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Project Title:	Development of a Splice-Switching Antisense Oligonucleotide for the Treatment of Spinal Muscular Atrophy
Principal Investigator Name:	Christian Lorson
CONTRACT ORGANIZATION:	Shift Pharmaceuticals, 3908 Foxcreek Way, Columbia, MO
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PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland

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OCTOBER 2019	ANNUAL	30 SEP 2018 - 29 SEP 2019

4. TITLE AND SUBTITLE

Development of a Splice-Switching Antisense Oligonucleotide for the Treatment of Spinal Muscular Atrophy

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5b. GRANT NUMBER

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6. AUTHOR(S)

Christian Lorson

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5d. PROJECT NUMBER

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5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Shift Pharmaceuticals, 3908 Foxcreek Way
Columbia, MO

8. PERFORMING ORGANIZATION REPORT NUMBER

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13. SUPPLEMENTARY NOTES

14. **ABSTRACT: Spinal Muscular Atrophy (SMA), an Area of Encouragement for the CDMRP Medical Technology/Therapeutic Development, is a devastating neurodegenerative disease and is the leading genetic cause of infantile death worldwide, and therefore commonly affects families within the armed services, often with devastating emotional and financial consequences. SMA is marked by severe neurodegeneration and skeletal muscle wasting, similar to the muscle wasting and weakness in active duty soldiers recovering from combat injuries and/or environmental toxins. Additionally, SMA is remarkably similar to ALS, a deadly neurological disease that has been shown to have an increased frequency in Gulf War veterans. The current project focuses upon the pre-clinical development of a uniquely targeted nucleic acid-based therapeutic to treat all forms of SMA. Our strategy is comprehensive and continues to lead toward a therapeutic that could have immediate as well as longterm positive impacts upon active and retired service members and their families struggling with SMA. The gene responsible for SMA is called survival motor neuron-1 (SMN1). SMN2 is nearly identical to SMN1, however, mutations in SMN2 have no clinical consequence if SMN1 is retained. The reason why SMN2 cannot prevent disease development in the absence of SMN1 is that the majority of SMN2-derived transcripts are alternatively spliced, resulting in a truncated and unstable protein. The presence of SMN2 opens the door to a number of exciting therapeutic strategies, including alternative splicing modulation of SMN2 exon 7. In this project, we will build upon our previous "basic" molecular biology findings that identified "Element 1" (E1) as a potent repressor of SMN2 exon 7 inclusion. The molecular genetic context makes SMA especially attractive for nucleic acid-based therapeutics designed to modulate pre-mRNA splicing for several reasons**

15. SUBJECT TERMS
NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	21	19b. TELEPHONE NUMBER *(include area code)*

1. Accomplishments: The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project identify these dates and show actual completion dates or the percentage of completion.

The overarching goal of this project is to provide critical pre-clinical data as we move our lead compound, $E1^{v1.11}$, to an IND submission with the FDA.
Aim 1: Dose-escalation in SMA mice
Aim 2: SMN protein levels in SMN2 mice
Aim 3: PK/tox studies in CD-1 mice
Aim 4: Examine compound stability
Aim 5: IND application

During the second reporting period (starting April 2019), Shift Pharmaceuticals worked on each of the 5 grant aims as described below:

- Aim 1: We conducted dose-range finding efficacy studies in the SMNΔ7 mouse model and correlated the findings with a comprehensive published study on Spinraza (Passini, 2011).

- Aim 2: We initiated studies in *SMN2* mice to determine how long SMN protein is elevated. Mice were ordered and treated, tissue from 4 of 6 timed cohorts were collected, with remaining 2 cohorts completing at the end of October and November, respectively. Analysis of SMN protein levels will be conducted after all animals have completed the study.

- Aim 3: We continued the preliminary pharmacokinetics and toxicity studies in newborn CD-1 mice. The toxicity studies were completed and the pharmacokinetic study analysis is in progress.

- Aim 4: We continued to examine our research-grade $E1^{v1.11}$ used in our research studies. Since our animal research studies are almost complete, we manufactured pre-GMP drug substance material to use in GLP animal studies for our IND application (Aim 5). Comparable data from our research and pre-GMP material was obtained to bridge the research studies with the GLP studies.

- Aim 5: IND application. Since data from the mouse model studies demonstrate significant extended survival and wildtype mouse toxicity studies indicate a broad safe profile, we believe we are ahead of our development timeline such that GLP animal studies that will enable the IND submission can begin in early 2020. We developed a list of nonclinical studies that we believe are needed to support the IND and eventual New Drug Application (NDA) for chronic IT administration in a pediatric patient population, based on the clinical development program, review of FDA's Summary Basis of Approval for several approved compounds to understand what the Agency required for market approval, review of related publications, and work with several nonclinical CROs. To mitigate risk in our programs, we requested and were granted a Written Response Only meeting with FDA. We expect to receive FDA's guidance and/or agreement on our proposed nonclinical and drug substance/drug product programs in November 2020.

The team continues to meet in person quarterly and holds weekly phone calls to assess the progress, including a weekly "team" call on Mondays and a weekly "science" call on Fridays. On the following pages, a more detailed breakdown of our progress on each aim is included.

Aim 1: Dose escalation in SMA mice

Shift has demonstrated dose-dependent efficacy of E1$^{v1.11}$ in the SMNΔ7 (*Smn*$^{-/-}$; *SMN*$^{hi/hi}$; *SMNΔ7*$^{+/+}$) mouse model based on survival, time-to-right performance, and weight gain. Following intracerebroventricular (ICV) administration of E1$^{v1.11}$ at doses up to 2000 µg within 36 hours of birth, survival was significantly extended in the SMNΔ7 mice (Figure 1-1), time-to-right performance improved, and weight gain elevated.

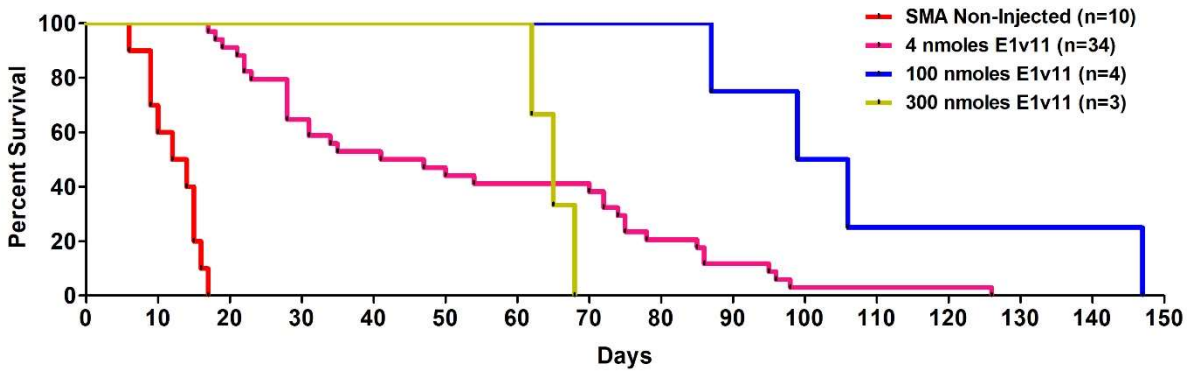

Figure 1-1 **Effects of E1$^{v1.11}$ in SMA mice.** Life span for SMNΔ7 mice for a dose-ranging study following single ICV injections on post-natal (P1) of E1$^{v1.11}$ (27 µg [4 nmol], 670 µg [100 nmol], and 2000 µg [300 nmol]).

