INVESTIGATOR'S BROCHURE

ELAMIPRETIDE INJECTION FOR INTRAVENOUS OR SUBCUTANEOUS ADMINISTRATION

(also known as MTP-131)

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TABLE OF CONTENTS FOR THE INVESTIGATOR BROCHURE

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Definition or Explanation
ACS	acute coronary syndrome
ADE	adverse device effect
ADR	adverse drug reaction
AE	adverse event
ADP	adenosine diphosphate
AKI	acute kidney injury
Ai	time-velocity integral representing left atrial contraction
AMD	age-related macular degeneration
AR	area at risk
AR/LV	ratio of the mass of the area at risk to the left ventricular mass
ARAS	atherosclerotic renal artery stenosis
ATP	adenosine triphosphate
AUC	area under the plasma concentration-time curve
$AUC_{0\text{-}inf}$	area under the plasma concentration-time curve from time zero to infinity
$AUC_{0\text{-}24}$	area under the plasma concentration-time curve from 0 to 24 hours
AUC_{last}	area under the plasma concentration-time curve at the last observation
^{14}C	8-neutron/6-proton isotope of carbon (radioactive)
CAO	coronary artery occlusion
CHF	congestive heart failure
C_{5min}	plasma concentration at 5 min post-dose
C_{max}	maximum plasma concentration
CsA	cyclosporine A
CYP	cytochrome P450
CYP1A2	cytochrome P450 1A2 enzyme
CYP2C9	cytochrome P450 2C9 enzyme
CYP2C19	cytochrome P450 2C19 enzyme
CYP2D6	cytochrome P450 2D6 enzyme
CYP2E1	cytochrome P450 2E1 enzyme
CYP3A4	cytochrome P450 3A4 enzyme
DDI	drug-drug interaction
DG/GD	gestational day

Abbreviation	Definition or Explanation
DT	deceleration time of early mitral inflow velocity
ECG	electrocardiogram
EDV	end diastolic volume
EDWS	end diastolic circumferential wall stress
EF	ejection fraction
Ei	time-velocity integral of the mitral inflow velocity waveform representing early filling
Elamipretide Delivery System	Elamipretide Injection Cartridge, elamipretide pen injector and single-use needle
ESV	end systolic volume
ETC	electron transport chain
EV	ejection volume
FA	Friedreich's Ataxia
FXN	frataxin
GLP	Good Laboratory Practices
hERG	human ether-à-go-go-related gene
IA	infarct area
IA/AR	ratio of the mass of the area of infarcted tissue to the mass of the area at risk (ischemia)
IC_{50}	half maximal inhibitory concentration
IFU	Instructions for Use
IgE	Immunoglobulin E
IMM	inner mitochondrial membrane
IP	Elamipretide Injection or Elamipretide Delivery System
IR	ischemia reperfusion
IRI	ischemia-reperfusion injury
ISR	injection site reaction
IV	intravenous
LC-MS/MS	liquid chromatography tandem mass spectrometry
LV	left ventricular
M1	tripeptide metabolite of elamipretide (also referred to as SPI-09-No.29)
M2	dipeptide metabolite of elamipretide (also referred to as SPI-09-No.30)
MedDRA	Medical Dictionary for Regulatory Activities

Abbreviation	Definition or Explanation
mtDNA	mitochondrial DNA
mPTP	mitochondrial permeability transition pore
MTP-131	elamipretide
NOEL	no-observed-effect level
NOAEL	no-observed-adverse-effect level
NYHA	New York Heart Association
NZW	New Zealand White
PA	peak mitral inflow velocity during left atrial contraction
PBS	phosphate-buffered saline
PCI	percutaneous coronary intervention
PE	peak mitral flow velocity in early diastole
PK	pharmacokinetic(s)
PMM	Primary Mitochondrial Myopathy
PMMSA	Primary Mitochondrial Myopathy Symptom Assessment
PT	preferred term
PTRA	percutaneous transluminal renal angioplasty
QTc	corrected QT interval on the electrocardiogram
QTcB	corrected QT interval on the electrocardiogram using the Bazett formula
QTcF	corrected QT interval on the electrocardiogram using the Fridericia formula
QTcV	corrected QT interval on the electrocardiogram using the Van de Water's formula
RA	risk assessment
ROS	reactive oxygen species
SAE	serious adverse event
SC	subcutaneous
SOC	System Organ Class
STEMI	ST-segment elevation myocardial infarction
SUSAR	Suspected unexpected serious adverse reaction
SV	stroke volume
SWFI	sterile water for injection
τ	tau, relaxation time constant
$t_{½}$	plasma terminal phase half-life

Abbreviation	Definition or Explanation
TEAE	treatment-emergent adverse event
T_{max}	time to/of maximum plasma concentration
TK	toxicokinetic(s)
UFH	unfractionated heparin
V_d	volume of distribution
V_{ss}	volume of distribution at steady state

1. SUMMARY

Stealth BioTherapeutics Inc. (SBT) is a biopharmaceutical company with a focus on treating mitochondrial dysfunction in both common and rare diseases. Elamipretide is being developed in various drug product presentations for that purpose. This Investigator's Brochure (IB) contains details for elamipretide injection for intravenous (IV) and subcutaneous (SC) administration and elamipretide injection for SC administration with or without the Elamipretide Delivery System.

1.1. Pharmaceutical Properties and Formulation

Elamipretide (also known as MTP-131 and SS-31) is an aromatic-cationic tetrapeptide that readily penetrates cell membranes and transiently localizes to the inner mitochondrial membrane (IMM). Elamipretide for parenteral administration has been supplied as:

- a lyophilized powder in a single-dose glass vial for reconstitution with sterile water for injection (SWFI) or saline and diluted with sterile saline for IV infusion;
- a ready-to-use sterile aqueous elamipretide injection solution in a single-patient-use, single-dose or multi-dose glass vial for SC administration or further diluted with sterile saline for IV infusion;
- a ready-to-use sterile aqueous elamipretide injection solution in a single-patient-use, multi-dose glass cartridge for SC administration for use in combination with the elamipretide pen injector and single-use pen needle (Elamipretide Delivery System) to administer a fixed dose. This cartridge and delivery system is no longer being supplied nor in development.

The testing of elamipretide encompasses a program of nonclinical and clinical studies. Throughout the development program, different naming conventions for the active molecule have been used: MTP-131 (elamipretide acetate or HCl salts) and SS-31 (elamipretide acetate salt) and elamipretide (elamipretide acetate or HCl salts). Test article SS-31 was used in early development, primarily in the pharmacology studies, whereas MTP-131 (acetate and HCl) were used in later development and all toxicology studies. Data suggest that the 3 salt forms of the peptide perform similarly in all *in vitro* and *in vivo* studies. Therefore, the peptide is generally referred to in this document as elamipretide as a free base.

1.2. Nonclinical Studies

Elamipretide was comprehensively evaluated in a series of pharmacology, metabolism, pharmacokinetic (PK) and toxicological nonclinical studies. Elamipretide was effective in multiple models of cardiac disease and skeletal muscle dysfunction and has been active across all species tested to date, including mouse, rat, guinea pig, rabbit, dog, sheep, and pig. Based on results from a battery of secondary and safety pharmacology studies, elamipretide is not expected to cause any adverse off-target pharmacodynamic effects at therapeutic concentrations.

Elamipretide did not cause end-organ toxicity at any dosage tested in either rats or dogs. Systemic toxicity at high doses was manifested primarily by acute and transient clinical signs, which were mediated by histaminergic-like reactions. Effects were associated with maximum elamipretide plasma concentration (C_{max}) and were rapidly reversible as plasma concentrations of elamipretide (and histamine) decreased. Dose administration was not associated with any adverse effects on cardiovascular, respiratory or central nervous system function; off-target non-adverse effects were limited to transient decrease of blood pressure and heart rate, consistent with histaminergic-like reactions. In all studies, the severity of the effects was proportional to C_{max} for elamipretide; thus, the safety margin is estimated based on C_{max}, and not area under the plasma-concentration-time curve (AUC). The plasma elamipretide threshold concentration for clinically relevant adverse effects appears to be approximately 20,000 ng/mL in both rats and dogs, which is more than 10-fold higher than the maximum observed human exposures at clinical doses.

Intravenous administration of elamipretide to rats and dogs was well tolerated at the administration site. Local injection site reactions evident upon SC administration varied with species, dose, and dose concentration.

Elamipretide was negative for genotoxicity in the full battery of tests and caused no significant hemolysis or inhibition of receptor binding. Elamipretide was not associated with adverse effects on fertility or embryo-fetal development. Similarly, elamipretide did not have any adverse effects on post-natal (juvenile) development.

Elamipretide is metabolized via sequential C-terminal degradation to the tripeptide M1 and the dipeptide M2. The apparent plasma half-life ($t_{½}$) of M1 was comparable to that of elamipretide, whereas $t_{½}$ of M2 was longer than that of elamipretide. No sex difference was evident for either metabolite. The 2 metabolites were evaluated for systemic toxicity and *in vivo* genotoxicity. In addition, *in silico* analysis for genotoxic structural alerts was conducted on new impurities introduced in the modified manufacturing process for elamipretide HCl. This analysis confirmed the absence of structural alerts for genotoxicity. When tested directly, both M1 and M2 were negative for gene mutation, for receptor binding, and rat mast cell degranulation. Systemic exposure to the metabolites in rats and dogs was not related to any toxicity in acute, subchronic, or chronic studies. Neither M1 nor M2 metabolites showed biological activity when evaluated in an *ex vivo* guinea pig heart model. At a concentration of 1 µM, neither metabolite provided myocardial protection against ischemic reperfusion injury.

The possible drug interactions of elamipretide and its metabolites M1 and M2 were investigated in a series of *in vitro* and *in vivo* experiments including cytochrome P450 (CYP) inhibition studies with elamipretide using human liver microsomes or recombinant human enzymes, *in vivo* CYP induction study in rats, *in vitro* studies for human transporter inhibition, CYP inhibition and CYP induction, *in vitro* interaction studies of elamipretide with human MATE1 and MATE2-K uptake transporters, and *in vitro* substrate interaction of elamipretide with human MATE1, MATE2-K, OAT1, OAT3 and OCT2 uptake transporters. Results demonstrate elamipretide shows little to no inhibition or inductive effects on human CYP enzymes. Elamipretide showed

no inhibition of human transporter proteins OCT2, BCRP, OAT1, OAT3, OATP1B1, OATP1B3, p-glycoprotein or BCRP. Elamipretide was not a substrate of MATE1, OAT1, OAT3, and OCT2 uptake transporters. However, significant inhibition of substrate uptake by elamipretide was observed for MATE1 transporter and elamipretide may be a substrate for MATE2-K uptake transporter. Therefore, with the possible exception of MATE1 inhibition, and the possibility that elamipretide is a MATE2-K substrate, elamipretide is unlikely to affect the protein binding, metabolism, excretion, or uptake of concomitantly administered drugs.

1.3. Clinical Trials

To date, data from 30 completed clinical trials with parenteral elamipretide (16 with the IV formulation and 14 with the SC formulation). This IB provides information on the parenteral products and the studies conducted with elamipretide injection solution for SC administration and elamipretide reconstituted solution for IV infusion administration only.

Of the 301 subjects exposed to single IV doses of elamipretide (ranging from 0.005 mg/kg/hour to 0.25 mg/kg/hr, typically administered over 2 to 4 hours), 100 were healthy adult subjects and 201 were patients with various cardiovascular, renal, and skeletal conditions.

Of the 229 subjects exposed to multiple IV doses of elamipretide, 23 were healthy subjects with normal renal function or varying degrees of renal impairment who received 0.25 mg/kg/hr for 1 hour daily for 7 days, 24 were healthy subjects who received 56 to 140 mg elamipretide over 1 hour daily for 5 days, 27 were subjects with primary mitochondrial myopathy (PMM) who received 0.01 mg/kg/hr to 0.25 mg/kg/hr administered over 2 hours daily for 5 days, and 155 subjects hospitalized with congestion due to heart failure who received 20 mg administered over 1 hour daily for 7 days.

Data are available from 56 healthy subjects who have been exposed to a single SC dose and from 143 healthy subjects who have been exposed to multiple SC doses (up to 8 total days of exposure) of elamipretide, ranging from 2 mg to 400 mg. Additionally, 493 subjects have been exposed to multiple SC doses of elamipretide for ≥8 days: 254 subjects with PMM, 157 subjects with age-related macular degeneration (AMD), 12 subjects with Barth syndrome, 23 subjects with stable heart failure with preserved ejection fraction, and 47 subjects with stable heart failure with reduced ejection fraction.

1.4. Clinical Pharmacology

Following single and multiple doses given via IV infusion, ranging from 0.010 mg/kg/hr to a fixed dose of 140 mg, elamipretide showed dose-proportional exposure (mean area under the plasma concentration-time curve from time zero to infinity [$AUC_{0\text{-}inf}$] and C_{max}). The mean elamipretide exposures were independent of day of administration, indicating no significant accumulation when administered daily. There appeared to be a low plasma clearance (130 mL/kg/hr) of approximately 15% of hepatic or renal blood flow and a small volume of distribution (300 mL/kg, less than the total body water volume). The plasma $t_{1/2}$ of elamipretide was approximately 3 to 4 hours, independent of dose, across all studies of elamipretide given via IV infusion.

Following single and multiple SC doses ranging from 2 to 80 mg, elamipretide showed dose proportional exposure via $AUC_{0\text{-}24}$ and C_{max}. Elamipretide T_{max} occurred at approximately 1 hour post-dose, independent of dose, across all studies of elamipretide given via SC injection. Elamipretide exposures were independent of day of administration, indicating no significant accumulation when administered daily. Plasma half-life of elamipretide was approximately 3 hours in doses greater than 6 mg. In the dose cohorts receiving 40 mg as either a 1 mL injection of elamipretide at 40 mg/mL, a 0.5 mL injection of elamipretide at 80 mg/mL, or a 0.25 mL injection of elamipretide at 160 mg/mL, elamipretide demonstrated a potential for saturation at the injection site with high concentration dose formulations (160 mg/mL).

Studies to assess the PK of elamipretide and its metabolites in subjects with varying degrees of renal impairment demonstrated that subjects had increased exposure (AUC_{last}), to elamipretide and both metabolites, which increased proportionally to the degree of renal failure.

A study to assess the PK of elamipretide administered SC as 2 different salt forms (acetate and HCl) and 2 different locations (abdomen and thigh) demonstrated no clinically significant changes in the safety or PK profile of elamipretide.

There are no gender differences in the PK profile of elamipretide in healthy adult subjects and no drug-drug interactions (DDIs) have been identified to date.

1.5. Clinical Safety

Parenteral administration of elamipretide was assessed following single and multiple IV and SC administrations as described in Appendix 1 and Appendix 2. Dose levels studied ranged from approximately 0.7 mg/day to 400 mg/day. There were no apparent differences between the safety profiles of IV or SC elamipretide dosing routes except for injection site reactions which were widely reported in subjects receiving SC elamipretide.

Differences (≥2% difference) in systemic treatment-emergent adverse events (TEAEs) reported in elamipretide- and placebo-treated subjects, summarized by dosing duration, is displayed in Table 1.

Table 1 Summary of Systemic TEAEs Reported in >5% of Elamipretide-treated Subjects with Greater Frequency (≥ 2% difference) in Elamipretide-treated Subjects Compared with Placebo-treated Subjects

	Elamipretide Number (%)	Placebo Number (%)
Repeat dose ≤8 days	**n=372**	**n=231**
Headache	5.1%	2.6%
Repeat dose >8 days	**n=493**	**n=258**
Dizziness	5.7%	3.0%
Headache	7.7%	4.3%
Nasopharyngitis	6.3%	1.9%
Upper respiratory tract infection	6.9%	3.5%
Eosinophil count increased	5.5%	0%

Additionally, the absolute eosinophil count increased several-fold from baseline in the majority of patients that administered elamipretide longer than 28 days. The eosinophil values typically return to normal upon chronic administration of elamipretide. This elevation in eosinophils was not associated with clinical manifestations or changes in other laboratory parameters.

In SPICP-103, high-dose IV infusions (56 to 140 mg/day) of elamipretide were administered daily for 5 days in healthy subjects. Four subjects reported events of paraesthesia or oral paraesthesia. These events generally occurred immediately after the end of the drug infusion, at the T_{max} of elamipretide. Three of these AEs occurred in the 140 mg cohort, and 1 occurred in the 112 mg cohort. All were reported to be mild in severity, resolved within 2 hours of symptom onset, and were not associated with hypersensitivity reactions.

Injection site reactions were reported in the majority of subjects receiving elamipretide by SC injection in any study. Detailed characterization of the injection site reactions occurring in single-dose SC clinical trials demonstrates that mild erythema, swelling, pain, and pruritus are the most commonly reported signs and symptoms and that pain and bruising may also be experienced. Generally, the injection site reactions resolved within 4 hours of elamipretide administration. In the longer-term studies reporting of injection site reaction commonly included the signs and symptoms previously mentioned as well as injection site bruising, induration, urticaria, haemorrhage, and mass. In most subjects, the tolerability of the injection site reactions was not problematic and did not require treatment, however, some subjects have been treated with topical and systemic antihistamines and/or topical corticosteroids in order to manage the impact of the signs and symptoms. A Phase 1 study, SPICP-106, examined various potential interventions that may lead to mitigation of ISRs and confirmed the impact of those interventions on the systemic absorption of elamipretide from the SC tissue. Subject or caregiver retraining on proper injection technique should also be considered. In 2 subjects, injection site reactions evolved into wounds that in turn became infected. In one subject, upon healing, some of the wounds left evidence of scar formation. For both subjects contributing factors were present. In clinical trials > 8 days in duration with data available approximately 10% of subjects receiving

elamipretide via SC injection have discontinued study drug treatment due to injection site reactions.

Three potential cases of hypersensitivity / allergic reactions have been reported, including one reported as a suspected unexpected serious adverse reaction (SUSAR) in an Expanded Access/Early Access Program (EAP). There does not appear to be a clear association between the occurrence of these events and duration of exposure to elamipretide. Investigators and subjects should be aware of the potential for hypersensitivity / allergic reactions with elamipretide.

All additional assessments of safety (including vital signs and laboratory, ECG, and physical examination findings) across all clinical studies have been unremarkable.

In subjects with renal impairment who received elamipretide, exposure (as measured by AUC) to elamipretide and both of its metabolites, M1 and M2, increased proportionally to the degree of renal impairment; however, there was no evidence of increased toxicity as a consequence of impaired renal function. Similarly, in the DDI studies carried out to date, co-administration of elamipretide with aspirin, with clopidogrel, or with UFH did not indicate a change in the nature, severity, or frequency of AEs to the safety profile of either elamipretide or the comparator.

A supratherapeutic dosing regimen of elamipretide, 80 mg every 3 hours for 5 doses, was utilized in a thorough QTc study (TQT) to evaluate the QT/QTc interval prolongation and proarrhythmic potential of elamipretide in healthy subjects. The results showed no impact on the QTc interval up to plasma concentration of approximately 4200 ng/mL for elamipretide, approximately 3300 ng/mL for M1, and approximately 300 ng/mL for M2.

Across all studies, with either IV infusion and SC formulation, there have been no reports of exposure during lactation, overdoses, or abuse or misuse. There has been one report of exposure in a pregnant partner of a subject treated with elamipretide with a normal outcome.

1.6. Clinical Efficacy

The efficacy of elamipretide has been evaluated in multiple patient populations. A summary of efficacy results for individual studies is presented in Appendix 2.

2. INTRODUCTION

2.1. Elamipretide

Elamipretide injection is being developed by SBT for both common and rare diseases caused by mitochondrial dysfunction.

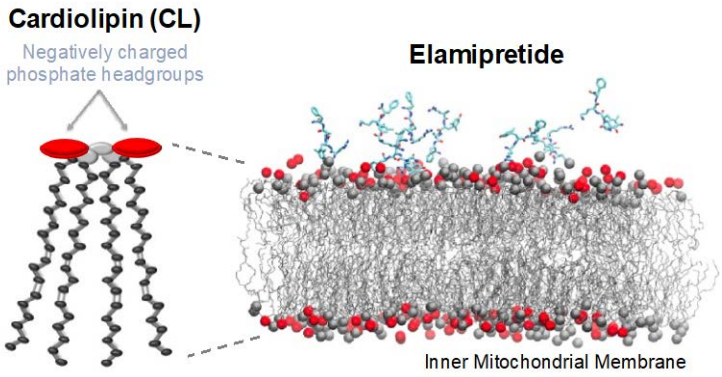
2.2. Mechanism of Action

Elamipretide is a small peptide that targets the inner membrane (IMM) where energy production occurs in the mitochondria. Studies from pathological human tissues and many different animal models of disease have shown improvements in mitochondrial structure and function with elamipretide. In these studies, the consequence of improving mitochondrial bioenergetics led to improved cellular and organ function (Manczak M et al, 2010; Birk AV et al, 2013; Dai D-F et al, 2013; Eirin A et al, 2014; Siegel MP et al, 2013).

Elamipretide binds reversibly to cardiolipin, a phospholipid that comprises the IMM and is unique to mitochondrial membranes. Elamipretide has been shown to influence mitochondrial structure and function via its interaction with cardiolipin. Elamipretide diffuses across the outer mitochondrial membrane and, when proximate with the inner mitochondrial membrane, its positively charged residues interact electrostatically with the anionic headgroups of cardiolipin, increasing local concentration levels, and its nonpolar side chains interact hydrophobically with the acyl chains (Figure 1) (adapted from Mitchell W et al, 2020). This electrostatic/hydrophobic binding modulates the surface electrostatics of the inner membrane to facilitate increases in lipid packing, membrane curvature and membrane surface area. The consequences of the effects of elamipretide on the inner mitochondrial membrane include augmenting cristae formation, improving the assembly/activity of membrane protein complexes, and ultimately normalizing oxidative phosphorylation in diseases states despite disease-induced cardiolipin deficit (Mitchell W et al, 2020; Allen ME et al, 2020).

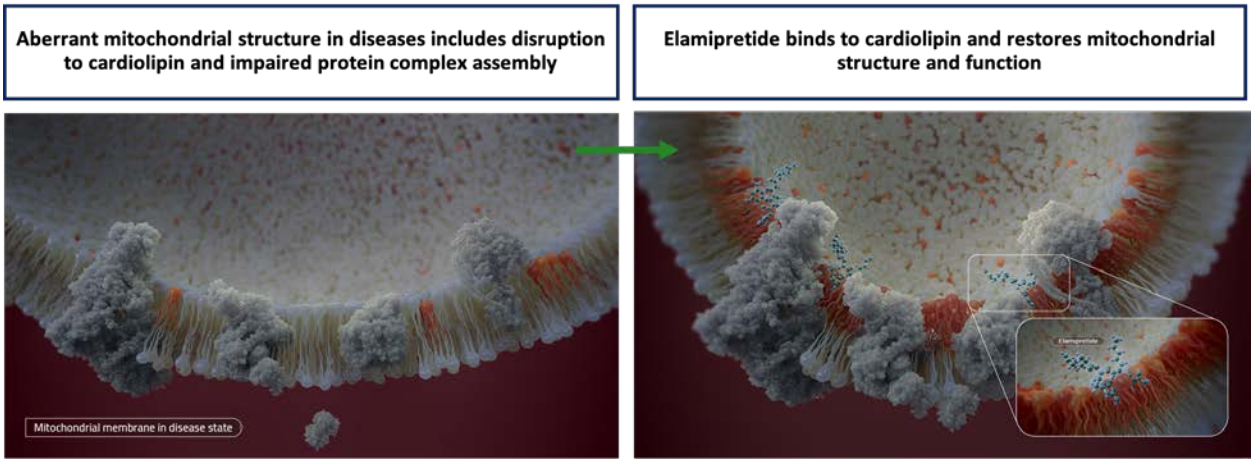
Cardiolipin influences nearly every aspect of the dynamic energy network required to maintain bioenergetic homeostasis, and a lack of cardiolipin is incompatible with life. Cardiolipin is obligatory for mitochondrial biogenesis and turnover, the import of nuclear-encoded proteins, organization and replication of mitochondrial DNA, and the activity of the electron transport system. The centrality of cardiolipin in mitochondrial physiology highlights its potential as a key therapeutic target across diseases (Chicco AJ and Sparagna GC, 2007; Brown DA et al, 2017; Sabbah HN et al, 2020).

Figure 1 Interaction of Elamipretide with Cardiolipin in the IMM



SBT has demonstrated that treatment of cells or isolated organs undergoing oxidative stress with elamipretide can maintain the normal morphology of the IMM and the association of the ETC complexes within the IMM, protecting them from degradation in the presence of increased reactive oxygen species (ROS) (Figure 2). Numerous studies have shown an ensuing improvement in various downstream consequences of mitochondrial dysfunction following treatment with elamipretide, including reduced fibrosis, inflammation, and cell death.

Figure 2 Improved Mitochondrial Structure and Function Across Pathologies with Elamipretide



Elamipretide has been extensively studied in multiple preclinical studies and in clinical trials for diseases involving mitochondrial dysfunction (Manczak M et al, 2010; Birk AV et al, 2013; Dai DF et al, 2013; Eirin A et al, 2014; Siegel MP et al, 2013). Conversely, elamipretide is expected

to have minimal or no effect in disease models in which mitochondrial dysfunction is not critical to pathogenesis or disease etiology.

Multiple peer-reviewed publications from more than 20 independent laboratories demonstrate elamipretide consistently improves mitochondrial, cellular, and organ function in both *in vitro* and *in vivo* disease models for which mitochondrial dysfunction is understood to be an important component. This includes cardiovascular, ophthalmic, metabolic, skeletal muscle, neurodegenerative, and genetic mitochondrial diseases.

