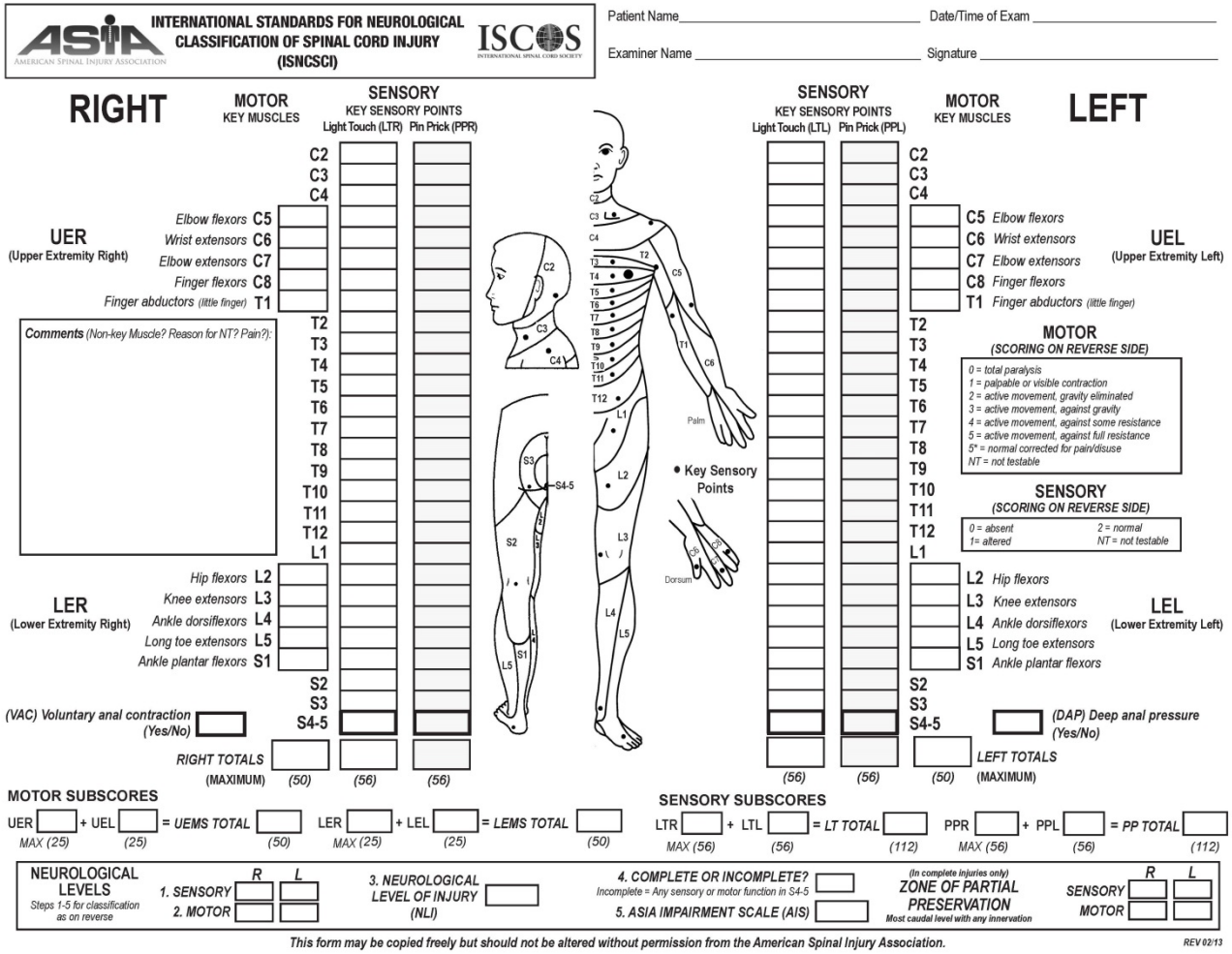


Figure 13-1 (contd.): ISNCSCI Scoring System (Back Side)

Muscle Function Grading

0 = total paralysis

1 = palpable or visible contraction

2 = active movement, full range of motion (ROM) with gravity eliminated

3 = active movement, full ROM against gravity

4 = active movement, full ROM against gravity and moderate resistance in a muscle specific position

5 = (normal) active movement, full ROM against gravity and full resistance in a functional muscle position expected from an otherwise unimpaired person

5* = (normal) active movement, full ROM against gravity and sufficient resistance to be considered normal if identified inhibiting factors (i.e. pain, disuse) were not present