Shift compared the survival results in SMNΔ7 mice from E1$^{v1.11}$ with published data from a comprehensive study of Spinraza (ASO-10-27) (Passini, 2011). The published data demonstrated that Spinraza's maximum mean life-span extension was achieved at 4 µg, resulting in a mean life span of ~10 days longer than untreated SMA animals. In comparison, E1$^{v1.11}$ administered at 670 µg extended survival an average of 95 days more than untreated SMNΔ7 mice (Figure 1-2). Spinraza doses up to 8 µg extended survival; however, doses of 16 µg or higher resulted in a significant death rate within 24 hours of administration. In contrast, E1$^{v1.11}$ has an effective concentration range that spans greater than 10-fold and did not elicit any overt toxicity effects at high doses.

Together, with safety data described below, E1$^{v1.11}$ may have greater efficacy potential and a broader safety window compared with Spinraza. Therefore, Shift plans to move forward with an IND-enabling program.

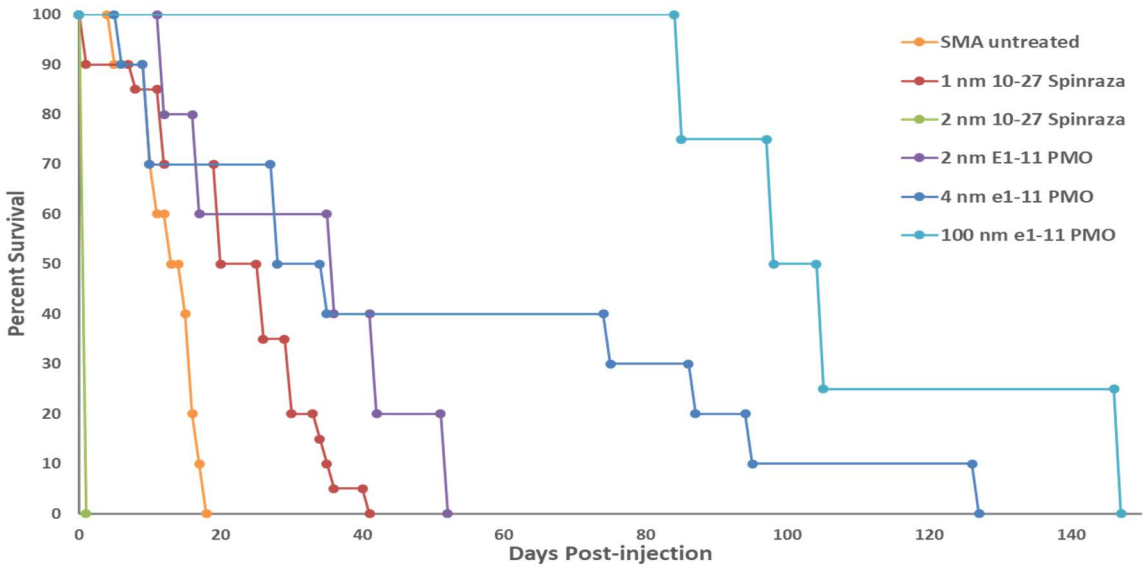

Figure 1-2 **Efficacy comparison of Spinraza (ASO-10-27) and E1$^{v1.11}$ in SMNΔ7 mice.** In each cohort, a single bolus injection of ASO was delivered at post-natal day 1 (P1) via intracerebroventricular injection. Untreated and Spinraza (ASO-10-27; Passini, 2011) treated with 8 µg (1 nmole) or 16 µg (2 nmole) are shown (orange, red, and green, respectively). E1$^{v1.11}$ treated with 13, 27, and 670 µg (2, 4, and 100 nmole) are shown.

Aim 2: SMN protein levels in *SMN2* mice

A pharmacodynamic study is currently being conducted to determine how long SMN protein is elevated in disease-relevant tissues following a single injection of E1$^{v1.11}$ into neonatal *SMN2* transgenic mice. The *SMN2* transgenic mouse is being used to assess SMN protein levels because these animals are asymptomatic and disease-free; however, they express the human genomic cassette expressing *SMN2*. The human *SMN2* gene maintains the appropriate splicing patterns that allows for the analysis of compounds that modulate exon 7 splicing, such as E1$^{v1.11}$. As a standard practice, using the "unaffected" mice eliminates any potential complications related to the SMA pathology.

In this study, *SMN2* transgenic mice were injected with 0, 27, 134, and 670 µg of E1$^{v1.11}$ on post-natal day 2 (P2). As a control, CD-1 mice were injected with 670 µg of E1$^{v1.11}$ on P2. Tissue samples from *SMN2* transgenic mice are being collected at: 1hr, 4 hr, 24 hr, P10, P30, P60, and P90 post-dose and assessed for pharmacodynamic parameters. Tissue samples from CD-1 mice are being collected on P10 and P90. This study is in progress; tissue from 4 of 6 timed cohorts have been collected with the remaining 2 cohorts completing at the end of October and November, respectively, and tissue assessed in December 2019 and Q1/2020.

We have obtained and are optimizing the Abcam SMN ELISA kit for detecting SMN protein in tissue homogenates. Figure 2 is a standard curve from a method development assay.

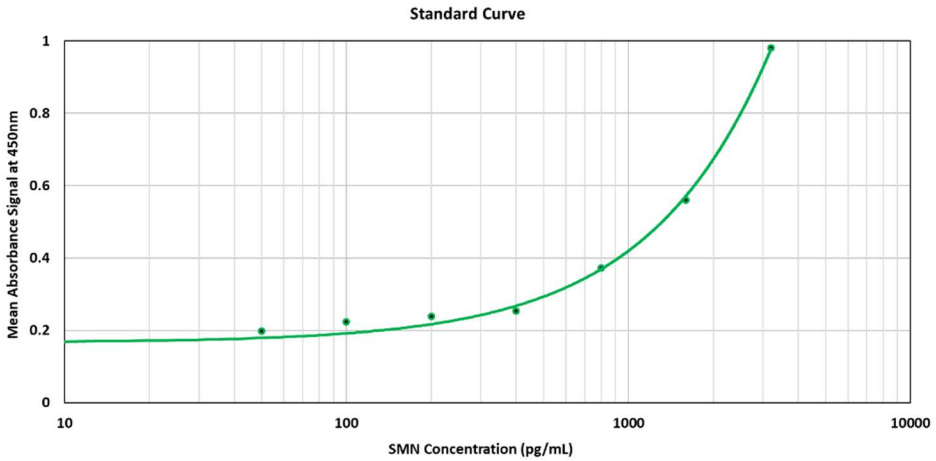

Figure 2 **Standard curve from Abcam SMN ELISA kit** for detecting SMN protein (pg/mL) in tissue homogenates.

