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Elamipretide HCl, Injection

Application Number: NDA 215244

Treatment of Patients with Barth Syndrome

Cardiovascular and Renal Drugs Advisory Committee Meeting

Briefing Document

Meeting Date: 10 October 2024

Document version: 10 September 2024

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Table 1 Definitions

ATP	Adenosine triphosphate, the cellular unit of energy
BL	Baseline
BSA	Body surface area
BSF	Barth Syndrome Foundation, the global patient advocacy group representing individuals and families living with Barth syndrome
BTHS-SA	Barth syndrome symptom assessment
CARDIOMAN	A Phase 2 clinical trial of bezafibrate for the treatment of Barth syndrome conducted at University Hospital, Bristol, UK in which 11 subjects were enrolled (Dabner 2021). The trial failed to meet its primary or secondary endpoints.
CGI-C	Clinician Global Impression of Change
CGI-S	Clinician Global Impression of Symptoms
CHF	Chronic heart failure
CL	Cardiolipin, a mitochondrial phospholipid
COPD	Chronic obstructive pulmonary disease
Cristae	Curved sections of the IMM, housing the electron transport chain
DCMA	Dilated cardiomyopathy with ataxia syndrome, an ultra-rare disease of cardiolipin deficiency, with overlapping pathognomonic and phenotypic characteristics to Barth syndrome.
DMD	Duchenne muscular dystrophy
ECMO	Extra-corporeal membrane oxygenation
Elamipretide	A mitochondrial protective agent that interacts with CL in the inner mitochondrial membrane (H-D-Arg-Tyr(2,6-diMe)-Lys-Phe-NH2; D-Arginyl-2,6-dimethyl-L-tyrosyl-L-lysyl-L-phenylalaninamide). Also referred to as SS-31 and MTP-131.
5XSST	5-times sit-to-stand test
HHD	Hand-held dynamometry
HFpEF	Heart failure with preserved ejection fraction
Johns Hopkins	Johns Hopkins University, including the Barth Syndrome Multidisciplinary Clinic at Kennedy Krieger Institute
ID	Interventional data
IMM	Inner mitochondrial membrane
IND	Investigational new drug application
LV	Left ventricular
LV-EDVI	LV end-diastolic volume, indexed to BL BSA
LV-ESVI	LV end-systolic volume, indexed to BL BSA
LV-SVI	LV stroke volume, indexed to BL BSA
MCID	Minimal clinically important difference
MDRI	A multi-domain responder index utilized as an endpoint in NH CONTROL STUDY
MLCL	Monolysocardiolipin, an immature form of CL that is elevated in Barth syndrome
MLCL:CL ratio	The ratio of abnormal MLCL to normal CL that is diagnostic for Barth syndrome and has been associated with phenotypic severity
Muscle strength by HHD	Muscle strength measured by HHD

NH	Natural history
NH Control Study	A Phase 3 retrospective NH control clinical trial (also known as SPIBA-001)
NHD	Natural history data
OLE	Open-label extension
PASE	Professional affairs and stakeholder engagement
PCPC protocol	Patient and caregiver perception of change protocol
PFDD	Patient focused drug development
PGI-S	Patient Global Impression of Symptoms
PMM	Primary mitochondrial myopathy
PROMIS Fatigue	Patient reported outcome measurement information system (PROMIS) Fatigue Short Form
ROS	Reactive oxygen species
r	Spearman's correlation coefficient
Senger's syndrome	An ultra-rare disease of cardiolipin deficiency, with overlapping pathognomonic and phenotypic characteristics to Barth syndrome
Stealth	Stealth BioTherapeutics (the sponsor and developer of elamipretide)
6MWT	Six-minute Walk test
SWAY Balance	SWAY Balance Application, a mobile balance app created by Sway Medical as a postural balance and reaction time test used by clinicians to identify balance deficits.
Tafazzin	An acyltransferase required for assembly of mature CL
TAZ	*TAZ* (also called tafazzin) is an X-linked nuclear gene (G4.5) that encodes tafazzin. A pathogenic mutation of *TAZ* causes Barth syndrome.
TAZKD	The tafazzin knockdown (TAZKD) mouse model, a tetracycline inducible shRNA-mediated knock-in mutation mouse model of Barth syndrome.
TAZPOWER Study	A Phase 2 placebo-controlled crossover clinical trial (also known as SPIBA-201 Part 1)
TAZPOWER Extension Study	A Phase 2 OLE clinical trial prospectively designed to assess long-term outcomes relative to baseline controls (also known as SPIBA-201 Part 2)
2D echo	Two-dimensional echocardiogram
3D echo	Three-dimensional echocardiogram
US	United States of America
VAD	Ventricular assist device (also known as Berlin heart)
VOP	Voice of the Patient Report
X-linked	Appearing on the X-chromosome
Z-score	Z-scores play a crucial role in pediatric practice, where due to somatic growth a single reference range cannot be applied across patients of vastly different sizes and ages. Z-scores express how many SD above (positive values) or below (negative values) a given measurement lies with respect to the size-specific mean.

Executive Summary

The **KEY TAKE AWAYS** we hope to convey are that:

(i) **elamipretide has demonstrated substantial evidence of effectiveness** for the treatment of Barth syndrome with **consistent and durable findings observed on all parameters of disease** (function and feel);

(ii) **elamipretide has a benign safety profile** characterized across all indications studied with over 400 patient safety years of exposure; and

(iii) the **potential benefits of elamipretide and the urgency and severity of the unmet medical need** suggest that any residual uncertainty regarding benefit is best addressed post-approval.

This briefing document has been prepared for the Cardiovascular and Renal Drugs Advisory Committee's review of elamipretide for the treatment of Barth syndrome. It presents results from the elamipretide Barth syndrome development program, along with discussion and analyses of the findings from all studies that support the proposed indication for elamipretide for the treatment of Barth syndrome.

Elamipretide (also known as SS-31) is a small mitochondrial-targeted tetrapeptide that interacts with cardiolipin (CL), a mitochondrial phospholipid essential for human life. This interaction improves lipid packing in the inner mitochondrial membrane (IMM), thereby normalizing mitochondrial structure and function in a variety of disease states. The drug has been studied in various diseases of mitochondrial dysfunction. Its safety has been characterized through >400 patient safety years of exposure and demonstrates a benign safety profile. The current NDA relates to the evaluation of the safety and effectiveness of elamipretide in treating individuals living with Barth syndrome.

Barth syndrome is a serious ultra-rare progressive X-linked genetic mitochondrial disease that affects an estimated 130 individuals in the U.S. The disease typically presents in infancy or early childhood, has a devastating impact on day-to-day functioning of affected individuals, and is associated with a severely reduced life expectancy marked by frequent premature deaths, 85% of which occur by age 5. Most who survive early childhood do not survive their fourth decade and live lives limited by debilitating progressive muscle myopathy and associated fatigue. As contextualized by patients and their caregivers, the muscle myopathy is so severe that it affects daily living. One affected "13-year-old must sit in the shopping cart as he doesn't have the stamina to make it throughout the store" and some men report not having the strength to use a bathroom without help (VOP 2019). Similarly, the fatigue is so debilitating that "As I get older and older, I feel like my fatigue is getting worse and worse; and I wonder, 'Where does it [end]?

Does it cap at where I just can't move ever?'" (VOP 2019). The progressive myopathy severely constrains normal activities including playing with friends, going to school, working a job and basic self-care. There are no existing treatments approved or in clinical development for Barth syndrome, highlighting the significant medical need for new agents to manage this condition.

The genetic defect in Barth syndrome results in a severe deficiency in CL. Affected individuals have only 5 to 30% of normal levels of mature CL, and instead have elevated levels of an immature form of cardiolipin called monolysocardiolipin (MLCL). In 2014, patient advocates from the Barth Syndrome Foundation (BSF) asked Stealth BioTherapeutics (Stealth, we, or us) to commence the first ever clinical development efforts in this disease due to elamipretide's demonstrated cardiolipin-protective effects. The mechanistic rationale for elamipretide, a cardiolipin-targeted mitochondrial therapeutic, for the treatment of Barth syndrome, a mitochondrial disease of cardiolipin deficiency, was clear: elamipretide was considered to have the potential to compensate for the cardiolipin deficit central to Barth syndrome disease pathology.

Development of novel drugs for ultra-rare patient populations presents inherent challenges, because "certain aspects of drug development that are feasible for common diseases"—including identifying sufficient patients for well-powered, randomized, placebo-controlled trials— "may not be feasible for rare diseases …[such] that development challenges are often greater with increasing rarity of the disease."[1] Indeed, identifying and recruiting enough patients to meet conventional powering standards and regulatory expectations presented the most significant challenge to the development of elamipretide for Barth syndrome.

Stealth, together with the FDA, experts in the field and patient support groups, explored multiple avenues to generate sufficient evidence to meet the regulatory standard of substantial evidence of effectiveness for approval of this much needed therapy. These efforts necessarily included the conduct of relatively small clinical trials with innovative trial designs and choices of controls. They also required learnings and adjustments along the way, particularly since TAZPOWER (SPIBA-201 Part 1), which was the first clinical trial ever conducted in Barth syndrome, did not meet its primary objectives. In hindsight, we surmised that this was attributable to too short a duration of treatment (3-months). Nonetheless, we continued to collaborate with the FDA and experts in conducting the TAZPOWER Extension (SPIBA-201 Part 2) and NH Control studies to generate substantial evidence of effectiveness in a scientifically rigorous manner. Notably, our

[1] FDA Draft Guidance for Industry, Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products, Dec 2019.

studies collectively evaluated a significant proportion of the total US Barth syndrome patient population and together represent the largest clinical studies ever conducted in Barth syndrome. These studies collectively involved almost 25% of the entire US patient population (n=31 out of ~130 affected individuals) and over 45% of eligible patients in the US (i.e., transplant-free individuals in the target age-range, assessing n=31 out of n=66 nationwide). [2]

Elamipretide has been shown to demonstrate benefits in Barth syndrome, with statistically significant and clinically meaningful improvements observed on both clinical and pharmacodynamic endpoints relative to both a historical control, in the NH Control study (Section 2.5), and a baseline control, in the TAZPOWER Extension study (Section 2.4.3). Changes observed in these studies are consistently positive, durable over time, and completely unexpected relative to the progressive decline seen in the well-established natural history of the disease.

Furthermore, these benefits can be achieved at minimal to no risk. The safety of elamipretide (Section 3) has been evaluated in over 1,500 subjects (including healthy volunteers and subjects with other diseases of mitochondrial dysfunction) with over 400 patient-years of exposure and a duration of exposure of up to 3.5 years in clinical trials and up to 7 years for patients with Barth syndrome who continued therapy in our expanded access program (EAP). Elamipretide has demonstrated a benign safety profile with only mild to moderate injection site reactions (which can be treated with topical steroids) as the most common adverse event, and no observations of off-target effects or end-organ toxicity. These data support the conclusion that the potential risks of elamipretide are modest. This supports a favorable benefit-risk determination even if any potential uncertainty about the level of evidence supporting benefit remains.

There is an urgent need for a therapy that can slow the progression of Barth syndrome given the irreversible and universally progressive nature of this disease that both shortens and severely limits the lives of affected children and young adults. The Barth syndrome community has acknowledged that it is willing to tolerate any remaining uncertainty as to the benefit of elamipretide, noting the absence of alternative therapies for treating this debilitating disease. Thirty-six doctors and medical professionals, including Dr. Peter Barth, for whom the disease is

[2] BSF advised Stealth that as of September 30, 2020 (the year the NH Control study was conducted), there were 79 affected US individuals ≥12-years-old, of whom approximately 16% had heart transplants, leaving 66 potentially evaluable subjects. 51 of the 66 potentially evaluable subjects were assessed in the elamipretide development program: (i) 12 were enrolled in the TAZPOWER study, (ii) 19 were prognostically matched as controls in the NH Control study, and (iii) demographic and functional performance data were assessed for 20 who were ineligible for the NH Control study due to lack of longitudinal data (Figure 3; Table 9).

eponymous, and Dr. Colin Steward, who founded and ran the only other Barth syndrome multi-disciplinary clinic worldwide other than Johns Hopkins, advocated through interactions with the FDA between 2020 and 2022 to expedite review of the elamipretide data for purposes of ensuring access to therapy for patients living with Barth syndrome.[3]

One pathway for accelerating access is an expanded access program (EAP), an FDA-recognized pathway for patients with serious or immediately life-threatening diseases to gain access to investigational medical products outside of clinical trials when no comparable or satisfactory alternative therapy options are available. Patients with Barth syndrome can receive access to elamipretide under Stealth's EAP via (i) an Intermediate Expanded Access Protocol approved by the FDA in 2021 to support continued provision of therapy to the TAZPOWER Extension study participants and other individuals ≥12-years-old with Barth syndrome, (ii) Individual Patient Expanded Access IND applications, which are available for licensed physicians to use for expanded access requests for individual patients, and (iii) Emergency Use IND applications, which are requests to use an unapproved experimental drug in an emergency situation where there isn't enough time to submit an IND application. The process is not easy; it requires families and healthcare providers to generate and maintain significant record-keeping and careful oversight. Despite these challenges, Stealth has received requests for expanded access for many affected individuals (44 patients) living in 10 countries worldwide. 22 affected patients worldwide have received access to elamipretide through this EAP.

The volume of these EAP requests has increased exponentially since 2023. Particularly noteworthy are the number of requests for infants in acute cardiac distress, since we had previously understood that only a few children a year are born and diagnosed with Barth

[3] The following experts in the care and treatment of Barth syndrome and other cardiac diseases wrote to the FDA between 2020 and 2022 to express this opinion: Drs. William Abraham (Ohio State), Peter G. Barth (Univ of Amsterdam), Mary Ann Bonilla (St Joseph Medical Center), Michael Bristow (Univ of CO), Todd Cade (Duke), Kathryn Chatfield (Univ of CO), Maryanne Chrisant (Joe DiMaggio Children's), Bruce Cohen (Akron Children's), Gerald Cox (Boston Children's), Linda Cripe (Nationwide Children's), Gregory Enns (Stanford), Brian Feingold (Univ of Pitt), Jaya Ganesh (Icahn/Mt Sinai), Michael Gewitz (Maria Fareri Children's), Amy Goldstein (CHOP), Barry Greenberg (UCSD), Grant Hatch (Univ of Manitoba), Michio Hirano (Columbia), Kan Hor (Nationwide Children's), John Jefferies (Univ of TN), Amel Karaa (MGH), Mary Kay Koenig (McGovern Med School), Christoph Maack (Wurzburg), Kim McBride (Nationwide Children's), Colin Phoon (Hassenfield/NYU Langone), William Pu (Boston Children's), Tony Sabbah (Henry Ford), Michael Schlame (NYU Langone), Brian Stauffer (Univ of CO), Colin Steward (Bristol Univ/NHS Barth Syndrome Service), Arnold Strauss (Univ of Cincinnati), Carolyn Taylor (MUSC), Reid Thompson (Johns Hopkins), Jim Udelson (Tufts), Hilary Vernon (Johns Hopkins) and Jerry Vockley (Univ of Pitt). None of these individuals were compensated by Stealth for their endorsement.

syndrome in the United States. The FDA has approved every Individual Patient or Emergency IND request submitted, including 10 cases involving children <2-years-old, one of which required delivery of elamipretide to (b) (6) before the child was even born. That child received elamipretide starting at 6-days-old in the newborn intensive care unit and continuing through eventual discharge (>6-months exposure to date), and his doctor recently applauded the FDA for its swift action in approving the Emergency IND.[4] To approve these requests, FDA must determine that the potential patient benefit justifies the potential risks of treatment, at least in this controlled setting. Overall, our EAP experience suggests that availability of elamipretide may improve diagnosis and care of affected individuals.[5] We surmise that increased awareness is improving diagnosis, resulting ultimately in better patient care.

The totality of evidence presented in the NDA provides substantial evidence of the safety and effectiveness of elamipretide for the treatment of Barth syndrome. As clearly demonstrated in the forest plots below, on every parameter assessed, over a four-year period, compared to both baseline (Figure 1) and natural history (Figure 2) controls, benefits favoring treatment over control were observed. Importantly, beneficial changes were durable – which is completely contrary to the well-established uniformly progressive natural history of the disease. Based on

[4] In an (b) (6) letter to Drs. Califf and Thompson, (b) (6) said: "We credit the addition of elamipretide to the standard of care and non-disease specific treatments as potentially averting crisis and contributing to a positive transition to outpatient status for a seriously ill patient. I sincerely hope that elamipretide can be made available as a prescribable resource to treat my future patients with Barth syndrome. Most importantly, I hope that there will be continued access for this young boy whom I believe that it has benefited."