NT = not testable (i.e. due to immobilization, severe pain such that the patient cannot be graded, amputation of limb, or contracture of > 50% of the normal range of motion)

Sensory Grading

0 = Absent

1 = Altered, either decreased/impaired sensation or hypersensitivity

2 = Normal

NT = Not testable

Non Key Muscle Functions (optional)

May be used to assign a motor level to differentiate AIS B vs. C

Movement	Root level
Shoulder: Flexion, extension, abduction, adduction, internal and external rotation **Elbow:** Supination	C5
Elbow: Pronation **Wrist:** Flexion	C6
Finger: Flexion at proximal joint, extension. **Thumb:** Flexion, extension and abduction in plane of thumb	C7
Finger: Flexion at MCP joint **Thumb:** Opposition, adduction and abduction perpendicular to palm	C8
Finger: Abduction of the index finger	T1
Hip: Adduction	L2
Hip: External rotation	L3
Hip: Extension, abduction, internal rotation **Knee:** Flexion **Ankle:** Inversion and eversion **Toe:** MP and IP extension	L4
Hallux and Toe: DIP and PIP flexion and abduction	L5
Hallux: Adduction	S1

ASIA Impairment Scale (AIS)

A = Complete. No sensory or motor function is preserved in the sacral segments S4-5.

B = Sensory Incomplete. Sensory but not motor function is preserved below the neurological level and includes the sacral segments S4-5 (light touch or pin prick at S4-5 or deep anal pressure) AND no motor function is preserved more than three levels below the motor level on either side of the body.

C = Motor Incomplete. Motor function is preserved below the neurological level**, and more than half of key muscle functions below the neurological level of injury (NLI) have a muscle grade less than 3 (Grades 0-2).

D = Motor Incomplete. Motor function is preserved below the neurological level**, and at least half (half or more) of key muscle functions below the NLI have a muscle grade ≥ 3.

E = Normal. If sensation and motor function as tested with the ISNCSCI are graded as normal in all segments, and the patient had prior deficits, then the AIS grade is E. Someone without an initial SCI does not receive an AIS grade.

** For an individual to receive a grade of C or D, i.e. motor incomplete status, they must have either (1) voluntary anal sphincter contraction or (2) sacral sensory sparing with sparing of motor function more than three levels below the motor level for that side of the body. The International Standards at this time allows even non-key muscle function more than 3 levels below the motor level to be used in determining motor incomplete status (AIS B versus C).

NOTE: When assessing the extent of motor sparing below the level for distinguishing between AIS B and C, the ***motor level*** on each side is used; whereas to differentiate between AIS C and D (based on proportion of key muscle functions with strength grade 3 or greater) the ***neurological level of injury*** is used.

ASIA
AMERICAN SPINAL INJURY ASSOCIATION

INTERNATIONAL STANDARDS FOR NEUROLOGICAL CLASSIFICATION OF SPINAL CORD INJURY

ISCOS
INTERNATIONAL SPINAL CORD SOCIETY

Steps in Classification

The following order is recommended for determining the classification of individuals with SCI.

1. Determine sensory levels for right and left sides.
The sensory level is the most caudal, intact dermatome for both pin prick and light touch sensation.

2. Determine motor levels for right and left sides.
Defined by the lowest key muscle function that has a grade of at least 3 (on supine testing), providing the key muscle functions represented by segments above that level are judged to be intact (graded as a 5).
Note: in regions where there is no myotome to test, the motor level is presumed to be the same as the sensory level, if testable motor function above that level is also normal.

3. Determine the neurological level of injury (NLI)
This refers to the most caudal segment of the cord with intact sensation and antigravity (3 or more) muscle function strength, provided that there is normal (intact) sensory and motor function rostrally respectively.
The NLI is the most cephalad of the sensory and motor levels determined in steps 1 and 2.

4. Determine whether the injury is Complete or Incomplete.
(i.e. absence or presence of sacral sparing)
*If voluntary anal contraction = **No** AND all S4-5 sensory scores = **0** AND deep anal pressure = **No,** then injury is **Complete.***
*Otherwise, injury is **Incomplete.***

5. Determine ASIA Impairment Scale (AIS) Grade:

Is injury Complete? If YES, AIS=A and can record ZPP (lowest dermatome or myotome on each side with some preservation)

NO ↓

Is injury Motor Complete? If YES, AIS=B

NO ↓ (No=voluntary anal contraction OR motor function more than three levels below the motor level on a given side, if the patient has sensory incomplete classification)

Are at least half (half or more) of the key muscles below the neurological level of injury graded 3 or better?