2.3. Nonclinical Studies and Translation

Elamipretide has been shown to protect or improve mitochondrial morphology in multiple nonclinical models, and this has resulted in functional improvement in various highly energy dependent organ systems. Specifically, treatment with elamipretide has improved skeletal muscle function, heart function, and kidney function in both nonclinical models and clinical studies and has improved visual function in several nonclinical models (Szeto HH and Birk AV, 2014; Alam NM et al, 2015).

3. PHYSICAL, CHEMICAL, AND PHARMACEUTICAL PROPERTIES AND FORMULATIONS

3.1. Chemical Properties

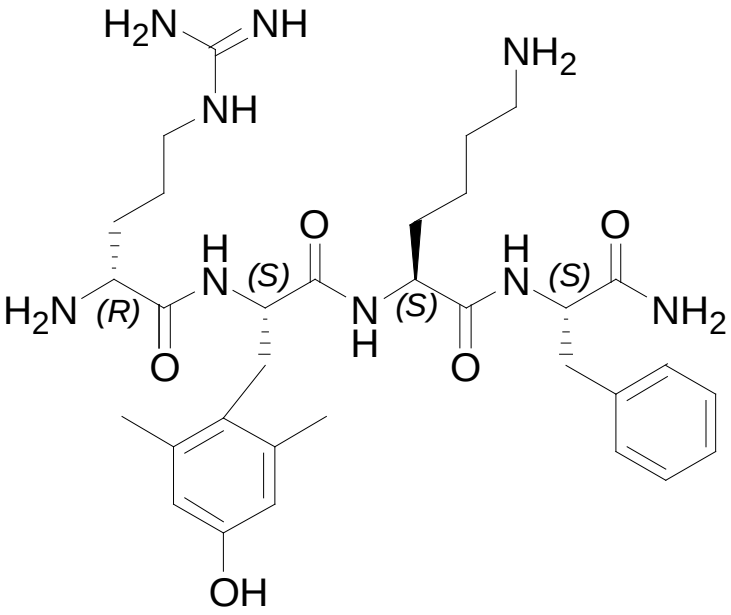
3.1.1. Chemical Name

D-Arginyl-2',6'-dimethyl-L-tyrosyl- L –lysyl- L –phenylalaninamide (free base)

3.1.2. Structural Formula

The structural formula of elamipretide (free base) is provided in Figure 3.

Figure 3 Structural Formula of Elamipretide



3.1.3. Molecular Formula

$C_{32}H_{49}N_9O_5$ (free base)

3.1.4. Molecular Weight

639.8 (free base)

3.2. Formulations

Elamipretide for parenteral administration is supplied for clinical trials as:

- a ready-to-use sterile aqueous elamipretide injection solution in a single-patient-use, single-dose or multi-dose glass vial for SC administration or further diluted with sterile saline for IV infusion

3.2.1. Description, Composition, and Storage of Elamipretide Injection Solution

Early clinical trials were conducted with Elamipretide for Injection, a lyophilized powder supplied in a single-dose glass vial containing 20 mg of study drug with conventional excipients for lyophilized drug products including bulking agent for formation of lyophilization cake, salt for tonicity and acid/base for pH adjustment, and antioxidant to stabilize elamipretide drug substance. Elamipretide for Injection was reconstituted with SWFI or saline and further diluted to the required volume for infusion.

Elamipretide Injection is currently supplied as a ready-to-use sterile aqueous injection solution in a single-patient-use, single-dose or multi-dose glass vial.

Elamipretide Injection sterile solution is a ready-to-use liquid solution supplied in a single-patient-use, single-dose or multi-dose glass vial. There are 2 different strengths and formulations of the single patient, singe dose vial: 1) a 10 mg/mL concentration (4 mL extractable fill volume) with sodium chloride (NaCl) and sodium phosphate, monohydrate monobasic; 2) a 40 mg/mL concentration (1 mL extractable fill volume) with NaCl. The solutions are suitable for IV infusion administration by IV infusion, following dilution in sterile saline, or by SC injection. The single-patient use multi-dose container is an 80 mg/mL concentration (up to 3.75 mL extractable fill volume) with sodium phosphate monohydrate monobasic and benzyl alcohol.

Stability data supports long-term storage of elamipretide injection at refrigerated storage (2 to 8ºC) for at least 36 months. Lot specific shelf-life/expiry dating is provided for any material shipped to clinical testing sites. Data also support the exposure of elamipretide injection to room temperature condition (approximately 25°C) for short durations. Elamipretide injection remains chemically stable in 0.9% saline infusion bags for up to 4 hours at room temperature and up to 12 hours at refrigerated temperature following further dilution for IV infusion.

4. NONCLINICAL STUDIES

The testing of elamipretide encompassed a program of nonclinical studies. Throughout the IB, MTP-131 (elamipretide acetate or HCl salts) and SS-31 (trifluoroacetate salt) were employed. Test article SS-31 was used in early development, primarily in the pharmacology studies, whereas MTP-131 (acetate and HCl) was used in later development and all toxicology studies. Data suggest that the 3 salt forms of the peptide perform similarly in all *in vitro* and *in vivo* studies. Therefore, the peptide is generally referred to in this document as elamipretide.

4.1. Nonclinical Pharmacology

Nonclinical pharmacology has been investigated in multiple disease state models; both *in vitro* and *in vivo*. In addition, safety pharmacology studies have also been conducted. Major findings from these studies are discussed below.

4.1.1. Cardiology

Pre- and post-ischemic treatment with elamipretide (1 nM or 100 nM) was found to improve myocardial contractile activity and relative infarct size of isolated guinea pig hearts in myocardial ischemia reperfusion (IR) models. Results are summarized in Table 2.

Table 2 Elamipretide Effect in Myocardial Ischemia Reperfusion Models

Study	Concentration (nM)	Treatment	Findings
PD-001	1, 100	Pre- and post-ischemia	Treatment with 1 nM elamipretide improved myocardial contractile activity ($p<0.001$)
SPIRIIV-N109	1, 0.01, 0.001	Pre- and post-ischemia	Treatment with elamipretide decreased relative infarct size at doses of 1 nM (38.8% reduction, $p=0.015$), 0.01 nM, (42.8%, $p=0.094$) and 0.001 nM (38.1%, $p=0.035$)

4.1.1.1. Acute Cardiac Injury

The therapeutic effect of parenteral elamipretide was determined in a series of myocardial infarct studies. The exposures to elamipretide in the nonclinical models of myocardial infarct for which drug concentrations are available are shown in Table 3.

Table 3 Elamipretide Exposure in Myocardial Infarct Pharmacology Studies

Study	Species	Route	Dosages (mg/kg/h)	n	AUC_{last} (ng•h/mL)	Elamipretide C_{max} (ng/mL)	Reduction in infarct Size %
SPIRIIV-N103	Rabbit	IV	0.05 x 3.3 h 0.10 x 3.3 h 1.00 x 3.3 h	4 4 2	205 324 3,339	65 106 1,060	42 20 33
			All animals	10			32*
SPIRIIV-N110	Sheep	IV	0.005 x 3.5 h 0.025 x 3.5 h 0.050 x 3.5 h 0.100 x 3.5 h 0.500 x 3.5 h	8 8 11 2 4	136 805 1,274 2,873 16,563	22 130 209 461 2,710	25* 3 16* 6 17
			All animals	33			14*
SPI-QTE-10-01	Rabbit	IV	0.05 x 3 h 0.05 x 1 h 0.025 x 1 h 0.005 x 1 h	7 8 8 8	ND	61 31 38 15	49* 50* 37* 9
SPIRIIV-N112	Rabbit	IV	0.05 x 3 - 3.33 h	49	340	112	11 No-reflow: 21*

Abbreviations: AUC_{last} = Area under the plasma concentration-time curve at the last observation; C_{max} = maximum concentration in plasma; h = hour(s); IV = intravenous; ND = not determined.

* Statistically significant at $p<0.05$

4.1.1.2. Heart Failure

In a canine model of advanced heart failure (produced by multiple sequential intracoronary microembolizations until the animal's left ventricle [LV] ejection fraction [EF] was <30%), the animals were treated with either elamipretide or placebo (*SPI-HFH-11-01*) for 3 months (subchronic). Effects of both acute and subchronic administration of elamipretide were examined. Significant findings are reported in Table 4.

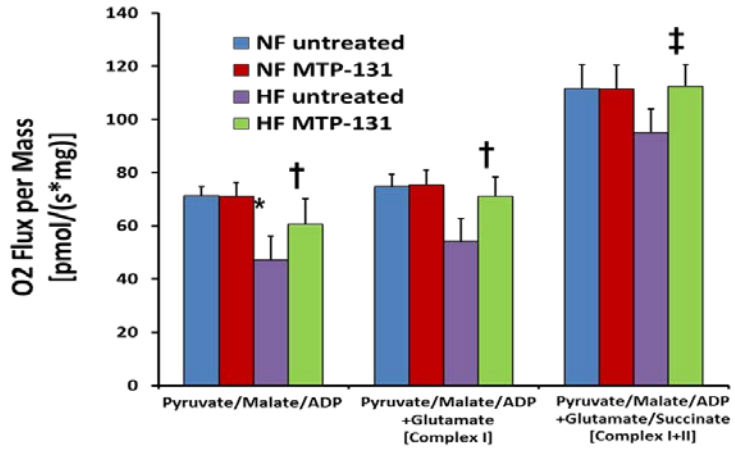
Table 4 Findings Observed in Canine Model of Heart Failure Following Elamipretide Treatment

Treatment Duration	Dosage	ROA	Results
4-hour	0.05 mg/kg/h	IV infusion	Elamipretide increased cardiac output (p=0.009) and stroke volume (p=0.028). Elamipretide significantly decreased LV end systolic volume (ESV; p=0.006) and increased LV ejection fraction (p=0.012), fractional area shortening (p=0.014), and stroke volume (SV; p=0.028). Elamipretide improved the Ei/Ai ratio (p=0.012) and deceleration time of early mitral inflow velocity (DT; p=0.048).
90 days	0.5 mg/kg/d	SC	Elamipretide significantly increased peak LV dP/dt (p=0.01), increased cardiac output (p=0.07) and stroke volume (p=0.13). Elamipretide decreased ESV (p=0.08) and significantly increased EF (p=0.001) and fractional area shortening (p=0.05). Elamipretide improved Ei/Ai (p=0.35) and EDWS (p=0.11). Elamipretide significantly increased slow-twitch, fatigue-resistant type-I fibers (p=0.022). Elamipretide significantly reduced glycolytic fast-twitch type-II fibers (p=0.022) and significantly the ratio of type-I to type-II fibers (p=0.036). Elamipretide significantly increased the State-3 respiration of myocyte mitochondria (p<0.05) and mitochondria membrane potential (p<0.05). Elamipretide reduced opening of the mPTP, improved activity and expression of cytochrome c oxidase, and decreased the amount of cytochrome c in the cytosol. Improvements were associated with a significant increase in ATP synthesis (p<0.05) and the ratio of ATP/ADP in the cells (p<0.05).

Abbreviations: ADP = adenosine diphosphate; Ai = time-velocity integral representing left atrial contraction; ATP = adenosine 5´-triphosphate; DT = deceleration time of early mitral inflow velocity; EDWS = end diastolic circumferential wall stress; Ei = time-velocity integral of the mitral inflow velocity waveform representing early filling; ESV = end systolic volume; LV = left ventricle; mPTP = mitochondrial permeability transition pore; ROA = route of administration; SV = stroke volume

Heart failure is known to involve mitochondrial dysfunction. In studies of human heart tissue explanted from 21 pediatric and adult heart transplant patients with congenital, ischemic, or non-ischemic heart failure undergoing transplant at the University of Colorado, exposure to elamipretide significantly improved mitochondrial respiration to levels comparable to those measured in healthy heart tissue, but it did not increase mitochondrial respiration above baseline levels in 15 non-failing donor hearts deemed unsuitable for transplant (Stauffer E et al, 2016). These observations in human heart tissue, particularly that from pediatric patients with congenital heart failure (n=6 nonfailing, n=10 failing), also suggest elamipretide's potential to improve the congenital cardiomyopathy associated with FA (Figure 6).

Figure 4 Effect of Elamipretide on Respiration in Mitochondria Isolated from Normal and Failing Hearts



NF – Non-failing heart, HF – Failing heart

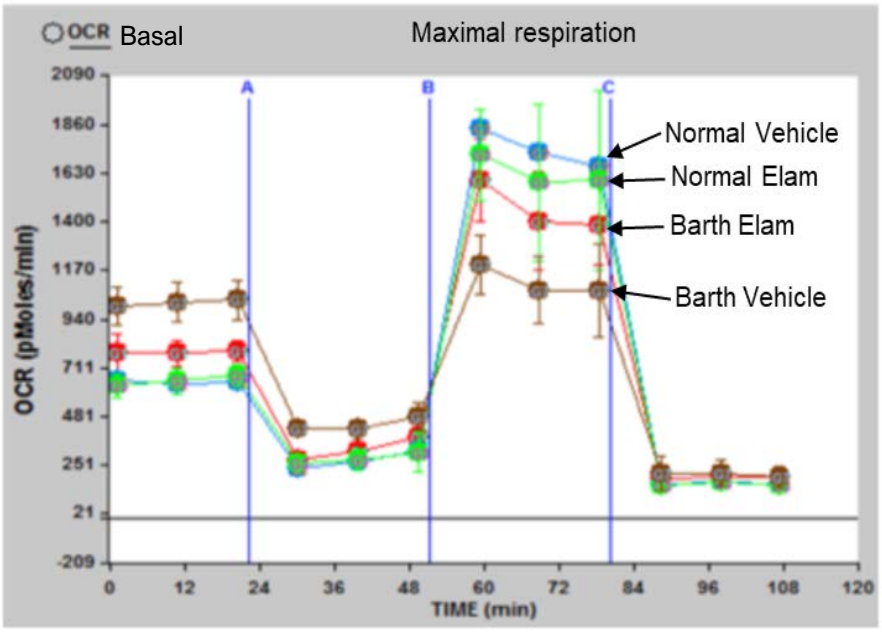
4.1.1.3. Barth Syndrome Cardiomyopathy

4.1.1.3.1. Human Barth iPSC-derived Cardiomyocytes

To investigate the effect of elamipretide on respiration in cells with abnormal cardiolipin content, iPSC-derived cardiomyocytes generated from human subjects with Barth syndrome were compared with non-Barth syndrome cardiomyocytes (normal) (Wang G et al, 2016).

Oxygen consumption by these same cardiomyocytes was measured under various conditions in multi-well plates using an instrument designed to assess mitochondrial function (Seahorse technology). Oxygen consumption rates (OCR) are shown in Figure 7. During the initial 'resting' or basal state, the OCR was higher in cardiomyocytes derived from Barth subjects relative to control, i.e., these cells were consuming more oxygen to maintain a basal energy level, which suggests inefficient mitochondrial function. This difference found for Barth-derived cells at a basal respiration rate was partially ameliorated by elamipretide. Of greater significance, when cells were forced to respire at a maximum rate, Barth-derived cells had a decrease of 30% – 40% in OCR relative to non-Barth cells. This deficit was improved substantially with elamipretide treatment (1 μM). Elamipretide had no effect on OCR at maximal respiration in cardiomyocytes derived from non-Barth syndrome subjects (Wang G et al, 2016). Also, of importance is the difference between the OCR at maximal versus basal respiration rate. This difference is often referred to as the 'reserve capacity' available for mitochondria to respond under stress conditions that could be induced in cardiomyocytes by strenuous activity, for example. Cardiomyocytes from Barth subjects had little to no such reserve capacity when treated with vehicle only; however, the reserve capacity was substantially improved with elamipretide treatment.

Figure 5 Elamipretide Improved Mitochondrial Function in iPSC-derived Cardiomyocytes from Barth vs. Normal Human Subjects



Abbreviations: ELAM = elamipredide; OCR = oxygen consumption rates.

4.1.2. Skeletal Muscle

Elamipretide was effective in nonclinical models of both skeletal and cardiac muscle dysfunctions. Elamipretide restored ATP production in the skeletal muscle of old (27 months) mice to levels comparable to those found in young (5 months) mice 1 hour after a single administration, which translated into increased muscle endurance (Siegel MP et al, 2013). In a rat model of muscle wasting, elamipretide reduced functional loss of the diaphragm muscles after the animals were placed on a ventilator for 12 hours (Powers S et al, 2011) and in another study, elamipretide prevented casting-induced skeletal muscle atrophy via protecting mitochondrial function (Talbert E et al, 2013).

In a study using a mouse model of immobilization induced skeletal muscle atrophy, it was shown that elamipretide rescued skeletal muscle from disuse-induced atrophy via prevention of mitochondrial ROS production (Min K et al, 2011).

These improvements in mitochondrial function were also observed in non-clinical studies using patient fibroblasts from nPMD (data on file) and translated into improved ATP generation in muscles from aged individuals (Roshanravan B et al, 2020).

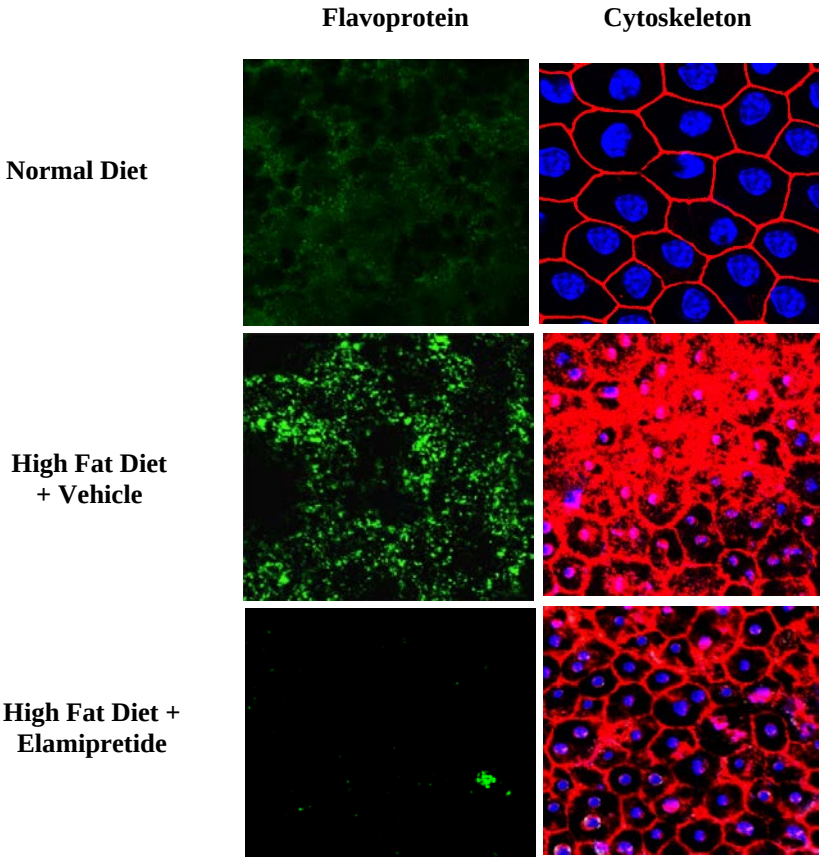
4.1.3. Dry Aged-related Macular Degeneration

Elamipretide was effective in reducing retinal levels of oxidative stress, cytoskeletal abnormalities, and pathologic deposition of extracellular matrix protein in the sub-RPE layer in both rodent and rabbit models of dry age-related macular degeneration (dry AMD) induced by metabolic stress or the environmental toxin hydroquinone. Data are summarized in Table 5 and exemplary data are presented in Figure 8.

Table 5 Summary of Findings in Preclinical Models of Dry AMD Treated with Elamipretide

Model	Induction	Elamipretide Dosing	Results
Chronic: Aged human ApoE4 knock-in mice	Animals aged to 16 months, then fed high fat diet for 4 months	Daily SC at 3mg/kg/day for final 30 days of experiment	Reduced oxidative damage to retinal flavoproteins; reduction in cytoskeletal derangement; reduction of sub-RPE deposits; improved A wave and B wave amplitude in ERG
Acute: Normal C57/Bl6 Mice	Subconjunctival HQ injection of 75 mM HQ q 4 days x 3	SC at 3mg/kg dosed once 24 hours before animal sacrifice	Reduction of retinal ROS; reduction in cytoskeletal derangement; reduction of extracellular matrix protein deposits
Acute: Dutch Belted Rabbits	Subconjunctival HQ injection of 250 mM HQ q 4 days x 3	SC at 3mg/kg dosed once 24 hours before animal sacrifice	Reduction of retinal ROS; reduction in cytoskeletal derangement; reduction of extracellular matrix protein deposits

Figure 6 Elamipretide Reverses Retinal Oxidative Damage and Cytoskeletal Derangement in a Mouse Model of Dry AMD

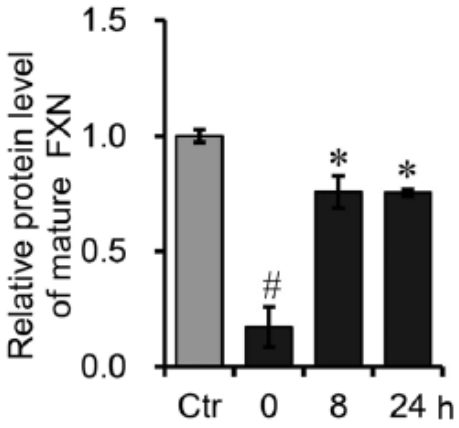


Depicted are confocal images of retinal flavoprotein autofluorescence, indicative of oxidative damage, and phalloidin staining of the actin cytoskeleton in normal or ApoE4 transgenic knock-in mice fed a high fat diet treated with vehicle or elamipretide. Adapted from S. Cousins, Duke Eye Center, Angiogenesis, Exudative, and Degeneration, February 2017, Miami, FL

4.1.4. Friedreich's Ataxia

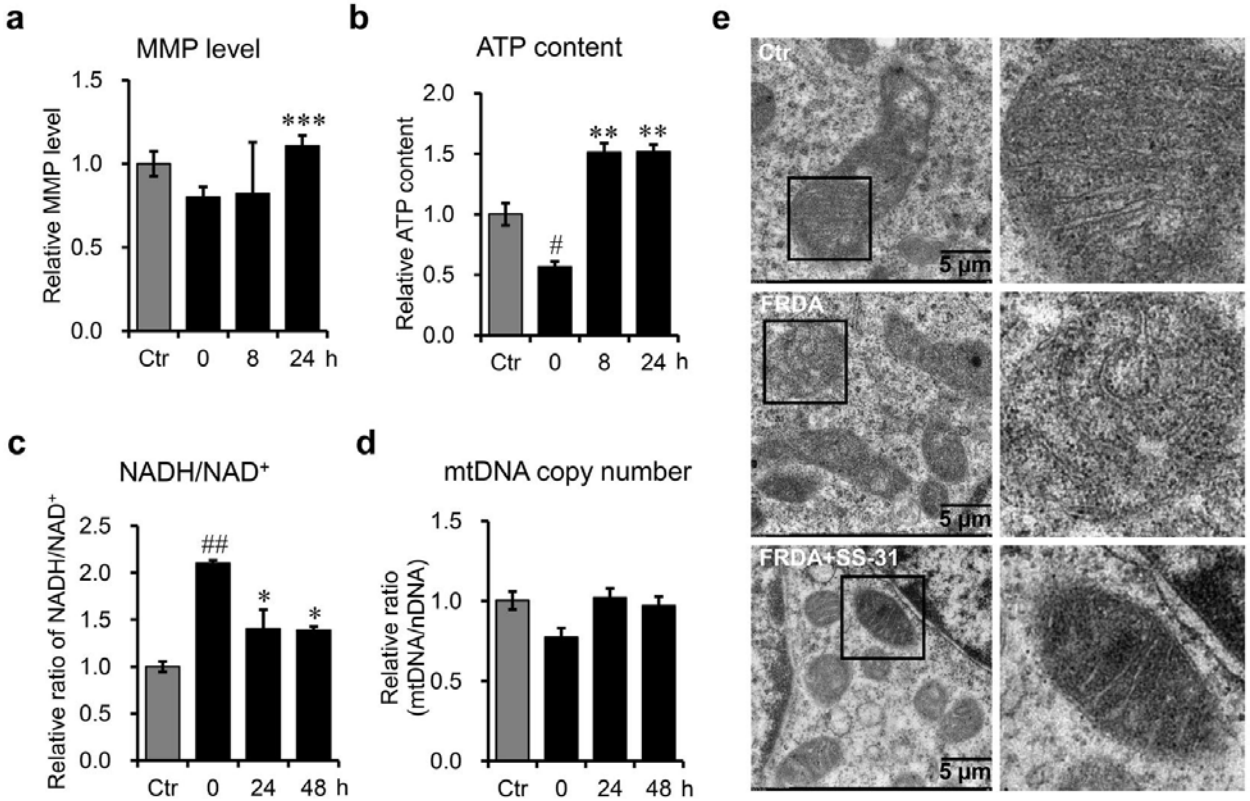
In a cell-based experiment in FA patient-derived cells, treatment with elamipretide was shown to increase the expression of frataxin in both lymphoblasts and fibroblasts in a dose dependent manner, as shown below in Figure 9 (Zhao H et al, 2017). In addition to increasing expression of frataxin, elamipretide was also found to increase the enzymatic activities of the iron-sulphur enzymes, including aconitase and complex II and III of the respiratory chain, and to improve mitochondrial membrane potential, ATP content, NAD+/NADH, and the morphology of mitochondria, as shown below in Figure 10 (Zhao H et al, 2017).

Figure 7 Frataxin Level After Elamipretide Treatment



Quantification of the FXN protein level after elamipretide treatment in lymphoblasts derived from a FA patient reaching 75% of healthy control. Values represent mean ±SEM (n=3, each duplicates). A one-way ANOVA was carried out, *p<0.05 compared with Ctr; *p<0.05 compared with the untreated cells (t=0). GAPDH was used as an internal control.