Aim 3: Perform preliminary pharmacokinetics/toxicity studies

For Aim 3, Shift conducted a series of studies in neonate CD-1 mice to obtain toxicity and pharmacokinetic data following a single-dose, ICV administration of E1$^{v1.11}$ ranging from 13-2000 µg. Results from the studies demonstrate minimal difference between 0 and 2000 µg doses in toxicology based upon: observation, weight gain, organ weight, comprehensive clinical pathology, and histopathology. With no deaths or drug-related toxicities from doses up to 2000 µg, it appears E1$^{v1.11}$ has a broader safety window compared with Spinraza. With the completion of these studies, Shift has adequate data to proceed with the GLP safety studies that will support the opening of an IND.

Single-Dose, ICV Dose-Range-Finding Toxicity Studies in Juvenile CD-1 Mice

Newborn CD-1 mice were randomly selected at birth for E1$^{v1.11}$ dosing groups of 0, 13, 27, 134, 670, 1340, and 2000 µg. CD-1 mice received a single ICV injection of E1$^{v1.11}$ of the designated concentration on P2 (within 36 hours of birth), with 2000 µg being administered in two 1000 µg doses approximately 8 hours apart. Mice were allowed to recover for 14 days post-injection. The following parameters were evaluated in these studies. The toxicity studies have been completed with results described below. The pharmacokinetic study analysis is in progress and should be completed by the next progress report.

- Clinical observations: behavior, body and organ weights
- Clinical pathology:
  - Hematology: complete blood counts, RBC with reticulocyte count, WBC with differentiation, and platelet parameters
  - Clinical chemistry: blood urea nitrogen (BUN), creatinine, creatine kinase (CK), total protein, albumin, globulins, phosphorus, sodium, chloride, potassium, total CO_2, glucose, cholesterol, triglycerides, lactate dehydrogenase, calcium, total bilirubin, aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT)
- Histopathology: brain, liver, kidney, spleen, and heart
- Pharmacokinetics: E1$^{v1.11}$ levels in cerebral spinal fluid and tissue (brain, liver, kidney, heart, lung, kidney, spleen, liver, muscle).

Following administration, there were no acute deaths and the treatment was well-tolerated. There were no deaths reported during the study nor drug-related toxicities observed among any of the mice. Initial weights at birth of the individual mice were similar, with very small variations between litter mates. Mothers were attentive and nurturing. By P9-P10, all mice were active and responsive to intervention. Mouse growth and weight gain progressed for all cohorts throughout the study, with 2000 µg groups trending at slightly lower rates. During the weight measurements, mice were huddling together and

responded with normal movements. By P13-P14, mice were grooming themselves and had a clean fur. There were no lack of movements, abnormal activity, or hunched posture observed in any of the individual mice. Additionally, all mice were responsive and did not show signs of dehydration (loss in skin elasticity). At P14 post injection, animals were harvested for blood analysis and tissue samples. Organ weights were assessed by raw organ weight, percent ratio of organ to total body weight, and percent ratio of organ to brain weight. Organ weights fluctuated between dosing groups with no consistent dose-related effect observed.

Comprehensive Clinical Pathology

Overall, no test article related changes are observed. For most of the hematology and clinical chemistry parameters, no clinically relevant changes or trends were observed between treatment groups (Figure 3-1) or between gender. Most of the observed changes were of negligible magnitude, sporadic in nature and consistent with biologic variation. Changes in WBC parameters may indicate an inflammatory stimulus, but the changes do not appear to be dose dependent, are of small magnitude, and lack microscopic correlates. Confounding factors include pre-analytical sample interference (i.e. clotting or red blood cell rupture [i.e., hemolysis]) and age-related resulting in changes as noted in the report. The clinical pathology report, prepared by Comparative Clinical Pathology Services, is included as an attachment.

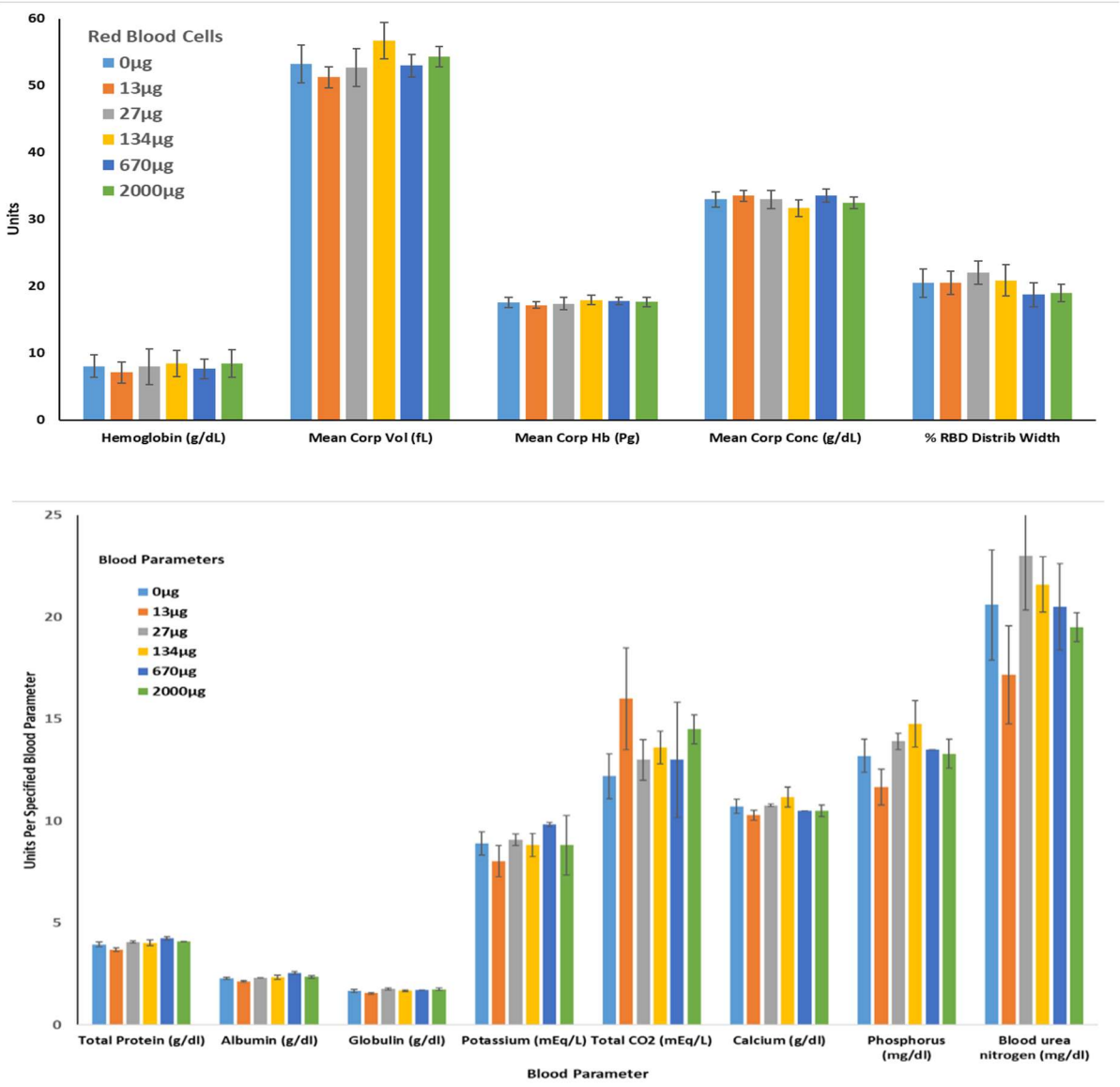

Figure 3-1 **Representative blood chemistries from CD-1 mice** 14 days after a single dose of 0, 13, 27, 134, 670, 2000 µg of E1$^{v1.11}$ on P1.