NO ↓ AIS=C

YES ↓ AIS=D

If sensation and motor function is normal in all segments, AIS=E
Note: AIS E is used in follow-up testing when an individual with a documented SCI has recovered normal function. If at initial testing no deficits are found, the individual is neurologically intact; the ASIA Impairment Scale does not apply.

Appendix E: Magnetic Resonance Imaging Procedures

Background

MRI will be performed to accomplish several objectives, including:

- determination of eligibility for study participation
- identification of the target site for AST-OPC1 injection
- evaluation of the injection site for safety
- evaluation of distant regions of the CNS for safety
- acquisition of data for possible research analyses

The imaging protocol for the clinical safety evaluation was designed with consideration given to the results of the nonclinical biodistribution studies of AST-OPC1 following injection into the cervical spinal cord as described in Section 1.2.4.

Based on these data, all MRI exams will include imaging of the cervical spine. Sagittal views of the cervical spine will include a field of view that extends from the top of the cerebellum to the upper thoracic spine (approximately T1-T2 level). Selected MRI exams (baseline, Day 365) will also include imaging of the brain. All MRI exams will include imaging with and without gadolinium-DTPA contrast to detect, evaluate, and monitor any potentially enlarging abnormal cysts or masses.

Standard Imaging Parameters

All MR imaging should be performed on systems with a static magnetic field strength of at least 1.5 Tesla in order to ensure that high-resolution images with good signal-to-noise can be obtained. All MR images of the spine should be obtained with a phased array receive coil (or equivalent) for the same reason as above.

Suggested imaging sequences for spine MRI include:

- Sagittal T1-weighted spin-echo and T2-weighted fast spin-echo
  - field of view: top of cerebellum to upper thoracic spine (approx. T1-T2)
  - slice thickness: 3 mm + 0.3 mm gap between slices
  - T1 scans: TR/TE/nex: 600/20/2
  - T2 scans: TR/TE/ETL/nex: 2000-3000/80-100/4-8/2-4
  - recommendations to minimize artifacts due metallic implants: turn off fat suppression and increase receiver bandwidth to maximum allowed by the system

- Axial T1-weighted spin-echo and T2-weighted fast spin-echo
  - Number of slices: 16, with middle slices at the level of the injury epicenter
  - Slice thickness: 4 mm + 1 mm gap between slices
  - T1 scans: TR/TE/nex: 600/20/2
  - T2 scans: TR/TE/ETL/nex: 2000-3000/80-100/4-8/2-4
  - Recommendations to minimize artifacts due metallic implants: turn off fat suppression and increase receiver bandwidth to maximum allowed by the system

All MRI exams will include postcontrast (gadolinium-DTPA) T1-weighted images in the sagittal and axial planes with the same parameters used for the respective pre-contrast images (unless the subject is allergic to contrast).

Suggested MRI scans of the brain include routine T1-weighted and T2-weighted scans in the sagittal and axial planes, plus postcontrast T1-weighted images.

Central Reading of MRI Scans

All study-driven MRI reading will be performed by a central group of neuroradiologists with extensive experience in MRI evaluation of spinal cord trauma. The images will be sent to the central group electronically via an imaging contract research organization.

The poststabilization screening/baseline MRI scan will be used to determine the whether the following eligibility criteria are met:

- single spinal cord lesion on a post-stabilization magnetic resonance imaging (MRI) scan, with sufficient visualization of the spinal cord injury epicenter and lesion margins to enable post-injection safety monitoring
- absence of anatomical transection or significant laceration
- spinal cord lesion with anteroposterior diameter of the spinal cord ≥ 2 mm at point of maximal compression on a midline sagittal image from a poststabilization MRI

The screening/baseline MRI will also be used to make a preliminary determination of the target site for AST-OPC1 injection. This site should be near the caudal margin of the injury epicenter, and within damaged, but nonhemorrhagic tissue.

Several additional parameters may be measured for future research analyses. These may include:

- Length of contusion (ie, maximal extent of petechial hemorrhages)
- Presence/absence of intramedullary hemorrhage
- Length of intramedullary hemorrhage

- Length of edema
- Percent compression at epicenter

Following AST-OPC1 injection, the MR images will be assessed according to standard practice and with specific attention to the following potential risks:

- intramedullary hemorrhage, CSF leak, epidural abscess, infection
- evidence of expanding cyst or mass at the injection site or elsewhere in CNS
- evidence of inflammatory lesion(s) at injection site or elsewhere in CNS
- evidence of CSF flow obstruction