[5] As another example, in a (b) (6) letter to Drs. Califf and Cavazzoni, (b) (6) , reported on the utility of elamipretide for a newborn diagnosed with Barth syndrome. (b) (6) said: "Understanding that elamipretide is not yet approved for the treatment of Barth syndrome, we are grateful that you provided the opportunity for us to access this investigational therapy. We cannot solely attribute [our patient's] recovery to elamipretide given he was receiving other heart failure therapies at the same time, but his transition from potentially needing a heart transplant to having normal heart function is remarkable. Based on our experience with this one patient, we believe elamipretide can be an important addition to the resources available to treat this devastating disease". Additionally, on (b) (6) (b) (6) wrote to Dr. Cavazzoni to express her opinion that "elamipretide was instrumental in successfully bridging [a] very ill infant to a successful transplant, and he remains on therapy post-transplant." (b) (6) conveyed that the (b) (6) care team was concerned that the 2-week-old boy "would not survive the wait for transplant with his very poor cardiac function" when they sought emergency access to elamipretide in (b) (6) . In their expert opinion, elamipretide "was a critical tool in our arsenal to stabilize and support this child through transplant, and we view the potential benefit afforded by elamipretide relative to its known risks as clearly supporting a decision to make this drug immediate available to patients and prescribing physicians battling with this devastating pediatric disease."

these findings, "it could fairly and responsibly be concluded by [qualified] experts[6] that [elamipretide] will have the effect it purports or is represented to have" as a treatment for Barth syndrome.[7] Mechanistically, the findings make sense: (i) elamipretide has been shown across multiple disease models to compensate for the CL deficit that is lethal in the setting of Barth syndrome, and (ii) clinically, elamipretide improved abnormal cardiolipin ratios, which are known to be associated with phenotypic severity, for all the TAZPOWER Extension study patients (**Section 2.4.3.1**). Patients contemporaneously contextualized the meaningfulness of the findings in terms of how they feel and function in their daily lives under a protocol designed to capture patient perspectives regarding trial participation (the PCPC protocol) (**Section 2.4.3.3.3**). No material safety concerns are associated with elamipretide (**Section 3**). Taken together, there is a strong basis to conclude that elamipretide is effective in treating Barth syndrome and that the benefits of treatment outweigh any potential risks. Furthermore, in the context of this ultra-rare and serious condition, the totality of the evidence should be considered and deemed to meet FDA's substantial evidence of effectiveness standard.

[6] Supra notes **3**, **4** and **5.**

[7] FDA Draft Guidance for Industry, Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products, Dec 2019.

Figure 1 Forest Plot of TAZPOWER Extension Study Data at Final Study Visit (Week 168) Comparing Elamipretide to Baseline Controls

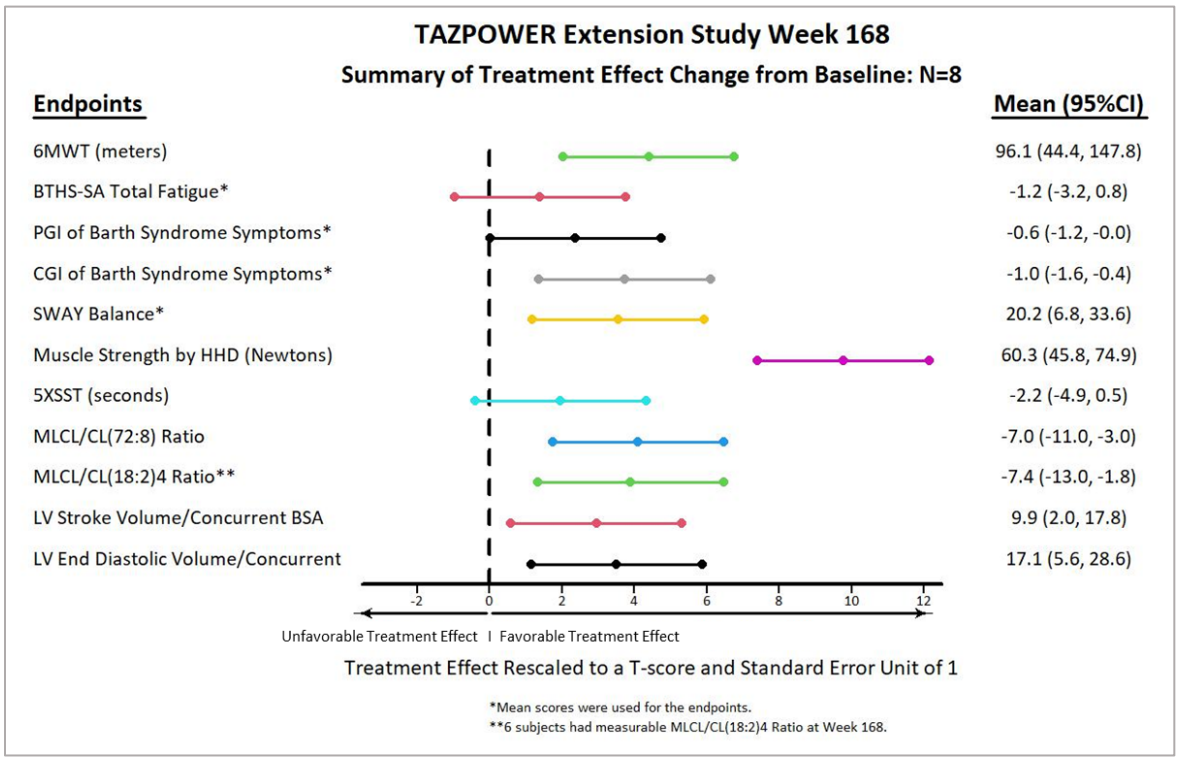
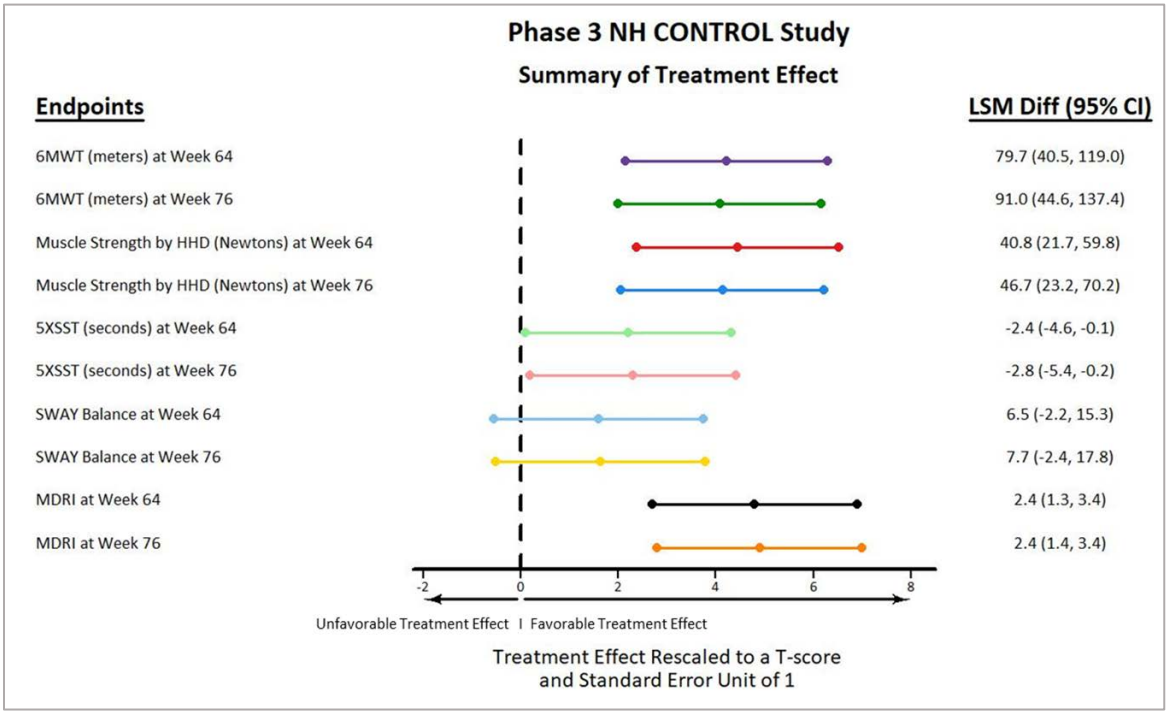


Figure 2 Forest Plot of NH Control Study Data Comparing Elamipretide to NH Controls



Our commitment to the Barth syndrome patient community will not stop here. We are acutely aware that additional data is desirable. In a disease this rare, where it is impossible to conduct large placebo-controlled outcome trials, a registry approach enrolling both treated and untreated patients is the only feasible way to understand the full impact of elamipretide on the long-term trajectory of Barth syndrome. We have committed to establish a registry post-approval to better understand the potential of elamipretide on outcomes and help inform the development of new treatment options for this devastating disease (**Section 4.1**).

1 Introduction

Barth syndrome (3-methylglutaconic aciduria type II, MIM 300394) is an ultra-rare,[8] serious, progressive, and ultimately fatal X-linked mitochondrial disease. The disease is caused by defects in the *TAZ* (G4.5) gene resulting in a severe (up to 95%) deficit in the mitochondrial phospholipid, cardiolipin (CL), leading to mitochondrial dysfunction and signs and symptoms of disease. For a video on the mechanism of disease, click or type www.stealthbt.com/mod. Prevalence is estimated at one case per million male population (Miller 2019), with approximately 130 affected individuals living in the United States and 250 affected individuals living worldwide (Voice of the Patient Report). The disease is diagnosed by the ratio of abnormal, structurally immature MLCL to mature CL (MLCL:CL ratio) or, increasingly, genetic testing.

1.1 Unmet Medical Need

Barth syndrome is often embryonically lethal (Steward 2010) and otherwise typically presents in infancy or early childhood. Eighty-five percent of deaths due to the disease occur by age five, typically due to cardiac dysfunction. For those who survive early childhood, the disease results in a dramatically shortened lifespan that is severely compromised by extreme exercise intolerance, muscle weakness, debilitating fatigue, neutropenia, and cardiac dysfunction. All affected individuals have lifelong challenges that affect them from infancy through adulthood (for those who survive into adulthood), for example (VOP 2019):

- “Nursing was exhausting for him. He tired very quickly and would often work up a sweat;”
- A pre-teen: “is not strong enough to open up a bag of chips [or] pop off the top of a ketchup bottle;” and
- a 36-year-old says: “As I have gotten older, my fatigue continues to get worse. Some days, I'm too tired to leave home. I need to plan ahead and pace myself for how many things I can do or I can schedule in one day or even in the same week. After activities, I feel worse and must rest, sometimes for the rest of the day. Sometimes, I feel so exhausted that I am sick.”

[8] Europe defines ultra-rare conditions as those affecting one patient per 50,000 people; there is no consensus definition in the US..

Frequent hospitalizations and medical interventions including for eating and digestive issues, complications of muscle weakness such as scoliosis, neutropenia and other symptoms also impact quality of life. For example, the son of BSF's cofounder "was hospitalized eight times for a total of 114 days when he was 21, and over the span of his short 28-year life endured 47 overnight hospitalizations for a total of 564 days" (VOP 2019).

No therapy has been approved for Barth syndrome and currently no other therapy is known to be in clinical development, making the need for the timely approval of this drug all that more pressing.

1.2 Proposed Indication

The proposed indication is: *Elamipretide HCl is indicated for the treatment of patients with Barth syndrome.*

1.3 Brief Overview of Development Program

A serendipitous discovery. Elamipretide is part of a class of compounds serendipitously discovered by researchers at Weill Cornell in 2005 (Szeto 2014). Researchers targeting receptors in the brain mistakenly dosed a rodent in the hind limb instead of intrathecally. When activity was unexpectedly observed, the compound was radiolabeled and found to target mitochondria, a hitherto undruggable target. Elamipretide has since been shown to bind reversibly with both CL and MLCL in the inner mitochondrial membrane to improve lipid packing, thereby compensating for and protecting against CL deficiency and ROS-mediated CL degradation in diseases of mitochondrial dysfunction. An extensive body of published literature shows that elamipretide restores normal mitochondrial bioenergetic functioning and improves organ function, particularly in high energy-demanding organs including the heart and the muscle system.

Patient-centered drug development. In 2014, representatives of BSF and the Kennedy Krieger Institute at Johns Hopkins (Johns Hopkins), which is one of only two multi-disciplinary clinics treating Barth syndrome worldwide, approached Stealth to propose development of elamipretide for Barth syndrome. Elamipretide was already in late-stage development for other rare mitochondrial diseases. Stealth spent several years conducting nonclinical studies, characterizing the natural history of Barth syndrome, developing a Barth syndrome specific patient-reported outcome assessment (the BTHS-SA) (Contesse_2019) and engaging with patients and experts in the field to inform a logical development plan for this ultra-rare condition, before initiating clinical development in 2017. Key aspects of the development plan, including endpoint selection, were informed by extensive discussions and alignment with the FDA on a related Phase 3

development program in primary mitochondrial myopathy (PMM) which was overseen under the same investigational new drug application (IND).

TAZPOWER, the first ever clinical trial in Barth syndrome. In 2017, Stealth initiated the TAZPOWER study, the first clinical trial ever conducted in Barth syndrome. As the first trial ever conducted in the disease, the duration of treatment and study endpoints were also being assessed for the first time. The trial was a double-blind, placebo-controlled crossover trial, in which all patients received elamipretide or placebo once daily SC for twelve weeks before crossing over to receive the opposite treatment after a four-week wash-out period during which no treatment was administered. This ambitious trial failed to meet its defined primary endpoints, which were a difference between the elamipretide and placebo groups in distance walked on the 6MWT and a patient-reported assessment of fatigue at 12-weeks; however, small improvements in indexed left ventricular end diastolic volume (LV EDV) were observed in ten of the twelve subjects following elamipretide administration, prompting further assessment of the cardiac natural history and supporting longer-term follow-up. Stealth and its expert consultants conjectured that the three-month treatment duration in this trial may not have been long enough to realize a full treatment benefit.

Rationale for continuation of development. All TAZPOWER subjects were eligible to continue into the TAZPOWER Extension study, an up to 192-week open-label extension (OLE) trial prospectively designed to evaluate long-term trends in efficacy relative to the patients' pre-randomization baseline ("baseline controls"). At the time of the TAZPOWER read-out, 10 subjects had already enrolled in the TAZPOWER Extension Study, and evidence of efficacy with longer-term therapy was observed at early timepoints. The principal investigator also reported meaningful observable changes. This aligned with patients' and caregivers' positive perspectives which were prospectively evaluated under a separate protocol conducted while patients remained blinded to treatment assignment (the "PCPC protocol"), in which improvement was reported for most (9 out of 10) participating subjects. Stealth accordingly elected to continue the TAZPOWER Extension study as planned and engaged with FDA to align on next steps for further development.

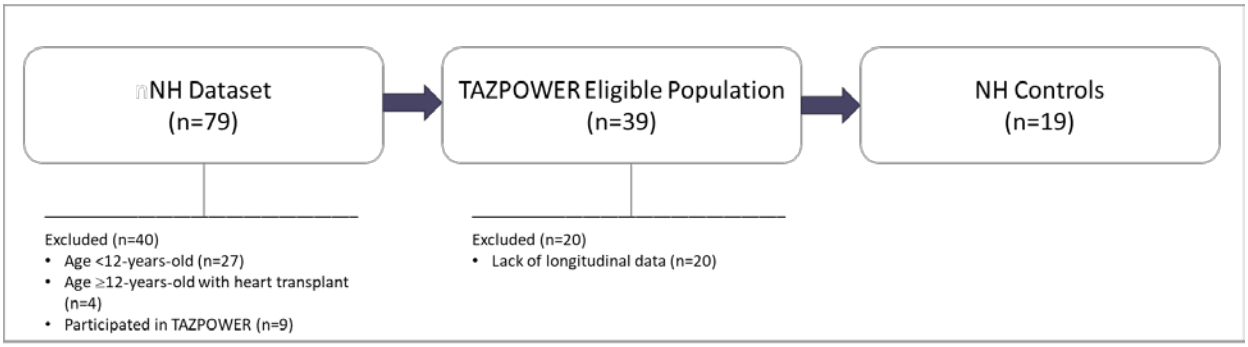
TAZPOWER Extension, a baseline-controlled open-label trial. During the TAZPOWER Extension study, in which ten subjects were evaluated for up to 24-weeks and eight subjects were evaluated for up to 168-weeks (two subjects withdrew by week 24 because of injection site reactions (ISRs)), large and durable improvements from TAZPOWER baseline were observed on functional assessments, patient and clinician reported symptom assessments, and pharmacodynamic endpoints (Thompson 2024).