Histopathology

No treatment related histological findings were detected in the tissues examined. No adverse drug effects were noted. Some spurious, incidental, and background findings were observed, but not considered treatment-related (Figure 3-2). As neonates, the CD-1 mice were not fasted prior to euthanasia, and this may have contributed to the variation in hepatocellular vacuolation. Renal tubular dilation/lumen expansion may be associated with underdeveloped neonatal renal function. Internal nuclei in peripheral and axial muscles may be associated with muscular remodeling and development of neonates. These findings did not appear to be related to treatment group and/or were considered within normal limits for neonatal rodents. The histopathology report is included as an attachment.

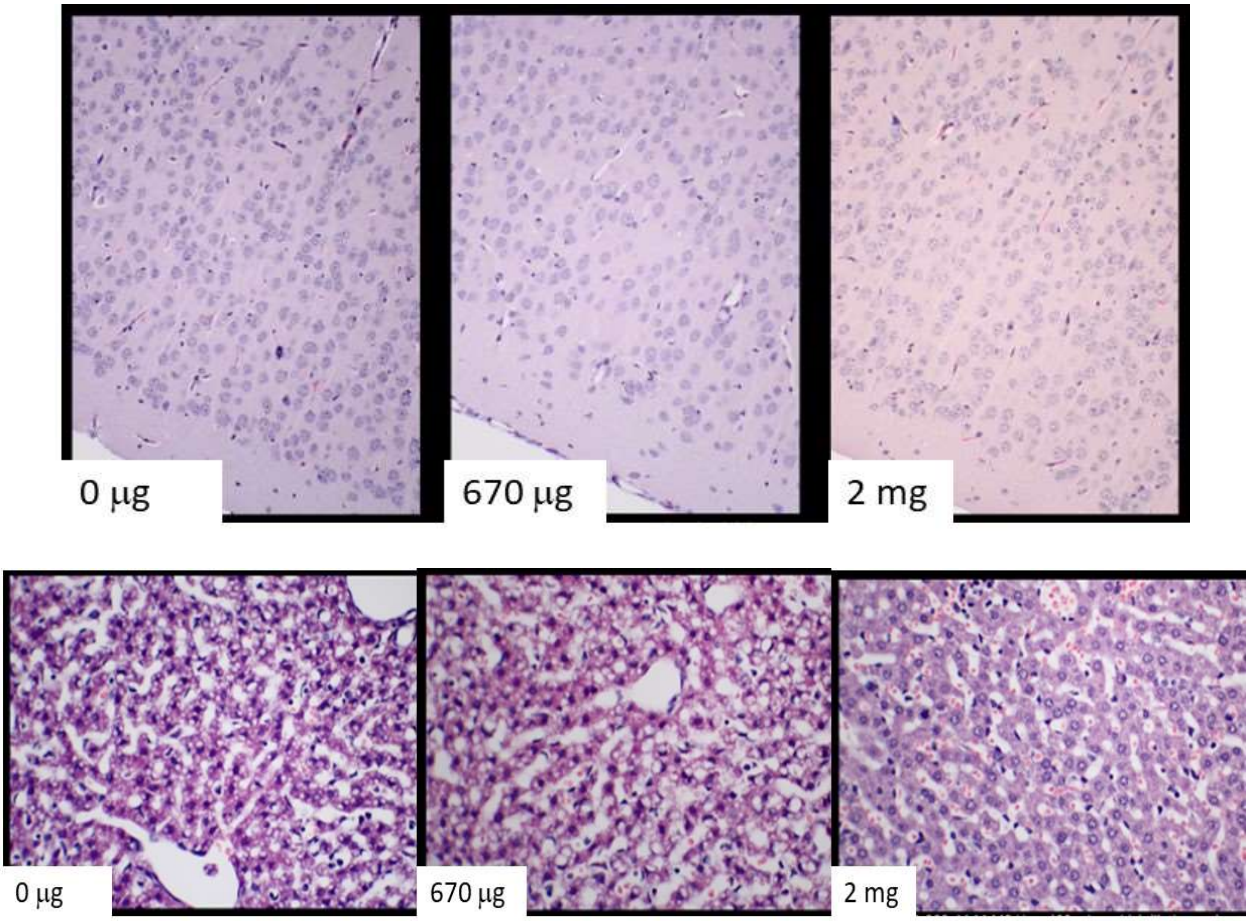

Figure 3-2 **Representative tissues sections** from CD-1 mice 14 days after a single ICV dose of 0, 13, 27, 134, 670, 1340, 2000 µg E1$^{v1.11}$ within 36 hours of birth. All images are 200x magnification. (TOP) Brain. (BOTTOM) Liver.

Pharmacokinetics

CD-1 mice were used to assess pharmacokinetics of E1$^{v1.11}$ following a single injection of 0, 27, 134, 670 µg of E1$^{v1.11}$ within 36 hours of birth. Cerebral spinal fluid and tissue (brain, liver, kidney, heart, lung, kidney, spleen, liver, muscle) samples were be collected at 0, 15 min, 60 min, 2 hr, 4 hr, 12 hr, 24 hr, and 72 hr post dose to assess E1$^{v1.11}$ levels with an ELISA method. Shift is currently developing the bioanalytical methods and optimizing the assay. Shift has constructed standard curves and QC concentrations for spiked mouse serum, and brain, kidney, and liver homogenates from treated mice. Preliminary method development results indicate that three different tissue homogenates spiked with E1$^{v1.11}$ diluted into the standards and QCs concentrations with mouse serum were similar (Figure 3-3). This suggests the E1$^{v1.11}$ /anti- E1$^{v1.11}$ PTO interact/protect similarly in different homogenate. These results provided confidence for the assay's ability to measure concentrations of E1$^{v1.11}$ similarly throughout tissues for this study. Preliminary pharmacokinetic results demonstrated a dose-response dependency in the mouse. E1$^{v1.11}$ levels were present in the brain, which increased quickly and decreased over 24-72 hrs. E1$^{v1.11}$ was identified in the liver and kidney, but in a lower concentration compared with the brain. The analysis of the remaining tissues samples is currently in progress.