Figure 8 Improvements in FA Cells After Elamipretide Treatment.

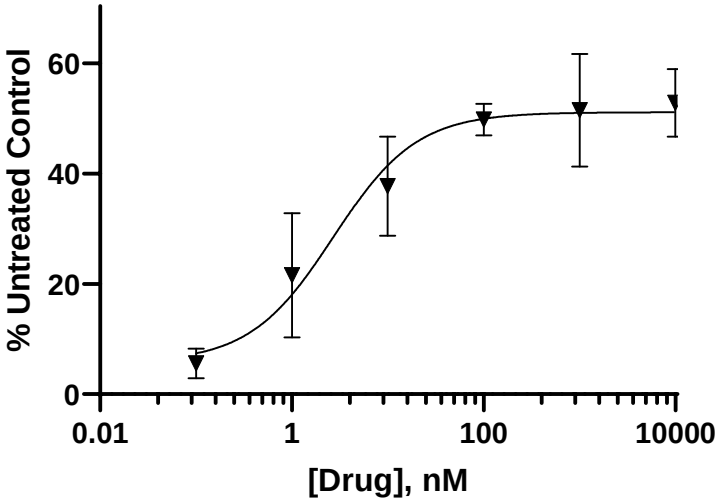


Elamipretide (SS-31) improves the quality and quantity of mitochondria in FA cells after elamipreitde treatment.
(a) Mitochondrial membrane potential (MMP), examined with a fluorescent probe JC-1. (b) ATP content.
(c) Ratio of NADH/NAD+. (d) Relative copy number of mitochondrial DNA (mtDNA) normalized to nuclear DNA (nDNA). CytB represents mtDNA genome; β-actin represents nuclear genome. Values represent mean ± SEM (n = 4, each duplicates). (e) Mitochondrial morphology revealed by electron transmission microscopy. Ctr: GM15849; FA: GM15850; FA+SS-31: FA cells treated with elamipretide for 24 h. Values represent mean ± SEM (n = 3, each duplicates). A one-way ANOVA was carried out, #p < 0.05, compared with Ctr. *p < 0.05, **p < 0.01, ***p < 0.001, compared with the untreated FA cells.

Additionally, a significant protective dose response to elamipretide was identified in a cell death model utilizing FA patient cells. These results depicted in

Figure 11 indicate differential susceptibility of frataxin deficient cells to ferroptosis may be mitigated by elamipretide treatment. These findings suggest that elamipretide may not only improve levels of mature frataxin in FA patient derived cells, but also address many of the downstream manifestations of cellular dysfunction that result from FA-associated frataxin deficiencies.

Figure 9 Elamipretide Protects FA Patient Fibroblasts from Ferroptotic Cell Death Induced by Iron Overload and Glutathione Peroxidase Inhibition



Ferroptotic cell death was induced by sequential treatment of primary FA fibroblasts with ferric ammonium citrate and L-Buthionine Sulfoximine (BSO) for 72 hours. Elamipretide was added at doses from 0.1 to 10,000nM two hours following BSO treatment. Cell death was quantified by MTT assay. These unpublished data obtained via collaboration with Dr. Robert Wilson, Children's Hospital of Philadelphia.

4.2. Pharmacology of Metabolites

The biologic activity of the 2 elamipretide metabolites, the M1 tripeptide and M2 dipeptide, seen in human plasma was assessed in the isolated guinea pig heart model (*SPI-ECU-10-01*). Infarct masses in isolated guinea pig hearts were evaluated following global (no-flow) ischemia for 20 minutes followed by coronary reperfusion for 2 hours with elamipretide metabolites, M1 (0.1 nM, 1 µM), or M2 (0.1 nM, 1 µM). The design of the study was similar to that of *SPIRIIV-N109*, in which elamipretide was active at 0.001 nM. At a concentration of 1 µM, neither metabolite provided myocardial protection against ischemia-reperfusion injury (IRI).

4.3. Secondary Pharmacology

Based on the *in vitro* receptor screens conducted, elamipretide and its metabolites are not expected to have off-target pharmacological effects at therapeutic concentrations.

The binding affinity of elamipretide at numerous receptors was evaluated in 2 separate and independent studies (*SPIRIIV-N105* and *SPI-CER-17-01*). At an elamipretide concentration of 10 μM (6.4 μg/mL), no significant binding (defined as ≥50% inhibition of binding) was observed except for 2 receptors: Neuropeptide Y Y_1 and Opiate κ (~65%) in study *SPI-CER-17-01*. Although significant inhibition was observed for these receptors, the concentration used represents 5 times the clinical C_{max} and suggests low potential for adverse effect in humans.

No direct interaction between elamipretide and antidiuretic hormone (ADH also known as vasopressin) was evident. Elamipretide, at concentrations of 0.1 and 10 μM (0.064 and 6.4 μg/mL), did not bind to vasopressin receptors (V_1 or V_2) nor did it act as a direct V_1 or V_2 agonist or antagonist (*SPI-CER-10-01*).

Elamipretide exhibited minimal potential to inhibit kinase activity (*SPI-CER-17-02*) at a concentration of 10 μM.

Furthermore, elamipretide metabolites, M1 and M2, were negative in a battery of human *in vitro* receptor binding assays at a concentration of 10 μM (*SPI-CER-11-02*). Specifically, both metabolites were negative in the vasopressin receptor binding assays (*SPI-CER-11-01*) at concentrations up to 10 μM (4.9 μg/mL for M1 and 3.6 μg/mL for M2).

4.4. Safety Pharmacology

In a non-GLP study (*CP-0002379*), elamipretide caused no significant inhibition of the human ether-à-go-go-related gene (hERG) channel current at concentrations up to 100 μM (64 μg/mL).

Elamipretide had no adverse effect on cardiovascular and respiratory function in dogs, following a 7-hour continuous IV infusion at doses up to 10 mg/kg/h (*SPIRIIV-N107*). Elamipretide was related to release of histamine that was associated with peripheral vasodilation and minimal decreases in blood pressure and increased heart rate at 10 mg/kg/h. No QT prolongation was evident. All changes were reversible, and no significant elamipretide-mediated adverse cardiovascular or respiratory effects were observed at up to 10 mg/kg/h in the dog.

The absence of adverse effects on the cardiovascular system was confirmed in a follow-up study in which elamipretide was administered in dogs by SC injection at up to 30 mg/kg (*MTP-131-TOX004*). Elamipretide was not associated with clinical signs of toxicity, or changes in body temperature, QRS complex, or QT interval corrected by the Van Der Water's formula (QTcV) intervals at any dose level. Elamipretide-related effects were seen only at 30 mg/kg and were limited to transient changes in blood pressure, pulse pressure, heart rate, and shortening of the PR, RR, and QT intervals. Effects typically peaked at 25- to 40-minutes post-dose and resolved by 55-minutes post-dose. Due to the transient nature of the findings and the absence of clinical signs, the 30 mg/kg dosage was considered to be non-adverse.

Subcutaneous administration of elamipretide at doses up to 100 mg/kg to rats had no effects on respiratory frequency, tidal volume or minute volume (*MTP-131-TOX005*) or on the behavioral, physiological or neurological state (*MTP-131-TOX006*).

4.5. Pharmacokinetics and Drug Metabolism in Animals

The PK of elamipretide has been investigated in both *in vivo* and *in vitro* studies. Plasma PK after single IV or SC administration was evaluated in rats, dogs, and monkeys; PK after IV infusion was evaluated in rats and dogs. Toxicokinetic (TK) data have also been obtained in the repeat-dose IV bolus and SC toxicity studies in rats and dogs. *In vitro* studies included measurement of elamipretide binding to proteins in dog and human plasma. The distribution of unlabeled, radioactive iodine ^{125}I-labeled, and radioactive carbon ^{14}C-labeled elamipretide was evaluated in the rat. Distribution of ^{125}I-labeled elamipretide was also evaluated in mice. *In vitro* stability studies of elamipretide were conducted in plasma and liver microsomes from mice, rats, dogs, monkeys, and humans. *In vivo* metabolism of ^{125}I-labeled elamipretide was assessed in mice and rats; plasma, selected tissues, and excreta were analyzed. In addition, the PK of elamipretide metabolites was determined after administration of elamipretide to rats and dogs. Excretion was assessed in rats using unlabeled and ^{125}I-labeled elamipretide. The inhibitory potential of elamipretide on the activity of drug-metabolizing enzyme cytochrome P450 (CYP) was evaluated using recombinant enzymes or pooled human hepatic microsomes. In addition, a preliminary study of CYP enzyme induction was conducted after administration of elamipretide to rats.

The *in vivo* studies were conducted primarily in Sprague-Dawley rats and beagle dogs, which characterized the absorption, distribution, metabolism, and excretion of elamipretide. In addition, the TK analyses for the pivotal repeat-dose toxicity studies of elamipretide were performed in full compliance with Good Laboratory Practice (GLP) regulations.

4.5.1. Pharmacokinetics

A series of single- and repeat-dose IV and SC studies have been conducted to characterize the plasma PK and SC bioavailability of elamipretide. The species and strains used in these studies have been Sprague-Dawley rats, beagle dogs, and cynomolgus monkeys. These species include those employed in the definitive 35-day IV bolus, 28-day and 90-day, and 26-week (rat) and 39-week (dog) SC toxicity studies.

4.5.1.1. Intravenous Administration

After a single IV bolus dose of elamipretide to rats, dogs, and monkeys, the plasma clearance was generally less than 25% of the hepatic and renal blood flow and the volume of distribution was less than total body water. The terminal half-life was approximately 1 hour in the rat and the monkey, and 2 hours in the dog. No sex differences were seen. The PK parameters are summarized as overall means for males and females combined in Table 6.

Table 6 Plasma PK Parameters of Elamipretide Following Single IV Administration to Rats, Dogs, and Monkeys

Species (Sex)	Dose (mg/kg)[a,b]	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{0\text{-}inf}$ (ng.hr/mL)	$t_{½}$ (hr)	CL (L/hr/kg)	V_{ss} (L/kg)
Rat[c,f] (M/F)	1.0	0.033	4,162	2,125	0.80	0.478	0.288
Dog[d,f]	1.0	0.083	2,735	2,235	1.96	0.464	0.433
	1.0 + 1mg/kg/h x 2h	0.33	2,472	5,819	1.55	0.534	0.824
	3.0 + 3mg/kg/h x 2h	0.25	7,827	20,507	1.58	0.459	0.785
	0.5 + 3mg/kg/h x 2h	2.00	3,927	9,361	2.40	0.694	1.188
Monkey[e,g]	1.0	0.083	3,291	3,290	1.05	0.304	0.323

Abbreviations: $AUC_{0\text{-}inf}$ = Area under the plasma concentration-time curve from time 0 to infinity, CL = Clearance, C_{max} = Maximum plasma concentration, T_{max} = Time C_{max} occurred, $t_{½}$ = Terminal half-life, V_{ss} = Volume of distribution at steady state

a Vehicle consisted of elamipretide in 0.01M PBS, pH = 7.0.

b All doses are expressed in terms of net peptide content of elamipretide.

c Single-dose rat (Sprague-Dawley) data from RP- MTP-131-002_RPK.

d Single-dose dog (beagle) data from RP-MTP-131-003_DPK and RP-MTP-131-013_DPK.

e Single-dose monkey (cynomolgus) data from RP-SPI-DMPK-SS-31-007.

f Rat and dog parameters represent mean for equal numbers of males and females.

g Monkey parameters derived from males only.

The TK parameters for elamipretide (and its 2 metabolites) after daily IV bolus injection for 35 days to rats and dogs were investigated (*SPI-CIT-13-01, SPI-CIT-13-02*). In both species, the high-dose group received elamipretide at the mid-dose level for the first week and then received the high-dose level for the remaining 4 weeks. In rats, steady-state C_{max} and $AUC_{0\text{-}24}$ of elamipretide increased in a dose-proportional manner. When determined, elamipretide plasma clearance (CL), volume of distribution at steady state (V_{ss}) and terminal half-life ($t_{½}$) remained unchanged across the dose levels. Plasma clearance values ranged from 422.9 to 816.4 mL/h/kg, and V_{ss} values ranged from 193.4 to 490.9 mL/kg. In dogs, steady-state exposure to elamipretide increased with dose. No sex-related differences or drug accumulation was evident in either species.

Steady-state exposure of M1 and M2 increased with elamipretide dose. Exposure to M1 was similar to or lower than exposure to elamipretide and the apparent terminal $t_{½}$ of M1 was comparable to that of elamipretide. Metabolite M2 appeared to be a minor metabolite in rats, with relative $AUC_{0\text{-}24}$ values ranging from 3.8 to 6.5%; in contrast, the relative $AUC_{0\text{-}24}$ values for M2 in dogs ranged from 15 to 45%. In each species, the $t_{½}$ of M2 was longer than that of elamipretide. No sex-related difference in the TK parameters for M1 or M2 was seen in rats or dogs. Table 7 lists major PK parameters in rats and dogs.

Table 7 Plasma PK Parameters of Elamipretide Following IV Administration to Rats and Dogs for 35 Days

Species	Elamipretide Dose	Analyte	C_{5min} / C_{max}[a] (ng/mL)	AUC_{last} (ng·h/mL)	$t_{½}$ (hr)
Rat[b]	10 mg/kg/day	Elamipretide	45,875	24,465	1.38
		M1	9,058	24,020	0.79
		M2	229	1,538	13.9
Dog[c]	10 mg/kg/day	Elamipretide	39,800	24,300	5.0
		M1	21,250	22,050	8.7
		M2	321	2,915	56.0[d]

Abbreviations: AUC_{last} = area under the plasma concentration-time curve from hour 0 to the time of the last measurable concentration; C5 min = plasma concentration at 5 minutes; C_{max} = maximum concentration in plasma; M1 = tripeptide C-terminus degradation product of elamipretide; M2 = dipeptide C-terminus degradation product of elamipretide; $t_{1/2}$ = terminal half-life.

[a] C_{5min} for elamipretide and C_{max} for metabolites M1 and M2. [b] SPI-CIT-13-01. [c] SPI-CIT-13-02. [d] male dog only

4.5.1.2. Subcutaneous Administration

Single-dose PK studies showed that elamipretide was rapidly absorbed following SC injection. Data are shown in Table 8. Peak plasma concentration was generally observed within 0.5-hour post-dose. The SC bioavailability was estimated to be 35% in rats and ~80% in dogs and monkeys. Dose proportionality was seen over a single dose from 1 to 10 mg/kg in rats and dogs. No sex differences were noted.

Table 8 Plasma PK Parameters of Elamipretide Following Single SC Administration to Rats, Dogs, and Monkeys

Species (Sex)	Dose (mg/kg)[a,b]	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{0\text{-}inf}$ (ng.hr/mL)	$t_{½}$ (hr)	CL (L/hr/kg)	V_{ss} (L/kg)	F (%)
Rat[c,f] (M/F)	1.0	0.25	628	817	0.59	1.29	NA	38.4
	3.0	0.25	1,827	2,264	0.78	1.38	NA	35.5
	10.0	0.33	3,553	5,210	0.82	1.99	NA	24.5
Dog[d,f]	1.0	0.50	763	1,729	1.65	0.60	NA	78.1
	3.0	0.50	2,698	5,278	1.91	0.59	NA	78.8
	10.0	0.75	8,546	19,728	1.31	0.53	NA	88.4
Monkey[e,g]	1.0	0.75	1,289	2,674	0.67	0.377	0.501	81.4

Abbreviations: $AUC_{0\text{-}inf}$ = Area under the plasma concentration-time curve from time 0 to infinity, CL = Clearance, corrected for fraction of drug reaching the circulation, C_{max} = Maximum plasma concentration of elamipretide, F = Bioavailability, NA = Not applicable or not available, T_{max} = Time C_{max} occurred, $t_{½}$ = Terminal half-life, V_{ss} = Volume of distribution at steady state, corrected for fraction of drug reaching the circulation

a Vehicle consisted of elamipretide in 0.01M PBS, pH = 7.0.

b All doses are expressed in terms of net peptide content of elamipretide.

c Single-dose rat (Sprague-Dawley) data from RP- MTP-131-002_RPK.

d Single-dose dog (beagle) data from RP-MTP-131-003_DPK.

e Single-dose monkey (cynomolgus) data from RP-SPI-DMPK-SS-31-007.

f Rat and dog parameters represent mean for equal numbers of males and females.

g Monkey parameters derived from males only.

The TK of elamipretide were investigated in rats and dogs that received daily SC injections for 28 and 90 days (*MTP-131-TOX008, MTP-131-TOX002; SPI-RIC-13-03, SPI-RIC-13-04*) provided confirmation of the findings from shorter duration studies.

The TK parameters for elamipretide, M1 and M2 following daily SC injections of elamipretide in the rat and dog for 26-weeks (*SPI-CIT-15-03*) and 39-weeks (*SPI-CIT-15-02*), respectively are summarized in Table 9 and Table 10.

SPI-CIT-15-03: In the rat, the TK evaluation of elamipretide in plasma following single and repeated once daily SC administration to Sprague-Dawley rats at doses of 10, 20 and 40 mg/kg/day from Day 1 to Day 7, and 5, 10 and 15 mg/kg/day from Day 8 onwards, revealed that generally, the mean maximum plasma concentrations of elamipretide were achieved 0.5-hour post-dose for all dose levels and for both sexes. On all sampling days, the increase in exposure to elamipretide between the low- and the mid-dose was approximately dose proportional whereas it was slightly lower than dose proportional between the low-and the high-dose when using mean C_{max}. Between Day 19 and Day 182, there was no clear evidence of accumulation of parent, M1 or M2 following repeated SC administration of elamipretide for all dose levels, suggestive of achievement of steady state on Day 19. There were no clear differences in the kinetics of parent, M1 or M2 observed upon comparison of C_{max} and AUC_{last}

between sexes following single and multiple administrations. The PK data are summarized in Table 9.

SPI-CIT-15-02: In the dog, the TK of elamipretide and its metabolites (M1 and M2) were assessed in male and female beagle dogs at dose levels of 2.5, 10, and 20 mg/kg/day following a single SC administration (Day 1) and following repeated SC administrations (Days 133 and 273) at dose levels of 2.5, 5 and 10 mg/kg/day starting from Day 79/80. Plasma exposure of elamipretide and metabolites was generally dose-proportional throughout the study. With repeated administration, compared to Day 1, there was no impact on overall plasma exposure to elamipretide, while there was a slightly reduced exposure to M1 over time, and slightly increased exposure to M2 at the low dose of 2.5 mg/kg/day, only. There were no clear sex differences in parent, M1 or M2 kinetics in all treated groups, and no apparent impact of histamine pretreatment on the TK of elamipretide and its metabolites. The PK data are summarized in Table 10.

Table 9 Mean Plasma TK Parameters for Elamipretide, M1 and M2 on Day 182 Following Daily SC Administration of Elamipretide to Rats (male and female combined) for 26-Weeks (SPI-CIT-15-03)

Elamipretide Dose (mg/kg/day)	Analyte	T_{max} (hr)	C_{max} (ng/mL)	AUC_{last} (ng·hr/mL)	$t_{½}$ (hr)	Relative % Parent AUC_{last}
5.0	Elamipretide	0.50	3,650	7,795	0.895	100
	M1	3.00	5,240	22,850	ND	293
	M2	4.50	128	1,054	ND	13.7
10.0	Elamipretide	0.50	4,920	15,100	1.19	100
	M1	4.50	6,570	38,550	ND	255
	M2	4.50	211	1,900	ND	12.6
15.0	Elamipretide	0.75	6,875	23,850	0.89*	100
	M1	4.50	7,385	43,000	ND	182
	M2	4.50	260	2,470	ND	10.4

Abbreviations: AUC_{last} = Area under the plasma concentration-time curve at the last observation, C_{max} = Maximum plasma concentration, F = Female, M = Male, ND = Not Determined, T_{max} = Time C_{max} occurred, $t_{½}$ = Terminal half-life
* Female only

Table 10 Mean Plasma TK Parameters for Elamipretide, M1, and M2 on Day 273 Following Daily SC Administration of Elamipretide to Dogs (male and female combined) for 39-Weeks (SPI-CIT-15-02)

Elamipretide Dose (mg/kg/day)	Analyte	T_{max} (hr)	C_{max} (ng/mL)	AUC_{last} (ng·h/mL)	$t_{½}$ (hr)	Relative % Parent AUC_{last}
2.5	Elamipretide	0.38	3,465	6,815	7.60*	100
	M1	1.00	701	2,715	6.21	41.5
	M2	2.00	126	2,710	ND	32.7
5.0	Elamipretide	0.50	6,225	10,950	ND	100
	M1	1.00	764	2,775	ND	25.4
	M2	3.01	194	3,455	ND	31.6
10.0	Elamipretide	0.50	13,000	21,100	4.74*	100
	M1	1.00	1,700	5,500	ND	26.1
	M2	1.00	312	5,085	ND	24.1

Abbreviations: AUC_{last} = Area under the plasma concentration-time curve at the last observation, C_{max} = Maximum plasma concentration, F = Female, M = Male, ND = Not Determined, T_{max} = Time C_{max} occurred, $t_{½}$ = Terminal half-life
* Males only

SPI-APT-16-01: The TK of elamipretide and metabolites following administration of elamipretide acetate, elamipretide HCl and elamipretide HCl LTS (degraded form of elamipretide HCl obtained by Long-Term Storage simulation at 60ºC for 1 week) were compared in a GLP toxicity study. Test articles were administered by SC injection to male and female Sprague-Dawley rats at 15 mg/kg/day (acetate and HCl) or 5 and 15 mg/kg/day (HCl LTS) for 28 days. Generally, no significant differences in elamipretide, M1, and M2 systemic exposure were observed after single and repeat administration in all treatment groups. There were no clear sex differences in parent, M1 or M2 kinetics in all treated groups. Median T_{max} for elamipretide occurred between 0.5 and 1 hours after dosing on both Day 1 and Day 28 in both sexes in all treatment groups. Key TK parameters following daily SC injections of test article are summarized in Table 11.

Table 11 Mean Toxicokinetic Parameters in Rats (Male and Female Combined) Following Repeat Subcutaneous Administration of Elamipretide Acetate, Elamipretide HCl, or Elamipretide HCl LTS Once Daily for 28 Days (SPI-APT-16-01)

	Elamipretide			Metabolite M1			Metabolite M2		
Analyte	AUC_{0-t} (ng.h/mL)	C_{max} (ng/mL)	*T_{max} (h)	AUC_{0-t} (ng.h/mL)	C_{max} (ng/mL)	*T_{max} (h)	AUC_{0-t} (ng.h/mL)	C_{max} (ng/mL)	*T_{max} (h)
Elamipretide HCl LTS (5 mg/kg/day)	4,650	2,905	0.50	20,300	6,935	2.00	777	128	2.00
Elamipretide HCl LTS (15 mg/kg/day)	16,950	6,640	1.00	31,950	6,215	3.00	2,005	198	3.00
Elamipretide HCl (15 mg/kg/day)	14,750	4,890	1.00	24,550	5,900	4.00	1,980	194.5	4.00
Elamipretide Acetate (15 mg/kg/day)	12,900	5,250	1.00	32,050	7,795	3.00	2,210	259	3.00

Abbreviations: AUC_{0-t} = Area under the plasma concentration-time curve from the start of dosing (0) to the last quantifiable time point (t), C_{max} = Maximum plasma concentration, *T_{max} (median presented) = Time C_{max} occurred

SPI-CIT-16-04: The TK of elamipretide and metabolites was evaluated in juvenile male and female Sprague-Dawley rats that received elamipretide by once-daily SC injection from *post-partum* Days 7 to 63 (PPD7 to PPD63) at dose levels of 5, 10, and 15 mg/kg/day, with TK reported for each dose level. With males and females combined, plasma exposure to elamipretide was generally dose proportional, with exceptions at the high dose where AUC_{last} showed greater increases. Once normalized to elamipretide, the systemic exposure of all 3 analytes (elamipretide, M1, and M2), was similar across groups on PPD7, while on PPD63, M1 was the highest in the low dose group and similar in the mid and high dose groups. Metabolite-to-parent molar ratios suggested high conversion of elamipretide to M1, whereas very low for M2. Key TK parameters following daily SC injections of elamipretide are summarized in Table 12.

Table 12 Mean Toxicokinetic Parameters in Rats (Male and Female Combined) Following Repeat Subcutaneous Administration of Elamipretide in Neonatal Status Advancing into Juvenile Status Rats (male and female combined) Once Daily for 8 Weeks (SPI-CIT-16-04)

Analyte	Elamipretide			Metabolite M1			Metabolite M2		
Elamipretide Dose Level (mg/kg/day)	$AUC_{0\text{-last}}$ (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0\text{-last}}$ (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0\text{-last}}$ (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)
Post-Partum Day 7									
5	9,250	5,310	0.5	10,950	3,245	0.75	732	77	2.0
	19,000	9,435	0.375	23,400	6,735	1.0	1,755	177	3.0
15	31,000	14,850	0.5	42,150	10,950	1.0	2,425	259	3.0
Post-Partum Day 63									
5	6,995	3,870	0.5	7,635	4,075	2.0	694	96.5	2.0
10	22,800	10,070	0.5	8,475	3,595	2.0	929.5	107	3.0
15	43,450	12,250	0.5	12,050	5,240	3.0	1,415	208.5	2.0

Abbreviations: $AUC_{0\text{-last}}$ = Area under the plasma concentration-time curve from the start of dosing (0) to the last quantifiable time point, C_{max} = Maximum plasma concentration, F = female, M = male, T_{max} (median presented) = Time C_{max} occurred

4.5.2. Distribution

No clinically or statistically significant *in vitro* elamipretide plasma protein binding was observed in dog and human plasma at concentrations ranging from 200 to 10,000 ng/mL (*RP-MTP-131-007-PPB*). Plasma protein binding was not evaluated in rat plasma because elamipretide was not stable under the experimental conditions at 37°C.