NH Control pivotal study utilized an external historical control. Stealth conducted the NH Control study, a Phase 3 natural history control study, to establish an appropriate control for the TAZPOWER Extension study open-label data (Hornby 2022).

The NH Control trial design was inspired by FDA's 2017 approval of Brineura for CLN2 batten disease, a rare lysosomal storage disorder affecting about 14,000 people worldwide, based on a single-arm, open-label trial with a retrospective natural history control. Following the Brineura approval, FDA published guidance[9] which Stealth followed closely in designing the NH Control study.

The NH Control study utilized subjects from a 79-patient longitudinal natural history cohort compiled by the Johns Hopkins team and representing more than half of the US Barth syndrome patient population. The cohort included 9 TAZPOWER subjects and an additional 39 affected eligible individuals ≥12-years-old, 19 of whom had longitudinal data meeting the inclusion criteria for the NH Control study (**Figure 3)**. These 19 patients were prognostically matched as historical controls to TAZPOWER Extension subjects (n=8 long term Part 2 participants, with sensitivity analyses conducted on all 12 TAZPOWER subjects). Together, the 12 TAZPOWER subjects and 19 NH control subjects represent >45% of the eligible US patient population (see **note 2**) . Stealth also characterized demographic and functional assessment data for the 20 patients who did not have longitudinal data meeting study inclusion criteria (**Table 9**), thereby establishing comparability of key parameters of clinical impairment for >75% of the US eligible patient population..

Figure 3 NH Control Cohort Selection



The NH Control study met its primary (6MWT) and most secondary endpoints at the time periods of primary interest, corresponding to weeks 36 and 48 of the TAZPOWER Extension study (Section 2.4.3). In addition, based on FDA's recommendation, Stealth conducted a post-hoc analysis corresponding to week 72 of the TAZPOWER Extension study which demonstrated a benefit on indexed left ventricular stroke volume (LV-SVI) relative to (n=12) historical controls for whom longitudinal echo data was available.

Cardiac natural history and other confirmatory evidence. Infants and young children with Barth syndrome can present with dilated cardiomyopathy (Sabbah 2023). Stealth's work to elucidate the cardiac natural history for older patients (≥12-years-old) established that for those who survive early infancy, the bioenergetically starved heart may begin to compensate by developing a phenotype with a smaller left ventricle reminiscent of heart failure with preserved ejection fraction (HFpEF) or non-obstructive hypertrophic cardiomyopathy (Sabbah 2023). This hypertrophic-like phenotype, which was observed in all TAZPOWER study participants, is common in mitochondrial cardiomyopathies (Braunwald 2023; Arbustini_2018; Sabbah 2023). In older patients with this cardiac phenotype, LV-SVI is expected to decline with age (Sabbah 2023, Chowdhury 2022). Other confirmatory evidence includes mechanistic and nonclinical evidence, compassionate use case studies, including in several infants presenting in acute heart failure,[10] and the PCPC protocol.

1.4 Regulatory Interactions

Stealth and FDA interacted extensively on this development program, meeting more than twenty times between 2018 and 2023 to discuss approaches that could be considered to collect sufficient data given the ultra-rare nature of Barth syndrome (see Appendix A Table 1). Over this time, FDA sought guidance from four different Center for New Drug Evaluation (CDER) review divisions, beginning with the Division of Neurology Products (DNP), which recommended a transfer to the Division of Gastroenterology and Inborn Errors of Metabolism (DGIEP) in 2019 following DGIEP's approval of Brineura which relied on published natural history, and eventually to the Division of Cardiology and Nephrology (DCN) in 2021 to help interpret the

[9] FDA Draft Guidance to Industry, Rare Diseases: Natural History Studies for Drug Development, March 2019. Key aspects of this guidance relating to the use of historical controls for serious, ultra-rare diseases were reiterated in FDA Draft Guidance to Industry, Rare Diseases: Considerations for the Development of Drugs and Biological Products, December, 2023.

[10] Notably, Stealth has received 44 requests for expanded access from a dozen US hospitals and 10 foreign countries. FDA has approved every expanded access request submitted since mid-2022.

emerging cardiac signal. Ultimately, Stealth and the FDA discussed approximately a dozen proposed protocols for collecting additional data from TAZPOWER and de novo trial participants, on a pre- or post-approval basis, utilizing both clinical and biomarker endpoints. The primary and overarching regulatory hurdle despite the desire for more data was whether it would be ethical to conduct additional controlled trials that all understood would be underpowered (i.e., too few patients available to participate because of the rareness of the condition), and therefore insufficient to answer key questions reliably.[11]

FDA also met extensively with BSF. FDA attended a Patient Focused Drug Development (PFDD) meeting in 2018, a Professional Affairs and Stakeholder Engagement (PASE) meeting in 2019, a PASE meeting in 2021, and Patient Listening Sessions in 2022 and 2024. The purpose of these meetings was to gain better understanding of the serious nature of Barth syndrome, the severity of the unmet need, the urgent need for new treatments, the paucity of patients available to participate in clinical research despite the extensive work conducted by BSF to identify patients globally, and the patient community's tolerance of risk in the context of uncertainty of benefit.

1.5 Regulatory Basis for Approval

Substantial evidence of effectiveness for elamipretide for the treatment of Barth syndrome has been demonstrated relative to historical (NH Control study) and baseline (TAZPOWER Extension study) controls, both of which meet the regulatory definition of "a small externally controlled trial with an outcome markedly superior to the well-established natural history of a disease [that] may provide a compelling case for drug effectiveness."[12]

"FDA has determined that it is appropriate to exercise the broadest flexibility in applying the statutory standards, while preserving appropriate standards of safety and effectiveness, for products that are being developed to treat severely debilitating or life-threatening rare

[11] For example, FDA advised Dr. Rachel Sherman, former Principal Deputy Commissioner of FDA and an advisor to Stealth, that 21CFR 312.42(b)(2)(ii) describes grounds for a clinical hold when a phase 2 or 3 study protocol "is deficient in design to meet its stated objectives," noting that "If a study intended to confirm clinical benefit is underpowered to do so, then it is deficient in design." [Correspondence, November 27, 2023]

[12] Draft Guidance for Industry, Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products, December 2019; Draft Guidance for Industry, Rare Diseases: Natural History Studies for Drug Development, March 2019.

diseases."[13] This is particularly appropriate when it is not feasible or would not be considered ethical to use a concurrent placebo control, such as in the setting of Barth syndrome.[14] Here, the totality of the evidence approach is appropriate and is overwhelmingly supportive of a finding of effectiveness, despite the limitations of sample size. Moreover, although the trial sizes are small, the subjects studied collectively exceed 45% of the US evaluable patient population (see **note 2**).

The FDA asked Stealth to specify the regulatory basis for approval. Stealth and its advisors[15] contend that the totality of the evidence, including positive externally controlled data from the NH Control study, baseline-controlled data from the TAZPOWER Extension study and confirmatory evidence, taken together, support a traditional (full) approval. However, as there are multiple examples in which the Agency has chosen a pathway different from that proposed by the sponsor, Stealth notes that an approval under Subpart H based on LV-SVI, indexed LV end diastolic volume (LV-EDVI), or intermediate clinical endpoints could also be appropriate.

[13] Draft Guidance for Industry, Rare Diseases: Considerations for the Development of Drugs and Biological Products, December 2023.

[14] Supra at note 10.

[15] Memorandum re CRITICAL ANALYSIS OF SPIBA-001 from Dr. Jeffrey Brown to Sponsor, dated January 6, 2024 (Appendix B Figure 1); Memorandum, dated January 6, 2024, from Dr Rachel Sherman to Sponsor.

FDA acknowledges that uncertainty in the body of evidence available to inform regulatory decision-making is sometimes inevitable, such that a judgment must be made as to whether a drug's likely benefits outweigh the risks.[16] During the FDA Patient Listening Session held with the Barth Syndrome Foundation (BSF) on March 2, 2021, individuals living with Barth syndrome and their direct caregivers reported that the need for a treatment, even if it comes with greater uncertainty, is warranted. In the context of an overwhelmingly positive safety profile supported by studies across indications, the risk expectation associated with use of elamipretide is low. As such, a decision granting approval is supported by (i) the totality of the evidence demonstrating substantial evidence of effectiveness of elamipretide as a treatment for Barth syndrome, (ii) the patient community's tolerance for risk of uncertainty of benefit, (iii) the medical community's repeated request for[17] and FDA's endorsement of expanded access therapy, and (iv) the demonstrated safety profile of elamipretide, taken together, support a conclusion that the observed benefits outweigh the demonstrated risks despite any residual uncertainty due to the necessarily small dataset in this ultra-rare disease.

2 Efficacy

2.1 Barth Syndrome

2.1.1 Mechanism of Disease

Cardiolipin (CL) is an essential phospholipid within the inner mitochondrial membrane (IMM). This conically-shaped phospholipid is critical to establish both the curved architecture of the curves, or cristae, of the IMM (Figure 4) and the proper formation of the protein complexes that form the electron transport chain (Figure 5). The proper organization, or packing, of CL molecules enables the function of the electron transport chain, which generates cellular energy in the form of adenosine triphosphate (ATP) (Figure 5).

[16] Draft Guidance for Industry, Benefit-Risk Assessment for New Drug and Biological Products, Oct 2023.

[17] Supra notes **3**, 6 and **5**.

Figure 4 Curved Cristae Architecture of the IMM (Cardiolipin Depicted in Orange)

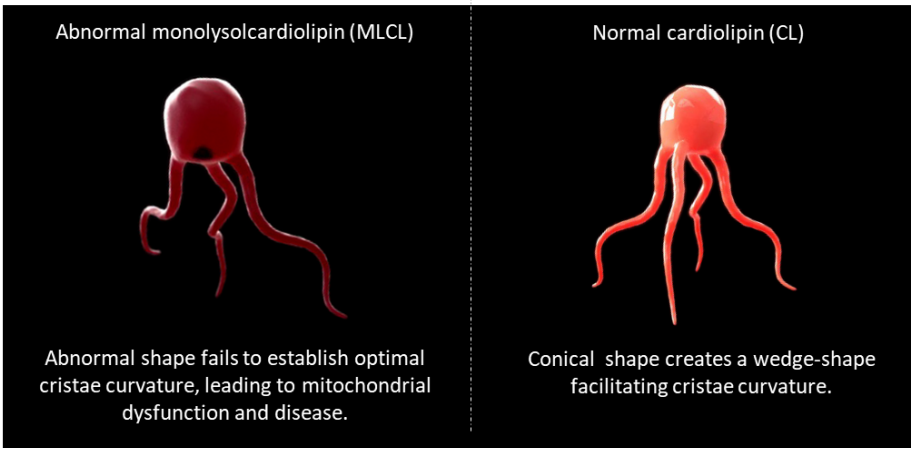
Figure 5 Mitochondrial Cristae House the Protein Complexes of the Electron Transport Chain, Optimizing Efficient Oxidative Phosphorylation (Cardiolipin Depicted in Orange)

Cardiolipin is made *de novo* through a series of reactions. When fully mature and functional, it contains four fatty acid (acyl) side chains. *TAZ* is a nuclear gene encoding for tafazzin, an acyltransferase that enables the final step in CL maturation. Mutations in *TAZ* lead to loss of mature CL in Barth syndrome. Loss of mature CL disrupts the form and function of the IMM, ultimately impairing cellular energy production.

A direct consequence of the *TAZ* genetic defect is a reduction of structurally mature CL (Figure 6 right) and an increase in structurally immature MLCL (Figure 6 left) (Vreken 2000) leading to mitochondrial dysfunction (Bertero 2020; Paradies 2014) and broad metabolic dysregulation

(Sandlers 2016). For a video tutorial on the mechanism of disease, click or type www.stealthbt.com/mod or scan the QR code below.

Figure 6 Abnormal Monolysocardiolipin (MLCL) Versus Normal Cardiolipin (CL)



These bioenergetic deficits contribute to key clinical manifestations of the disease, including progressive **skeletal myopathy** and **cardiomyopathy**. Skeletal muscle requires an approximate 100-fold increase in ATP production to meet the energy requirements of exercise. In Barth syndrome, mitochondrial respiration cannot increase to meet increased demand during exercise (Wang 2016; Gang 2016). The failing heart has been shown to attempt to compensate for this bioenergetic deficit, including by reducing reliance on dysfunctional mitochondrial fatty acid oxidation (Russo 2022) and relying more heavily on glycolysis, which is also impaired (Chowdhury 2023). In some animal models of Barth syndrome, this has been shown to result in a hypertrophic (Greenwell 2022) or HFpEF-like cardiac phenotype (Bertero 2020), although animal models are known to be heterogeneous for onset or existence of cardiac dysfunction (Pu_2021).

Diagnosis. The disease can be diagnosed by measuring the ratio of abnormal MLCL to normal CL (referred to as the MLCL:CL ratio) or by genetic testing for variants in the *TAZ* gene.

2.1.2 Clinical Burden of Disease

The clinical manifestations of Barth syndrome include skeletal muscle myopathy, fatigue, neutropenia, feeding problems, cardiomyopathy, pain, growth abnormalities, and a severely shortened life span, with 85% of deaths occurring by age 5. For individuals who survive early childhood, myopathy is progressive and can be debilitating and life expectancy remains shortened, with only some individuals surviving into their late forties (Pang 2022).

The devastating impact of the disease on affected families can, perhaps, be best contextualized by comments on the recent (December 2023) petition submitted by BSF to FDA; for example, one signatory explained that: "I have a 4-year-old son who has been through hell and back already at a young age. He is the 8th child in my family with this disorder and the ONLY one to survive. Give people like my son a fair chance to live as normal of a life as possible. To not be in so much pain, to gain weight, to be able to do things like basic hygiene care and not have to take a break or be in pain." (signatory from Racine, Wisconsin).[18] Other comments included on the petition are similarly illuminative of the tragic impact of this disease on affected individuals and families.

2.1.2.1 MLCL:CL Ratio

The cellular signature of the disease is the abnormally high MLCL and abnormally low CL caused by the TAZ deficiency. The MLCL:CL ratio has been associated with clinical severity, with lower (less abnormal) MLCL:CL ratios accompanying a less severe clinical phenotype (Bowron 2015). As discussed in **Section 2.4.3.1**, elamipretide-mediated improvements in the MLCL:CL ratio were observed starting at week 12 of TAZPOWER Extension (after 24-weeks exposure to elamipretide). To contextualize these changes, FDA asked Stealth to characterize the anticipated change over time in the MLCL:CL ratio (Type A meeting, June 2023). The resulting analysis demonstrated that the MLCL:CL ratio does not decrease (improve) over time absent therapeutic intervention (Table 2).

[18] Text of petition is accessible at https://www.regulations.gov/document/FDA-2023-P-5634-0001 or www.nottooraretocare.org at Updates & Resources tab.

Table 2 Ratio of Abnormal to Normal Cardiolipin Not Expected to Change Over Time

	JHU Natural History Cohort (N=15)*			TAZPOWER Extension Study (ITT Population, N=10)		
	Age at BL	**BL MLCL:CL**	**% Δ**	**Age at BL**	**BL MLCL:CL**	**% Δ**
Mean	11.3	7.5	0%	19	8.13	-82%
SD	10.55	4.61	52%	7.20	5.20	11%
Median	7.0	6.0	-14%	16.5	8.4	-83%
Min, Max	2.0, 34.0	3.0, 19.0	-84%, 100%	12, 35	2.6, 14.0	-94%, -58%

* Change assessed over average 5.5 years

Stealth worked with collaborators at Johns Hopkins to analyze MLCL:CL samples from 55 affected individuals, 15 of whom had multiple (longitudinal) samples. In the JHU NH Study (n=15), over an average evaluation interval of 5.5 years (range 1–8.5 y), the mean (SD) baseline MLCL:CL ratio was 7.5 (4.61) (range 3–19) and the mean % change (SD) in the MLCL:CL ratio was 0% (52%) (range −84%-100%). As noted above, this analysis demonstrates that the MLCL:CL ratio is not expected to improve over time in the natural course of the disease. In fact, after adjusting for time, the longitudinal natural history cohort demonstrated a mean 6% increase (worsening) of these ratios, which stands in contrast to the improvements observed in these ratios with elamipretide therapy (Section 2.4.3.1).