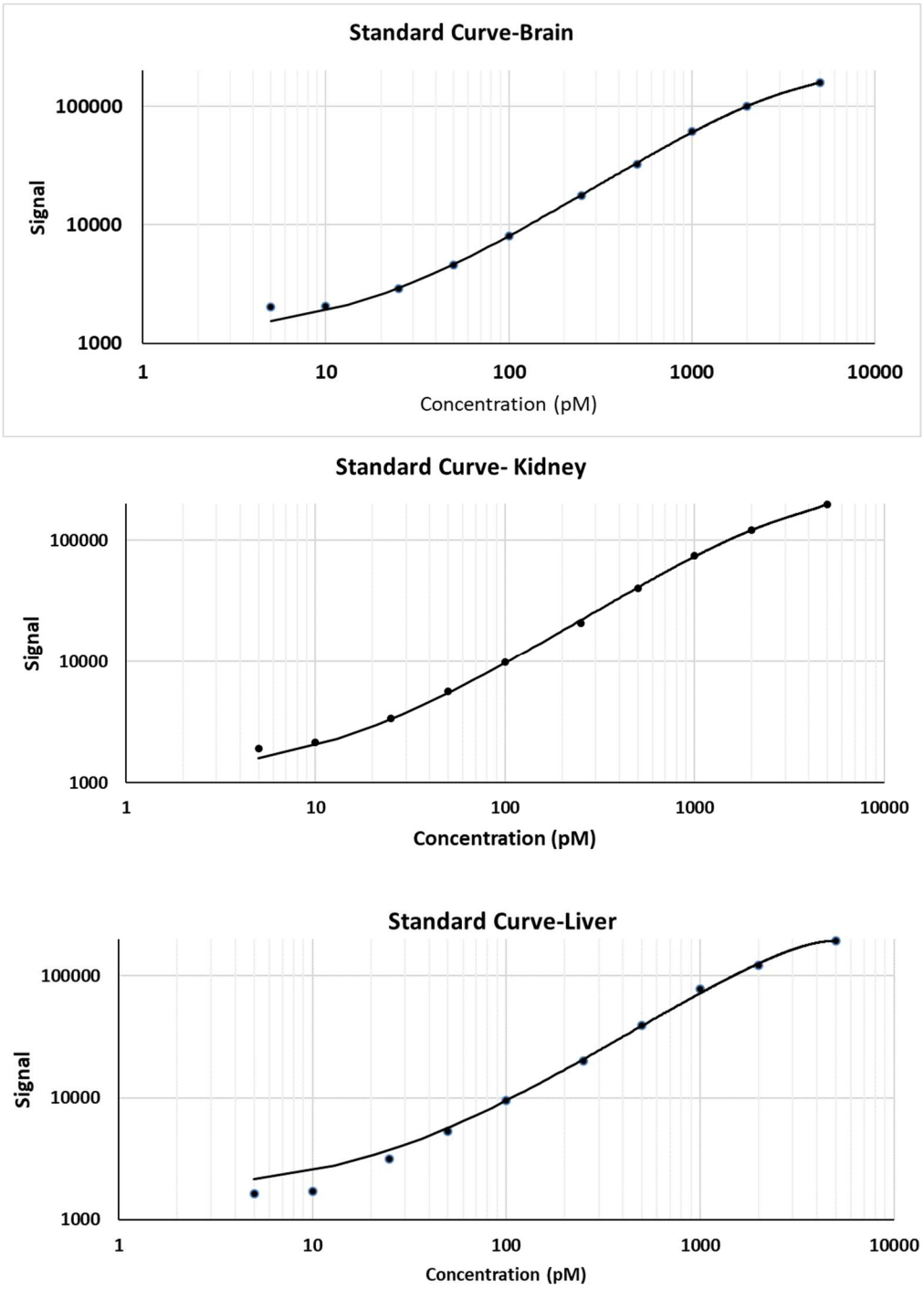

Figure 3-3 **Standard E1$^{v1.11}$ concentration curves**. Diluted E1$^{v1.11}$ homogenate to 100,000 pM before dilution of standard curve in mouse serum for (TOP) brain, (MIDDLE) kidney, and (BOTTOM) liver.

Aim 4: Detection and analysis of the E1$^{v1.11}$ PMO for purity and stability studies.

The E1$^{v1.11}$ research material used to conduct the animal studies presented in this update was further characterized during this reporting period. Characterization of the research material is needed to bridge the data from research studies to the IND-enabling program that will be conducted with pre-GMP material.

Since data from the mouse model studies demonstrate significant extended survival and wildtype mouse toxicity studies indicate a broad safe profile, Shift believes we are ahead of our development timeline such that GLP animal studies to enable submission an IND (Aim 5) can begin in early 2020. For these studies, pre-GMP material is needed. We have completed manufacturing of the pre-GMP drug substance material and analytical methods development. Figure 4-1 shows the HPLC purity assessment of a batch of pre-GMP E1$^{v1.11}$ drug substance. The synthetic approach for this material was optimized, as was the post-synthesis purification protocol.