The ^{125}I-elamipretide-related radioactivity was rapidly distributed following SC administration to rats and mice (Radio DE-001). Most of the ^{125}I-elamipretide-related radioactivity distributed into organs involved in urinary excretion (kidney, urinary bladder and urine), with lower concentrations detected in circulation (plasma).

Tissue distribution was assessed in rats receiving a single 4-hour IV infusion of 0.5 mg/kg/hr ^{14}C-elamipretide (SPI-XBL-12-01). Elamipretide had insignificant association to blood cells and was located primarily in the plasma compartment. Kidney exposure to drug-derived radioactivity was high (~50-fold greater than plasma), with a radioactivity $t_{1/2}$ similar to that of plasma, consistent with renal clearance of ^{14}C-elamipretide and its metabolites. Potentially therapeutic concentrations of elamipretide were found in the eyes, heart, skeletal muscle and brain with concentrations of drug-derived radioactivity decreasing immediately following cessation of infusion, which suggests no noteworthy tissue retention.

A follow-up study was conducted to determine the concentrations of ^{14}C-elamipretide-derived radioactivity within the substructures of selected tissues (*SPI-XBL-12-02*). Rats received a single IV infusion of ^{14}C-elamipretide at 0.5 mg/kg/hr for 4 hours. Distribution of ^{14}C-elamipretide-derived radioactivity was similar to the previous data however certain substructures within the brain, eyes and heart were exposed to substantially higher concentrations of elamipretide and/or its metabolites than would be implied by simple gross or average quantification of ^{14}C-elamipretide-derived radioactivity.

4.5.3. Metabolism

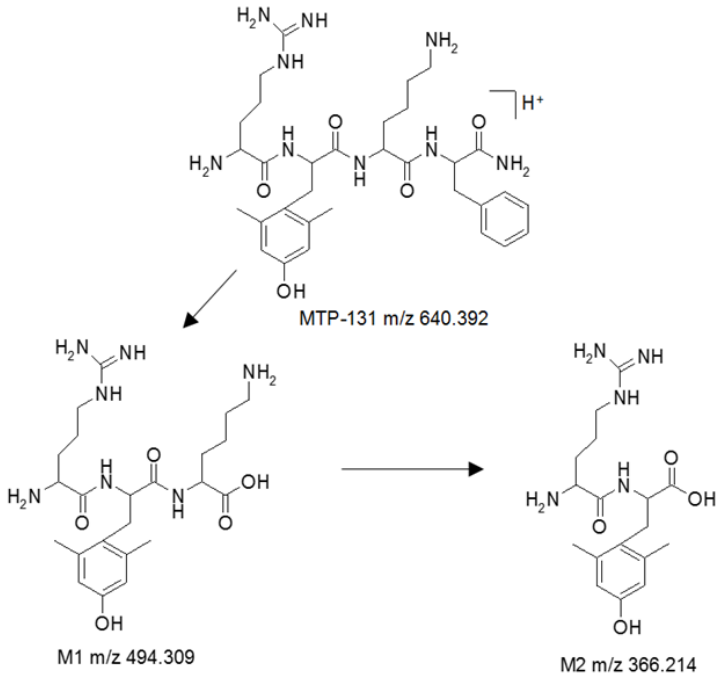
In vitro plasma stability of elamipretide (trifluoroacetate salt) was determined across species (*SPI-DMPK-SS31-004*). Plasma spiked with elamipretide was incubated for 6 hours at 37°C and analyzed by LC-MS/MS. At 1 µg/mL, the *in vitro* t½ values were 3.59, 1.78, 7.69, 4.25, and 30.8 hours in plasma from mouse, rat, dog, monkey, and human, respectively. Similar results were obtained at 10 µg/mL demonstrating that elamipretide was stable in dog, monkey and human plasma, but was rapidly degraded in rat and mouse plasma. Based on these results, rat samples collected for PK or TK analysis were acidified with formic acid to stabilize the elamipretide.

Comparative metabolism and metabolic stability of elamipretide across species have been investigated using hepatic microsomal systems. In study *RP-MTP-131-006-DDI_MS*, the metabolic stability of elamipretide (3 µM) was determined in the incubations of pooled liver microsomes prepared from the male mouse (CD-1), rat (Sprague-Dawley), dog (beagle), monkey (cynomolgus), and male/female human donors. Incubations were conducted in the presence of fortified NADPH (co-factor of oxidative metabolism). Results indicated that 97.4%, 97.5%, 55.7%, 92.1% and 97.2% of elamipretide remained at the end of 60-min incubations with hepatic microsomes from human, monkey, dog, rat, and mouse, respectively. In an experiment in which the incubation was conducted at a higher elamipretide concentration (50 µM) for metabolite search using UPLC-Q/TOF-MS methods (*RP-MTP-131-005_RME*), none of the potential hydrolytic metabolites were found.

In vivo metabolite profiles were evaluated in rats and mice receiving SC administration of ^{125}I-labeled elamipretide (*Radio DE-001*). Rats each received 0.69 mg (3318 kBq, ~3 mg/kg), whereas each mouse received 0.126 mg (606 kBq, ~4.2 mg/kg). In both species, parent compound and the 2 major metabolites (M1 and M2) were found in plasma, urine, liver and kidney homogenates.

The identity of the 2 metabolites was subsequently characterized after dosing with unlabeled elamipretide (3 mg/kg, SC) to rats (*RP-MTP-131-005_RME*). Based on the molecular weight and mass spectral fragmentation patterns along with chromatographic retention times, these 2 metabolites were consistent as products from C-terminal degradation. Metabolite M1 was identified as D-Arg-Dmt-Lys-OH, and M2 was identified as D-Arg-Dmt-OH. The proposed metabolic pathway of elamipretide is presented in Figure 12.

Figure 10 Proposed Metabolic Pathway of Elamipretide in Rats



M1 = tripeptide C-terminus degradation product of elamipretide; M2 = dipeptide C-terminus degradation product of elamipretide; MTP-131 = elamipretide. Source: RP-MTP-131-005_RME

4.5.4. Excretion

In rats, elamipretide and its metabolites were excreted primarily in urine; the excretion was considered rapid and complete. Routes of excretion and elimination of drug-related material were examined in the rat following SC administration of ^{125}I-labeled elamipretide (0.69 mg, 3318 kBq per rat, approximately 3 mg/kg) (*Radio DE-001*). The ^{125}I-elamipretide-related radioactivity was primarily excreted in urine, with minimal present in feces. Cumulative recovery through 36 hours after SC administration was approximately 90% and 3.7% in urine and feces, respectively. Excreted radioactivity in bile was low, approximately 1% of the dose. Metabolite profiling of the urine samples showed that the majority of the drug-related radioactivity was excreted as metabolites.

4.5.5. Pharmacokinetic Drug Interactions

The potential of elamipretide or its metabolites M1 and M2 to inhibit human CYP was evaluated in a series of *in vitro* and *in vivo* experiments using specific probe substrate assays and recombinant human enzymes (CYP1A2, 2C19, 2D6, 2E1, and 3A4) or human liver microsomes (CYP2C9) (*RP-MTP-131-006-DDI-Inhib*). Elamipretide did not show any inhibitory effects on CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP2E1 at concentrations up to 200 μM, and up to 10 μM for CYP1A1, CYP2B6, and CYP2C8 (*SPI-CER-17-03*). Minimal inhibition was

observed for CYP3A4-dependent activities, with estimated IC_{50} values greater than 200 µM. This weak inhibitory effect is unlikely to be clinically significant.

Elamipretide exhibited no potential to induce hepatic CYP enzyme activities (*RP-MTP-131-006-DDI-Induc*). Activities of CYP1A, CYP2C, CYP2D, and CYP3A of the elamipretide-treated group were 0.86, 0.92, 1.04, and 0.98 times those of the vehicle control group, respectively. Thus, elamipretide had no significant inductive effects on the hepatic CYP1A, CYP2C, CYP2D and CYP3A enzymes in the rat. In human hepatocytes, elamipretide did not induce CYP1A2, CYP2B6 or CYP3A4 at doses up to 100 µM (*SPI-CER-17-03*).

In a series of *in vitro* studies assessing substrate uptake across a range of transporters, elamipretide at 250 µM did not inhibit BCRP activity in CPT-P1 cells or P-gp in Caco-2 cells (*SPI-ABS-11-01*). Similarly, elamipretide (10 µM) exhibited no inhibitory activity when incubated with CHO-K1 cells expressing recombinant human transporter proteins OCT2, BCRP, OAT1, OAT3, OATP1B1, or OATP1B3, or MDR1-MDCKII cells expressing P-gp (*SPI-CER-17-03*). No inhibition of substrate uptake was apparent after incubation of elamipretide (10 and 100 µM) with MDCKII cells expressing MATE2-K (*SPI-SOL-17-01*). There was no substrate interaction with human MATE1, OAT1, OAT3 and OCT2 uptake transporters however, it is possible that elamipretide may be a MATE2-K substrate (*SPI-SOL-18-01*).

Significant inhibition of substrate (where significant is defined as ≥50%) by elamipretide was observed only for the MATE1 transporter (*SPI-SOL-17-01, SPI-SOL-17-02*). Elamipretide inhibited MATE1-mediated metformin accumulation with a calculated IC_{50} of 3.53 µM (*SPI-SOL-17-02*), a concentration 1.8 times the anticipated plasma C_{max} (~2 µM) for a 40 mg/kg dose. Given that MATE1 is expressed in the kidney, and elamipretide exhibits high concentrations in the kidney, further investigation is needed to better understand the clinical importance of this finding. Thus, except for MATE1 inhibition, and the possibility that elamipretide is a MATE2-K substrate, elamipretide is unlikely to affect the protein binding, metabolism, elimination, or uptake of concomitantly administered drugs.

4.6. Toxicology

The safety of elamipretide has been characterized in a series of single- and repeat-dose studies in rats and dogs using IV and SC routes of administration. Additional studies have characterized local tolerability in rats and rabbits. Elamipretide has been evaluated for potential genotoxicity in a full battery of tests; in addition, its metabolites, M1 and M2, have been tested for bacterial mutagenesis in the Ames test and have been qualified in the rat micronucleus test. Other completed studies of toxicology include an *in vitro* hemolysis test, an assessment of pulmonary effects when administered in combination with morphine, reproductive toxicology studies in rats and rabbits, a juvenile (postnatal) toxicity study in rats and a 28-day bridging study in rats comparing elamipretide HCl to elamipretide acetate.

The design of the repeat-dose toxicity studies was appropriate for assessment of systemic and local toxicity after IV bolus or SC administration, as well as reversibility of any effects after SC administration. In addition, these studies determined the TK profile of elamipretide and, in some cases, its metabolites M1 and M2, using appropriate analytical methods.

4.6.1. Intravenous Administration

A number of toxicity studies have been conducted with IV administration, either as an infusion or slow bolus injection, in both rats and dogs. Elamipretide was well-tolerated (no observed effect level) up to doses of 3 mg/kg in both species. Animals administered doses above 3 mg/kg demonstrated histaminergic-like effects which included reddening and swelling of the muzzle and extremities, tremors, labored breathing and behavioral abnormalities. These effects generally worsened with increasing dose, appeared to be temporally related to elamipretide C_{max} and spontaneously resolved within 1 hour post-dose. Elamipretide-related animal deaths were reported at dose levels >30 mg/kg/hr in either species. There were no elamipretide-related end-organ toxicities described in any IV study.

Confirmation of the involvement of histamine in most studies was clearly demonstrated in the IV cardiovascular safety study in dogs, detailed in Section 4.4.

The findings from the longest duration IV administration studies are detailed below.

In a GLP toxicity study (*SPI-CIT-13-01*), elamipretide was administered to rats by slow IV bolus injection once daily for 35 consecutive days. Dose levels were 0 (placebo), 1, 3, and 10 mg/kg/day. Rats in the high-dose group were given 3 mg/kg for 1 week prior to a dose increase to 10 mg/kg for the remaining 4 weeks of the study (in an attempt to overcome any histaminergic reactions). Elamipretide had no effects on mortality, body weight, food consumption, ophthalmology, hematology, coagulation, clinical chemistry, urinalysis, organ weights, or macroscopic or microscopic parameters. Elamipretide-related effects were limited to transient histaminergic-like clinical signs observed in some rats receiving 10 mg/kg/day. Findings included excessive scratching, tremors, salivation, uncoordinated gait, partly closed eyes, lying on cage bottom, sneezing, and wheezing. These findings were transient and reversible each day. The no-observed-adverse-effect-level (NOAEL) was the high dose, 10 mg/kg for 4 weeks (preceded by 7 days at 3 mg/kg/day); Table 7 lists major PK parameters.

In a GLP toxicity study (*SPI-CIT-13-02*), elamipretide was administered to dogs via slow bolus IV injection once daily for 35 consecutive days. Dose levels were 0 (placebo), 1, 3, and 10 mg/kg/day. To overcome histaminergic reactions, dogs in the high-dose group were given 3 mg/kg for 1 week prior to a dose increase to 10 mg/kg for the remaining 4 weeks of the study. Elamipretide had no effects on mortality, body weight, food consumption, ECG, ophthalmology, hematology, coagulation, clinical chemistry, urinalysis, organ weight, or macroscopic or microscopic parameters. Treatment resulted in skin reddening and skin swelling (including pinna[e], periorbital[s], muzzle, ventral cervical region, lip and ear canal[s]) in most elamipretide-dosed animals of all groups. Other minimal transient histaminergic-like clinical

signs considered related to elamipretide treatment included eyes partly closed (1 dog at 1 mg/kg); excessive licking (3 mg/kg), increased vocalization (3 and 3/10 mg/kg); and retching, salivation, increased aggressiveness, and tremors (3/10 mg/kg). In general, these observations commenced soon after dosing and dissipated within 1 hour following dosing. The high dose, 10 mg/kg/day, was considered the NOAEL; Table 7 lists major PK parameters.

4.6.2. Subcutaneous (SC) Administration

A number of toxicity studies have been conducted with SC administration in both rats and dogs. Elamipretide was well-tolerated (no observed effect level) up to doses of approximately 10 mg/kg in the rat and 3 mg/kg in the dog. Animals administered dose levels above 10/3 mg/kg in rats/dogs demonstrated systemic histaminergic-like effects including reddening and swelling of the muzzle and extremities, tremors, labored breathing and behavioral observations. These effects generally worsened with increasing dose, appeared to be temporally related to elamipretide C_{max} and spontaneously resolved within 1-hour post-dose. Local administration site reactions were noted throughout the SC toxicity program with increasing severity related to increased dose concentration. Histopathologic findings at the injection sites included cell infiltration, hemorrhage, tissue necrosis, myofiber degeneration and fibrosis and all findings were consistent with inflammation. All studies where recovery groups were included demonstrated partial or complete recovery from the histopathologic findings indicating their reversibility. No elamipretide-related animal deaths were reported at dose levels up to 30 mg/kg/day in either species. There was no elamipretide-related end-organ toxicities described in any SC study. The findings from the longest duration SC administration studies and a 28-day SC bridging study are detailed below.

4.6.2.1. 26-Week SC Study in Rat

The local and systemic toxicity of elamipretide were assessed, in a GLP toxicity study (*SPI-CIT-15-03*), following once daily SC administration to rats for 26-weeks. Doses were 0 (placebo), 10/5, 20/10, and 40/15 mg/kg/day. Initial dose levels of 10, 20 and 40 mg/kg/day at an injection concentration of 20 mg/mL were decreased to 5, 10 and 15 mg/kg/day at an injection concentration of 6 mg/mL starting on Day 8 of dosing due to minor ulceration at the injection sites. There was 1 unscheduled euthanasia in a female control rat, and 1 male rat at each dose of 10/5 and 40/15 mg/kg/day of elamipretide were found dead. The examination of the euthanized control female rat revealed that it had lymphoma in the bone marrow. An exact cause of death was not determined for either male rat but there were no elamipretide-related macroscopic or microscopic changes in either rat related to death.

No systemic toxicity was apparent at any elamipretide dose tested at dose levels up to 40/15 mg/kg/day. The predominant clinical observation was minor ulceration at the injections site, which resulted in a dose reduction. Additional clinical signs included transient histaminergic-like responses that resolved within 1-hour post-dose.

At necropsy, the primary macroscopic observation in the majority of control and elamipretide-treated animals was subcutis red/hemorrhagic areas at the injection sites that correlated with the last injection. There were no signs of necrosis or muscle damage. There were no adverse elamipretide-related effects on organ weights.

There were no histopathologic findings to indicate a systemic effect of elamipretide in tissues after 6 months of daily SC dosing in rats. The histopathologic findings at the injection site correlated with macroscopic observations and included minimal to moderate SC hemorrhage, inflammation, fibrosis, and myofibril degeneration/regeneration and/or minimal to mild epidermal crust. These findings were observed in all treatment groups including the control group, indicating that the changes were partially procedure-related however, the incidence and/or severity of these microscopic changes were increased in rats dosed with 40/15 mg/kg/day of elamipretide when compared to control rats.

Following a 4-week non-treatment recovery period, the incidence and/or severity of the fibrosis were lower in recovery rats compared to main rats indicating incomplete but progressive ongoing reversal. All other findings were incidental and sporadic with no clear dose-response.

The local NOAEL was 5 mg/kg/day in a 6 mg/mL dose concentration, while the systemic NOAEL was 15 mg/kg/day in a 6 mg/mL dose concentration; TK parameters for this study are listed in Table 9.

4.6.2.2. 39-Week SC Study in Dog

The local and systemic toxicity of elamipretide were assessed, in a GLP toxicity study (*SPI-CIT-15-02*), following once daily SC administration to dogs for 39-weeks. Doses were 0 (placebo), 2.5, 10/5, and 20/10 mg/kg/day. From Day 15 to 92, 4 dogs in the mid-dose (3M and 1F) group and 9 dogs in the high-dose group (3M and 6F) began to show acute, transient (5- to 15-minutes post-elamipretide administration) clinical signs of injection site reactions (irritation/pain resulting in vocalization).

During this time, doses were decreased to 2.5, 5 and 10 mg/kg/day and the dose concentration was decreased to 20 mg/mL and then to 10 mg/mL. Although, these dose and dose concentration changes alleviated the vocalization and irritation in some animals, it did not alleviate the symptoms in 4 dogs in the mid-dose group (2M and 2F) and 6 dogs in the high-dose group (2M and 4F). Beginning on Day 108 these animals were administered an intramuscular (IM) dose of 4 mg/kg diphenhydramine (DPH) 30-minutes prior to elamipretide administration. The pretreatment with DPH alleviated the symptoms in the majority of these animals, however, 2 mid-dose dogs (1M and 1F) and 2 high-dose dogs (1M and 1F) required additional treatment with 1 mg/kg ranitidine IM injection. Treatment with the antihistamines alleviated the vocalization and allowed for dosing to continue for the remainder of the study.

There were no mortalities related to elamipretide during the study and there were no significant ocular changes nor any changes in ECG, clinical chemistry, hematology, coagulation or urine parameters in any elamipretide-treated animal compared to either baseline or control dogs.

At necropsy, elamipretide did not result in any macroscopic organ toxicity and the major macroscopic finding, in both control and elamipretide-treated animals, was subcutis red/hemorrhagic areas at the injection site that correlated with the last injection. There were no signs of necrosis or muscle damage. There were no histopathologic changes considered related to the systemic toxicity of elamipretide and no target organs identified. There was a tendency towards higher severity of inflammation at the SC injection sites of all groups dosed with elamipretide compared to controls. In addition, there was a higher severity and/or incidence of hemorrhages at the SC injection sites in animals dosed with elamipretide, specifically at the high-dose level when compared to the control groups. These findings were likely due to local pruritus and/or irritation at the injection sites. Furthermore, there were no macroscopic or microscopic differences between dogs that received antihistamines compared either to animals within the same group (that did not receive antihistamines) or to control dogs that received or did not receive antihistamines.

Following the 4-week recovery period, the majority of the microscopic changes described at the injection sites, such as hemorrhages and inflammation, including some procedure-related changes were either reversed or reversing, with only minimal to mild persistence of subcutaneous inflammation in both control and high dose animals, suggesting that with a longer recovery period, the inflammatory change would likely be fully reversible.

The local NOAEL was the low-dose, 2.5 mg/kg/day in a 10 mg/mL dose concentration, while the systemic NOAEL was the high-dose 10 mg/kg/day in a 10 mg/mL concentration; TK parameters for this study are listed in Table 10.

4.6.2.3. 28-Day SC Bridging Toxicity Study in Rats

The local and systemic toxicity of impurities present in elamipretide drug substance produced by 2 different synthetic routes were addressed in a 28-day GLP toxicity study in rats. Two test articles, elamipretide HCl produced by solution-phase synthesis and elamipretide HCl LTS (a solution containing degradation products generated by simulated Long-Term Storage) were compared with those of elamipretide acetate produced by solid-phase synthesis. In this GLP toxicity study (*SPI-APT-16-01*), each of the test articles was administered once daily by SC injection to rats for 28 days. Doses were 0 (placebo), 5 and 15 mg/kg/day.

There were no mortalities related to any of the elamipretide test articles during the study, and there were no significant ocular changes nor any changes in ECG, clinical chemistry, hematology, coagulation or urine parameters in any elamipretide-treated animal compared to either baseline or control rats.

At necropsy, macroscopic findings included areas of SC discoloration at the injection sites of many animals in all groups (including vehicles); these findings were considered to be procedure-related (repeated SC injection) and not test article related. The primary microscopic findings observed were non-elamipretide-related inflammatory and reactive lesions at the injection sites of many animals in all groups (including vehicles). The lesions consisted of focal SC hemorrhage seen primarily in main test animals and subcutaneous mononuclear cell infiltrates. The findings at the injection sites were deemed to be a consequence of repeated SC injections and not elamipretide-related.

After the treatment-free period, most findings were resolving or had resolved. The capillary/fibrous activation was still present, whereby the connective fibers appeared to be more condensed than in main test animals. This finding is deemed a sequelae of previously induced injection trauma that represent a minor scar formation. The remainder of findings were within the range of normal background lesions that may be recorded in animals of this strain and age.

Elamipretide HCl, elamipretide HCl (LTS), and elamipretide acetate were all well tolerated and provided comparable systemic exposures to elamipretide, and metabolites M1 and M2 after single and repeat administration at all doses tested. The NOAEL was 15 mg/kg/day for all test articles; PK parameters for this study are listed in Table 11. The data (PK and histopathology) from this study confirmed that the effects from elamipretide HCl and elamipretide HCl (LTS) were indistinguishable from those observed for elamipretide acetate.

4.6.3. Genotoxicity

Elamipretide was negative for gene mutations in the Ames assay and was negative for chromosomal aberrations in the *in vitro* mammalian cell (Chinese hamster ovary) assay (*MTP-131-TOX007*); both assays were conducted with and without rat S9 activation. Elamipretide was also negative in the *in vivo* rat micronucleus study at SC doses up to 500 mg/kg (*MTP-131-TOX007*). In addition, *in silico* analysis for genotoxic structural alerts was conducted on new impurities introduced in the modified manufacturing process for elamipretide HCl. This analysis confirmed the absence of structural alerts for genotoxicity.

The 2 major metabolites of elamipretide, the tripeptide M1 and the dipeptide M2 were also negative for bacterial mutagenicity (*SPI-COV-11-01* and *SPI-COV-11-02*). In addition, the 2 major metabolites were qualified during the rat bone marrow erythrocyte micronucleus test of elamipretide; exposure to the metabolites at the dose levels tested exceeded that anticipated in humans at the recommended dose (0.10 mg/kg).

4.6.4. Carcinogenicity Studies

No carcinogenicity studies have been conducted to date.

4.6.5. Reproductive and Development Toxicity

Elamipretide has not been associated with adverse effects on fertility, embryo-fetal, peri-natal, or postnatal development; data from the reproductive studies are summarized in Table 13. As a precaution to mitigate any potential reproductive risks, all investigational studies will mandate pregnancy testing for women of child-bearing potential, exclusion of those found to be pregnant, and the use of effective contraception for all participants during trial participation.