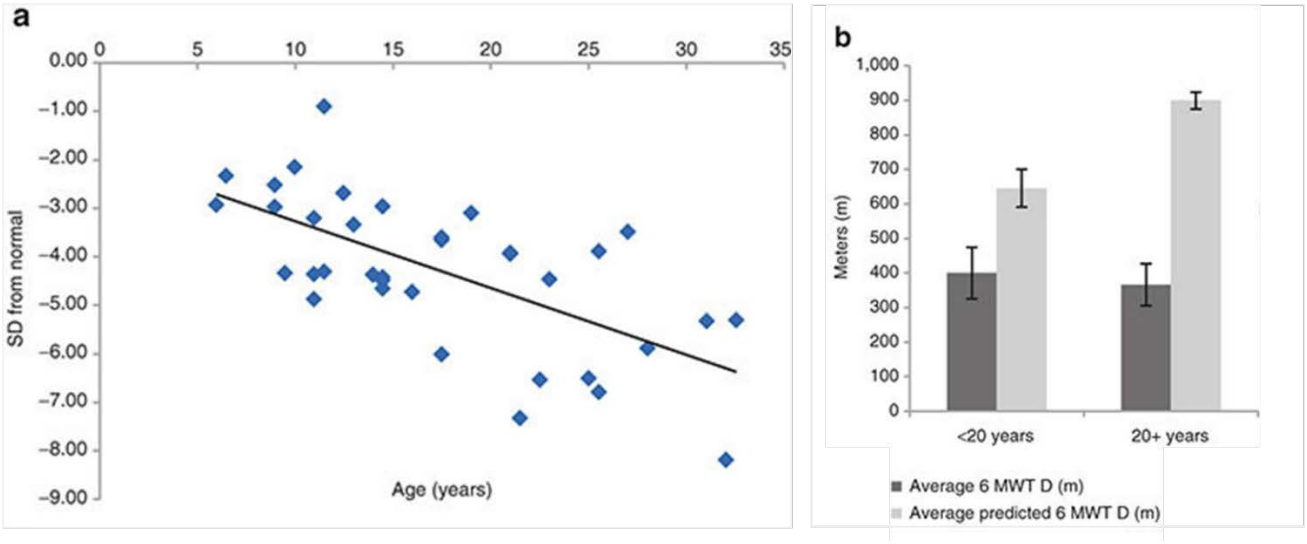
2.1.2.2 Myopathic Burden of Disease

Individuals with Barth syndrome have only 35 to 50% the exercise tolerance of their peers (Spencer 2011), and muscle strength is also significantly lower (Bittel 2018; Bohnert 2021). Exercise tolerance does not improve with age; rather, myopathic symptoms are well-documented and progressive as patients age during their limited lifespan and cannot be overcome by increases in desire or effort (Thompson 2016; Bittel 2018; VOP 2019). This exercise intolerance has been associated with both cardiac and skeletal muscle bioenergetic impairments (Spencer 2011; Bashir 2017; Bertero 2021).

The 6MWT, a recognized objective evaluation of functional exercise capacity, assesses the global and integrated responses of multiple systems involved during exercise including the pulmonary, cardiovascular, circulatory, neuromuscular, and metabolic systems. In Barth syndrome, the 6MWT has been studied longitudinally to assess the impact of the disease on exercise capacity and has been shown to correlate with measures of quality of life including daily

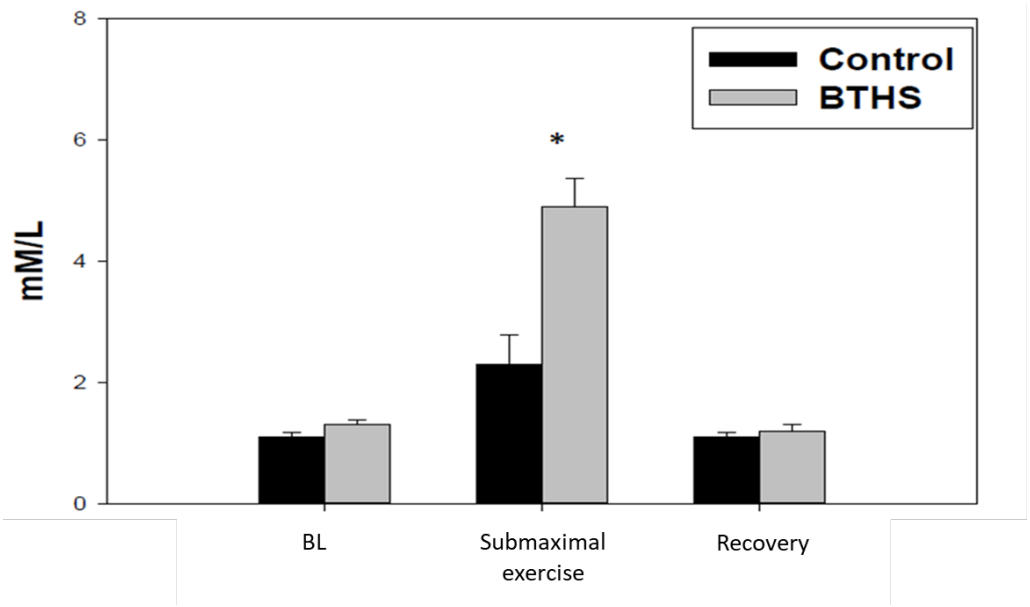
activity, and reported dyspnea and fatigue (Thompson 2024; Hornby 2019). Compared to height-matched controls, patients with Barth syndrome (n=34) have significantly reduced 6MWT distances, with a mean of 387 meters (mean z-score of −4.39 [−0.9 to −8.18]) (Figure 7) (Thompson 2016). Older individuals (20-32 years) tend to have the worst performance, walking only 41% of the predicted normal value as compared to younger individuals (4–19 years) who walk 62% of the predicted normal value (Figure 7) (Thompson 2016). Muscle weakness has similarly been found to become more pronounced with increased age (Thompson 2016). Taken together, these findings are consistent with reports that myopathy is progressive and increasingly debilitating in early to mid-adulthood ((VOP 2019).

Figure 7 Barth Syndrome (a) 6MWT Standard Deviation from Normal Controls and (b) Average 6MWT Distance versus Predicted Distance



As in other mitochondrial myopathies, deficiencies in mitochondrial respiration lead to increased reliance on anaerobic metabolism to produce ATP. In Barth syndrome, this leads to elevated lactic acidosis even during submaximal exercise. Affected individuals are physiologically incapable of buffering lactic acid levels even during submaximal exercise (Figure 8) (Cade 2017). Thus, despite increased effort, patients with Barth syndrome have been shown to be unable to overcome these deficits (Cade 2017), in contrast to subjects with other cardiomyopathies (De Maeyer 2013).

Figure 8 Barth Syndrome Plasma Lactate Concentration at Rest, Submaximal Exercise and Recovery



2.1.2.3 Cardiomyopathic Presentation

Cardiomyopathy, which occurs in approximately 90% of males with Barth syndrome, is often diagnosed within the first year of life (Sabbah 2023). In a recent publication, Dr. Eugene Braunwald remarked that mitochondrial cardiomyopathies including Barth syndrome "most frequently cause…non-obstructive hypertrophic cardiomyopathy and heart failure with preserved ejection fraction. Ventricular dilatation with reduced ejection fraction, leading to dilated cardiomyopathy, may occur (usually late in the course of the condition). Mitochondrial cardiomyopathy may also be responsible for ventricular non-compaction, a variety of arrhythmias, disturbances in conduction and sudden cardiac death" (Braunwald_2023). While this is consistent with animal models (Greenwell 2022, Bertero 2020), and is reflective of the cardiac phenotype observed in TAZPOWER, the literature paints a more complicated picture of a temporal evolution of the cardiac phenotype (Sabbah 2023), as discussed briefly below.

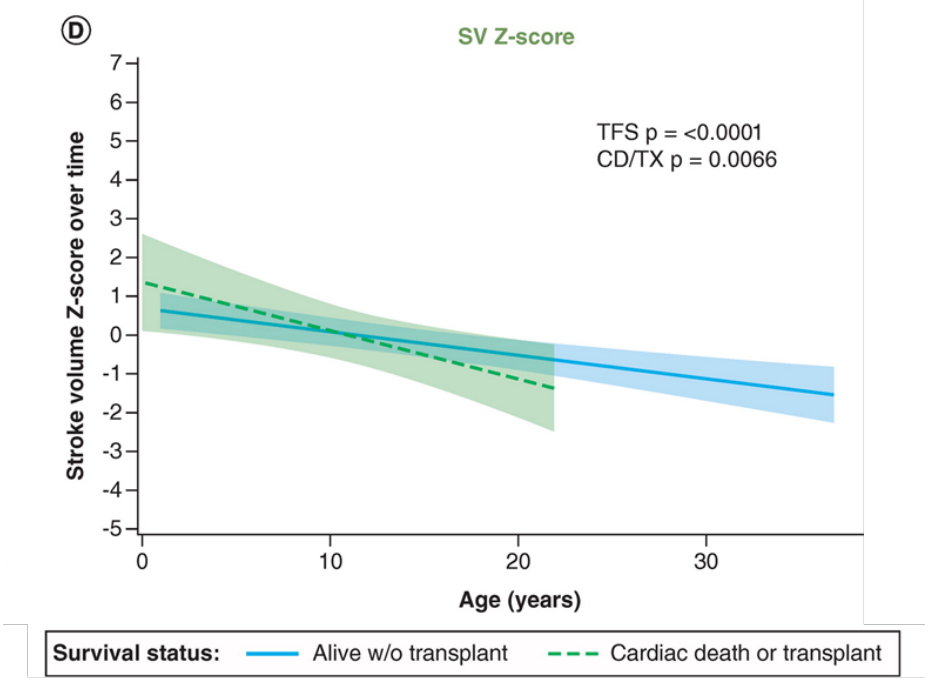
Infants can be born with dilated cardiomyopathy, which often leads to transplant or death in infancy or early childhood (Sabbah 2020). This is likely due to (i) bioenergetic crisis as the neonate heart attempts (and likely fails) the normal shift from glycolysis to fatty acid oxidation as a primary source of energy and/or (ii) a response to childhood infections, during which the bioenergetically fragile heart is known to quickly decompensate (for example, in the emergency access case of the baby with Barth syndrome treated at CHOP, see Section 2.7, his only

presenting symptom before being found unresponsive in his crib was a cold). Left ventricular (LV) noncompaction characterized by LV trabeculations with associated wall motion abnormalities, endocardial fibroelastosis, hypertrophic cardiomyopathy, as well as an apical form of hypertrophic cardiomyopathy have also been reported (Sabbah 2020).

It is reported in the literature that survivors of early childhood enter a so-called cardiac "honeymoon period" during which parameters of LV function "normalize," before reemergence of cardiac dysfunction in the years leading up to puberty. A recent review of a large cardiac NH database of 45 affected individuals followed for up to 15-years reveals that various parameters of cardiac function worsen over time, suggesting that the bioenergetically deprived heart may undergo compensatory remodeling during this period (Chowdhury 2022; Sabbah 2023).

Prepubescence through puberty is the next highest risk period following early childhood. By about age 10 years, affected children develop chronic fatigue, substantially reduced exercise capability, worsening of cardiomyopathy, potentially lethal cardiac arrhythmias, and diminished body mass (Towbin 2017; Raja 2017). As previously noted, emerging data characterizing the later years of the disease suggest an expected decline in LV volumes (LV ESV and LV EDV z-scores decline) suggestive of small left ventricles, and a declining LV-SVI z-score has also been characterized in patients ≥12-years-old (Chowdhury 2022; Sabbah 2023) (Figure 9). These data suggest a gradual evolution into a non-obstructive hypertrophic or HFpEF-like phenotype characteristic of mitochondrial cardiomyopathies (Braunwald 2023; Bates 2012; Florian 2015; Vakrou 2014).

Figure 9 Changes in LV SV with Age



In short, while the cardiac phenotype appears variable, it is apparent that LV SVI is low and declines with age, and it seems probable that the predominant phenotype in older individuals is a hypertrophic-like phenotype characterized by small LV volumes without diastolic dysfunction until worsening disease provokes decompensation and dilation.

2.2 Rationale for Development of Elamipretide for Barth Syndrome

2.2.1 Elamipretide Mechanism of Action

Elamipretide (MTP-131, SS-31), a first-in-class mitochondrial targeting agent, has been shown to improve cell viability and organ function across a spectrum of diseases including cardiovascular, renal, metabolic, skeletal muscle, neurodegenerative, and genetic mitochondrial disease (Birk 2013, Dai 2013, Eirin 2016). Elamipretide readily penetrates cell membranes and localizes to the IMM of the mitochondria, where it targets and reversibly binds to CL, a pathognomonic deficiency of which causes signs and symptoms of Barth syndrome.

Elamipretide compensates for CL deficit by aggregating CL and improving lipid packing within the IMM, restoring cristae curvature and the structure and function of energy-generating proteins in the electron transport chain. This has been shown to result in improved mitochondrial

respiration and reduced oxidative stress leading to end-organ benefit across models. Across all models assessed, elamipretide has been shown to have no measurable effect on normally functioning mitochondria – if CL lipid packing and resulting IMM cristae curvature is optimized, then elamipretide's interaction with CL does not result in further improvement or have any off-target effects.

2.2.2 Nonclinical Investigation

Stealth conducted nonclinical work in cell and animal models of Barth syndrome and in models of CL deficiency to inform elamipretide's therapeutic potential. In a model of the phospholipid bilayer of the inner mitochondrial membrane in which various levels of CL deficiency were tested, treatment with elamipretide compensated for an up to 30% deficit in normal CL (Barth syndrome causes a 70-95% deficit) and interacted with both MLCL and CL to improve inner membrane structure and function (Mitchell 2019; Mitchell 2020; Mitchell 2022; Allen 2020). The concentration of elamipretide utilized in these models corresponds to concentrations expected with a 20-30mg/day dose for a person weighting 70 kg. Elamipretide-mediated normalization of abnormally high MLCL levels relative to low CL levels has been observed in models of age-related cardiac disease (Campbell 2020), and elamipretide-mediated preservation of cardiolipin content has been observed in two separate large animal models of cardiomyopathy (Sabbah 2020; Eirin 2016), with comparable dosing parameters across studies.

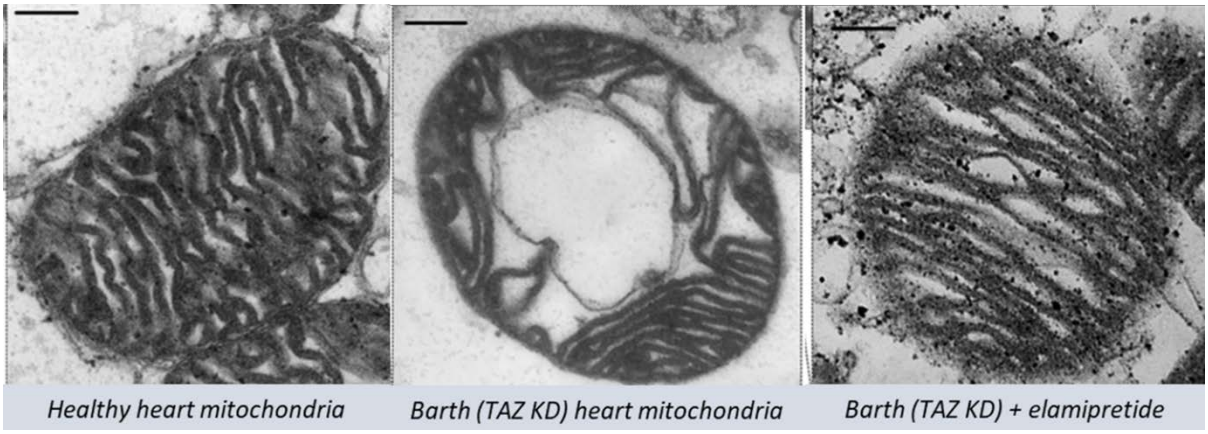
A commonly studied animal model of Barth syndrome is a mouse model in which the tafazzin gene is knocked down by doxycycline induction (TAZKD model) (other models include yeast, flies, or zebrafish) (Pu 2021). Cardiac abnormalities have been reported to be highly variable in the TAZKD model, typically demonstrating a mild, late adult-onset dilated or hypertrophic (HFpEF-like) cardiomyopathy if induced in chow or, if induced through drinking water in utero, demonstrating pre- and perinatal lethality (Ren 2019; Bertero 2021; Russo 2022; Pu 2021). Differences have also been noted between male and female mice and hetero- and homozygotes (Wang 2016; Tomczewski 2023).