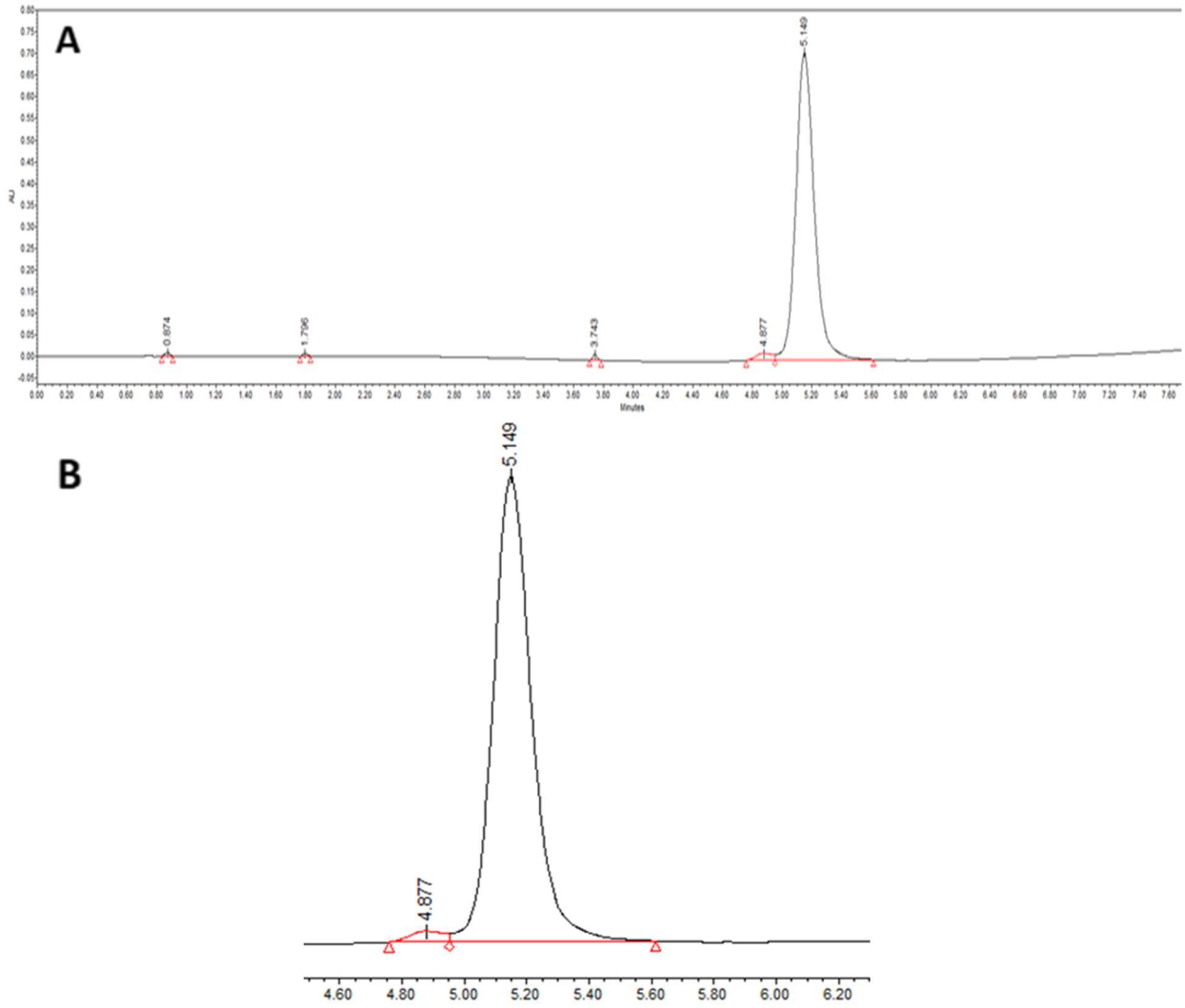

Figure 4-1 **HPLC Chromatogram of E1$^{v1.11}$** (A) HPLC Chromatogram of E1$^{v1.11}$ after purification (Abs: 260 nm) of GLP material. (B) Enlargement of same chromatogram with the area around 5.149 minutes.

Each of the peaks in Figure 4-1 were automatically quantitated using the Waters UPLC software an provided in Table 1. The peak at 4.877 min was identified using mass spectrometry and is shown in Figure 4-1. The conversion of one -N(CH$_3$)$_2$ group to a -OH (at low amounts) is a bi-product of the reaction chemistry and a very small chemical change to the molecule. Due to the large molecular weight and size of E1$^{v1.11}$, the fully optimized purification protocols are unable to separate the two molecules. Thus, all previous studies and future studies are used with the mixture of these two compounds. Figure 4-2 is a mass spectrum of the two eluted peaks shown in the HPLC Chromatogram in Figure 4-1.

Table 1 HPLC Peak Results				
Peak	**Retention Time (minutes)**	**Peak Area**	**Peak Height**	**% Area**
1	0.874	23,666	10,825	0.3195
2	1.796	16,620	8,233	0.2244
3	3.743	25,119	13,186	0.3392
4	4.877	149,849	20,088	2.0233
5	5.149	7,190,963	834,112	97.0936

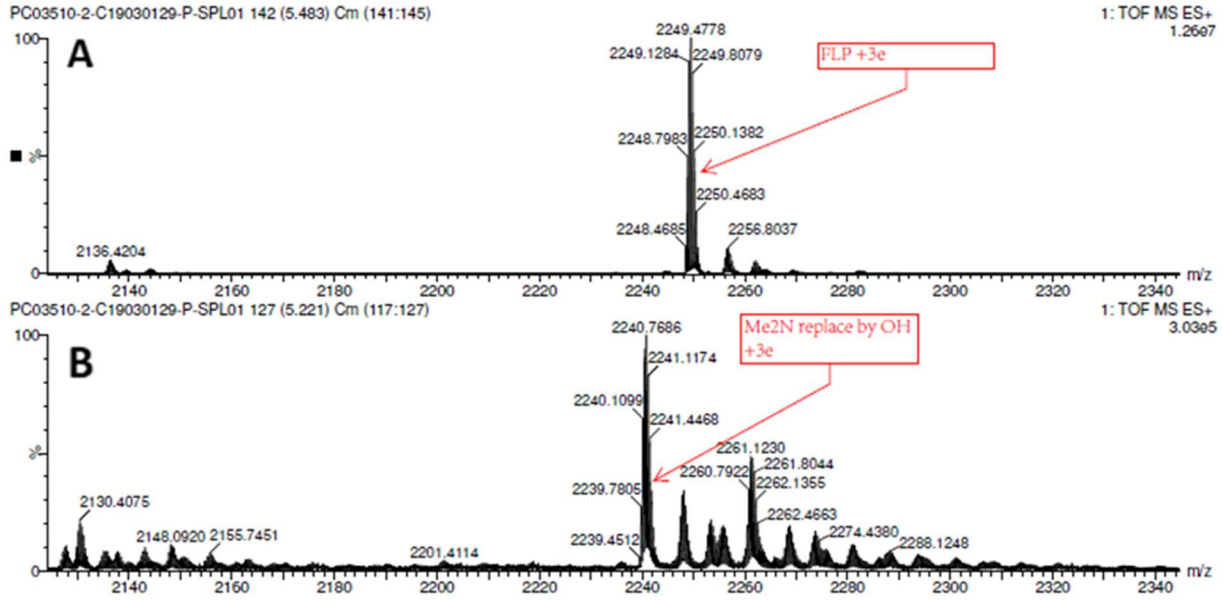

Figure 4-2 **Mass Spectrum of Eluted Solvent.** Mass spectrum of the eluted peaks (as shown in the HPLC Chromatogram in Figure 4-1) of the parent compound (A) and small impurity (B). This impurity at 4.877 minutes is E1$^{v1.11}$ with one -N(CH3)$_2$ group replaced by a -OH (mass/charge 2240.8).

Aim 5: IND application

Data from the mouse model studies demonstrate significant extended survival and wildtype mouse toxicity studies indicate a broad safe profile, which puts Shift ahead of our development timeline. Therefore, we began working on Aim 5, which included preparing for nonclinical GLP studies and initiating interactions with FDA.

Because SMA is a rare, severely debilitating, and life-threatening disease whose patient population is more than 50% pediatric patients, the IND-enabling program requires specialized nonclinical studies to support an intrathecal administration in predominantly young patients. Shift developed a list of nonclinical studies we believe are needed to support the IND (Table 2) and eventual New Drug Application (NDA) for chronic IT administration in a pediatric patient population, based on the clinical development program, review of FDA's Summary Basis of Approval for several approved compounds to understand what the Agency required for market approval, review of related publications, and work with several nonclinical CROs.

To mitigate risk in our development program and ensure that Shift conducts the appropriate animal studies, we are obtaining FDA guidance and agreement prior to conducting the IND-enabling studies. On September 5, 2019, Shift submitted a Type C (Consultation) meeting request to FDA's Center for drug Evaluation and Research, Division of Neurology Products. This meeting was granted as a Written Response Only. On October 3, 2019, we submitted the FDA meeting package and expect to receive FDA's guidance and/or agreement on our proposed nonclinical and CMC program around November 20.

Once we know what FDA will require in an IND-enabling program, we will initiate the GLP studies in late 2019 or Q1/2020.