Table 13 Summary of Reproductive Studies

Study Type	Species/ Strain	ROA	Dosing Duration	Dosage (mg/kg/day)	Sex/No. per Group	C_{max} (ng/mL) 1	AUC (ng·hr/mL) 1	Findings
Fertility[a]*	Rat/Sprague Dawley	SC	See Footnote[f]	0, 2, 6, 20	25M/ 25F	ND	ND	No adverse effects on fertility in M and F rats
Embryo-fetal Development DRF[b]	Sprague-Dawley Rat	1-h IV infusion	GD7 to GD17	0, 30, 100	4F	ND	ND	Maternal: Decreased body weights (10, 30 mg/kg/day); deaths first or second day of dosing (100 mg/kg/day). Developmental: No effects at any dose.
Embryo-fetal Development[c]*	Sprague-Dawley Rat	1-h IV infusion	GD7 to GD17	0, 1, 3, 10	25F	Maternal: 3,940 ± 306 Developmental: 12,600 ± 1100	Maternal: 4300 ± 271 Developmental: 14,200 ± 898	Maternal: Reduction in body weight gain (10 mg/kg/day), NOAEL = 3 mg/kg/day. Developmental: No toxicity at any dose, NOAEL = 10 mg/kg/day.
Embryo-fetal Development[d]*	NZW Rabbit	4-h IV infusion	GD7 to GD19	0, 5, 15, 50	22F	Maternal: 855 ± 122 Developmental: 10,000 ± 927	Maternal: 3020 ± 210 Developmental: 35,900 ± 4370	Maternal: Reduction in food consumption and body weight gain (15, 50 mg/kg/day); NOAEL=5 mg/kg/day. Developmental: No toxicity at any dose; NOAEL=50 mg/kg/day.
Preliminary Embryo-fetal Development[e]	NZW Rabbit	4-h IV infusion	GD7 to GD19	0, 10, 25, 50, 100	4F	ND	ND	Maternal: adverse clinical observations, body weight loss and early euthanasia (100 mg/kg/day). Developmental: No effects at any dose.
Effects on Pre-, Postnatal Development and Maternal Function[g,]*	Sprague-Dawley Rat	SC	See footnote[h]	0,5,10,15	88F in F0 generation:80M/80F in F1 generation	ND	ND	Test item-related clinical signs were limited to non-adverse transient local reactions of low to moderate severity at injection sites in all treated groups with a dose-related trend. NOAEL = 15 mg/kg/day (the maximum dose)

Abbreviations: AUC = area under the plasma concentration-time curve; C_{max} = maximum concentration in plasma; DRF = dose range finding; F = female; GLP = Good Laboratory Practices; GD = gestation day; h = hour(s); IV = intravenous; M = male; No. = number; ND = not determined; NOAEL = no-observed-adverse-effect level; ROA = route of administration; SC = subcutaneous; * = GLP

1 Maternal Cmax and AUC are reported at the maternal NOAEL, while Developmental Cmax and AUC are reported at the developmental NOAEL

[a] SPI-CRL-14-05. [b] SPI-CRL-14-03. [c] SPI-CRL-14-04. [d] SPI-CRL-14-02. [e] SPI-CRL-14-01. [f] M: 4 weeks prior to mating, through mating, until the day before euthanasia. F; 15 days prior to mating through GD7, [g] SPI-CRL-19-01 [h] F: GD6 to weaning on Lactation Day (LD)20

4.6.5.1. Peri-Natal and Post-Natal Development

The effects of elamipretide (HCl) exposure on pregnancy, parturition, and lactation of the maternal animals and on embryo-fetal, and peri-, and post-natal development of the rat and subsequent reproductive performance of the offspring were evaluated when elamipretide HCl was administered to inseminated females from Gestation Day (GD)6 to weaning on Lactation Day (LD)20 (*SPI-CRL-19-01*).

Elamipretide, was administered by daily subcutaneous injection (SC) at dose levels of 5, 10, and 15 mg/kg/day (at dose volumes of 0.83, 1.67, and 2.5 mL/kg, respectively) to groups of 22 mated female Sprague-Dawley rats from GD6 to LD20 inclusive. An additional group received the control item (sterile physiological saline [0.9% NaCl]) at the dose volume of 2.5 mL/kg.

No test item-related death occurred among the F0 females and the selected F1 generation.

Test item-related clinical signs were limited to non-adverse transient local reactions of low to moderate severity at injection sites in all treated groups with a dose-related trend. These findings, which consisted primarily of erythema and scabbing, correlated with non-adverse macroscopic observations at necropsy.

No test item-related effect on mean body weight gain and food consumption of the F0 females was noted.

No test item-related effect on gestation length, pre-birth loss, and pup viability from birth through weaning was noted.

Under the conditions of the study, daily SC administration of elamipretide to inseminated female Sprague-Dawley rats from GD6 to LD20 at dose levels of 5, 10, and 15 mg/kg/day was well tolerated with only non-adverse local reactions at the injection sites at all doses.

Maternal treatment had no effect on embryo-fetal, peri-, and post-natal development, and reproductive performance of the offspring up to 15 mg/kg/day. Therefore, the No Observed Adverse Effect Level (NOAEL) for both maternal and embryo fetal, peri-, and post-natal development of the offspring was 15 mg/kg/day.

4.6.5.2. Postnatal Development

The toxicity and toxicokinetic (TK) profile of elamipretide was determined following SC administration to the neonatal Sprague-Dawley rat once daily for 8 weeks; in addition, the reversibility, persistence, or delayed occurrence of any changes was assessed during a 7-week post-dose period. Parameters evaluated included growth, development, and subsequent reproductive performance (*SPI-CIT-16-04*).

The test and control items were administered SC to neonatal rats once daily from *post-partum* Days 7 to 63 (PPD7 to PPD63). After the end of the dosing period, Main males and females were terminated on PPD64 for post mortem evaluations. Recovery animals were allotted a treatment-free period of approximately 21 days (PPD64 to PPD85), followed by a 14-day pre-mating period (PPD83 to PPD98), and then mated for up to a 2-week period (PPD96 to PPD112). Following the mating period, Recovery males and non-mated Recovery females were necropsied

(Day 113). Mated Recovery females were kept on study from GD0 to GD14, and then necropsied.

Blood samples for plasma concentration analysis of elamipretide and metabolites (M1 and M2) were collected from a subset of TK animals on PPD7 and PPD63 at pre-dose and 0.25, 0.5, 1, 3, and 12 hours post-dose.

Subcutaneous administration of elamipretide, at doses up to 15 mg/kg/day, to neonatal male and female Sprague-Dawley rats from PPD7 to PPD63 was well tolerated.

During the dosing period, clinical, gross, and microscopic observations were noted at the injection sites of all groups including controls; however, a mild increase in the incidence and severity of microscopic hemorrhage, mononuclear inflammation, and myocyte necrosis/regeneration at the injection sites was considered related to elamipretide. Injection site findings were fully recoverable following a 7-week recovery period.

Elamipretide administration had no effect on body weight, food consumption, or clinical pathology parameters. Indices of physical development, including ocular and bone density, general developmental performances (including sensory, reflexological, behavioral, and functional assessments), and estrous cycle, were not affected by treatment at any dose level.

Elamipretide administration had no effect on reproductive performance. Parental performance (mean days to mating, and male mating, male fertility, and conception rate indices) was unchanged by elamipretide administration. At the cesarean sections on GD14, no elamipretide-related effects on the number of corpora lutea, number of implantation sites, and live or dead embryos (live embryos index) were observed. The male reproductive assessment (spermatozoa motility, count, concentration, and morphology) was also without significant elamipretide-related changes.

In summary, SC administration of elamipretide to neonatal Sprague-Dawley rats once daily for 8 weeks (PPD 7 to PPD 63) at dose levels of 5, 10, and 15 mg/kg/day was well tolerated. Elamipretide-related findings were limited to slightly increased histologic injection site reactions at the high dose that were reversible over a 7-week recovery period. Growth, development, and reproductive performance were considered essentially unaffected at all dose levels. Based on the results of this study, the NOAEL was considered to be 15 mg/kg/day; TK parameters for this study are listed in Table 12.

4.6.6. Local Tolerance

In the single- and repeat-dose toxicity studies, IV administration of elamipretide to rats and dogs was well tolerated at the administration site, as detailed in Section 4.6.1. No adverse local effects were observed upon once-daily IV administration of elamipretide formulated in saline at concentrations up to 50 mg/mL.

Subcutaneous tolerability was assessed in several dedicated studies in rat (*SPI-RIC-13-02*, *SPI-RIC-14-01*, *12-1303-N1*), and rabbit (*NCSED2009017*). Local tolerance was also determined as part of the single- and repeat-dose SC toxicity studies (see Section 4.6.2). In all studies, elamipretide caused similar injection site reactions, consistent with inflammation, which were concentration and volume dependent. Microscopic findings consisted of SC hemorrhage, myofibril degeneration/regeneration, muscle and collagen necrosis, edema, perivascular mononuclear cell infiltrates and serocellular epidermal exudate. All injection site findings were considered reversible in the recovery groups where such groups were utilized.

4.6.7. Other Toxicity Studies

Elamipretide did not cause hemolysis when incubated at 1, 3, 6, or 10 mg/mL (in normal saline) with rat, dog, or human whole blood (*SPIRIIV-N104*).

Elamipretide had no synergistic, additive or inhibitory effects on morphine's effects on respiratory function. Elamipretide (6 and 20 mg/kg 2-hour IV infusion) was given to conscious unrestrained rats to assess DDIs with morphine on respiratory function after a single 10-mg/kg IV dose of morphine (*SPI-MPI-10-01*). No physiologically- or statistically-meaningful differences in respiratory rate, tidal volume, minute volume, inspiratory time, inspiratory pause, expiratory time, and expiratory pause were found between morphine-treated rats receiving saline vs. elamipretide.

At clinically relevant concentrations elamipretide and its metabolites M1 and M2 did not induce in vitro histamine release from mast cells harvested from Sprague-Dawley rats (*SPI-BTS-15-01*, *SPI-BTS-17-01*).

5. EFFECTS IN HUMANS

5.1. Design of Clinical Trials

Data are available from 30 parenteral elamipretide studies conducted in healthy adult subjects and numerous patient populations. Data reported are exported from Tables, Figures, and Listings generated for these studies. The clinical development of parenteral elamipretide has included 2 routes of administration: elamipretide dosed via IV infusion and elamipretide dosed via SC injection.

An overview of clinical trials evaluating parenteral elamipretide in healthy subjects by dosing regimen (IV and SC) is presented in Appendix 1. A brief description of trials in patient populations are listed below. Tabular summaries of these trials are presented in Appendix 2.

- SPIRI-201 (EMBRACE) was a Phase 2a, randomized, double-blind, placebo-controlled trial. Subjects (n= 297) aged 18 to 84 years with first-time acute, anterior wall STEMI scheduled to undergo primary PCI and stenting received a single IV infusion of 0.05 mg/kg/hr elamipretide/placebo starting 15 to 60 minutes before PCI procedure and continuing for 60 minutes post-procedure in ACS.

- SPIRI-225 (EVOLVE) was Phase 2a, single-center, randomized, double-blind, placebo-controlled trial. AKI subjects (n=16) aged 40 to 80 years with ARAS who were undergoing PTRA and stenting received a single IV infusion, 0.05 mg/kg/hr elamipretide/placebo starting 15 to 30 minutes before PTRA and continuing for 3 hours post-procedure.

- SPIHF-101 (PREVIEW) was Phase 1, a single-center, randomized, double-blind, placebo-controlled, single ascending dose trial. Subjects (n=36) aged 45 to 80 years with stable NYHA Class II-III congestive CHF with LVEF ≤ 35% received a single 4-hour IV infusion; ascending doses of 0.005, 0.05, 0.25 mg/kg/hr elamipretide/placebo.

- SPIHF-201 (PROGRESS) was a Phase 2, randomized, double-blind, placebo-controlled trial. Subjects (n=71) aged 40 to 80 years with stable heart failure with reduced ejection fraction LVEF ≤35% received single daily 4 or 40 mg SC injections of elamipretide/placebo for 28 days (1:1:1 randomization).

- SPIHF-203 (RESTORE) was a Phase 2, randomized, double-blind, placebo-controlled trial. Subjects (n=47) aged 45 to 80 years with stable heart failure with preserved ejection fraction LVEF ≥45% received single daily 40 mg SC injections of elamipretide/placebo for 28 days.

- SPIHF-204 (IDDEA-HF) was a Phase 2, randomized, double-blind, placebo-controlled trial. Subjects (n=308) aged 18 years and older who were hospitalized with congestion

due to heart failure received single daily 20 mg IV infusions of elamipretide/placebo over 60 minutes for 7 days.

- SPITM-201 (MOTION) was a Phase 2a, randomized, double-blind, placebo-controlled trial. Subjects (n=40) aged ≥60 to ≤85 years with evidence of skeletal muscle mitochondrial dysfunction received a single IV infusion of placebo/elamipretide 0.25 mg/kg/hr at a rate of 60 mL/hr for 2 hours.

- SPIMM-201 (MMPOWER) was a Phase 1/2, multicenter, randomized, double-blind, placebo-controlled, multiple ascending dose trial. Subjects (n=36) aged ≥16 and ≤65 years with PMM received placebo/multiple ascending IV dose 0.01, 0.10, 0.25 mg/kg/hr elamipretide administered as a single daily dose over 2 hours for 5 consecutive days.

- SPIMM-202 (MMPOWER-2) was a Phase 2, multicenter, randomized, double-blind, placebo-controlled, crossover trial. Subjects (n=30) aged ≥16 and ≤65 years with PMM previously treated in SPIMM-201 received single daily 40 mg SC injections of elamipretide/placebo for 28 days in Treatment Period 1 followed by single daily 40 mg SC injections of the opposite treatment (40 mg elamipretide/placebo) in Treatment Period 2 (separated by 4-week washout period).

- SPIMM-203 (MMPOWER-OLE) was a Phase 2, multicenter, open-label extension trial. Subjects (n=28) aged ≥16 to ≤65 years with PMM previously treated in SPIMM-201 and/or SPIMM-202 received single daily 40 mg/mL SC injections of elamipretide/placebo for up to 260 weeks.

- SPIMM-301 (MMPOWER-3) was a Phase 3, multicenter, double-blind, parallel-group, placebo-controlled trial followed by an open-label treatment extension. Subjects aged ≥16 to ≤80 years with PMM received in Part 1 single daily 40 mg/mL SC injections of elamipretide/placebo for up to 24 weeks; in Part 2 received single daily 40 mg/mL SC injections of elamipretide for up to 144 weeks.

- SPIBA-201 (TAZPOWER) was a multicenter, Phase 2, randomized, double-blind, placebo-controlled, crossover trial followed by an open-label treatment extension. Subjects (n=12) aged ≥12 years with genetically confirmed Barth syndrome received in Part 1 single daily 40 mg SC injections of elamipretide/placebo for 84 days in Treatment Period 1 followed by single daily 40 mg SC injections of the opposite treatment (40 mg elamipretide/placebo) in Treatment Period 2 (separated by 4-week washout period); in Part 2 single daily 40 mg/mL SC injections of elamipretide for up to 168 weeks..

- SPIAM-101 (ReCLAIM) was a Phase 1, open-label, single site trial. Subjects (n=40) with dry AMD ≥55 years of age with either high risk drusen without geographic atrophy (GA) or with noncentral GA received single daily 40 mg/mL SC injections of elamipretide for 168 days (24 weeks).

- SPIAM-202 (ReCLAIM-2) was a Phase 2, double-masked, placebo-controlled trial. Subjects with dry AMD ≥55 years of age with non-central GA received single daily 40 mg SC injections of elamipretide or placebo for 48 weeks.

- SPIMD-301 (NuPOWER) is a Phase 3, double-blinded, placebo-controlled trial. Subjects with primary mitochondrial disease resulting from pathogenic nuclear DNA nutations (nPMD) will receive single daily 60 mg SC injections of elamipretide or placebo for 48 weeks. This trial is currently recruiting subjects.

5.2. Pharmacokinetics and Drug Metabolism in Humans

5.2.1. Analytical Methods

Methods for the analysis of elamipretide and its metabolites, M1 and M2, in human plasma and urine have been developed and validated. Methods employ protein precipitation extraction from plasma followed by LC-MS/MS assay with stable-isotope labeled internal standards.

5.2.2. Pharmacokinetics

A summary of maximum dose tested, and exposures achieved are presented in Table 14. Additional PK properties for elamipretide are provided in Table 15. An overview of the Phase 1 PK studies with IV or SC elamipretide and intrinsic factors are summarized in Table 16. The steady-state IV and SC PK studies performed to date are detailed in Appendix 1 and Appendix 2.

Table 14 Maximum Dose Tested and Exposures Achieved

Maximum Dose Level Tested	Elamipretide Exposure Achieved
Single Dose	
SC: 80 mg	Mean C_{max}: 1,810 ng/mL (CV=17.1%)
	Mean AUC_{0-24}: 6,980 ng.hr/mL (CV=24.1%)
IV: 0.25 mg/kg/hr as a 4 hour infusion (TDD 1.0 mg/kg)	Mean C_{max}: 1,655 ng/mL (CV=7.3%)
	AUC_{0-last}: 8,270 ng.hr/mL (CV=7.3%)
Multiple Dose	
SC acute dosing: 80 mg q3h x5 doses	Mean C_{max}: 4,053 ng/mL (CV=27.3%)
	Mean $AUCD5_{0-24}$: 13,308 ng.hr/mL (CV=21.6%)
SC daily dosing: 80 mg x 7 days	Mean C_{max}: 1,800 ng/mL (CV=34.6%)
	Mean AUC_{0-24}: 6,960 ng.hr/mL (CV=25.8%)
IV daily dosing: 140 mg x 5 days	Mean C_{max}: 7,233 ng/mL (CV=22.9%)
	Mean AUC_{0-24}: 15,883 ng.hr/mL (CV=18.2%)

Table 15 Elamipretide PK Properties

Range of Linear PK	SC: 2 – 80 mg IV: 0.01 – 1.75 mg/kg
Accumulation at Steady State	SC 80 mg/day x 7 days: Accumulation ratio = 1.00 (CV=20.8%) IV 140 mg/day x 5 days: Accumulation ratio = 0.95 (CV=10.7%)
Absorption (SC Dosing)	
Absolute bioavailability	~90%
T_{max}	Median T_{max}: 0.75 hr
Distribution	
Vd/F or Vd	SC Mean Vd/F = 54.5 L (CV=14.9%) IV as 4-hour infusion Vd = 322 mL/kg (CV=3.9%)
% Protein bound	Mean bound elamipretide = 38.7% (CV=7.8%)
Metabolism	Sequential C-terminal degradation in plasma to the tripeptide M1 and the dipeptide M2 No hepatic metabolism demonstrated with elamipretide
Elimination	
Route	Renal: ~100% as elamipretide, M1, and M2
Terminal $t_{1/2}$	Between 3 and 4 hours

Table 16 Overview of Phase 1 PK Studies with IV or SC Elamipretide and Intrinsic Factors

<table>
<tr><td>Age</td><td>No apparent effect of age: Age range in PK studies 20 – 80 years</td></tr>
<tr><td>Sex</td><td>No apparent effect of sex: approximate sex distribution (%) in PK studies below
<table>
<tr><td>Male</td><td>Female</td></tr>
<tr><td>60%</td><td>40%</td></tr>
</table></td></tr>
<tr><td>Race</td><td>No apparent effect of race: approximate race distribution (%) in PK studies below
<table>
<tr><td>White</td><td>Black/African American</td><td>Asian</td><td>Other</td></tr>
<tr><td>82.6%</td><td>14.5%</td><td>1.7%</td><td>1.3%</td></tr>
</table></td></tr>
<tr><td>Hepatic Impairment</td><td>Effect of hepatic impairment on PK not studied – no hepatic metabolism demonstrated with elamipretide</td></tr>
<tr><td>Renal Impairment</td><td>Elamipretide and its metabolites are virtually entirely excreted by the renal route, the impact of varying degrees of renal function (defined using baseline 24-hr creatinine clearance) has been studied in single- and repeat-dose studies. Key PK parameters established in the 7-day repeat dose study of elamipretide (0.25 mg/kg/day as a 1-hour IV infusion) (SPICP-101) is presented below and key findings summarized:
• Elamipretide
o AUC_{0-24} increases significantly as renal function decreases
o C_{max} is observed to increase without reaching statistical significance as renal function decreases
o Small amount of accumulation observed in severe renal impairment
• M1
o AUC_{0-24} increases significantly as renal function decreases
o C_{max} increases significantly as renal function decreases
o Accumulation observed in severe renal impairment
• M2
o AUC_{0-24} increases significantly as renal function decreases
o C_{max} increases significantly as renal function decreases
o Accumulation observed in severe renal impairment</td></tr>
</table>

Renal Impairment

Table 16 Overview of Phase 1 PK Studies with IV or SC Elamipretide and Intrinsic Factors (continued)

SPICP-101: Summary Table of Key Mean PK Parameters Following 7-days Repeat Daily Dosing of Elamipretide (0.25 mg/kg/hr x 1hr [approx. 18.4 mg[a]]) in Subjects with Varying Degrees of Renal Impairment

			Elamipretide			M1			M2		
Renal Group [CrCL mL/min]		**n**	**T_{max} (h)**	**C_{max} (ng/mL)**	**AUC_{0-24h} (ng.h/mL)**	**T_{max} (h)**	**C_{max} (ng/mL)**	**AUC_{0-24h} (ng.h/mL)**	**T_{max} (h)**	**C_{max} (ng/mL)**	**AUC_{0-24h} (ng.h/mL)**
Normal [≥ 90]	Day 1	6	1	921	2409	2	201	1662	6	22.2	393
	Day 7	6	1	938	2355	2	201	1637	6	35.1	635
	Acc. Ratio[b]				0.98			0.98			1.62
Mild [60-89]	Day 1	6	1	950	3223	4	239	2919	12	35.9	682
	Day 7	6	1	950	3319	3	266	3342	8	74.6	1561
	Acc. Ratio[b]				1.03			1.14			2.29
	% Exposure Mild:Normal (Day 7)				*141*			*204*			*246*
Moderate [30-59]	Day 1	6	1	963	3884	4	193	2805	12	36.4	661
	Day 7	6	1	1020	4196	3	248	3743	8	108	2384
	Acc. Ratio[b]				1.08			1.33			3.61
	% Exposure Moderate:Normal (Day 7)				*178*			*229*			*375*
Severe [< 30]	Day 1	5	1	956	4627	8	237	4015	18	60	994
	Day 7	5	1	1010	5333	6	356	6395	12	242	5336
	Acc. Ratio[b]				1.15			1.59			5.37
	% Exposure Severe:Normal (Day 7)				*226*			*391*			*840*

[a] 0.25 mg/kg/day = 18.4 mg/day in an 73.4 kg person (mean body weight value at Day-1 in SPICP-101)
[b] Accumulation ratio = $AUC_{0-24h\ (Day\ 7)}/AUC_{0-24h\ (Day1)}$

5.2.2.1. Additional Extrinsic Factors

No drug-drug interactions have been noted and data is consistent across the study populations, including subjects with acute cardiac disease (SPIRI-201), stable heart failure (SPIHF-101), AKI undergoing PTRA (SPIRI-225), and PMM (SPIMM-201). See Section 4.5.5 for data pertaining to nonclinical PK drug interaction data.

In SPICP-106, the impact of applying ice to the injection site location pre-and post-administration of SC elamipretide, 60 mg, on the PK parameters was statistically significant. There was a reduction in both plasma C_{max} and AUC_{0-6} (C_{max} was reduced by approximately 23% [$p = 0.0003$], AUC_{0-6} was reduced by approximately 12% [$p < 0.0001$]), likely due to vascular constriction. Similarly, the application of ice demonstrated a statistically significant reduction in both the C_{max} and AUC_{0-6} of M1 (C_{max} was reduced by approximately 11% [$p = 0.0028$], AUC_{0-6} was reduced by approximately 11% [$p < 0.0001$]) and on the AUC_{0-6} of M2 (AUC_{0-6} was reduced by approximately 12% [$p = 0.0135$]).

5.2.2.2. Relative Bioavailability—Impact of Formulation and Injection Site Location

SPICP-105 was a two-arm study to evaluate the PK, safety, and tolerability of two formulations and two injection site locations of SC elamipretide in healthy volunteers. This crossover study was conducted to test the relative bioavailability and comparability of the following 3 dosing regimens:

- Regimen A1: a single 40 mg elamipretide (acetate formulation) dose via SC injection into the abdomen;
- Regimen B1: a single 40 mg elamipretide (HCl formulation) dose via SC injection into the abdomen;
- Regimen C1: a single 40 mg elamipretide (HCl formulation) dose via SC injection into the thigh.

Adjusted geometric means for C_{max} (ng/mL) were 1660 for elamipretide acetate 40 mg in the abdomen, 1440 for elamipretide HCl in the abdomen, and 1340 for elamipretide HCl in the thigh. Adjusted geometric means for AUC_{0-24} (ng.h/mL) were 4210 for elamipretide acetate 40 mg in the abdomen, 3940 for elamipretide HCl in the abdomen, and 3740 for elamipretide HCl in the thigh.

The GMRs (90% CI) for the comparisons of interest were 93.62% (92.30%, 94.95%) and 94.92% (93.58%, 96.27%) for formulation (ie, HCl/acetate) and location effects (ie, thigh/abdomen), respectively. While the differences for each of these comparisons were statistically significant, the 90% CI were within the standard bioequivalence criteria of 80.00% to 125.00%, suggesting that any differences were of no clinical significance. The PK profile of metabolites M1 and M2 was also comparable in all dosing regimens.

5.2.2.3. QT/QTc Interval Prolongation and Proarrhythmic Potential of Elamipretide

SPICP-102 was a Phase 1 trial to evaluate the QT/QTc interval prolongation and proarrhythmic potential of elamipretide in healthy subjects. This was a 3-way crossover study in which healthy subjects were administered elamipretide 80 mg every 3 hours for 5 doses, placebo, and open-label moxifloxacin in separate treatment periods. Serial electrocardiograms (ECGs) were extracted from continuous Holter monitors at baseline (pre-dose on Day 1 in each period) and for 84 hours post-dosing (72 hours after the fifth dose of elamipretide).

Elamipretide, administered every 3 hours for 5 doses, did not have a clinically relevant effect on ECG parameters (ie, heart rate and PR, QRS, and QTcF intervals). Using the concentration-QTc analysis, a QTcF effect (ΔΔQTcF) exceeding 10 ms can be excluded within the observed elamipretide plasma concentrations up to approximately 4,200 ng/mL, approximately 3,300 ng/mL for M-1, and approximately 300 ng/mL for M2. Assay sensitivity was demonstrated by the QT effect of moxifloxacin with a statistically significant slope of the concentration-ΔΔQTc relationship and the lower bound of the 2-sided 90% CI of the predicted effect at the observed geometric C_{max} above 5 ms. These results represent a solidly negative thorough QT study.