Mitochondrial cardiac abnormalities including disturbed sarcomere organization, mitochondrial proliferation, myofibrillar disarray, mitophagy, and mitochondria-associated membrane abnormalities have been consistently observed in TAZKD mice (Ren 2019, Gang 2016, Russo 2022, Russo 2024). Given the mechanism of action of elamipretide, and the variability of the cardiac presentation in rodents, cardiac mitochondrial function was expected to be most predictive of potential clinical benefit.

In a TAZKD model with clear cardiomyocyte mitochondrial dysfunction, treatment with elamipretide improved IMM morphology and organization of the protein complexes in the

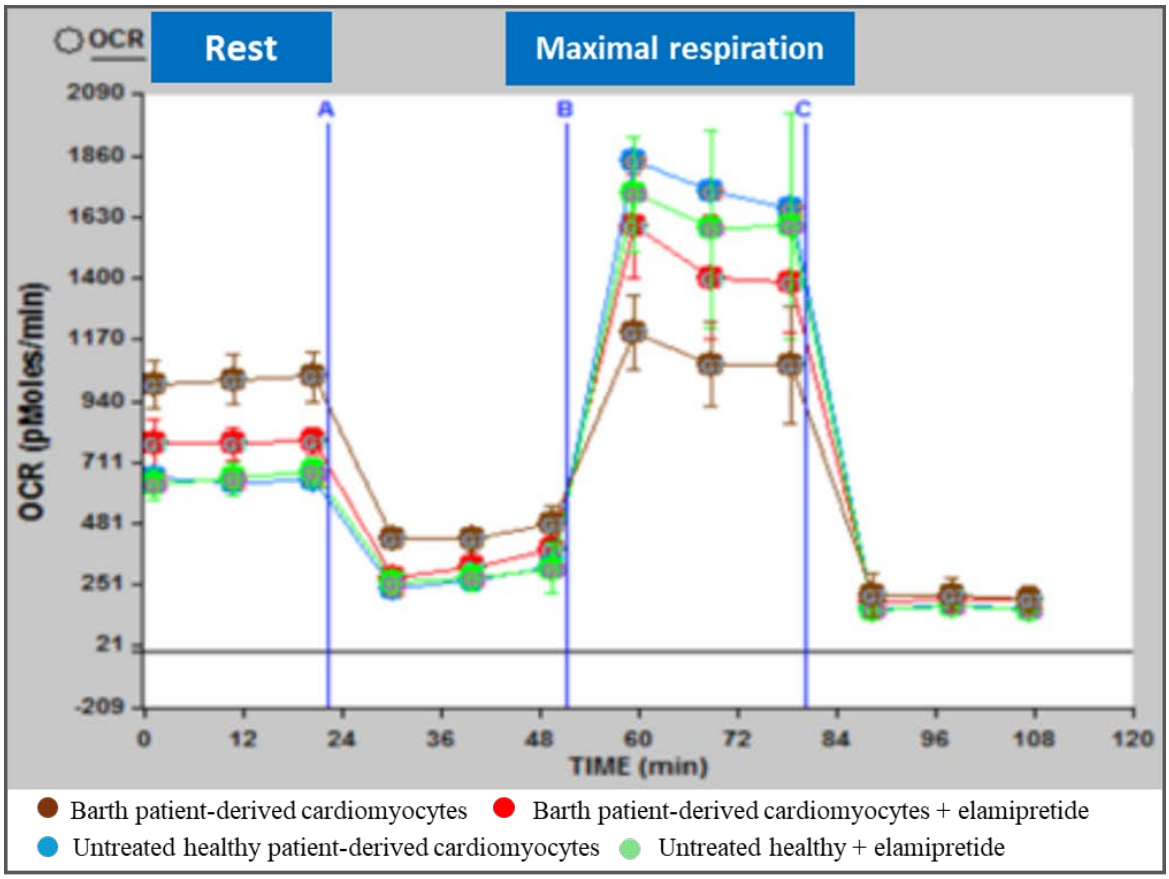
electron transport chain, leading to improved cardiac mitochondrial respiration (Russo 2022; Russo 2024). Elamipretide was dosed at 3mg/kg/day in this model (and at 3-5 mg/kg/day across mouse models), which scales allometrically to approximately 20-30mg/d for a 70kg person (consistent with the clinical dose utilized in TAZPOWER and TAZPOWER Extension). As shown in Figure 10 below, which depicts cross sections of cardiac mitochondria from normal mice (left), TAZKD mice (middle), and TAZKD treated with elamipretide (right) imaged with electron microscopy, elamipretide restores healthy mitochondria IMM architecture, depicted by the lines showing the folding of the IMM within the organelle.

Figure 10 Elamipretide Normalizes Mitochondrial Morphology in TAZKD Mouse Model of Barth Syndrome



In Barth syndrome patient-derived cardiomyocytes, mitochondria are unable to respire more in response to energy demand, meaning they lack the innate capacity to reach the maximal respiration necessary to support healthy heart function during exercise (Wang 2016). Treatment with elamipretide helped compensate for this respiratory deficit (Wang 2016; Gang 2016). As shown in Figure 11, Barth syndrome patient-derived cardiomyocytes (in **brown**) are expending close to maximal effort even at rest (left panel), and during maximal respiration (middle panel) are unable to increase respiration to meet the energy requirements of exercise. Treatment with elamipretide (in **red**) improves mitochondrial efficiency at rest and increases work capacity during maximal respiration. Consistent with published data across numerous other models, elamipretide (in **green**) has no effect on healthy controls (in **blue**); i.e., there is no suprapharmacologic effect of the drug.

Figure 11 Elamipretide Improves Mitochondrial Respiration in Patient-derived Cardiomyocytes



Elamipretide-mediated improvement in cardiac respiration has also been observed in human heart tissue from transplant patients (Chatfield 2019) and in beating cardiomyocytes derived from peripheral blood mononuclear cells from children with dilated cardiomyopathy with ataxia syndrome, an ultra-rare autosomal recessive disease linked to Barth syndrome, where normalization of fragmented mitochondrial ultrastructure was also observed (Rohani 2019).

Elamipretide has been dosed with once-daily administration across most animal models. In the setting of genetic mitochondrial diseases like Barth syndrome, this approach recognizes that when CL deficiencies are caused by a pathogenic mutation, the insult is ongoing as cardiolipin and mitochondrial proteins turn over. In several non-genetic disease models, the durability of effect of elamipretide-mediated improvements has been characterized post-withdrawal. For example, in a canine model of chronic heart failure, elamipretide-mediated improvements in left ventricular fractional shortening took nearly 7 days to dissipate following dosing, and in old (24 months of age) mice with left ventricular diastolic dysfunction, elamipretide-mediated

improvements in heart function were maintained for up to 2 weeks after withdrawal of elamipretide (Sabbah 2022). This therapeutic durability is thought to be explained by the structural, functional, and biochemical benefits of chronic elamipretide therapy, which may potentially normalize cardiolipin half-life and alter aspects of disease trajectory.

We hypothesized that elamipretide could (i) stabilize and extend the half-life of mature CL, with the potential to improve MLCL:CL ratio, (ii) improve cardiac mitochondrial function, and (iii) improve muscle mitochondrial function. By this mechanism, it was thought that elamipretide could improve the muscle myopathy and, if present, the cardiomyopathy characteristic of Barth syndrome. Conversely, neutropenia, which is effectively managed by GMCSF (Steward 2019), was not a focus of the development program because (i) the link between defective CL maturation and neutropenia remains unclear (Sohn 2021) and (ii) the neutropenia is not peripheral but rather occurs in the bone marrow, which may be inaccessible to therapy.

See Table 3 for a summary of nonclinical data in CL-deficient or Barth syndrome related models supporting the therapeutic potential of elamipretide as a treatment for Barth syndrome. These data exist within a broader class of data across models of cardiac and skeletal muscle dysfunction which provide further support for therapeutic potential (see, e.g., Sabbah 2020).

Table 3 Nonclinical Studies Supporting the Use of Elamipretide in Barth Syndrome

Model/Publication details	Findings
Barth syndrome (including CL deficient membrane) models	
Biomimetic IMM (25%-50% CL deficit)/Allen 2020	Elamipretide attenuated loss of membrane surface area.
Lipid modeling system/ Mitchell 2019; Mitchell 2020; Mitchell 2022	Elamipretide improved lipid packing, membrane curvature, membrane surface area, cristae formation, super-complex association, and oxidative phosphorylation through interaction with CL and MLCL.
Barth syndrome iPSC-derived cardiomyocytes (80% reduction in CL; 8-10-fold increase in MLCL)/Wang 2016	Elamipretide improved respiration in human cells from Barth syndrome subjects, particularly during maximal respiration.
Cas9 edited isogenic human iPSC cardiomyocytes with *TAZ* mutation/ Wang 2016	Elamipretide significantly decreased ROS levels.
TAZ-deficient cells/Anzmann 2021	Elamipretide up-regulated NDUFA1 (codes for essential complex I component reduced in Barth syndrome), with associated improvement in protein expression. Elamipretide ameliorated deficiencies in PARL and PGAM5 (affect mitochondrial quality control, known to be deficient in and thought to be central to the cardiac pathogenesis of Barth syndrome).
Doxycycline-treated TAZKD mice (~90% CL reduction; 10-fold MLCL	Observed improvements in fractional shortening and histology (ventricle diameter and degree of fibrosis); insignificant across the

Model/Publication details	Findings
increase; variable cardiac presentation)/ Wang 2016	full cohort due to variability resulting from sex and genotype (hetero vs. homozygotes).
TAZKD mice (lower respiratory rates; 80% reduction in tafazzin)/Russo 2022; Russo 2024	Elamipretide improved cardiac mitochondrial respiratory capacity, promoted super complex organization, and improved mitochondrial morphology.
Related diseases of CL deficiency	
DCMA patient-derived iPSC-beating cardiomyocytes/Rohani 2019	Elamipretide reversed mitochondrial abnormalities (fragmented and abnormally shaped mitochondria) associated with an imbalanced isoform ratio of OPA1, a regulator of mitochondrial fusion..
DCMA patient fibroblasts/Machiraju 2019	Elamipretide significantly improved overall mitochondrial structure, decreased ROS generation, and improved L-OPA1 expression and L-OPA1/S-OPA1 ratios (thought to improve mitochondrial fusion).

The underlying pathology of Barth syndrome (i.e., due to CL deficiency) is well-understood. The TAZKD model recapitulates established consequences of CL deficit at the cellular level in Barth syndrome, including severe mitochondrial dysfunction in cardiomyocytes. Cellular models (Barth syndrome iPSC-derived cardiomyocytes, Cas9 edited isogenic human iPSC cardiomyocytes with TAZ mutation, and TAZ-deficient cells) are similarly reflective of severe mitochondrial dysfunction. Accordingly, the data demonstrating elamipretide's therapeutic effect in these models are confirmatory of the clinical benefit observed and contribute to the totality of the evidence favoring a determination of effectiveness of elamipretide in humans.

2.3 Barth Syndrome Clinical Development Program

Stealth conducted three separate clinical trials in Barth syndrome. The first, the TAZPOWER study, was a double-blind, placebo-controlled, crossover trial. The second, the TAZPOWER Extension study, was an open-label extension prospectively designed to evaluate safety and long-term trends in efficacy compared to baseline controls. The third, the NH Control study, was a Phase 3 study prospectively designed to establish external historical controls for the TAZPOWER Extension study data. In addition, concurrent with the TAZPOWER study, Stealth conducted a prospective, blinded, independent, qualitative study to explore the experiences of participants and observations of their caregivers during the TAZPOWER study (the "PCPC protocol"). While multiple other trial designs were proposed to and discussed with the FDA, none of these was deemed by the FDA to be appropriate (in most cases due to low statistical power) to generate additional evidence of effectiveness.

We worked closely with BSF and the Johns Hopkins team to design our clinical development program. Starting in 2012, well before initiation of our development efforts, the Johns Hopkins team compiled a longitudinal natural history cohort eventually including up to 79 affected

individuals (more than 50% of the US Barth syndrome patient population) to inform endpoints for clinical monitoring and therapeutic development (Hornby 2019; Thompson 2016). The design of TAZPOWER was also informed by clinical experience and regulatory interactions in the context of our Phase 3 development of elamipretide for a related disease of primary mitochondrial myopathy (PMM) for which extensive regulatory discussions under the same IND had occurred, clinical data was supportive, and the 6MWT and a Stealth-developed disease-specific fatigue scale were the agreed family of primary endpoints.

2.4 TAZPOWER and TAZPOWER Extension Studies

2.4.1 Design

Key design features are depicted in Figure 12 and summarized in Table 4.

Figure 12 Schema of TAZPOWER and TAZPOWER Extension Studies

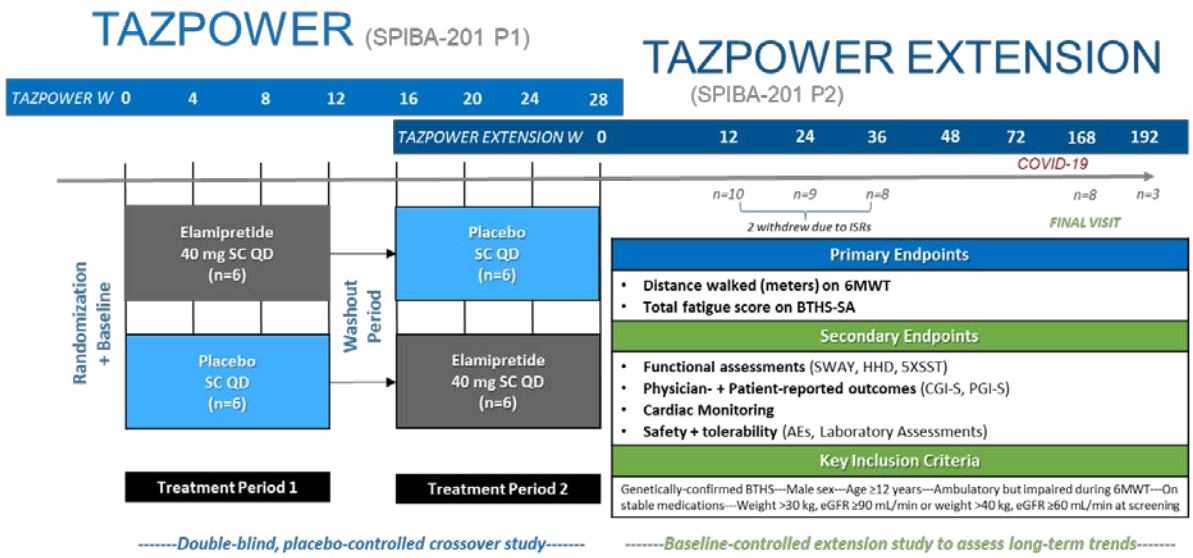


Table 4 Key Design Features of TAZPOWER and TAZPOWER Extension

Study Objectives
TAZPOWER study. The primary objective was to evaluate the effect of single daily SC doses of 40 mg elamipretide administered for 12 weeks in subjects with Barth syndrome on distance walked (meters) during the 6MWT and Total Fatigue on the BTHS SA. Secondary objectives were to evaluate the effect of single daily SC doses of 40 mg elamipretide administered for 12 weeks in subjects with Barth syndrome as measured by change in secondary functional assessments, patient, caregiver and clinician reported outcomes, biomarkers including the MLCL:CL ratio, 2 D and 3 D echocardiographic measurements and accelerometry counts. An additional objective was to evaluate the safety and tolerability of single daily SC doses of 40 mg elamipretide administered for 12 weeks in subjects with Barth syndrome.
TAZPOWER Extension study. The primary objective was to assess the long-term safety and tolerability of single daily SC doses of 40 mg elamipretide for up to 192 weeks (about 3 and a half years). Secondary objectives were to evaluate longitudinal trends of single daily SC doses of 40 mg elamipretide administered for up to 192 weeks in subjects with Barth syndrome assessed utilizing the same endpoints studied in TAZPOWER. Longitudinal trends were considered important to inform durability of potential therapeutic benefit, particularly for outcomes, including cardiac outcomes such as death which occur infrequently and over a long-time period. Since these typically require large interventional and placebo cohorts to ascertain effect, longitudinal trends in echo assessments of structural or functional cardiac changes were of interest given the small changes observed in Part 1. This treatment period also allowed for detection of less-frequent AEs.
Site Selection
Conducted at a single site, Johns Hopkins, which hosts one of only two multi-disciplinary centers focused on treating Barth syndrome worldwide. The Barth Syndrome Clinic at Kennedy Krieger Institute at Johns Hopkins is known to see most affected individuals in the United States for routine outpatient monitoring visits. No other sites were identified in the continental United States. Stealth also approached NHS National Barth Syndrome Service at University Hospital in in Bristol, UK, the only other multi-disciplinary center treating Barth syndrome worldwide, which declined study participation because of its planned initiation of a trial of bezafibrate for Barth syndrome (the CARDIOMAN trial, which failed to show any benefit).
Patient Enrollment and Inclusion Criteria
TAZPOWER study. The minimum age of 12-years was informed by safety considerations and a desire to minimize variability in trial assessments, most of which were considered unsuitable for younger children. The number of patients was limited by availability. BSF advised that there were approximately 66 affected US individuals ≥12-years-old who had not received heart transplants (an exclusion criteria). After extensive outreach by BSF and the investigator, 16 subjects were screened (~25% of the eligible US patient population; see **note 2**)) and 12 subjects were enrolled.
TAZPOWER Extension study. All participants in the TAZPOWER study were eligible to participate. 10 of 12 subjects chose to participate; 2 subjects withdrew early by week 24 and 8 subjects completed week 168, the last visit for which all long-term subjects had data. Both subjects who withdrew early have since requested consideration for EAP access.
Design
TAZPOWER study. The crossover design was selected to increase statistical power with subjects serving as their own control, given the anticipated scarcity of available subjects due to the ultra-rare nature of this disease. Subjects were randomized (in a ratio of 1:1) to receive single daily SC doses of 40 mg elamipretide or placebo for an initial 12-week treatment period, followed by a 4-week washout period in which no treatment was administered, followed by a second 12-week treatment period in which the opposite treatment was administered (i.e. subjects who received elamipretide in the first 12-week period received placebo in the second 12-week period, and vice versa).