Table 2 Nonclinical Safety Studies						
Type of Study	**Species**	**ROA**	**Dose Duration**	**Age at 1st Dose**	**Doses**	**GLP Status**
Studies to be Conducted Prior to the IND						
13-Week, SC Repeat-Dose Toxicity Study in Juvenile Rats with Recovery	Sprague-Dawley rat	SC	13 wks	<P10	0, low, mid, high	GLP
Single-dose, IT Dose-Range-Finding Toxicity Study in Juvenile Nonhuman Primate with Recovery	Cynomolgus monkey	IT	Single dose	12-18 mn	0, low, mid, high	Non GLP
13-Week, IT Repeat-Dose Toxicity Study in Juvenile Nonhuman Primate with Recovery	Cynomolgus monkey	IT	13 wks	12-18 mn	0, low, mid, high	GLP
Genotoxicity: Bacterial reverse mutation (Ames) test, In vitro micronucleus test, and In vivo micronucleus assay						GLP
Studies to be Conducted during Clinical Development to Support the NDA						
39-Week, Intermittent, Repeat-Dose Toxicity Study in Juvenile Nonhuman Primate with Recovery	Cynomolgus monkey	IT	39 wks	12-18 mn	0, low, mid, high	GLP
Fertility/Reproduction, Embryofetal, and Peri/Post-natal Developmental Toxicity (Rodent Segment I/II/III)	Sprague-Dawley rat	Per protocol	Per protocol	Per protocol	0, low, mid, high	GLP
Embryofetal Toxicity Study (Segment II)	New Zealand white rabbits	SC	12 days	Sexually mature	0, low, mid, high	GLP
ICV = intracerebroventricular IT = intrathecal	P = post-natal day ROA = route of administration			SC = subcutaneous		

References

Passini MA, Bu J, Richards AM, Kinnecom C, Sardi SP, Stanek LM, Hua Y, Rigo F, Matson J, Hung G, Kaye EM, Shihabuddin LS, Krainer AR, Bennett CF, and Cheng SH. Antisense oligonucleotides delivered to the mouse CNS ameliorate symptoms of severe spinal muscular atrophy. Sci Transl Med. 2011;3(72):72ra18.

Describe the Regulatory Protocol and Activity Status (if applicable).
Describe the Protocol and Activity Status for sections a-c, as applicable, using the format described for each section. If there is nothing significant to report during this reporting period, state "Nothing to Report."

(a) ***Human Use Regulatory Protocols***

TOTAL PROTOCOLS: *State the total number of human use protocols required to complete this project (e.g., 5 human subject research protocols will be required to complete the Statement of Work."). If not applicable, write "No human subjects research will be performed to complete the Statement of Work."*

PROTOCOL(S): *List the identifier and title for all human use protocols needed to complete the project. Include information about the approved target number for clinical significance, type of submission, type of approval with associated dates, and performance status.*

The following format shall be used:

Protocol (of total):

Protocol [HRPO Assigned Number]:

Title:

Target required for clinical significance:

Target approved for clinical significance:

Submitted to and Approved by:

Provide bullet point list of protocol development, submission, amendments, and approvals (include IRB in addition to HRPO).

Status:

Report (i) progress on subject recruitment, screening, enrollment, completion, and numbers of each compared to original planned target(s), e.g., number of subjects enrolled versus total number proposed; (ii) amendments submitted to the IRB and USAMRMC HRPO for review; and (iii) any adverse event/unanticipated problems involving risks to subjects or others and actions or plans for mitigation.

TOTAL PROTOCOLS: *No human subjects research will be performed to complete the Statement of Work.*

PROTOCOL (of total):

Protocol [HRPO Assigned Number]:

Title:

Target required for clinical significance:

Target approved for clinical significance:

SUBMITTED TO AND APPROVED BY:

STATUS:

(i) Number of subjects recruited/original planned target:
Number of subjects screened/original planned target:
Number of patients enrolled/original planned target:
Number of patients completed/original planned target:

(ii) Report amendments submitted to the IRB and USAMRMC HRPO for review:

(iii) Adverse event/unanticipated problems involving risks to subjects or others and actions or plans for mitigation:

(b) Use of Human Cadavers for Research Development Test & Evaluation (RDT&E), Education or Training

"Cadaver" is defined as a deceased person or portion thereof, and is synonymous with the terms "human cadaver" and "post-mortem human subject" or "PMHS." The term includes organs, tissues, eyes, bones, arteries or other specimens obtained from an individual upon or after death. The term "cadaver" does not include portions of an individual person, such as organs, tissue or blood, that were removed while the individual was alive (for example, if a living person donated tissue for use in future research protocols, that tissue is not considered a "cadaver" under this policy, regardless of whether the donor is living or deceased at the time of tissue use).

***TOTAL ACTIVITIES**: State the total number of RDT&E, education or training activities that will involve cadavers. If not applicable, write "No RDT&E, education or training activities involving human cadavers will be performed to complete the Statement of Work (SOW)."*

***ACTIVITIES**: Provide the following information in a bulleted list for all RDT&E, education or training activities involving human cadavers conducted or supported during the quarter:*

- *Title of the RDT&E, education or training activity*
- *SOW task/aim associated with the activity*
- *Date the activity was conducted*
- *Identification of the organization's responsible individual (e.g., PI or individual primarily responsible for the activity's conduct)*
- *Brief description of the use(s) of cadavers in the activity and the total number of cadavers used during the reporting period*
- *Brief description of the Department of Army organization's involvement in the activity*
- *Status of document submission and approvals*
- *Problems encountered in the procurement, inventory, use, storage, transfer, transportation and disposition of cadavers used for RDT&E, education or training. Examples of problems include but are not limited to: loss of confidentiality of cadaveric donors, breach of security, and significant deviation from the approved protocol, failure to comply with state laws and/or institutional policies and public relations issues.*

***TOTAL ACTIVITIES**:*
No RDT&E, education or training activities involving human cadavers will be performed to complete the Statement of Work (SOW)

***ACTIVITES**:*

(c) Animal Use Regulatory Protocols

TOTAL PROTOCOL(S):

One (1) animal use research protocol will be required to complete the Statement of Work

PROTOCOL(S):

List the identifier and title for all animal use protocols needed to complete the project. Include information about the approved target number for statistical significance, type of submission, type of approval with associated dates, and performance status.

The following format shall be used:

Protocol (_ of _ total):

Protocol [ACURO Assigned Number]:

Title:

Target required for statistical significance:

Target approved for statistical significance:

Submitted to and Approved by:

Provide bullet point list of protocol development, submission, amendments, and approvals (include IACUC in addition to ACURO).

Status:

Provide bullet point list of performance and/or progress status relating to the above protocol and discuss any administrative, technical, or logistical issues that may impact performance or progress of the study (e.g. animal use protocol needs revision to minimize animal suffering, animal protocol modification to include additional staff) for the above ACURO approved protocol.

TOTAL PROTOCOL(S): 1

PROTOCOL (_ of _ total): 1

Protocol [ACURO Assigned Number]: PR171107.e001

Title: Therapeutic analysis in animal models of neurodegeneration

Target required for statistical significance:

Aim 1:

E1$^{v1.11}$ treated:	80 SMA mice
"Scrambled" ASO treated:	20 SMA mice
Vehicle treated:	20 SMA mice
Untreated:	20 SMA mice
Total:	140 SMA mice

Aim 2:

E1$^{v1.11}$ treated:	80 *SMN2* transgenic mice (20 per group)
"Scrambled" ASO treated:	20 *SMN2* transgenic mice
Vehicle treated:	20 *SMN2* transgenic mice
Untreated:	20 *SMN2* transgenic mice
Total:	140 *SMN2* transgenic mice

Aim 3:

E1$^{v1.11}$ 4 mM: 15 mice P10; 15 mice P30; 10 mice P50; 10 mice P100 (50 mice)
E1$^{v1.11}$ 50 mM: 15 mice P10; 15 mice P30; 10 mice P50; 10 mice P100 (50 mice)
E1$^{v1.11}$ 100 mM: 15 mice P10; 15 mice P30; 10 mice P50; 10 mice P100 (50 mice)
E1$^{v1.11}$ 500 mM: 15 mice P10; 15 mice P30; 10 mice P50; 10 mice P100 (50 mice)

Untreated: 15 mice P10; 15 mice P30; 10 mice P50; 10 mice P100 (50 mice)
"Scrambled" ASO 500 mM treated: 15 mice P10; 15 mice P30; 10 mice P50; 10 mice P100 (50 mice)

Total: 330 CD-1 mice

Target approved for statistical significance:
Aim 1: 140
Aim 2: 140
Aim 3: 330

SUBMITTED TO AND APPROVED BY:
This protocol has been previously submitted and reviewed by the ACURO and the IUCAC at MU.
MU Attending Veterinarian: Lon Dixon, DVM
IACUC Chair: Jeff Henegar, PhD

STATUS:
The ACURO and IACUC protocols were approved in 2018 and no additional changes have been made and no amendments have been needed. Mice have been ordered and will arrive at MU in February, 2019.