5.2.2.4. Additional Pharmacokinetics

Overall, there have been no sex-related differences observed in PK parameters in any clinical study with elamipretide. No drug-drug interactions have been noted and data are consistent across the study populations, including subjects with acute cardiac disease, stable heart failure, AKI undergoing PTRA, PMM, Barth syndrome, and AMD.

5.3. Clinical Safety (Cumulative Data)

Elamipretide was assessed following single and multiple IV and SC doses in 16 completed clinical pharmacology trials: 8 trials evaluated single doses of IV elamipretide (SPIRI-101, SPIRI-102, SPIRI-120, SPIRI-151, SPIRI-152, SPIRI-153, SPIRI-154, SPIRI-155), 2 trials evaluated multiple doses of IV elamipretide (SPICP-101, SPICP-103), 3 trials evaluated multiple doses of SC elamipretide (SPICP-102, SPICP-104, SPICP-105), 2 trials evaluated single and then multiple doses of SC elamipretide (SPISC-101, SPISC-102) and 1 trial (SPICP-106) evaluated 6 single doses of SC elamipretide (elamipretide alone and with 5 various potential ISR mitigation strategies) over the course of 18 days.

Data are available from 14 completed clinical trials in which elamipretide was studied in patient populations: a Phase 1 trial in subjects with stable CHF (SPIHF-101), a Phase 2 trial in subjects with stable heart failure with reduced ejection fraction (LVEF ≤35%) (HFrRF) (SPIHF-201), a Phase 2 trial in Subjects with Stable Heart Failure with Preserved Ejection Fraction (SPIHF-203), a Phase 2 trial in subjects who were hospitalized with congestion due to heart failure (SPIHF-204), a Phase 2a trial in subjects with ACS undergoing PCI for STEMI (SPIRI-201), a Phase 2a trial in subjects with AKI undergoing PTRA (SPIRI-225), a Phase 1/2 and a Phase 2 trial in subjects with PMM (SPIMM-201 and SPIMM-202, respectively), an open-label trial in subjects with PMM (SPIMM-203), a Phase 3 trial in patients with PMM (SPIMM-301), a Phase

2a trial in subjects over 60 years of age with evidence of skeletal muscle mitochondrial dysfunction (SPITM-201), a Phase 1 and a Phase 2 trial in subjects with age-related macular degeneration (AMD) (SPIAM-101, SPIAM-202), and a Phase 2 trial in subjects with Barth syndrome (SPIBA-201). The reviewed safety data from this study does not appear to impact the safety profile of elamipretide.

An overview of clinical trials evaluating parenteral elamipretide in healthy subjects by dosing regimen (IV and SC) is presented in Appendix 1. Summaries of trials evaluating parenteral elamipretide in patient populations are presented in Appendix 2.

The systemic and local (for SC dosing) safety profile is presented for single-dose trials, multiple-dose trials of ≤ 8 days in duration, and multiple-dose trials of > 8 days in duration. For the multiple-dose trials, the cutoff of ≤ 8 days and > 8 days in duration was selected due to previous trial design and to provide relevant safety information for chronic dosing (applicable to ongoing and future chronic-dosing trials). Data shown are n (%); percentages are based on N. Data is reported by MedDRA hierarchy System Organ Class (SOC) and Preferred Term (PT). If more than 1 AE was coded to the same PT for a subject, the subject was counted only once for that PT. If more than 1 PT was coded to the same SOC for a subject, the subject was counted only once for that SOC. Where severity is reported, if a subject has the same AE on multiple occasions the highest severity (severe > moderate > mild), highest drug relationship (related > probable > possibly related > unrelated/unlikely related), and longest duration recorded for the event is presented.

5.3.1. Systemic and Local Safety Profile in Subjects Enrolled in Single-Dose Trials

A brief overview of the key safety findings in subjects enrolled in the single-dose trials is presented in this section.

A summary of the most common (≥2%) systemic TEAEs occurring in elamipretide treated subjects from all the single dose studies is presented in Table 17. Of the systemic TEAEs reported in subjects receiving elamipretide, there were no TEAEs that differed more than 5% in frequency from subjects receiving placebo.

Table 17 Summary of Systemic TEAEs Reported in ≥2% Elamipretide-Treated Subjects in Single-Dose Trials

System Organ Class Preferred Term	**Elamipretide Number (%) of Subjects (n=356)**	**Placebo Number (%) of Subjects (n=252)**
Any TEAE	**180 (50.6)**	**143 (56.7)**
Cardiac Disorders		
Angina Pectoris	11 (3.1)	6 (2.4)
Cardiac Failure	28 (7.9)	31 (12.3)
Intracardiac Thrombus	10 (2.8)	10 (4.0)
Gastrointestinal Disorders		
Nausea	14 (3.9)	9 (3.6)
Vomiting	9 (2.5)	7 (2.8)
General Disorders and Administration Site Conditions		
Non-Cardiac Chest Pain	9 (2.5)	5 (2.0)
Metabolism and Nutrition Disorders		
Diabetes Mellitus	12 (3.4)	7 (2.8)
Hypercholesterolaemia	30 (8.4)	19 (7.5)
Hypokalaemia	27 (7.6)	27 (10.7)
Hyponatraemia	9 (2.5)	3 (1.2)
Musculoskeletal and Connective Tissue Disorders		
Back Pain	8 (2.2)	9 (3.6)
Nervous System Disorders		
Headache	16 (4.5)	5 (2.0)
Psychiatric Disorders		
Anxiety	9 (2.5)	15 (6.0)
Vascular Disorders		
Hypertension	12 (3.4)	10 (4.0)

Additionally, several systemic adverse drug reactions (ADRs) (TEAEs considered to be possible related, probably related, or related to elamipretide or placebo by the Investigator) have been reported in these trials. All ADRs reported in ≥1% of subjects in these trials are displayed in Table 18. It should be noted that hypokalemia occurred in elamipretide treated subjects at a similar frequency to placebo treated subjects and is a function of the subjects enrolled in SPIRI-201 who were hospitalized for acute STEMI.

Table 18 Summary of Systemic ADRs Reported in ≥1% Elamipretide-Treated Subjects in Single-Dose Trials

System Organ Class Preferred Term	**Elamipretide Number (%) of Subjects (n=356)**	**Placebo Number (%) of Subjects (n=252)**
All ADR	**52 (14.6)**	**28 (11.1)**
Gastrointestinal Disorders		
Nausea	4 (1.1)	3 (1.2)
Metabolism and Nutrition Disorders		
Hypokalaemia	13 (3.7)	13 (5.2)
Hyponatraemia	9 (2.5)	2 (0.8)
Nervous System Disorders		
Headache	8 (2.2)	1 (0.4)

Local tolerability of the SC injection of elamipretide has been widely reported in the relevant clinical studies. Of the trials that evaluated single doses of elamipretide therapy, SPISC-101 (Part 1) and SPISC-102 (Part 1) studied SC injection of elamipretide. The local adverse events of injection site reactions experienced by subjects in those trials are reported in Table 19.

Table 19 Summary of Local TEAEs Reported in ≥5% of SC Elamipretide-Treated Subjects in Single-Dose Trials

System Organ Class Preferred Term	**Elamipretide Number (%) of Subjects (n=56)**	**Placebo Number (%) of Subjects (n=21)**
Injection Site Erythema	54 (96.4)	2 (9.5)
Injection Site Pain	9 (16.1)	1 (4.8)
Injection Site Pruritus	22 (39.3)	0
Injection Site Reaction	3 (5.4)	1 (4.8)
Injection Site Swelling	35 (62.5)	0

Aside from the events reported in the text and tables presented above, there have been no additional findings in any safety parameter measured within the single-dose trials, including vital signs and laboratory, ECG and physical examination assessments.

5.3.2. Systemic and Local Safety Profile in Subjects Enrolled in Multiple-Dose Trials with Therapy ≤8 days

Ten trials have evaluated multiple doses of elamipretide therapy for less than or equal to 8 days. Descriptions of the trials, doses tested, and patient populations of SPICP-101, SPICP-102, SPICP-103, SPICP-104, SPICP-105, SPICP-106, SPISC-101 (Part 2), SPISC-102 (Part 2), SPIHF-204 and SPIMM-201 are presented in Appendix 1 and Appendix 2. The most common (≥ 2.0%) systemic TEAEs reported in subjects treated with elamipretide in these studies are displayed in Table 20. Headache (5.1%) and somnolence (2.2%) are the only TEAE which appears with a notably higher frequency (≥2% difference) in the elamipretide-treated group than in the placebo-treated group.

Table 20 Summary of Systemic TEAEs Reported in ≥2% of Elamipretide-Treated Subjects in Multiple-Dose Trials ≤8 Days

System Organ Class Preferred Term	**Elamipretide Number (%) of Subjects (n=372)**	**Placebo Number (%) of Subjects (n=231)**
All TEAE	**143 (38.4)**	**93 (40.3)**
Cardiac Disorders		
Cardiac failure	9 (2.4)	18 (7.8)
Nervous System Disorders		
Headache	19 (5.1)	6 (2.6)
Somnolence	8 (2.2)	0 (0.0)

In addition, in SPICP-103 specifically, 2 patients reported AEs of paraesthesia and 2 patients reported AEs of paraesthesia oral while on elamipretide, while 1 subject reported an AE of paraesthesia while receiving placebo. These events generally occurred immediately after the end of the drug infusion and correlated to the C_{max} of elamipretide. Three of these AEs occurred in the 140-mg cohort, and 1 occurred in the 112-mg cohort and where C_{max} was approximately 4-fold higher than that expected with the 40-mg dose level selected for the clinical studies. All were reported to be mild in severity, resolved within 2 hours of symptom onset, and were not associated with hypersensitivity reactions.

Additionally, there were no systemic ADRs occurring at a frequency ≥ 1% in these trials. Of the 8 trials that evaluated multiple doses of elamipretide therapy for less than or equal to 8 days, 5 trials, SPICP-102, SPICP-104, SPICP-105, SPISC-101 (Part 2), SPISC-102 (Part 2) studied SC administration of elamipretide. The local adverse events of injection site reactions experienced by subjects treated with elamipretide in SC trials are reported in Table 21.

Table 21 Summary of Local TEAEs Reported in ≥5% of SC Elamipretide-Treated Subjects in Multiple-Dose Trials ≤8 Days

System Organ Class Preferred Term	**Elamipretide Number (%) of Subjects (n=133)**	**Placebo Number (%) of Subjects (n=61)**
General Disorders and Administration Site Conditions	**126 (94.7)**	**7 (11.5)**
Injection Site Bruising	7 (5.3)	0
Injection Site Erythema	122 (91.7)	6 (9.8)
Injection Site Induration	10 (7.5)	0
Injection Site Pain	77 (57.9)	1 (1.6)
Injection Site Pruritus	84 (63.2)	0
Injection Site Swelling	106 (79.7)	1 (1.6)

Aside from the events reported in the text and tables presented above, there have been no additional findings in any safety parameter measured within the multiple-dose trials for ≤ 8 days, including vital signs and laboratory, ECG and physical examination assessments.

5.3.3. Systemic and Local Safety Profile in Subjects Enrolled in Multiple-Dose Trials with Therapy >8 Days

Eight trials have evaluated elamipretide therapy for longer than 8 days Descriptions of the trials and patient populations of SPIMM-202, SPIMM-203, SPIMM-301, SPIHF-201, SPIHF-203, SPIAM-101, SPIAM-202, SPIBA-201 are described in Appendix 2. The most common (>5.0%) TEAEs reported in these studies are displayed in Table 22.

Table 22 Summary of TEAEs Reported in ≥5% of Elamipretide-Treated Subjects in Multiple-Dose Trials >8 Days

System Organ Class Preferred Term	**Elamipretide Number (%) of Subjects (n=493)**	**Placebo Number (%) of Subjects (n=258)**
Any TEAE	**368 (74.6)**	**141 (54.7)**
Infections and Infestations		
Nasopharyngitis	31 (6.3)	5 (1.9)
Upper respiratory tract infection	34 (6.9)	9 (3.5)
Nervous System Disorders		
Dizziness	28 (5.7)	9 (3.5)
Headache	38 (7.7)	11 (4.3)
Investigations		
Eosinophil count increased	27 (5.5)	0 (0)

Additionally, laboratory data demonstrated elevations (>0.4 cells $x10^9$/L) in eosinophils with longer duration of therapy (>28 days) in a majority of subjects. The mean absolute eosinophil count has demonstrated to increase several-fold from baseline values. In general, the eosinophil counts appear to decrease to within normal limits with longer duration of elamipretide administration. These eosinophil counts return to pre-treatment levels after the end of elamipretide treatment. Elevations in eosinophils have not been reported to be associated with any clinical manifestations or changes in other laboratory parameters. Further, the increase in eosinophils was not related to changes in other white cells and total white cell counts are not significantly increased. No subjects have been withdrawn from treatment due to increased eosinophil counts. To further explore and to characterize these findings of increased eosinophils, several studies have incorporated collection of IgE values. There was no clear temporal relationship of IgE levels with an elevation in eosinophils. Likewise, there have been no clinical signs and symptoms associated with elevated IgE values.

Additionally, several ADRs have been reported in these trials. All ADRs reported in ≥1% or more subjects in these trials are displayed in Table 23.

Table 23 Summary of ADRs Reported in ≥1% Elamipretide Treated Subjects in Multiple-Dose Trials >8 Days

System Organ Class Preferred Term	**Elamipretide Number (%) of Subjects (n=493)**	**Placebo Number (%) of Subjects (n=258)**
Any ADR	**144 (29.2)**	**43 (16.7)**
Blood and Lymphatic System Disorders		
Eosinophilia	11 (2.2)	0 (0)
Gastrointestinal Disorders		
Nausea	9 (1.8)	4 (1.6)
General Disorders and Administration Site Conditions		
Fatigue	6 (1.2)	4 (1.6)
Investigations		
Eosinophil count increased	27 (5.5)	0 (0)
Nervous System Disorders		
Dizziness	10 (2.0)	2 (0.8)
Headache	14 (2.8)	6 (2.3)
Insomnia	5 (1.0)	1 (0.4)
Skin and Subcutaneous Tissue Disorders		
Erythema	5 (1.0)	0 (0)

All trials where treatment was given for greater than 8 days studied SC administration of elamipretide. The local adverse events of injection site reactions experienced by subjects treated with elamipretide are reported in Table 24.

Table 24 Summary of Local TEAEs Reported in ≥5% of SC Elamipretide Treated Subjects in Multiple-Dose Trials >8 Days

System Organ Class Preferred Term	**Elamipretide Number (%) of Subjects (n=493)**	**Placebo Number (%) of Subjects (n=258)**
Injection Site Bruising	65 (13.2)	32 (12.4)
Injection Site Erythema	283 (57.4)	36 (14.0)
Injection Site Haemorrhage	45 (9.1)	15 (5.8)
Injection Site Swelling	94 (19.1)	7 (2.7)
Injection Site Induration	136 (27.6)	12 (4.7)
Injection Site Mass	40 (8.1)	4 (1.6)
Injection Site Pain	157 (31.8)	32 (12.4)
Injection Site Pruritus	297 (60.2)	16 (6.2)
Injection Site Urticaria	64 (13.0)	0 (0)

In 2 subjects from the SPIMM-301 clinical trial, ISRs evolved into wounds that in turn became infected. In one subject, upon healing some of the wounds left evidence of scar formation. In this subject, further investigation revealed several contributing factors, including improper injection technique combined with possible deficient local hygiene and the use of non-medicinal topical products without permission of the investigator. For the other subject, the contributing factor was the longstanding irritation of the injection sites on thighs by the use of trousers with high plastic composition, combined with scratching and no timely reporting of the infected injection sites to

the investigator. Topical treatment was administered to both patients and close monitoring measures were taken by the investigators.

Aside from the events reported in the text and tables presented above, there have been no additional findings in any safety parameter measured within the trials including vital signs and laboratory, ECG and physical examination assessments.

5.3.4. Local Safety Characteristics of Subcutaneous Elamipretide

The severity and duration of the local tolerability of the SC injection of elamipretide was evaluated in SPISC-101 (Part 1) and SPISC-102 (Part 1). The local adverse events of injection site reactions experienced by subjects are reported in Table 25 by severity of reaction and in Table 26 by duration of reaction. The data demonstrate that injection site reactions are commonly reported in subjects receiving SC elamipretide and that, generally, symptoms are mild in nature and resolve within 4 hours of the injection being administered.

Table 25 Summary of Local TEAE Severity Reported in SC Elamipretide Treated Subjects in Single-Dose Trials

System Organ Class Preferred Term	Number (%) of Elamipretide Treated Subjects (n=56)		
	Severity		
	Mild	Moderate	Total
General disorders and administration site conditions	**44 (78.6)**	**10 (5.6)**	**54 (96.4)**
Injection Site Bruising	1 (1.8)	0	1 (1.8)
Injection Site Erythema	45 (80.4)	9 (16.1)	54 (96.5)
Injection Site Nodule	1 (1.8)	0	1 (1.8)
Injection Site Pain	9 (16.1)	0	9 (16.1)
Injection Site Pruritus	22 (39.3)	0	22 (39.3)
Injection Site Reaction	3 (5.4)	0	3 (5.4)
Injection Site Swelling	32 (57.1)	3 (5.4)	35 (62.5)

Table 26 Summary of Local TEAE Duration Reported in SC Elamipretide Treated Subjects in Single-Dose Trials

System Organ Class Preferred Term	Number (%) of Elamipretide Treated Subjects (n=56)				
	Duration				
	<0.5h	<1h	<4h	<24h	>24h
General disorders and administration site conditions	**17 (30.4)**	**28 (50.0)**	**35 (62.5)**	**6 (10.7)**	**3 (5.4)**
Injection Site Bruising	0	0	0	0	1 (1.8)
Injection Site Erythema	2 (3.6)	13 (23.2)	32 (57.1)	5 (8.9)	2 (3.6)
Injection Site Pain	5 (8.9)	3 (5.4)	1 (1.8)	0	0
Injection Site Pruritus	11 (19.6)	6 (10.7)	4 (7.1)	1 (1.8)	0
Injection Site Reaction	1 (1.8)	0	1 (1.8)	1 (1.8)	0
Injection Site Swelling	2 (3.6)	16 (28.6)	17 (30.4)	0	0

* Injection Site Nodule duration reported as "Unknown."

SPISC-101 and SPISC-102 employed variable dose solution concentrations and volumes however, additional clinical trials with elamipretide acetate have all been conducted at a fixed

dose solution concentration of 40 mg/mL with the exception of SPICP-105, which utilized an 80 mg/mL concentration. In all studies injections have been administered varying the 4 abdominal quadrants. SPICP-105 (Part 1) evaluated SC injections of elamipretide acetate (40 mg/mL) and elamipretide HCl (80 mg/mL) in both the abdomen and the thigh. The local adverse events of injection site reactions for these 3 dosing regimens are reported by severity in Table 27. The safety profile was comparable across salt forms, concentrations, and injection locations.

Table 27 Summary of Local TEAE Severity Differences Reported in SC Elamipretide Treated Subjects

System Organ Class Preferred Term	**Elamipretide Acetate 40mg Abdomen Number (%) of Subjects (n=32)**	**Elamipretide HCl 40mg Abdomen Number (%) of Subjects (n=32)**	**Elamipretide HCl 40mg Thigh Number (%) of Subjects (n=32)**
Severity	Mild	Mild	Mild
General disorders and administration site conditions	**30 (93.8)**	**30 (93.8)**	**30 (93.8)**
Injection Site Bruising	1 (3.1)	0	2 (6.3)
Injection Site Erythema	28 (87.5)	28 (87.5)	27 (84.4)
Injection Site Induration	1 (3.1)	0	0
Injection Site Nodule	0	1 (3.1)	0
Injection Site Pain	13 (40.6)	7 (21.9)	11 (34.4)
Injection Site Pruritus	11 (34.4)	20 (62.5)	19 (59.4)
Injection Site Swelling	16 (50.0)	14 (43.8)	28 (87.5)

SPICP-106 was conducted to assess the potential of various interventions to mitigate the effects reported following administration of elamipretide 60 mg via SC injection; 6 dosing regimens were investigated. ISRs were clinically assessed according to the DAIDS grading scheme prior to elamipretide administration and then at 0.5, 1, 2, 4, 6, 12, 24, and 48 hours after each elamipretide dose. At the same time points, subject self-reporting of ISR signs and symptoms were collected according to an adapted Self-Injection Assessment Questionnaire© (Keininger D. and Coteur G., 2011). Data recorded in Arms 2 – 6 were compared to data from Arm 1 (elamipretide alone) using either Chi-square or Fisher exact test for both clinical and subject self-assessed parameters.

Analysis of the data demonstrates:

- Arm 2 (Mometasone furoate, 0.1% ointment, applied once only under a hydrocolloid occlusive dressing (DuoDERM Extra Thin) 7 days prior to elamipretide administration)
    - Clinical Assessment: Reduced incidence of induration at 0.5-hour postdose ($p = 0.0031$) and a trend toward significance in reduction of incidence of pruritus at 0.5-hour postdose ($p = 0.0573$). A trend toward significance in reduction of incidence of induration at 1-hour postdose was also observed ($p = 0.0736$).
    - Subject self-assessment: A trend toward significance in reduction of bothersome itching at 1-hour postdose ($p = 0.1409$)
- Arm 3 (Ice applications at the injection site 5 minutes pre- and post-elamipretide administration)
    - Clinical Assessment: No statistically significant differences, or trends observed

    - Subject self-assessment: Reduced incidence of bothersome pain at 0.5-hour postdose (p = 0.0325). A trend toward significance in reduction of burning sensation at 0.5-hour postdose (p = 0.1409) and at 1-hour postdose (p = 0.0867), itching at 0.5-hour postdose (p = 0.0573), swelling at 0.5-hour postdose (p = 0.1409) and at 1-hour postdose (p = 0.1698), and redness at 1-hour postdose (p = 0.1409) when compared to Arm 1 at the same time point.
- Arms 4 (Tacrolimus, 0.1% ointment applied 15 minutes pre-elamipretide administration)
    - Clinical Assessment: No statistically significant differences, or trends observed
    - Subject self-assessment: A trend toward significance in reduction of incidence of burning sensation at 0.5-hour postdose (p = 0.1409) and at 1-hour postdose (p = 0.0867).
- Arm 5 (Doxepin, 5% cream, applied 15 minutes pre-elamipretide administration)
    - Clinical Assessment: No statistically significant differences, or trends observed
    - Subject self-assessment: No statistically significant differences, or trends observed.
- Arm 6 (Diphenhydramine, 50 mg po, taken 2 hours pre-elamipretide administration)
    - Clinical Assessment: Reduced incidence of induration at 1-hour postdose (p = 0.0198). A trend to significance in reduction of incidence of induration at 0.5-hour postdose was also observed (p = 0.0698)
    - Subject self-assessment: A trend toward significance in reduction of incidence of pain at 0.5- hour postdose (p = 0.1409), burning sensation at 1-hour postdose (p = 0.0867), and swelling at 1-hour postdose (p = 0.1698)

5.3.5. **Hypersensitivity / Allergic Reactions**

There have been 3 potential cases of hypersensitivity / allergic reactions resulting from treatment with elamipretide identified across all development programs.

For one subject in the SPIMM-301 clinical trial it was reported that a few minutes after administering the medication SC in the abdomen, subject had an itchy tongue feeling followed by red, swollen itchy fingers, and a red blotchy face around the nose and mouth and a throbbing headache. She did not have swelling in the mouth or pharynx and had no airway distress. The reaction started a few minutes after administration and the duration was approximately 10 minutes. No medical help was required.

For a second subject, in the SPIMM-203 clinical trial it was recorded that the subject complained of "throat closing like anaphylaxis feeling". The patient presented to the ER where the attending physician could not observe any objective signs or symptoms regarding the complaints. As a precaution, the administration of the study medication was discontinued in both patients.

On day 28 of treatment with 40mg SC elamipretide daily, one patient enrolled in an Individual Named Patient Program (NPP) in the elamipretide EAP developed an allergic reaction approximately 15 minutes after elamipretide injection. The allergic reaction manifested exclusively through an irritative cough, papular lesions on abdomen and erythematous rash on the upper body. No hypotension, other cardiovascular changes or respiratory distress were observed. An ambulance was called and the patient was treated according to anaphylactic shock protocol and transported to the emergency room where the subject was kept under surveillance

for 4 hours and discharged with no further treatment. The treating physician considered the event to meet the seriousness criterion of life threatening. The treating physician assessed the SAE as severe in intensity and probably related to elamipretide.

Stealth BioTherapeutics has established drug safety procedures to actively monitor and identify potential cases of hypersensitivity/allergic reactions, deemed as adverse events of special interest (AESI). These procedures are specific to each clinical trial or expanded access program. Potential cases may include systemic reactions and exclude local injection site reactions. In case of relevant events, investigators and treating physicians will be timely informed, following the safety guidelines.

5.3.6. Expanded Access Programs (EAPs)

To date, one SUSAR has been reported across the 77 patients treated in the EAPs (Section 5.3.5). In the opinion of the treating physicians, two SAEs assessed as "unlikely related" (including one death where an autopsy is not available), and six SAEs assessed as "unrelated" (including one death) have been reported. Overall, the safety data from the EAPs does not change the safety profile of elamipretide.

5.4. Marketing Experience

Not applicable; elamipretide injection is not a marketed product.

6. SUMMARY OF DATA AND GUIDANCE FOR THE INVESTIGATORS

Elamipretide injection and Elamipretide Delivery System are intended for use only as investigational products and should be used exclusively by selected Investigators who are experienced with conducting clinical research and in accordance with investigational protocols approved by an Institutional Review Board/ Ethical Committee.