An important hypothesis underlying this trial was that the pharmacology of the intervention would "wash out" and not carry over into the second treatment period for subjects randomized to elamipretide in the first treatment period. The 4-week washout was to reduce the likelihood of a carryover effect based on the known pharmacokinetics of elamipretide and anticipated turnover of mitochondria in skeletal muscle (7-days in rats). A longer wash-out period may have been necessary for cardiac endpoints because of the longer timeframe for turnover of mitochondria in the heart (30-days in rats) (Kumar 2019) as well as potential reverse remodeling effects observed preclinically. Another assumption, based on the treating cardiologist's evaluation that cardiac status was normal at BL, was that the intervention would not result in cardiac structural changes. Longitudinal trends in 2-D and 3-D echocardiograph measurements were prospectively included in Part 2 to capture any unexpected changes over time.
TAZPOWER Extension study. This was an open label, BL controlled assessment of the long-term safety and tolerability and longitudinal trends in efficacy of single daily SC doses of 40 mg elamipretide for up to 192 weeks in subjects with Barth syndrome. TAZPOWER study participants were eligible to participate.
Dose
The 40 mg daily SC dose was selected based upon data from MMPOWER, a Phase 2a dose escalation trial assessing 3 doses of IV-administered elamipretide or placebo over a 5-day period in patients with PMM. The trial demonstrated a dose-dependent increase in distance walked on the 6MWT with elamipretide treatment ($p = 0.014$). The highest level of functional improvement was observed at a dose equivalent to the 40 mg SC dose utilized in TAZPOWER (Karaa 2018). The 40 mg dose is also supported by allometric scaling of doses of elamipretide which demonstrated improvement in preclinical Barth syndrome models (Russo 2022, Russo 2024, Wang 2016) and to concentrations of elamipretide which demonstrated improvement in studies in explanted human heart tissue (Chatfield 2019), TAZ KO cells (Anzmann 2021), and DCMA patient-derived cells (Rohani 2019).
Choice of Control
TAZPOWER study. Patients were selected to serve as their own control to increase statistical power in the small sample size available for study participation.
TAZPOWER Extension study. Baseline controls were considered appropriate for long-term follow-up because Barth syndrome has been well-characterized as a progressive disease in which spontaneous improvement in exercise tolerance and/or muscle function has not been observed in patients ≥12-years-old. Conversely, studies have demonstrated the biological improbability of such improvement, with supervised aerobic exercise training only modestly (~5%) increasing exercise tolerance (VO2peak) in participants with Barth syndrome (Cade 2017) (compared to ~15–25% in other cardiomyopathies) (De Maeyer 2013).
Blinding
TAZPOWER study. Blinded treatment was used to reduce potential bias during data collection and evaluation of endpoints.
TAZPOWER Extension study. This was an open-label, baseline controlled clinical investigation.
Study Primary Endpoints
TAZPOWER study. The family of primary endpoints was comprised of the distance walked during the 6MWT and Total Fatigue on the BTHS-SA, a fit-for-purpose patient-reported outcome assessment developed by Stealth (Gwaltney 2021). These were chosen to capture both function and feel on assessments quantifying the severe exercise intolerance and debilitating fatigue identified as the most problematic symptoms of the disease (VOP 2019). A similar primary endpoint family had been agreed by the Division of Neurology Products (the review division for the PMM program housed under the same IND).
TAZPOWER Extension study. The TAZPOWER Extension study also utilized the 6MWT and BTHS-SA. However, since the BTHS-SA was collected only at study visits rather than daily during TAZPOWER Extension, it was not suitable as a primary assessment.

Study Secondary Endpoints
TAZPOWER study. The pre-specified secondary endpoints were muscle strength measured by HHD, 5XSST, 2-D and 3-D Echocardiographic measurements, accelerometry counts, SWAY Application Balance Assessment, PGI , CGI and CaGI Scales, the PROMIS short term fatigue scale, and biomarkers, including the MLCL:CL ratio. The statistical analysis plan specified a hierarchical approach to evaluate: (1) PGI-S [Q1] (severity of Barth syndrome symptoms), (2) CGI-S [Q1] (severity of Barth syndrome symptoms), (3) PROMIS Short Form Fatigue, (4) SWAY Balance Score, (5) Muscle strength as measured by HHD and (6) 5XSST. A prespecified subgroup analysis to assess whether patients with relatively more normal MLCL:CL ratios would respond more rapidly to elamipretide therapy was also conducted.
TAZPOWER Extension study. The TAZPOWER Extension study utilized all TAZPOWER study secondary endpoints except the CGI-C, PGI-C and accelerometry counts.
Statistical Methods
TAZPOWER study. A family wise alpha level of 0.05 was maintained for the primary endpoint family, using Hochberg's procedure at the primary time point of week 12 (i.e., measuring end of treatment on elamipretide versus end of treatment on placebo). This means that if either primary endpoint met a p value of ≤0.025, or both primary endpoints met a p value of ≤0.05, the study would be positive.
TAZPOWER Extension study. Continuous variables were summarized with descriptive statistics (the number of non-missing values, mean, standard deviation (SD), median, minimum, and maximum) and all categorical variables were summarized with frequency counts and percentages including a category of "missing," by treatment.
Sensitivity Analyses
TAZPOWER and TAZPOWER Extension studies. Additional analyses were performed at the request of the FDA to assess the robustness of the observed results. These included (i) analyses of the BORG scale of dyspnea and fatigue, collected with each 6MWT assessment, to ascertain whether increased 6MWT values were associated with increased effort (they were not), (ii) an independent overread of the 3-D echocardiographic assessments of LV volumes conducted by the imaging team at Cardiovascular Clinical Sciences (Tufts) (analysis was consistent with TAZPOWER and TAZPOWER Extension reported findings), (iii) an assessment of 6MWT adjusted for concurrent height, to adjust for growth (analysis was consistent with TAZPOWER and TAZPOWER Extension reported findings), (iv) assessment of all TAZPOWER Extension study endpoints relative to Part 1 placebo effect (analysis was consistent with TAZPOWER Extension reported findings, although the 6MWT improvement was reduced by ~30% after adjustment for placebo effect), and (v) assessment of LV volumes adjusted to index to concurrent body surface area (BSA) and concurrent z-scores (analyses were consistent with TAZPOWER and TAZPOWER Extension reported findings, although improvements observed prior to week 168 of TAZPOWER Extension study were generally within the variability of the assessment, by week 168 the effect size was large irrespective of these adjustments).
Duration
TAZPOWER study. Nonclinical data and early clinical data in PMM suggested that improvements on 6MWT and fatigue endpoints could occur within the 12-week treatment period. Stealth subsequently learned across all mitochondrial diseases that treatment duration needs to be longer to demonstrate organ system improvement. The choice of study duration was also informed by feasibility considerations after extensive conversations with the BSF Scientific Advisory Board, which approved the protocol design, and the principal investigator, who is a leading worldwide expert in the treatment of Barth syndrome.
TAZPOWER Extension study. 10 subjects chose to continue into the TAZPOWER Extension study, and 8 subjects remained enrolled through the close-out visit (all subjects completed week 168, 3 subjects completed week 192). Due to COVID-19, the amount of data generated between July 2020 (week 72 for most subjects) and study close-out (week 168 for most subjects) was limited. The study was terminated following receipt of a Refusal to File notification from FDA in October 2021. 7 of the 8 subjects were enrolled in EAP and 6 still remain on therapy. Both subjects who had an early termination visit have since requested EAP access.

2.4.2 TAZPOWER Study

2.4.2.1 Efficacy Summary

The TAZPOWER study was the first study ever conducted in Barth syndrome, and thus intended to inform both how to study this disease as well as the safety and efficacy of elamipretide. As such, it was not entirely surprising that it failed to meet its primary endpoints, which were distance walked on the 6MWT and a patient-reported assessment of fatigue, the BTHS-SA Total Fatigue scale, at 12 weeks.

Although the improvements observed in elamipretide-treated patients on the primary endpoints (43.1-meter improvement on 6MWT, 1.4-point reduction in fatigue) at 12 weeks directionally exceeded those observed with placebo (31.3-meter improvement on 6MWT, 1.2-point reduction in fatigue), the changes were not statistically significant. Indeed, the changes observed in both treatment arms on the 6MWT are within the range of an expected placebo effect as characterized in multiple clinical trials in heart failure (about 30-meters; Olsson 2005). No meaningful changes were observed in either treatment group on any secondary endpoints; for example, muscle strength by HHD improved by only 4.7 newtons (1 pound) with elamipretide treatment and 6.15 newtons (1.4 pounds) with placebo treatment and time to complete the 5XSST increased for both groups (by one second with elamipretide treatment and a half-second with placebo treatment). These findings provide important context for the improvements observed with long-term therapy in TAZPOWER Extension (Section 2.4.3), in that they corroborate the expectation that, during a placebo-controlled study, affected individuals were unable to increase their physical functioning due to hope or expectational bias.

An exploratory endpoint, the CGI-C, reflecting the investigator's perception of change in signs and symptoms of Barth syndrome, showed a mean 1-point improvement (SD 1.13) in the elamipretide treatment group relative to mean -0.3 decline in placebo (SD 0.49) (p=0.004). Ten of the twelve subjects demonstrated small improvements in left ventricular volumes assessed by 3-D echocardiogram following randomization to elamipretide, with associated small improvements in LV SVI. This finding was deemed worthy of further follow-up during open-label extension, because baseline LV volumes were extremely low in all patients as discussed further in Section 2.6 Notably, the improvements observed in LV volumes did not appear to "wash-out" following randomization to placebo, suggesting a potential carryover effect.

2.4.2.2 Rationale for Continued Development

Most (10 of 12) TAZPOWER subjects were already enrolled in the TAZPOWER Extension study at the time of the TAZPOWER data read-out. Stealth continued its development efforts despite the discouraging TAZPOWER findings primarily because (i) early TAZPOWER Extension study datapoints suggested that, with longer exposure to therapy, elamipretide-mediated changes may be observed, (ii) patients and caregivers reported perceived improvements in the PCPC protocol (Section 2.4.3.3.3), and (iii) the investigator reported quantifiable improvements. These, and subsequent findings, suggest that longer than 12-weeks of exposure may be required to observe a statistically meaningful effect of elamipretide in patients with Barth syndrome. Furthermore, based on apparent persistence of small LV changes, a potential carryover effect (i.e., failure to “wash-out” drug effect due to the crossover trial design) may have confounded the TAZPOWER study results.

2.4.3 TAZPOWER Extension Study

The TAZPOWER Extension study was an up to 196-week baseline-controlled open-label extension study in which all TAZPOWER study participants (n=12) were eligible to participate. The baseline utilized was the TAZPOWER study baseline, in recognition of the fact that all subjects entering the TAZPOWER Extension study were already exposed to 12-weeks of elamipretide therapy during TAZPOWER. Ten subjects entered the TAZPOWER Extension study; two of these subjects withdrew by week 24 due to injection site reactions, and eight of these subjects remained enrolled through week 168, which was the final visit for all patients. Three subjects completed a week 196 visit. The TAZPOWER Extension study utilized all TAZPOWER study endpoints except the CGI-C and PGI-C; however, the BTHS-SA was collected only at study visits rather than daily. Overall, starting by week 36 of the TAZPOWER Extension study, mean improvements were observed and maintained through the final visit at week 168 for most clinical endpoints including 6MWT, muscle strength, 5XSST, SWAY balance and CGI-S (Figure 22).

The discussion of the results is organized by (i) endpoints assessing the molecular physiology (MLCL:CL ratio) central to the disease (Section 2.4.3.1), (ii) endpoints assessing clinical function, including the 6MWT, which was part of the TAZPOWER primary endpoint family (Section 2.4.3.3), and (iii) endpoints assessing feel (Section 2.4.3.3) (including Total Fatigue on the BTHS-SA, which was part of the TAZPOWER primary endpoint family, and the PCPC Protocol, a separate protocol conducted to bring the patient voice into the development program in compliance with the 21st Century Cures Act, H.R. 34, 114th Cong. (2015)). A discussion of the physiological cardiac findings from the TAZPOWER Extension study (Section 2.6) follows the

discussion of the NH Control study (Section 2.5), which was designed to establish an external control for the TAZPOWER Extension study functional endpoints.

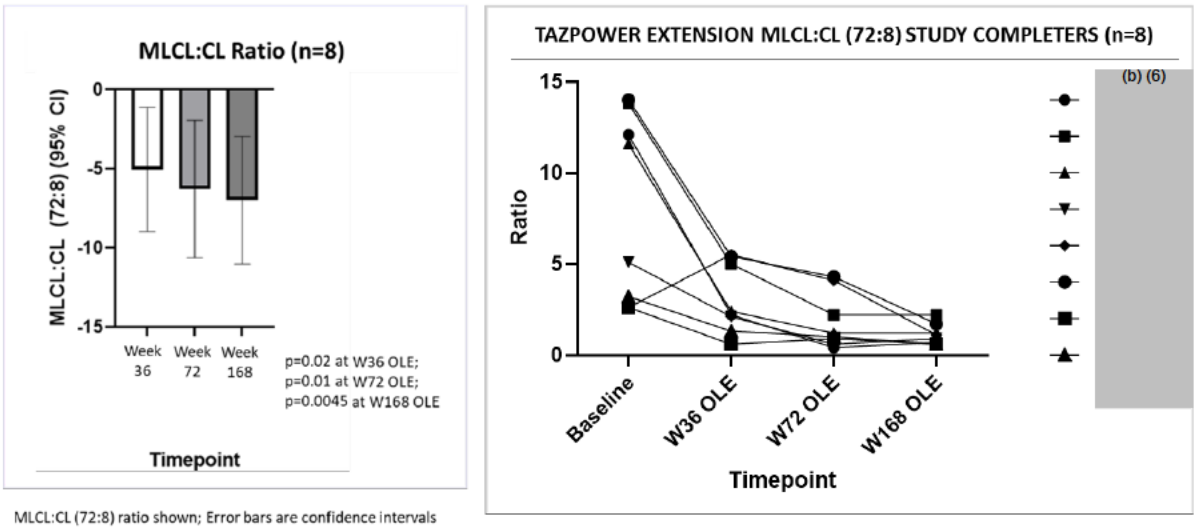
2.4.3.1 Improvement in MLCL:CL Ratio

Barth syndrome may be diagnosed by the ratio of abnormal MLCL to normal CL (the MLCL:CL ratio), which is abnormal in all affected individuals and pathognomonic for the disease. Although the MLCL:CL ratio was assessed as an exploratory biomarker in TAZPOWER Extension, we address it first in this discussion because it is fundamental to both the diagnosis and clinical manifestation of the disease as well as the mechanism of action of elamipretide.