We have started work on Aims 1-3. We have not experienced any technical issues or delays.

What do you plan to do during the next reporting period to accomplish the goals and objectives?

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

During the next reporting period, we plan to complete Aims 1, 2, and 3; receive FDA guidance on our proposed nonclinical IND-enabling studies and CMC programs; engage a nonclinical CRO to initiate GLP animal studies with pre-GMP material synthesized during Year 1 under this grant, and work with clinical CROs on the initial clinical study plans.

2. Products: List any products resulting from the project during the reporting period. If there are no products to report for the current quarter, state "Nothing to report."

Examples of products include:

- *publications, conference papers, and presentations;*
- *website(s) or other Internet site(s);*
- *technologies or techniques;*
- *inventions, patent applications, and/or licenses; and*
- *other products, such as data or databases, biospecimen collections, germplasm, audio or video products, software, models, educational aids or curricula, instruments or equipment, data and research material, clinical or educational interventions, or new business creation.*

Nothing to report.

3. Participants & Other Collaborating Organizations

What individuals have worked on the project?
Provide the following information for: (1) Project Directors (PDs)/ PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort).

Provide the name and identify the role the person played in the project. Indicate the nearest whole person month (Calendar, Academic, Summer) that the individual worked on the project. Show the most senior role in which the person worked on the project for any significant length of time. For example, if an undergraduate student graduated, entered graduate school, and continued to work on the project, show that person as a graduate student, preferably explaining the change in involvement.

Describe how this person contributed to the project. If information is unchanged from a previous submission, provide the name only and indicate "no change."

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*
Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*

Name: Christian Lorson
Project Role: PI (Shift Pharma)
Researcher Identifier: 0000-0002-1023-2169
Nearest person month worked: 3.6
Contribution to Project: Dr. Lorson is the PI at Shift Pharma. He is responsible for coordinating between Shift and MU and overseeing the overall project design and implementation.

Name: Steve O'Connor
Project Role: (Shift Pharma): CEO
Researcher Identifier: 0000-0002-9915-5787
Nearest person month worked: 5.6
Contribution to Project: Dr. O'Connor to manage the day-to-day research of the project. He is also responsible for chemistry, manufacturing, and controls program, including analytical methods development, data analysis, particularly for the LC/MS work for the PK analysis, product manufacturing, stability studies, as well as work with our sub-contractors and suppliers.

Name: Paul Morcos
Project Role: (Shift Pharma): Director of R&D
Researcher Identifier: 0000-0001-7415-6938
Nearest person month worked: 9.5
Contribution to Project: Dr. Morcos to manage the day-to-day research of the project. He will also direct the Morpholino sample protocols, assay development, and testing to be performed, as well as work with our sub-contractors and suppliers. Dr. Morcos will also be responsible for all data compilation and report writing.

Name: Diane Beatty
Project Role: (Shift Pharma): VP of Reg. Affairs and Product Development

Researcher Identifier: 0000-0001-9071-6384
Nearest person month worked: 11.5
Contribution to Project: Dr. Beatty is responsible for designing the studies to align with IND-enable studies, for coordinating the activities between Shift, MU, and the vendors supporting the studies, working with Dr. O'Connor for data analysis and compilation, and regulatory activities with FDA.

Name: Erkan (Erik) Osman (left MU and this project in June 2019)
Project Role: (MU): PI of the subaward
Researcher Identifier: 0000-0002-8887-8728
Nearest person month worked: 2.6
Contribution to Project: Dr. Osman is the PI on the subaward to MU. He has coordinated with Shift regarding ASO ordering, shipments, storage and handling, and has overseen the personnel at MU.

Name: Kerry David Farris (replaced Erkan Osman on the project)
Project Role: (MU): PI of the subaward
Researcher Identifier: 0000-0001-7270-5876
Nearest person month worked: 2.6
Contribution to Project: Dr. Farris is the PI on the subaward to MU. He has coordinated with Shift regarding ASO ordering, shipments, storage and handling, and has overseen the personnel at MU.

Name: Madeline Simon (Update per departure - off grant June, 2019)
Project Role: (MU): technical support
Researcher Identifier: 0000-0002-6936-908X
Nearest person month worked: 3.0
Contribution to Project: Research technician in charge of animal duties and routine colony tasks, and assists with tissue collection.

Name: Mona Kacher (replaced Madeline Simon on the project)
Project Role: (MU)
Researcher Identifier: 0000-0003-1744-6933
Nearest person month worked: 1.0
Contribution to Project: Research technician in charge of animal duties and routine colony tasks, and assists with tissue collection.

Name: Monique Lorson
Project Role: (MU): technical support
Researcher Identifier: 0000-0002-1772-0712
Nearest person month worked: 2.0
Contribution to Project: Coordinating animal duties, ordering, maintaining colony, organizing cages and breeding, as well as assisting with tissue collection.

Name: Toni Morcos
Project Role: (MU)
Researcher Identifier: 0000-0002-7729-070X
Nearest person month worked: 1.0
Contribution to Project: Assists in the lab for general lab related activities, assists with colony maintenance, PCR and genotyping.

4. Changes/Problems: The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

a. Actual Problems or delays and actions to resolve them

Provide a description of current problems or issues that may impede performance or progress of this project along with proposed corrective action. Also describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

For an award that includes the recruitment of human subjects for clinical research or a clinical trial, discuss any problems or barriers encountered, if applicable, and what has been done to mitigate those issues. Discussion may highlight enrollment problems, retention problems, and actions taken to increase enrollment and/or improve retention.

Nothing to Report

b. Anticipated Problems/Issues

Provide a description of anticipated problems or issues that have a potential to impede performance or progress. Also provide course of actions planned to mitigate problems or to take should the problem materialize.

Nothing to Report

5. Special Reporting Requirements:

Quad Charts: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

Appendix A.

Shift Pharmaceuticals Revised Budget as of October 2019.

At this time the overall spending is projected to remain unchanged. Please see details below.

Direct Costs		
	Direct Salaries + Fringe	$ 744,190
	Direct Supplies	406,279
	Consultants	175,000
	Subcontract	790,000
Total direct costs		2,115,469
Indirect costs (28%)		592,330
Total Budget		**$ 2,707,800**