6.1. Summary of Data Related to Safety

Parenteral administration of elamipretide was assessed following single and multiple IV infusion and SC administrations as described in Appendix 1 and Appendix 2. Dose levels studied ranged from approximately 0.7 mg/day to 300 mg/day. There were no apparent differences between the safety profiles of IV infusion or SC elamipretide dosing routes except for injection site reactions which were widely reported in subjects receiving SC elamipretide.

Differences (≥2% difference) in systemic TEAEs reported in elamipretide- and placebo-treated subjects, summarized by dosing duration is displayed in Table 28.

Table 28 Summary of Systemic TEAEs Reported in >5% of Elamipretide Treated Subjects with Greater Frequency (≥2% difference) in Elamipretide-treated Subjects Compared to Placebo-Treated Subjects

	Elamipretide Number (%)	Placebo Number (%)
Repeat dose ≤8 days	**n=372**	**n=231**
Headache	5.1%	2.6%
Repeat dose >8 days	**n=493**	**n=258**
Dizziness	5.7%	3.0%
Headache	7.7%	4.3%
Nasopharyngitis	6.3%	1.9%
Upper respiratory tract infection	6.9%	3.5%
Eosinophil count increased	5.5%	0%

Additionally, the absolute eosinophil count increased several-fold from baseline in the majority of patients that administered elamipretide longer than 28 days. The eosinophil values typically return to normal upon chronic administration of elamipretide. This elevation in eosinophils was not associated with clinical manifestations or changes in other laboratory parameters. While TEAEs of respiratory tract and other infections have been reported at greater frequency with elamipretide treatment than in placebo subjects, there is no evidence that this finding is related to eosinophil increases. A majority of those respiratory tract and other infections were reported in SPIAM-101 and SPIMM-203, both of which do not have a placebo comparator.

In SPICP-103, high dose IV infusions (56 to 140 mg/day) of elamipretide were administered daily for 5 days in healthy subjects. Four subjects reported events of paraesthesia or oral paraesthesia. These events generally occurred immediately after the end of the drug infusion, at

the T_{max} of elamipretide. Three of these AEs occurred in the 140 mg cohort, and 1 occurred in the 112 mg cohort. All were reported to be mild in severity, resolved within 2 hours of symptom onset, and were not associated with hypersensitivity reactions.

Injection site reactions were reported in the majority of subjects receiving elamipretide by SC injection in any study. Detailed characterization of the injection site reactions occurring in single dose SC clinical trials demonstrates that mild erythema, swelling, pain, and pruritus are the most commonly reported signs and symptoms and that pain and bruising may also be experienced. Generally, the injection site reactions resolved within 4 hours of elamipretide administration. In the longer-term studies reporting of injection site reaction commonly included the signs and symptoms previously mentioned as well as injection site bruising, induration, urticaria, haemorrhage and mass. In most subjects, the tolerability of the injection site reactions was not problematic and does not require treatment however, some subjects have been treated with topical and systemic antihistamines and/or topical corticosteroids in order to manage the impact of the signs and symptoms. In two subjects injection site reactions evolved into wounds that in turn became infected. In one subject, upon healing some of the wounds left evidence of scar formation. For both subjects contributing factors were present. In clinical trials > 8 days in duration with data available (completed or open-label), 21 (9.4%) subjects receiving elamipretide via SC injection have discontinued study drug treatment due to injection site reactions.

Three potential cases of hypersensitivity / allergic reactions have been reported. There does not appear to be a clear association between the occurrence of these events and duration of exposure to elamipretide. Investigators and subjects should be aware of the potential for hypersensitivity / allergic reactions with elamipretide.

All additional assessments of safety (including vital signs and laboratory, ECG and physical examination findings) across all clinical studies have been unremarkable.

In subjects with renal impairment who received elamipretide, exposure, as measured by AUC, to elamipretide and both of its metabolites (M1 and M2) increased proportionally to the degree of renal impairment, however, there was no evidence of increased toxicity as a consequence of impaired renal function. Similarly, in the DDI studies carried out to date, co-administration of elamipretide with aspirin, with clopidogrel, or with UFH did not indicate a change in the nature, severity or frequency of AEs to the safety profile of either elamipretide or the comparator.

A supratherapeutic dosing regimen of elamipretide, 80 mg every 3 hours for 5 doses, was utilized in a thorough QTc study (TQT) to evaluate the QT/QTc interval prolongation and proarrhythmic potential of elamipretide in healthy subjects. The results showed no impact on the QTc interval up to plasma concentration of approximately 4200 ng/mL for elamipretide, approximately 3300 ng/mL for M1, and approximately 300 ng/mL for M2.

6.2. Reference Safety Information

There are no serious adverse reactions (SARs) with elamipretide injection that are considered to be expected by the sponsor for the purpose of expedited global reporting at this stage of the development programs.

6.3. Recommended Safety Measures

6.3.1. Dosage and Administration

Elamipretide for parenteral administration is supplied for clinical trials as:

- a ready-to-use sterile aqueous elamipretide injection solution in a single-patient-use, single-dose or multi-dose glass vial for SC administration or further diluted with sterile saline for IV infusion

The specifics of dose administration (eg, dose level, frequency, duration, route of administration) should be in accordance with the clinical trial protocol.

It is important that investigators maintain good communication with their subjects and check regularly for use of proper injection technique and for any possible injection site reactions the subjects might develop. The subjects should follow an adequate hygiene regimen and not apply unauthorized topical products to their injection sites. During each study visit, it is very important that each subject have their injection sites assessed for signs of more severe injection site reactions.

6.3.2. Contraindications

Dosing with elamipretide is contraindicated in any subject who has sensitivity to elamipretide or any of its excipients.

6.3.3. Warnings and Precautions

6.3.3.1. Carcinogenicity and Genotoxicity

Carcinogenicity studies have not been performed to date.

Elamipretide and its metabolites were negative for genotoxicity in the full battery of tests and caused no significant hemolysis or inhibition of receptor binding. Elamipretide did not produce genotoxic effects in bacterial reverse mutation assays and showed no evidence of clastogenicity or aneuploidy.

6.3.3.2. Fertility, Pregnancy, and Lactation

Although elamipretide did not demonstrate fetal toxicity in nonclinical investigations, full precautions to mitigate any potential reproductive risks are applied for all clinical studies with

elamipretide. Studies will mandate pregnancy testing for women of child-bearing potential, exclusion of those found to be breastfeeding or pregnant, and the use of effective contraception for all participants during trial participation.

Across all studies, with either IV infusion and SC formulation, there have been no reports of pregnancy, exposure during lactation, overdoses, or abuse or misuse. There was one pregnancy in a subject's partner in the SPIMM-301 study. The subject had been administered 40 mg of elamipretide for 3 months and his partner became pregnant after the subject stopped treatment with elamipretide. The subject's partner delivered a healthy infant at full-term approximately 10 months after the last administered dose of elamipretide.

6.3.3.3. Elderly

Clinical studies with elamipretide have enrolled subjects up to the age of 80 years. There are no specific safety concerns relating to the geriatric population with the exception of aged-related renal impairment or significant inter-current illness. Subject eligibility will be defined within each specific study protocol.

6.3.3.4. Pediatrics

Clinical studies completed with elamipretide have enrolled subjects from the age 12 years and older and there are no specific safety concerns relating to the pediatric population. Subject eligibility will be defined within each specific study protocol.

6.3.3.5. Subjects with Specific Disease or Conditions

6.3.3.5.1. Renal Impairment

Elamipretide and its metabolites are excreted primarily through the kidneys. Subjects with renal dysfunction have been shown to have higher exposure to the drug and its metabolites which is proportionate to the level of dysfunction. Although these findings have not been associated with an increased incidence of reported AEs in single and multiple dose studies, caution should be exercised in treating subjects with renal dysfunction, especially those with creatinine clearance rates <30 mL/min.

Nonclinical toxicology studies provide adequate safety margins for elamipretide, even in subjects with severe renal impairment; however, due to increased retention of metabolite M2, the maximum dose of elamipretide in subjects with GFR >30 mL/min in chronic studies should generally be 80 mg/day. Subject eligibility and dosing guidance, including for subjects with a GFR $\leq$30 mL/min, will be defined within each specific study protocol.

6.3.3.5.2. Hepatic Impairment

The PK of elamipretide in patients with hepatic insufficiency has not been evaluated; however, nonclinical investigation demonstrates that elamipretide and its metabolites are not metabolized in the liver. Furthermore, they do not appear to inhibit or induce liver enzymes.

6.3.4. Injection Site Reactions

Mild to moderate ISRs are common following the elamipretide injections and in most subjects, the tolerability of the ISRs was not problematic and did not require treatment. The results of a clinical trial specially designed to investigate possible mitigation options indicate that certain medications including oral antihistamines and topical corticosteroids may significantly improve the signs and symptoms in the minority of burdensome ISR cases.

Investigators are invited to timely contact the Sponsor's medical monitor for individualized approach in cases of ISRs that are reported difficult to be tolerated by the subjects.

6.3.5. Drug-Drug Interactions

In preclinical studies, elamipretide resulted in significant inhibition (≥50%) for the MATE1 transporter. Given that MATE1 is expressed in the kidney further investigation is needed to better understand the clinical importance of this finding.

6.3.5.1. Cytochrome P450 Inhibition/Induction

In vitro studies of elamipretide show that it is a weak inhibitor of CYP3A4. Elamipretide did not show any inhibitory effects on CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP2E1 at concentrations up to 200 μM, and up to 10 μM for CYP1A1, CYP2B6, and CYP2C8. Minimal inhibition was observed for CYP3A4-dependent activities, with estimated IC_{50} values greater than 200 M. This weak inhibitory effect is unlikely to be clinically significant.

In a preliminary trial, elamipretide exhibited no potential to induce hepatic CYP enzyme activities. Elamipretide was administered to rats via SC injection at 10 mg/kg/day for 3 consecutive days, and enzyme activities of CYP isoforms were measured in liver microsomes. Elamipretide had no significant inductive effects on the hepatic CYP1A, CYP2C, CYP2D and CYP3A enzymes in the rat after 3 consecutive daily doses.

The investigator should refer to the clinical protocol for specific guidance on the use of concomitant medications and consumption of other substances (eg, grapefruit).

6.3.6. Overdose

To date, there have been no cases of overdose with elamipretide injection. In case of overdose, there is no antidote to elamipretide. Symptoms that present following overdose should be managed according to standard of care.

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APPENDIX 1. OVERVIEW OF CLINICAL TRIALS EVALUATING PARENTERAL ELAMIPRETIDE IN HEALTHY SUBJECTS

Trial Identifier	Trial Phase	Status	Country/Region	Trial Objectives	Subject Population	Number of Subjects Enrolled
Elamipretide for Intravenous Infusion						
SPIRI-101	1	Completed	US	Safety, tolerability, and PK	Healthy subjects aged ≥18 years	40
Trial Design: Double-blind, randomized, placebo-controlled, parallel-group. Dosing Regimen: Single IV infusion; ascending doses of 0.01, 0.025, 0.05, 0.10, or 0.25 mg/kg/hr elamipretide/placebo for 4 hours.						
SPIRI-102	1	Completed	US	Safety, tolerability, and PK	Healthy subjects aged 18-65 years	12
Trial Design: Open-label. Dosing Regimen: Single IV infusion; 0.05 mg/kg/hr elamipretide for 2 hours.						
SPIRI-151	1	Completed	US	PK and Antithrombotic Effects of Aspirin	Healthy subjects aged 18-65 years	9
Trial Design: Drug-drug interaction; Randomized, double-blind, 2-period, 2-sequence crossover. Dosing Regimen: 650 mg aspirin PO followed 4 hours later by a single IV infusion of 0.10 mg/kg/hr elamipretide/placebo for 4 hours.						
SPIRI-152	1	Completed	US	PK and Antithrombotic Effects of Clopidogrel	Healthy subjects, aged 18-65 years	8
Trial Design: Drug-drug interaction; Randomized, double-blind, 2-period, 2-sequence crossover. Dosing Regimen: 300 mg clopidogrel PO followed 4 hours later by a single IV infusion of 0.10 mg/kg/hr elamipretide/placebo for 4 hours.						
SPIRI-153	1	Completed	US	Effects of elamipretide on the Pharmacodynamic Effects of UFH, Safety	Healthy subjects aged 18-65 years	3 (terminated early)
Trial Design: Drug-drug-interaction; Randomized, double-blind crossover trial. Dosing Regimen: Single IV infusion; 0.25 mg/kg/hr elamipretide for 4 hours. Bolus of 60 U/kg UFH followed by constant infusion of 12 U/kg/hr for 6 hours.						
SPIRI-154	1	Completed	US	Safety and PK	Healthy subjects and subjects with mild, moderate, severe renal impairment; aged ≥18 years	24
Trial Design: Open-label. Dosing Regimen: Single IV infusion; 0.05 mg/kg/hr dose of elamipretide for 4 hours.						

Trial Identifier	Trial Phase	Status	Country/Region	Trial Objectives	Subject Population	Number of Subjects Enrolled
SPIRI-155	1	Completed	US	Pharmacodynamic effects of UFH co-administration with elamipretide, PK, and Safety	Healthy subjects aged 18-65 years	Part 1: 5 Part 2: 9
Trial Design: Part 1: Open-label Part 2: Drug-drug interaction; randomized, double-blind crossover. Dosing Regimen: Part 1: IV bolus infusion of 60 U/kg UFH followed by a continuous infusion of 12 U/kg/hr UFH for 12 hours Part 2: IV bolus infusion of 60 U/kg UFH followed by a continuous infusion of 12 U/kg/hr UFH for 12 hours; single IV infusion of 0.25 mg/kg/hr elamipretide/placebo for 4 hours beginning no later than 7 hours after start of UFH infusion.						
SPIRI-120	1	Completed	US	Effects of elamipretide on Cigarette Smoking-induced Endothelial Dysfunction	Male subjects aged 18-65 years subjects who smoke	6
Trial Design: Randomized, double-blind, 2 period, 2 sequence crossover trial. Dosing Regimen: Single IV infusion; 0.25 mg/kg/hr elamipretide/placebo for 4 hours; 2 doses given 1 week apart.						
SPICP-101	1	Completed	US	Safety and PK	Healthy subjects and subjects with mild, moderate, severe renal impairment; aged ≥18 years	23
Trial Design: Single-center, 7 day repeat dose, parallel-group, open-label. Dosing Regimen: 7 daily 1-hour IV infusions of 0.25 mg/kg/hr elamipretide.						
SPICP-103	1	Completed	US	Safety and PK	Healthy subjects aged 18 to 45	32
Trial Design: Single-center, randomized, 4-part dose-ascending study. Dosing Regimen: 5 daily 1-hour IV infusion of 56, 84, 112 or 140 mg elamipretide or placebo.						
Elamipretide for Subcutaneous Injection						
SPICP-102	1	Completed	US	Safety and PK	Healthy subjects aged 18 to 45	40
Trial Design: Single-center, randomized, 3-way cross-over study will enroll healthy volunteers. Subjects will participate in 3 Dosing periods and receive placebo, moxifloxacin, or elamipretide (80 mg) in each of 3 Dosing Regimens as described below. Dosing Regimen: Subjects received the following over the 3 treatment periods • Regimen A: Placebo (SC injection) every 3 hours for 5 doses • Regimen B: Placebo (SC injection) every 3 hours for 5 doses and a single open-label dose of moxifloxacin (400 mg oral) immediately following dose 5 • Regimen C: 80 mg elamipretide (SC injection) every 3 hours for 5 doses.						
SPISC-101	1	Completed	UK	Safety, Tolerability, PK, and Pharmacodynamics	Healthy subjects aged 18 to 65	Part 1: 47 Part 2: 36
Trial Design: Single-center, randomized, double-blind, placebo-controlled. Dosing Regimen: Part 1: Single SC injection: 2 mg, 6 mg, 12 mg, 20 mg, or 40 mg elamipretide/placebo in 1 mL; 20 mg elamipretide/placebo in 0.5 mL Part 2: Multiple SC injection: 6 or 40 mg elamipretide/placebo as 1 mL SC injection daily for 7 days; 20 mg elamipretide/placebo as 0.5 mL SC injection daily for 7 days.						

Trial Identifier	Trial Phase	Status	Country/Region	Trial Objectives	Subject Population	Number of Subjects Enrolled
SPISC-102	1	Completed	UK	Safety, Tolerability, PK, and Pharmacodynamics	Healthy subjects aged 18 to 65	30
Trial Design: Single-center, randomized, double-blind, placebo-controlled. Dosing Regimen: Part 1: Single SC injection: 40 or 80 mg elamipretide/placebo in 0.5 mL; 40 mg elamipretide/placebo in 0.25 mL Part 2: Multiple SC injection: 40 or 80 mg elamipretide/placebo in 0.5 mL SC injection daily for 7 days; 40 mg elamipretide/placebo in 0.25 mL SC injection daily for 7 days.						
SPICP-104	1	Completed	US	Safety and PK	Healthy subjects aged 18 to 55	8
Trial Design: Single-center, single-cohort, open-label study. Dosing Regimen: 60 mg (40 mg/mL) elamipretide via SC injection every 3 hours for 5 doses.						
SPICP-105	1	Completed	US	Safety and PK	Healthy subjects aged 18 to 55	Arm 1: 32 Arm 2: 9
Trial Design: Single-center, multiple arm, open-label. Dosing Regimen Arm 1: Three-way crossover, elamipretide acetate 40 mg in the abdomen, elamipretide HCl 40 mg in the abdomen, elamipretide HCl 40 mg in the thigh. Dosing Regimen Arm 2: 7 daily SC doses of 40 mg elamipretide HCl.						
SPICP-106	1	Completed	US	Safety and PK	Healthy subjects aged 18 to 65	10
Trial Design: Single-center, open-label. Dosing Regimen: 6-part crossover; Part 1 elamipretide 60 mg, Part 2 elamipretide 60 mg + mometasone ointment 0.1% with occlusive dressing, Part 3 elamipretide 60 mg + ice application to injection site, Part 4 elamipretide 60 mg + tacrolimus ointment 0.1%, Part 5 elamipretide 60 mg + doxepin cream 5%, Part 6 elamipretide 60 mg + diphenhydramine 50 mg.						

APPENDIX 2. OVERVIEW OF CLINICAL TRIALS EVALUATING PARENTERAL ELAMIPRETIDE IN PATIENT POPULATIONS

Trial Identifier	Trial Phase	Status	Country /Region	Trial Objectives	Subject Population	Number of Subjects Enrolled
Acute Cardiovascular Diseases						
SPIRI-201 (EMBRACE)	2a	Completed	US and Europe	Safety, Tolerability, and Efficacy	ACS subjects aged 18-84 years with first-time acute, anterior wall STEMI scheduled to undergo primary PCI and stenting	297
Trial Design: Randomized, double-blind, placebo-controlled. Dosing Regimen: Single IV infusion; 0.05 mg/kg/hr elamipretide/placebo starting 15 to 60 minutes before PCI procedure and continuing for 60 minutes post-procedure.						
Summary of Key Results: Efficacy: The primary objective of the trial was to determine if elamipretide could protect the heart from muscle damage that might occur when the previously blocked vessel is opened abruptly, as determined by CK-MB AUC over 72 hours. Of the 297 subjects enrolled, 179 were excluded from the primary analysis population mostly due to absence of complete arterial blockage at the time of initial angiography. The CK-MB AUC in elamipretide-treated subjects in this population was lower than placebo (6582 ng·h/mL versus 6738 ng·h/mL for placebo), although the difference did not achieve significance in these 118 subjects. Elamipretide did not reduce the secondary endpoint of infarct size measured by MRI on Day 4 nor at Day 30. Elamipretide did improve myocardial perfusion as determined by corrected TIMI frame count (79.7 vs 166.0, p=0.03), but not TIMI perfusion grade. Finally, elamipretide did not improve the composite clinical endpoint of death and new onset heart failure within 30 days (22.4% vs 28.3%, p=0.53) nor at 6 months (25.9% vs 28.3%, p=0.84) but trended toward a decrease in the incidence of new heart failure beginning within the first 24 hours following PCI but within the duration of the index hospitalization (13.8% vs 25.0%, p=0.16). Safety: There were no significant differences in subject safety and tolerability. The frequency and severity of reported AEs were consistent with those commonly experienced in this patient population and no trends were observed between elamipretide-treated and placebo groups. Additionally, there were no significant differences in arrhythmias, deaths, or SAEs, between elamipretide and placebo-treated subjects. For the elamipretide treatment group, 40.7% of TEAEs were assessed as mild, 29.3% were assessed as moderate, and 18% were assessed as severe. For the placebo group, 38.1% were assessed as mild, 32.7% were moderate, and 10.9% were severe. There were no notable findings in the review of the clinical laboratory, physical examination, vitals sign, and ECG data. Review of clinical laboratory data for low serum sodium levels revealed similar frequencies for each treatment group: 12.0% elamipretide versus 8.8% in the placebo arm.						
SPIRI-225 (EVOLVE)	2a	Completed	US	Safety and Efficacy	AKI subjects aged 40- 80 years with ARAS who are undergoing PTRA and stenting	16
Trial Design: Single-center, randomized, double-blind, placebo-controlled. Dosing Regimen: Single IV infusion, 0.05 mg/kg/hr elamipretide/placebo starting 15 to 30 minutes before PTRA and continuing for 3 hours post-procedure.						
Summary of Key Results: Efficacy: Efficacy results from an exploratory Phase 2a trial (SPIRI-225) evaluating the impact of a single IV dose of elamipretide in subjects with AKI undergoing renal revascularization to reduce blood pressure may be suggestive of a renal protective effect. The study was terminated early due to poor recruitment. The primary study endpoint of improvement in glomerular filtration rate, assessed by iothalamate clearance pre-PTRA and post-PTRA did not show a significant difference. However, PTRA and treatment with elamipretide demonstrated reduction in kidney hypoxia detected by blood oxygen-dependent MRI at 24 hours and an improvement in cortical perfusion and renal blood flow at 3 months. Estimated glomerular filtration rate increased more when comparing elamipretide-treated subjects to placebo-treated subjects at 3 months (p=0.11) (Saad A et al, 2017). Safety: The frequency and severity of reported AEs were consistent with those commonly experienced in this patient population and no trends were observed between elamipretide-treated and placebo groups. No events occurred in more than 1 subject treated						

Trial Identifier	Trial Phase	Status	Country /Region	Trial Objectives	Subject Population	Number of Subjects Enrolled
with elamipretide. There was 1 SAE of pseudoaneurysm of left brachial artery (severe, unrelated, resolved) in an elamipretide-treated subject.						
Chronic Cardiovascular Diseases						
SPIHF-101 (PREVIEW)	1	Completed	Bulgaria	Safety, Tolerability, PK, and PD	Subjects aged 45-80 years with stable NYHA Class II-III congestive CHF with LVEF ≤ 35%	36
Trial Design: Single center, randomized, double-blind, placebo-controlled, single ascending dose. Dosing Regimen: Single 4-hour IV infusion; ascending doses of 0.005, 0.05, 0.25 mg/kg/hr elamipretide/placebo.						
Summary of Key Results: Efficacy: The highest dose of elamipretide significantly reduced mean LV end systolic volume (absolute change from baseline -11 mL versus 2.8 mL for placebo; mean difference = -13.7 mL; p=0.005) and mean LV end diastolic volume (absolute change from baseline -15 mL versus 2.9 mL for placebo; mean difference = -17.9 mL; p=0.009) at the end of the infusion. The highest dose of elamipretide also reduced left atrial volume at all time points, as well as leading to an increase in right ventricular fractional area change at all time points. Finally, right-ventricular systolic pressure appeared to be reduced at most time points in both the medium and high dose elamipretide-treated subjects, as compared to placebo. Heart failure and exploratory biomarkers (plasma NT-pro-BNP, hs-CRP, urinary 8-isoprostane and 8-hydroxy-2-deoxyguanosine) were collected at Day 1 and on Day 2 pre- and 2 hours post-dose. There were no clear patterns or trends observed in mean serum creatinine levels or mean estimated glomerular filtration rate following treatment with either elamipretide or placebo. In addition, there were no clear patterns or trends observed in heart failure and exploratory biomarkers (plasma NT-pro-BNP, hs-CRP, urinary 8-isoprostane and 8-hydroxy-2-deoxyguanosine) collected at Day 1 and on Day 2 pre- and 2 hours post-dose following treatment with either elamipretide or placebo. Safety: Overall, single IV infusions of elamipretide were assessed in subjects with stable CHF. No SAEs or deaths were recorded. No TEAEs were reported in 12 placebo-treated subjects. A total of 4 TEAEs in 3 elamipretide-treated subjects were reported: 1 TEAE in the 0.005 mg/kg/h dose group and 3 TEAEs in the 0.05 mg/kg/h dose group. One subject treated with elamipretide 0.005 mg/kg/h experienced mild worsening renal failure on trial day 8 which was deemed by the Investigator to be unrelated to trial drug. One subject who received elamipretide 0.05 mg/kg/hr experienced 2 TEAEs (ie, dyspnea and tachycardia, both of moderate intensity) resulting in discontinuation of the infusion. Both were not felt to be related to trial drug by the Investigator. One subject in the intermediate dose group developed a low hemoglobin on day 8 and also was felt to the trial drug. There was no evidence of differences in changes for any of the clinical laboratory parameters (chemistry, hematology, and urinalysis), physical examination findings, ECG parameters, or vital signs among in either the elamipretide or matching placebo groups.						
SPIHF-201 (PROGRESS)	2	Completed	Italy, NL, UK	Efficacy, Safety, Tolerability, and PK	Subjects aged 40-80 with Stable Heart Failure with Reduced Ejection Fraction LVEF ≤ 40%	71
Trial Design: Randomized, Double-Blinded, Placebo-Controlled. Dosing Regimen: Single daily 4 or 40 mg SC injections of elamipretide/placebo for 28 days (1:1:1 randomization).						
Summary of Key Results: Efficacy: While a decrease from Baseline to Week 4 was observed in all groups for the primary endpoint, left ventricular end systolic volume (LV ESV), the differences between treatment groups were not statistically significant. There were improvements in quality of life detected in the patients that received 40 mg of elamipretide when compared to patients that received placebo (p=0.07), despite baseline differences. An improvement in the 6MWT distance was detected in the 40 mg group compared to placebo-treated patients, when adjusting for substantial baseline differences. Pharmacokinetics: No conclusions can be made regarding the PK profile of elamipretide or metabolites. Safety: Overall a total of 35 subjects (49.3%) had at least 1 TEAE; 1 subject had 2 TEAEs (nausea and fatigue) that led to discontinuation of study treatment (elamipretide 4 mg). All the 35 subjects reported a total of 65 TEAEs, of which 1 was an SAE. Subjects in the 40 mg group reported a greater number of TEAEs (29) in comparison to the 4 mg (17) and placebo group (19). The majority of TEAEs were considered unrelated to study treatment. A total of 19 TEAEs were considered related to study treatment (6 in subjects receiving elamipretide 4 mg, 9 in subjects receiving elamipretide 40 mg, and 4 in subjects receiving placebo), and 1 TESAE (1/65) of vestibular disorder was considered unrelated to study treatment (elamipretide 40 mg). Overall,						