This predictive diagnostic biomarker has been shown to be approximately 175 times higher in Barth syndrome fibroblasts relative to healthy controls (0.03–0.12 in healthy controls versus 5.4–13.8 in Barth syndrome) (VanWerkhoven 2016). This ratio has been found to have an association with, and possibly an impact on, several important aspects of clinical status, including functional exercise capacity and LV mass, and has been recommended as a viable biochemical target for long-term therapeutic monitoring (Bowron 2015; Thompson 2016; Kulik 2008). An FDA-requested analysis of the natural history progression of this ratio revealed that it is not expected to change over time (Table 2).

Two related ratios were assessed, the MLCL:L4-CL ratio and the MLCL:(72:8)-CL ratio. Of the two, the MLCL:(72:8)-CL ratio was expected to demonstrate lower variability and improved ease of quantification. Both ratios improved (lowered) for all TAZPOWER Extension study subjects starting at week 12 and continuing through week 168. Nominally significant improvements in the MLCL:L4-CL ratio were observed at week 12 (-7.8, p=0.003), week 24 (-7.0, p=0.03), week 48 (-7.1, p=0.04), week 72 (-17.9, p=0.03) and week 168 (-7.4, p=0.02). Nominally significant improvements in the MLCL:(72:8)-CL ratio were observed at week 12 (-5.9, p=0.002), week 24 (-6.0, p=0.003), week 36 (-5.1, p=0.02), week 48 (-5.5, p=0.007), week 72 (-5.7, p=0.03) and week 168 (-7.0, p=0.005). See Figure 13.

Figure 13 MLCL:CL Ratios Improved (Lowered)



During TAZPOWER, small changes in these ratios were observed following treatment with elamipretide, but these were not statistically significant. It is notable, however, and consistent with the hypothesis that longer than 12-weeks duration of elamipretide therapy may be required to detect benefit in Barth syndrome, that nominally significant changes in this biomarker emerged at week 12 of TAZPOWER extension, following 24 weeks of total elamipretide exposure (i.e., counting the 12-weeks of exposure during TAZPOWER). As discussed in Section 2.4.3.2, improvements in 6MWT and other functional endpoints were also observed starting at week 12 of TAZPOWER Extension.

We hypothesized, based on elamipretide's mechanism of action, that elamipretide would stabilize and extend the half-life of CL, thereby increasing concentrations of mature CL and improving the MLCL:CL ratio. It is clear from the data that elamipretide did, in fact, improve the abnormal cardiolipin ratios that are central to disease pathology. Moreover, the time course for improved clinical findings corresponded closely to the time course for this improved molecular pathology.

2.4.3.2 Functional Endpoints Improved in TAZPOWER Extension Study

FDA defines clinical benefit as a favorable effect on a meaningful aspect of how a patient feels (e.g., symptom relief), ***functions*** (e.g., improved mobility) or survives as a result of treatment.[19] In the TAZPOWER Extension study, as described below, durable ***functional improvements*** were observed on numerous assessments across numerous timepoints.

2.4.3.2.1 Improvements in 6MWT (Primary Endpoint)

The 6MWT is a sub-maximal exercise test validated to assess aerobic capacity and endurance and used in clinical trials in many disease areas as a primary endpoint supportive of regulatory approval. Stealth included 6MWT as part of the family of primary endpoints in TAZPOWER, and carried it over with the other functional endpoints as an endpoint of primary interest in TAZPOWER Extension, for the following reasons:

- The 6MWT was identified as a suitable endpoint for monitoring and therapy in Barth syndrome because performance on this assessment by affected individuals is severely impaired compared to height-matched normative controls and age-matched controls (Hornby 2019; Thompson 2016).

- All subjects in TAZPOWER Extension were severely compromised in their 6MWT at BL, walking only a mean 382.8 (57.11 SD) meters. A mean 6MWT distance of 637-meters is expected for healthy young adults (Halliday 2020).

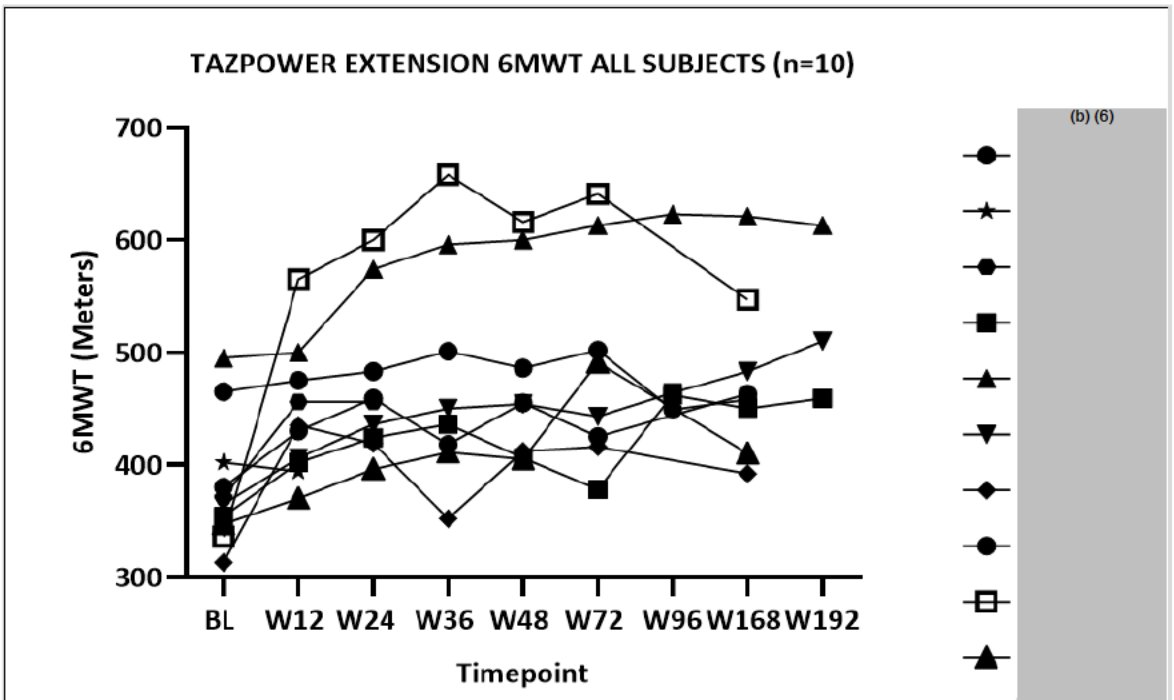
- There is ample regulatory precedent for approvals based on the 6MWT in both cardiac and neuromuscular diseases, with effect sizes ranging from 10 to 45 meters. FDA recommended that Stealth utilize the 6MWT as the primary endpoint for its Phase 3 clinical trial in primary mitochondrial myopathy (PMM), a related disease development program. FDA Guidance on Treatment for Heart Failure: Endpoints for Drug Development (2019) accepts that improvements in symptoms including walking distance can constitute evidence of effectiveness for a heart failure drug.

[19] E.g., FDA Draft Guidance to Industry: Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products, December 2019: “The Agency accepts clinical endpoints that reflect patient benefits (i.e., how patients feel, function, or survive) or validated surrogate endpoints (i.e., those that have been shown to predict a specific clinical benefit) as the basis for traditional approval.”

- The minimal clinically important difference (MCID) has been ascertained in the setting of COPD (35 meters) (Holland 2009, Puhan 2008), CHF Class II/III (30.1 meters) (Shoemaker 2013) and DMD (28.5-31.7 meters) (McDonald 2013). In our discussions with the FDA regarding our Phase 3 PMM program, we aligned that a 25-meter change would be clinically meaningful.

In the TAZPOWER Extension study, a nominally significant treatment benefit of long-term elamipretide therapy on exercise endurance as assessed by the 6MWT was observed at every timepoint assessed, i.e., Week 12 (60.5 meters; p = 0.02; n=10), Week 24 (91.2 meters; p = 0.004; n=9), Week 36 (95.9 meters; p = 0.02; n=8), Week 48 (97.4 meters; p = 0.01; n=8), Week 72 (106.8 meters; p = 0.01; n=8), Week 168 (96.1 meters; p = 0.003; n=8), and Week 192 (122.7 meters; p = 0.009; n = 3) (see Figure 14).

Figure 14 All TAZPOWER Extension Subjects 6MWT Performance [Figure Shows TAZPOWER BL and All TAZPOWER Extension Assessments]

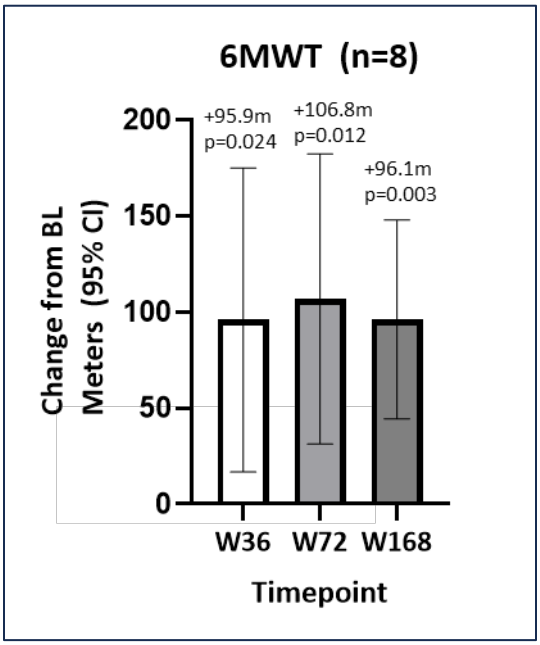


Subjects (b) (6) + (b) (6) had early termination visits on/before W24 + W36, respectively. Only 3 subjects completed W192; final study visit for all subjects was W168.

Ten of twelve TAZPOWER subjects opted to enter TAZPOWER Extension. Since enrollment in TAZPOWER Extension occurred at the end of TAZPOWER, all subjects in TAZPOWER Extension had up to 12-weeks exposure to elamipretide at the start of TAZPOWER Extension and, for subjects randomized to elamipretide in the second part of TAZPOWER, there was no interruption of study drug entering into TAZPOWER Extension. Two of the ten subjects in TAZPOWER Extension terminated early (by week 36) due to injection site reactions; both have since requested EAP access. For consistency of comparison, the period from week 36 through week 168, the final visit for all remaining subjects, is featured in this briefing book. Baseline refers to TAZPOWER baseline, as the last timepoint at which no subjects had been exposed to elamipretide.

From week 36 through week 168, the observed >90-meter mean improvement on 6MWT (>25% increase from baseline and 3X the 30.1-meter mean placebo effect observed during TAZPOWER study) was durable and nominally significant. Practically speaking, this improvement represents a gain of almost the length of a football field in distance walked in 6-minutes. The improvements were consistent across the cohort: four of the eight participants had a >100-meter improvement from BL; seven of the eight participants had a >60-meter improvement from BL; and the one participant who did not improve stopped treatment at week 48 **(**Figure 15**)**. An analysis of 6MWT improvements adjusted for concurrent height supports a conclusion that these changes were not driven by growth, consistent with the expected decline with age in the NH (Figure 7).

Figure 15 Change from BL in Distance Walked on 6MWT

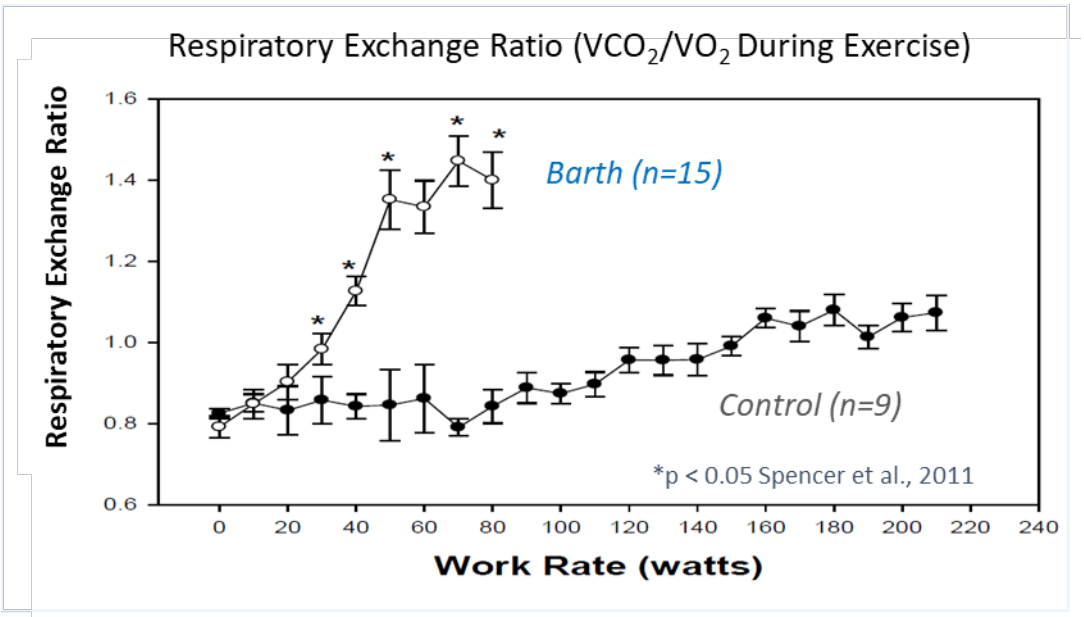


2.4.3.2.2 Potential Impact of Effort Bias

As with all functional endpoints, effort bias, which is how hard a patient is trying, can contribute to variability on the 6MWT in healthy individuals. This seems physiologically improbable in the setting of Barth syndrome, in which the severe and progressive exercise intolerance shares similar characteristics with mitochondrial myopathies and adult heart failure. Studies in Barth syndrome demonstrate that individuals affected by Barth syndrome cannot sustain increased mitochondrial respiration to support durable improvements in exercise (Spencer 2011).

In exercise-endurance training studies in which individuals affected by Barth syndrome were exposed to a supervised regime of exercise training over a 12-week period, a de minimis (5%) improvement in peak oxygen consumption was observed (Cade 2017). It was concluded that affected individuals are physiologically incapable of working harder, due to both diminished skeletal muscle oxygen extraction and utilization and impaired cardiac contractile reserve. To illustrate this, it is notable that respiratory exchange ratio is significantly higher in affected individuals versus controls even at very low levels of exercise intensity, indicating anaerobic energy supplementation at very low workloads (Figure 16).

Figure 16 Diminished Skeletal Muscle Oxygen Extraction Limits Exercise Capacity

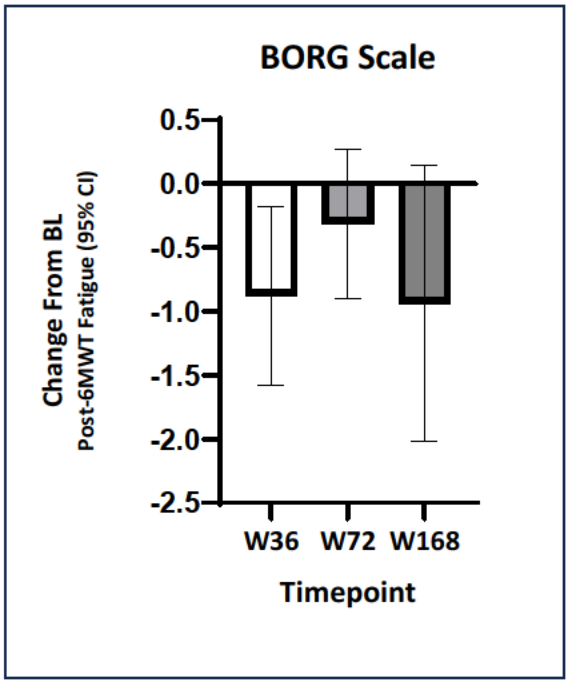


Indeed, there was little evidence of effort bias in the TAZPOWER controlled study. A 30.1-meter mean placebo effect was observed on the 6MWT (comparable to the placebo effect characterized in multiple heart failure trials; Olsson 2005, and as compared to a 43-meter mean effect observed with elamipretide) and no changes were observed in either treatment group on any other functional endpoints.