Trial Identifier	Trial Phase	Status	Country /Region	Trial Objectives	Subject Population	Number of Subjects Enrolled
24 subjects (33.8%) had TEAEs that were mild and 10 subjects (14.1%) had TEAEs with moderate intensity. One subject (1.4%) from the 40 mg group had a severe TEAE. There were no deaths in the study. The most common TEAEs (in treatment groups combined) by system organ class (SOC) were General Disorders and Administration Site Conditions (9 subjects [12.7%]) with fatigue (4 subjects [5.6%]) as the most common TEAE (by preferred term), followed by the SOC Infections and Infestations (7 subjects [9.9%]) with nasopharyngitis (4 subjects [5.6%]) as the most common TEAE (by preferred term), and the SOC Nervous System Disorders (6 subjects [8.5%]) with dizziness (3 subjects [4.2%]) as the most common TEAE (by preferred term). Overall a greater percentage of subjects (33.8%) experienced TEAEs considered mild in intensity; the proportion of subjects experiencing mild TEAEs was 48.0% in the 40 mg group, 33.3% in the placebo group, and 18.2% in the 4 mg group.						
SPIHF-203 (RESTORE)	2	Complete in clinic, pending final CSR	Germany Serbia	Efficacy, Safety, and Tolerability	Subjects aged 45-80 with Stable Heart Failure with Preserved Ejection Fraction LVEF ≥45%	47
Trial Design: Randomized, Double-Blinded, Placebo-Controlled. Dosing Regimen: Single daily 40 mg SC injections of elamipretide/placebo for 28 days.						
Summary of Key Results: Efficacy: The primary endpoint of the study was not met, but elamipretide administered subcutaneously at a dose of 40 mg once daily over 28 days did reduce septal E/e' at rest as compared with placebo in the mITT population. In addition, elamipretide-treated subjects exhibited a more marked reduction in E/e' during maximal stress. This phenomenon was also observed with the GLS endpoint. A separate independent SAP-guided analysis of the data revealed that left-ventricular volumes were increased in the elamipretide-treated subjects, but not in the placebo-treated subjects. This effect became more pronounced during exercise. This observation suggests that there may have been an effect observed on left-ventricular relaxation. In the setting of HFpEF, the fundamental issue is impairment in relaxation. Therefore, this observation of an increase in left-ventricular volumes with elamipretide may suggest potential benefits in this population. In fact, all imaging outcome measures exhibited a more pronounced effect during exercise. This may be highly relevant because the occurrence of symptoms in patients with HFpEF typically occurs during periods of exercise. Safety: Once daily SC administration of elamipretide at a dose of 40 mg was generally safe and well tolerated in subjects with HFpEF during this 4-week, placebo-controlled trial. Elamipretide was not associated with a higher percentage of moderate or severe AEs, however local intolerance reactions were more common in the elamipretide group. The most common AEs in elamipretide-treated subjects were injection site erythema (21.7%), application site erythema (17.4%), injection site swelling (17.4%), and injection site pruritus (8.7%). In the placebo group, no TEAEs occurred in more than 1 subject. One subject treated with placebo experienced acute myocardial infarction and subsequent sudden death during the study. There were no deaths, SAEs, or discontinuations due to AEs in the elamipretide group. One elamipretide-treated subject experienced 2 severe TEAEs (application site erythema and application site edema), each of which resolved the same day as onset with no counteractive treatment given. Treatment-related TEAEs were reported more often in the elamipretide group (52.2% vs 8.3% placebo) and were commonly application site or injection site reactions (e.g., erythema, swelling, pruritus). Analyses of clinical laboratory data and other safety data (vital signs, ECGs, and physical examinations) did not reveal any clinically relevant findings with elamipretide treatment.						
SPIHF-204 (IDDEA-HF)	2	Complete in clinic, pending final CSR	Europe	Efficacy, Safety, Tolerability, and PK	Subjects aged 18 years and older who are hospitalized with Congestion due to Heart Failure	308
Trial Design: Randomized, Double-Blind, Placebo-Controlled Trial. Dosing Regimen: Single daily 20 mg IV infusion of elamipretide/placebo over 60 minutes for 7 days.						
Summary of Key Results: Efficacy: The primary efficacy endpoint was change in NT-proBNP between Baseline and Day 8/Early Discharge. Mean (SD) values of log NT-proBNP at baseline were similar between the treatment groups. At Day 8, changes from baseline were also similar between the treatment groups. There was no statistically significant difference in slopes between the groups (p = 0.4136). While the study didn't meet its primary endpoint, changes in eGFR and mortality show promise for additional studies of elamipretide. Changes in eGFR from baseline to Day 3 and Day 8 were slightly larger in the elamipretide group than in the placebo group. Changes in weight from baseline to Day 3 and Day 8 were similar between the elamipretide group and the placebo						

Trial Identifier	Trial Phase	Status	Country /Region	Trial Objectives	Subject Population	Number of Subjects Enrolled
group. The incidence of specific treatment failures was similar between the treatment groups except for "Need to add new therapy or increase therapy of a thiazide" where more subjects in the placebo group (5.3%) met that criterion than did subjects in the elamipretide group (3.2%). A repeated measures analysis adjusted for baseline dose of furosemide and thiazide use and time showed no statistically significant difference in slopes between the treatment groups. Results of the exploratory analyses (orthopnea, index hospitalization, echocardiography, and specific laboratory values) showed no differences between the treatment groups. Safety: In general, treatment with elamipretide was safe and well tolerated and no clinically meaningful differences were seen in laboratory values, vital signs, or electrocardiograms. Overall, 4.9% of subjects (15/306 subjects) died during the study. Twice as many subjects in the placebo group died during the study (3.2% [5/155 subjects] in the elamipretide group versus 6.6% [10/151 subjects] in the placebo group). A total of 16.0% of subjects (49/306 subjects) experienced an SAE during the study. Overall, 41.5% of subjects experienced at least 1 AE during the study. The incidences were similar between the treatment groups (40.0% [62/155 subjects] in the elamipretide group and 43.1% [65/151 subjects] in the placebo group). The only AE that occurred in ≥ 5.0% of subjects overall was cardiac failure (8.8%, 27/306 subjects). The incidence of cardiac failure was smaller in the elamipretide group (5.8%, 9/155 subjects) than in the placebo group (11.9%, 18/151 subjects).						
Skeletal Muscle Mitochondrial Dysfunction						
SPITM-201 (MOTION)	2a	Completed	US	Efficacy	Elderly subjects aged ≥60 and ≤85 years with evidence of skeletal muscle mitochondrial dysfunction	40
Trial Design: Randomized, double-blind, placebo controlled. Dosing Regimen: Single IV infusion, 0.25 mg/kg/hr at a rate of 60 mL/hr for 2 hours.						
Summary of Key Results: Efficacy: Greater mean increases from Baseline in ATPmax, the primary endpoint, were observed in subjects treated with elamipretide (approximately 30%) compared to those who received placebo (approximately 12.5%). Subjects treated with elamipretide had a greater increase from Baseline in ATPmax at Day 1 Hour 2 (0.17 mM/sec) compared to those in the placebo group (0.12 mM/sec), but the treatment difference (0.06 mM/sec) was not significant (95% confident interval [CI] = -0.08, 0.21; p=0.382). A post-hoc re-analysis that removed an outlier in the placebo group resulted in a greater treatment difference (0.10 mM/sec [95% CI = -0.00, 0.21; p=0.056). Mean changes from Baseline in muscle FTI was approximately 2 to 4 times, but not significantly greater in subjects treated with elamipretide compared to placebo. Mean muscle FTI ranged for 0.43 to 0.83 N/sec in subjects treated with elamipretide compared to 0.10 to 0.44 N/sec in the placebo group. At all visits, muscle FTI was significantly correlated with ATP Max (r=0.27, p=0.004) Mean P/O and NAD levels generally increased from Baseline for both treatment groups during the trial. No significant differences were observed between subjects treated with elamipretide and those in the placebo group at either scheduled time points. Pharmacokinetics of elamipretide were unchanged from normal subjects in this patient population. Safety: There were no deaths, SAEs, or TEAEs resulting in withdrawal in this trial. There were 8 TEAEs reported in 5 (12.8%) subjects and most of the TEAEs were mild in severity. No single event was reported in more than 1 subject. Overall, there were no safety concerns with respect to clinical laboratory results, physical examination, vital signs, and ECG data.						
Genetically Confirmed Mitochondria Diseases						
SPIMM-201 (MMPOWER)	1/2	Completed	US	Safety, Tolerability, Efficacy, and PK	Subjects aged ≥16 and ≤65 years with PMM	36
Trial Design: Multicenter, randomized, double-blind, placebo-controlled, multiple ascending dose. Dosing Regimen: Multiple ascending IV doses 0.01, 0.10, 0.25 mg/kg/hr elamipretide/placebo administered as a single daily dose over 2 hours for 5 consecutive days.						
Summary of Key Results: Efficacy: The highest dose of elamipretide examined in this trial, 0.25 mg/kg/hr, was associated with significant improvement in skeletal muscle function compared to placebo, as measured by the 6MWT. Additionally, there was a positive dose response with						

Trial Identifier	Trial Phase	Status	Country /Region	Trial Objectives	Subject Population	Number of Subjects Enrolled
increasing dose of elamipretide as indicated in the linear trend test (p=0.014 on Day 5), with adjusted least squares mean distance of 13.5 m, 36.5 m, and 64.5 m further for subjects treated with 0.01, 0.10, and 0.25 mg/kg/hr elamipretide, respectively. In a post-hoc analysis which adjusted for gender and baseline disease walked, subjects treated with the highest dose of elamipretide walked an adjusted mean (ie, LS mean) distance of 48 meters further on Day 5 than subjects in the placebo group in the 6MWT compared to their Baseline assessment (p=0.030). Subgroup analysis of the 6MWT suggest that elamipretide is more effective in subjects with greater impairment at Screening, as those who initially walked <350 meter showed greater improvement following treatment compared to those who initially walked ≥350 meter. CPET, while not mandatory for all subjects, was used to assess skeletal muscle function. For all treatment groups, including placebo, there was an increase in aerobic capacity (peak VO2 max) and a decrease in ventilatory efficiency (peak VE/VCO2 slope). No CPET parameters were significantly different than placebo for any dose of elamipretide. Adjusted mean modified NMDAS total scores for current function and current clinical assessment did not change over time. Changes in biomarker data (plasma glutathione, plasma FGF-21, urine 8-isoprostane, and urine 8-hydroxy-2-deoxyguanosine) were not significantly different than placebo. Pharmacokinetics of elamipretide were unchanged from normal subjects in this patient population. Safety: Five days of daily IV elamipretide was well tolerated. At least 1 TEAE was reported by two-thirds of subjects. There were no deaths, serious adverse events (SAEs), or TEAEs resulting in withdrawal in this trial. The most common TEAE overall was headache in 6 (16.7%) subjects, followed by dizziness in 3 (8.3%) subjects. There were no treatment-related TEAEs that were severe in intensity. Overall, there were no apparent differences in TEAEs, vital signs, lab values, ECG changes, or suicide assessments among the 4 treatment groups.						
SPIMM-202 (MMPOWER-2)	2	Completed	US	Safety, Tolerability, and Efficacy	Subjects aged ≥16 and ≤65 years with PMM previously treated in SPIMM-201	30
Trial Design: Multicenter, randomized, double-blind, placebo-controlled, crossover. Dosing Regimen: Single daily 40 mg SC injections of elamipretide/placebo for 28 days in Treatment Period 1 followed by single daily 40 mg SC injections of the opposite treatment (40 mg elamipretide/placebo) in Treatment Period 2 (separated by 4-week washout period).						
Summary of Key Results: Efficacy: The primary efficacy measure of interest was mean difference in distance walked (meters) on the 6-minute walk test (6MWT) between elamipretide and placebo as evaluated at the end of treatment period assessments. Following treatment with single daily SC doses of 40 mg elamipretide for 4 weeks, subjects walked 19.8 m further during the 6MWT compared to those in the placebo group (p = 0.0833). Subgroup analysis based on distance walked in the 6MWT at Screening showed that the elamipretide group walked further at the end of treatment than the placebo group, with a larger treatment difference observed for subjects who walked <450 m at Screening (difference = 24.3 m; p = 0.1118) compared to those who walked ≥450 m at Screening (difference = 8.6 m; p = 0.5729). Secondary efficacy variables, evaluated in a similar manner, included the Primary Mitochondrial Myopathy Symptom Assessment (the PMMSA, formerly the Mitochondrial Disease Symptom Assessment) scores, the Neuro-QoL Fatigue-Short Form scores, the Patient Global Assessment (PGA) scores, the Physician Global Assessment (PhGA) scores, Triple Timed Up and Go (3TUG) Test time (seconds), wrist and hip Accelerometer counts, and exploratory biomarker parameters. The PMMSA assesses 10 symptoms experienced by patients with PMM on a 4-point scale. SBTanalyzed the results using pre-specified fatigue subscales focusing on the myopathic symptoms most commonly associated with PMM, namely, tiredness at rest, tiredness during activities, muscle weakness at rest, and muscle weakness during activities (Total Fatigue Score) as well as tiredness during activities and muscle weakness during activities (Total Fatigue During Activities Score). Throughout the study, the PMMSA Total Fatigue scores (a subscale) for the elamipretide group were statistically significantly lower compared to the placebo group. The greatest treatment difference was observed at the end of treatment (difference = 1.7; p = 0.0006). Similarly, while on elamipretide, subjects reported less Total Fatigue During Activities every week, with the greatest treatment difference observed at the end of treatment (difference = 0.8; p=0.002). In addition, the first time subjects completed the PMMSA, they were asked to identify which of the 10 items on the PMMSA questionnaire was the "most bothersome" symptom of their disease. The mean score for the most bothersome symptom was significantly lower for the elamipretide group compared to the placebo group at the end of treatment (difference = 0.3, p = 0.0111). At the end of treatment, improvements in several other patient-reported outcomes were observed. A significantly lower mean Neuro-QoL Fatigue Short Form Total score, indicating less severe fatigue symptoms, was observed with elamipretide compared to placebo (difference = 4.0; p = 0.0115). Additionally, there were significant treatment differences, favoring elamipretide, for 9 out of 19 Neuro-QoL Fatigue individual items. At the end of treatment with elamipretide, a significantly lower (indicative of						

Trial Identifier	Trial Phase	Status	Country /Region	Trial Objectives	Subject Population	Number of Subjects Enrolled
improvement) mean PGA score was observed for the elamipretide group compared to Placebo (difference = 0.3; p = 0.0421). A lower (indicative of improvement) mean PhGA score was observed in the elamipretide group compared to placebo (difference = 0.3, p = 0.0636). At the end of treatment, there were no significant treatment difference in the duration of the 3TUG Test between treatment groups, wrist or hip accelerometers counts, or concentrations of GDF-15, FGF-21, and glutathione. Safety: Twenty-eight days of daily SC elamipretide was generally well tolerated. Overall, at least 1 TEAE was reported by all (100%) subjects during elamipretide treatment and half (50%) of subjects while on placebo. There were no deaths or serious adverse events (SAEs). One subject withdrew from the trial due to moderate injection site pain while on elamipretide treatment. The most common TEAE (>10%) reported during elamipretide treatment were injection site reactions (characterized by erythema [56.7%], pruritus [46.7%], pain [20.0%], urticaria [20.0%], and irritation [10.0%]) and dizziness (10%). All other TEAEs were reported with similar frequency and severity in subjects receiving elamipretide when compared to placebo. Laboratory data demonstrated elevations (>0.45 cells x109/L) in eosinophils beginning at approximately 28 days after initiation of elamipretide treatment in 20.0% of subjects. These laboratory findings were neither reported to be associated with any clinical manifestations nor resulted in any reported TEAEs. In general, these elevations were demonstrated to have returned to within normal range or to baseline levels at the follow-up visit 2 weeks after the end of elamipretide treatment. There were no other identified safety concerns in this trial with respect to other clinical laboratory results, physical examinations, vital signs, ECG data or suicide assessments between the elamipretide and placebo treated periods.						
SPIMM-203 (MMPOWER-OLE)	2	Completed	US	Safety and tolerability	Subjects aged ≥16 and ≤65 years with PMM previously treated in SPIMM-201 and/or SPIMM-202	28
Trial Design: Multicenter, open-label extension trial. Dosing Regimen: Single daily 40 mg SC injections of elamipretide for up to 260 weeks.						
Summary of Key Results: Efficacy: An increase in the mean distance walked during the 6MWT compared to Baseline was observed after 13 weeks, however, this increase was not observed at subsequent visits (≥ Week 26). Safety: Overall elamipretide was well tolerated, with no deaths or treatment-related SAEs. All subjects had at least on TEAE but the majority of these events were mild to moderated in severity and not related to study treatment. There were no TEADEs.						
SPIMM-301 (MMPOWER-3)	3	Completed	North America and Europe	Efficacy, Safety and Tolerability	Subjects aged ≥16 and ≤80 years with PMM	Part 1: 218 Part 2: 196
Trial Design: Multicenter, double-blind, parallel-group, placebo-controlled trial followed by an open-label treatment extension. Dosing Regimen: Part 1: Single daily 40 mg SC injections of elamipretide/placebo for up to 24 weeks; Part 2: Single daily 40 mg SC injections of elamipretide for up to 144 weeks.						
Summary of Key Results: Efficacy: The distance walked (meters) during the 6MWT and Total Fatigue score on the PMMSA constituted the primary endpoint family. At Week 24, subjects randomized to elamipretide and placebo walked a mean distance of 15.33 m and 17.386 m farther during the 6MWT compared to baseline, respectively, with an adjusted mean difference between treatments of -3.185 m (95% CI: -18.693, 12.322); the difference was not significant. Similar results were noted in the sensitivity analysis (which used multiple imputation) and with the PP Population. At Week 24, the mean Total Fatigue Score (Q1 to Q4) was decreased from baseline for both treatment groups, with an adjusted mean difference of -0.07 (95% CI: -0.69, 0.54); the difference was not significant. Similar results were noted in the sensitivity analysis (which used multiple imputation) and with the PP Population. For the subgroup of subjects with a nDNA mutation, those randomized to elamipretide walked farther than those randomized to placebo at Week 24 for both the ITT Population (adjusted mean difference of 16.360 m; 95% CI: -18.498, 51.218; p = 0.3507) and the PP Population (adjusted mean difference of 24.113 m; 95% CI: -0.088, 48.314; p = 0.0508). Significant differences favoring elamipretide were noted at Week 4 for the ITT Population (adjusted mean difference of 22.387 m; p = 0.0419), and for overall during Part 1 for the PP Population (adjusted mean difference of 22.509 m; p = 0.0356). No significant treatment differences were noted with the mtDNA mutation subgroup.						

Trial Identifier	Trial Phase	Status	Country /Region	Trial Objectives	Subject Population	Number of Subjects Enrolled
In the exposure-response analysis, subjects with an nDNA mutation had an increase in the change and fractional change at Week 24 compared to the Day 1 (i.e., baseline) value for the 6MWT as a function of the elamipretide steady state area under the curve ($p = 0.0262$ and $p = 0.0345$, respectively). Safety: A higher proportion of subjects randomized to elamipretide had at least one TEAE (107 [98.2%] subjects) compared to those randomized to placebo (83 [76.1%] subjects). The majority of TEAEs were mild or moderate in severity. For elamipretide, the most common TEAEs were injection site erythema (86.2%) and injection site pruritus (75.2%), and for placebo, the most common TEAEs were injection site erythema (28.4%) and injection site pain (18.3%). No deaths or SAEs related to IMP occurred and the majority of TEAEs and TEADEs were mild to moderate in intensity. Mild elevations of eosinophils were observed in subjects treated with elamipretide.						
SPIMD-301 (NuPOWER)	3	Recruiting	Global	Efficacy, Safety and Tolerability	Subjects aged ≥18 with nPMD	130
Trial Design: Randomized, placebo-controlled, double-blinded, multi-center trial. Dosing Regimen: Single daily 60 mg SC injections of elamipretide/placebo for up to 48 weeks						
Summary of Key Results: Trial is currently ongoing.						
SPIBA-201 (TAZPOWER)	2	Completed, CSR pending	US	Safety, Tolerability, and Efficacy	Subjects aged ≥12 years with genetically confirmed Barth syndrome	Part 1: 12 Part 2: 10
Trial Design: Multicenter, randomized, double-blind, placebo-controlled, crossover trial followed by an open-label treatment extension. Dosing Regimen: Part 1: Single daily 40 mg SC injections of elamipretide/placebo for 84 days in Treatment Period 1 followed by single daily 40 mg SC injections of the opposite treatment (40 mg elamipretide/placebo) in Treatment Period 2 (separated by 4-week washout period); Part 2: Single daily 40 mg/mL SC injections of elamipretide for up to 168 weeks.						
Summary of Key Results: **Part 2 is currently ongoing. Part 1 data is presented below.** **Part 1** Efficacy: Data analysis is currently ongoing. Safety: There were no deaths, SAEs, or TEAEs resulting in withdrawal in this trial. All subjects experienced at least 1 treatment related TEAE. Overall, there were 121 TEAEs and 61 treatment related TEAEs. Higher rates of TEAEs and treatment related TEAEs were noted with elamipretide (100% for both) compared to placebo (83.3% and 66.7%, respectively). The majority of TEAEs were mild or moderate in severity. Injection site erythema and injection site pain were the most common treatment related TEAEs with a higher incidence in subjects treated with elamipretide (100.0% and 75.0%, respectively) compared to placebo (25.0% and 33.3%, respectively). With the exception of elevated eosinophil levels, laboratory values, vital signs, and ECG measured parameters were stable overall throughout Part 1 of the trial. Eosinophil levels shifted from normal to high values over time in the elamipretide treated group, returning to normal shortly after the end of the treatment, while they remained normal and stable in the placebo treated group.						
Ophthalmologic Diseases						
SPIAM-101 (ReCLAIM)	1	Completed	US	Safety and Tolerability	Dry AMD subjects ≥ 55 years of age with either high risk drusen without geographic atrophy (GA) or with noncentral GA	40
Trial Design: Open label, single site in approximately 40 subjects. Dosing Regimen: Single daily 40 mL SC injections of elamipretide for 168 days (24 weeks).						
Summary of Key Results: Efficacy: In this study, subcutaneous elamipretide was shown to be a feasible form of administration, with a mean 2.4 home visits needed for adequate training of this elderly population of subjects with intermediate AMD. In the HRD						

Trial Identifier	Trial Phase	Status	Country /Region	Trial Objectives	Subject Population	Number of Subjects Enrolled
cohort, elamipretide produced significant improvements in several functional outcomes, including BCVA, reading acuity, and quality-of-life measures. In each of these outcomes, improvements appeared to be more pronounced when testing was completed under LL conditions. In the NCGA cohort, elamipretide produced significant improvements in BCVA and reading acuity measures from baseline. In both of these outcomes, improvements appeared to be more pronounced when testing was completed under LL conditions. Quality-of-life measures showed significant improvements from baseline in the NCGA cohort. Examination of anatomic changes over time with elamipretide treatment in both cohorts, as measured by microperimetry and RPEDC thickness and volume, showed little difference from baseline at the Week 24 time point. Similarly, dark adaptation time did not vary significantly from baseline at Week 24. The lack of anatomic changes over time may be positive outcomes, given the progressive nature of this disease. For instance, the similarity of microperimetry results at baseline and Week 24 suggest that macular sensitivity was stable during the study period, which is inconsistent with the natural progression of the disease. However, this study was not designed to answer the question of whether it altered the natural state of this disease. Taken together, these data show that elamipretide improves functional outcomes, including improvements in vision, reading acuity, and quality of life, in both eyes with high-risk drusen and eyes with advanced geographic atrophy. Benefit appears more pronounced at LL conditions. Safety: The safety profile of elamipretide in this trial was favorable with no ocular or nonocular safety concerns, including no deaths, no treatment-related SAEs, and no severe TEAEs. In addition, neither vital signs, ECG results, nor IOP appeared to be impacted by the use of elamipretide in either cohort.						
SPIAM-202 (ReCLAIM-2)	2	Completed, CSR Pending	US	Safety, Tolerability, and Efficacy	Dry AMD subjects ≥ 55 years of age with non-central GA	Target Enrollment: 180
Trial Design: Randomized, placebo-controlled, double-masked, multi-center trial. Dosing Regimen: Single daily 40 mg SC injections of elamipretide/placebo for up to 48 weeks						
Summary of Key Results: Data analysis ongoing						