To further evaluate any effect of effort on functional assessments, we incorporated the modified BORG fatigue scale, an assessment in which patients are asked to numerically rate their pre- and post-exertional fatigue taking into consideration all sensations and feelings of physical stress and fatigue. In individuals affected by Barth syndrome, performance on the 6MWT has been correlated with fatigue on the BORG scale. Particularly for affected individuals aged 20 - 32 years, the difference between pre- and post-assessment fatigue was strongly correlated with 6MWT distance (r=0.47) (Hornby 2019; Thompson 2016). The BORG scale is recommended by the FDA as an endpoint supportive of efficacy in the setting of obstructive pulmonary disease (November 2007 Guidance for Industry).

For the 8 subjects who completed TAZPOWER Extension week 168, post-exertional fatigue at weeks 36 and 72 were essentially unchanged from BL and at week 168 were about 25% lower than BL (Figure 17). Indeed, the post BL change between pre- and post-exertional fatigue appeared to decrease over time from week 12 to week 168. This provides evidence that subjects did not perceive that they were exerting additional effort or “trying harder” to achieve the large improvement observed on the 6MWT.

Figure 17 Change from BL in Post-Exertional Fatigue

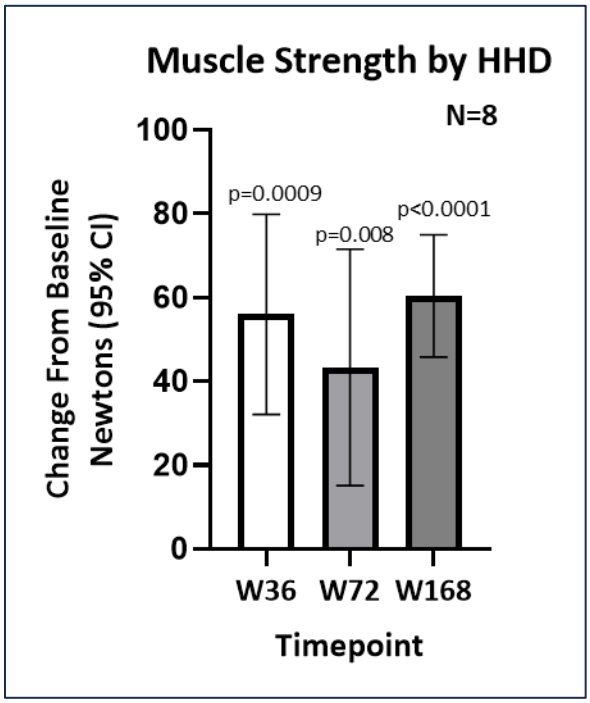


2.4.3.2.3 Improvements in Strength and Balance (Secondary Endpoints) in the TAZPOWER Extension Study

Muscular weakness, which primarily affects proximal limb muscles, has been identified as meaningfully affecting quality of life in Barth syndrome (VOP 2019). Testing of knee extensor muscle weakness by handheld dynamometry (HHD) was identified as an avenue for clinical assessment because it is impaired relative to controls in both pediatric and adult Barth syndrome patients, and, in adult patients, there is a strong negative correlation between knee extensor strength and age (Thompson 2016; Hornby 2019).

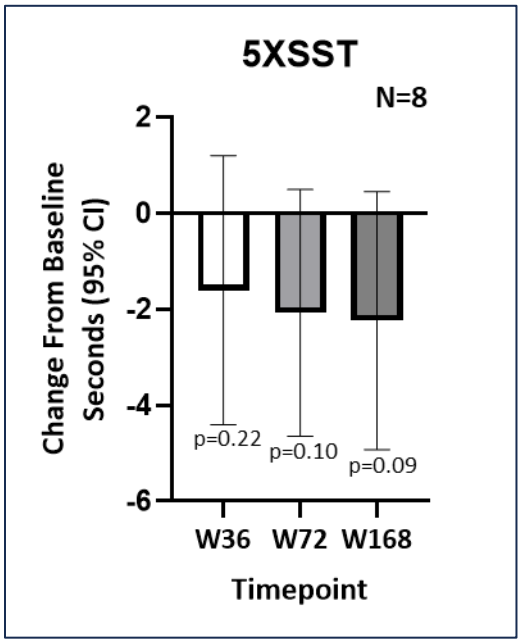
All TAZPOWER Extension study participants were extremely impaired at BL, demonstrating mean muscle strength of 126.60 (26.78 SD) newtons (about 28.5 pounds) as compared to a mean 202.5 newton (30.9 SD) reference value for 17-year-old boys (Hébert 2015). In the TAZPOWER Extension study, muscle strength by HHD (a secondary endpoint) improved by a mean 56 newtons (about 12.5 pounds) by Week 36 and by a mean 60 newtons (about 13.5 pounds) by Week 168, representing a nominally significant >45% improvement from BL (Figure 18). In the setting of DMD, the MCID has been reported as 2.1 pounds (9.34 newtons) (McDonald 2013).

Figure 18 Change from Baseline on Knee Extensor Muscle Strength Assessed by HHD



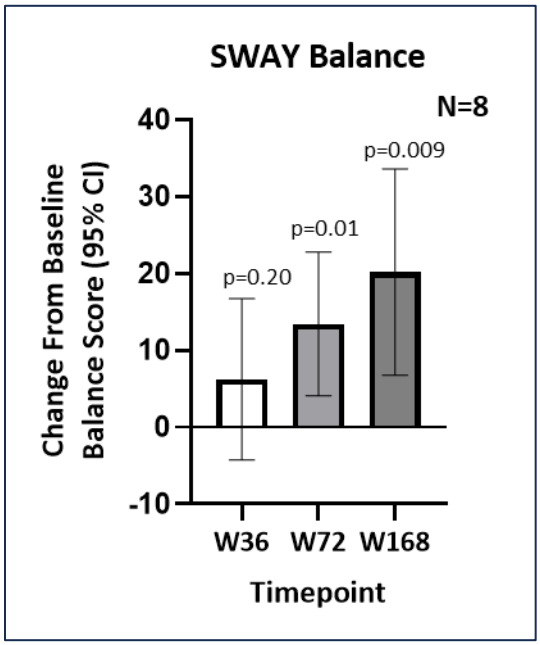
5XSST, a secondary endpoint, is also a test of proximal muscle weakness and lower extremity strength which is impaired in children and adults with Barth syndrome relative to control subjects (Hornby 2019). In this assessment, patients are asked to rise from a seated position five times in succession. This activity can be particularly challenging for individuals affected by Barth syndrome, impacting their ability to use a toilet independently, for example, or rise from a dinner table or school desk without assistance or significant effort. As expected, all TAZPOWER Extension subjects were impaired at BL, requiring a mean 12.3 (3.16 SD) seconds to complete the assessment versus the 6.5 (1.2 SD) seconds expected healthy 14–19-year-old individuals (PocketGuide). In the TAZPOWER Extension study, the time to complete the 5XSST decreased progressively over time, with >15% improvement from BL observed by Week 168, although these improvements were not significant (Figure 19). In the setting of stroke, depending on severity of ambulatory impairment, the MCID has been reported to be as low as 0.76 seconds (Martin 2021), and in the setting of COPD an MCID of 1.7 seconds has been reported (Jones 2013).

Figure 19 Change from Baseline on 5XSST



The SWAY Application Balance Assessment was developed in the setting of concussion to measure balance and motor reaction time on a scale of 0-100. This was also identified as an assessment of interest in the setting of Barth syndrome (Hornby 2019). All TAZPOWER subjects were impaired on this assessment with a mean BL score of 69.47 (20.07 SD) versus a mean 84.98 (12.51) for healthy 19-yr-old males. In the TAZPOWER Extension study, balance assessed by the SWAY Balance assessment (a secondary endpoint) improved over time (Figure 20), with >25% mean improvement from BL observed by Week 168.

Figure 20 Change from BL on Balance Measured by SWAY Application



2.4.3.3 Improvements on Endpoints Assessing How Patients Feel

As previously noted, FDA defines clinical benefit as a favorable effect on a meaningful aspect of ***how a patient feels*** (e.g., symptom relief), functions (e.g., improved mobility) or survives as a result of treatment. In the TAZPOWER Extension study (Section 2.4.3.3) and the PCPC Protocol (Section 2.4.3.3.3), ***improvements in how patients feel were observed on patient, caregiver and clinician reported assessments***.

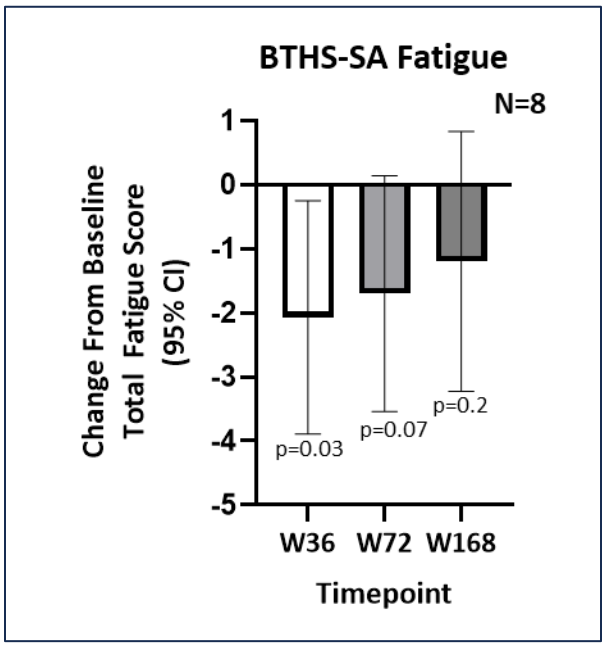
2.4.3.3.1 Fatigue Assessed by the BTHS-SA Showed Non-Significant Evidence of Improvement

As this was the first development program ever conducted in Barth syndrome, we were limited by the lack of Barth syndrome specific patient-reported outcome assessment tools. Thus, we undertook the design and validation of the BTHS-SA outcome assessment that ensured inclusion of the patient voice in the TAZPOWER and TAZPOWER Extension studies (Gwaltney 2021). During our initial interviews with patients, fatigue emerged as a symptom of primary importance. This was reiterated in the VOP 2019, where one patient explained that: “The only word in the English language that comes even close to describing how I feel is ‘depleted.’ I get so utterly exhausted that I have to really concentrate just to lift my arm,” and many participants described this “all-encompassing fatigue” as affecting virtually every aspect of their lives. The patient

community has subsequently embraced the term “Barth tired” to refer to this debilitating symptom (Reynolds 2022).

The BTHS-SA Total Fatigue scale was developed for daily assessment of muscle weakness and fatigue during activities and fatigue at rest, asking respondents to rate severity “at its worst” over the 24 hours prior to administration. This was part of the family of primary endpoints for TAZPOWER, for which powering assumptions were based on an effect size of -1.3. Although it was also assessed during the TAZPOWER Extension study, it was collected only sporadically (at trial visits or phone follow ups). Observed improvements ranged from 15% to 20% across timepoints (Figure 21).

Figure 21 Change from BL in Total Fatigue on BTHS-SA

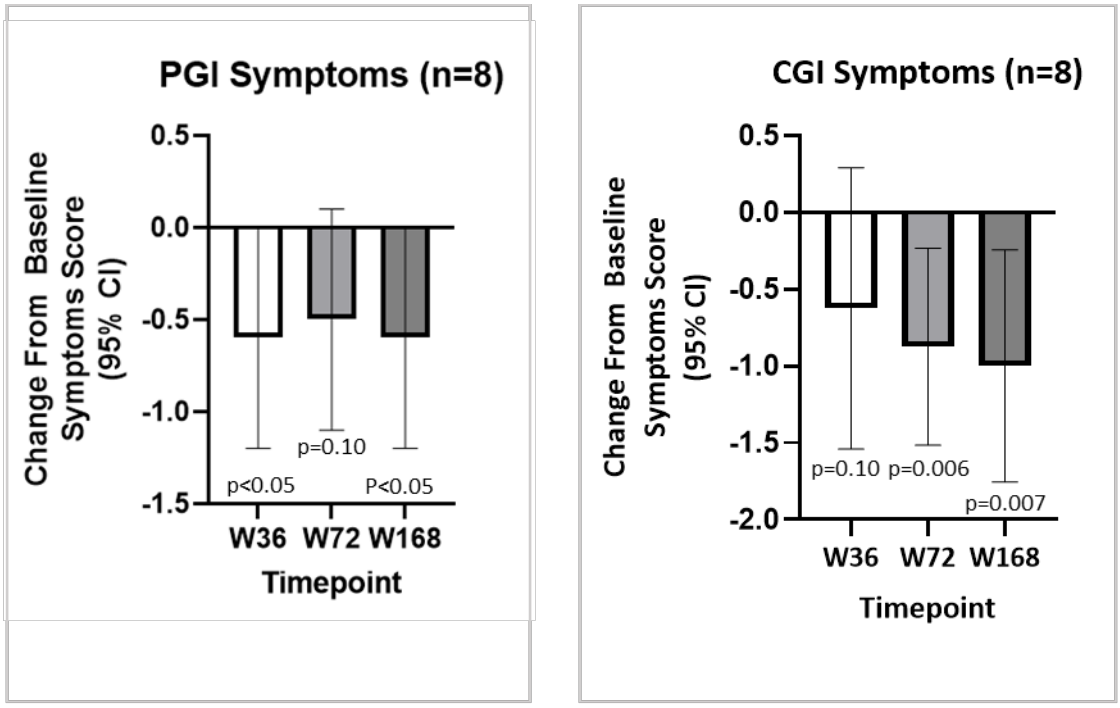


The PROMIS Fatigue short form scales are universal rather than disease-specific and assess fatigue for adolescents (Form 10a) and adults (Form 8a) over the past 7 days. Improvements were observed on the PROMIS Fatigue scales at each timepoint assessed during TAZPOWER Extension, culminating with an ~15% improvement from BL at week 168. None of the findings were significant.

2.4.3.3.2 Improvements Identified by Patient and Clinician Assessments

Patient and physician assessments of the severity of Barth syndrome symptoms were secondary endpoints. Patients assessed symptom severity on the Patient Global Impression of Symptoms Scale (PGI-S) by rating symptoms experienced over the prior week on a 0-4-point scale ranging from (0) for No Symptoms to (4) for Very Severe Symptoms. The clinician also assessed symptom severity on the Clinical Global Impression of Severity (CGI-S), by rating symptoms observed during a visit on a 0-4-point scale ranging from (0) for No Symptoms to (4) for Very Severe Symptoms (4). Both patients and the clinician reported improvements in symptom severity during TAZPOWER Extension, with PGI-S improving by >35% from BL and CGI-S improving by >70% from BL by Week 168 (Figure 22). At Week 168, the clinician assessed 5 out of 8 subjects as having no symptoms of Barth syndrome.

Figure 22 Change from BL in Clinician and Patient Reported Symptom Severity



PGI-S + CGI-S evaluate severity of symptoms on a scale of 0 (no symptoms) to 4 (very severe)

2.4.3.3.3 Patients and Caregivers Contextualized Meaningfulness of Perceived Improvements in the PCPC Protocol

The 21st Century Cures Act (PL 114-255) requires industry and FDA to incorporate patient experience data into new drug application reviews. The PCPC protocol was designed to comply with these requirements (Contesse 2019). Subsequent FDA guidance verified the utility of the approach adopted in the PCPC Protocol.[20]

All TAZPOWER subjects were invited to describe their experience during TAZPOWER study by participating in the PCPC Protocol. The study entailed a prespecified series of questions regarding patient and caregiver perception of changes, conducted while patients and caregivers remained blinded to TAZPOWER randomization. For patients who elected to continue into TAZPOWER Extension, all interviews were conducted after participants completed at least 12 weeks of TAZPOWER Extension. Patients and caregivers were invited to select a partner to videotape their response to the questions. Nine subjects and nine caregivers (representing ten subjects overall, since one caregiver participant was a father of two study participants) participated in the PCPC Protocol. An external team of two coders, who were blinded to study randomization, watched all the video interviews independently and added salient themes to a code list using open coding. The code lists were compiled to create a master codebook agreed by both coders. The coders each independently coded the transcripts then discussed and resolved all coding discrepancies. After finalizing the coding, the coders summarized the key themes in the data.

Overall, 9 of the 10 participating subjects and/or their caregivers reported improvements in energy levels (90%), stamina (90%), muscle strength (80%), appetite (70%), heat tolerance (70%), and ability to heal from wounds (60%). Some subjects also reported improvements in muscle pain (50%), fogginess (40%), ability to heal from sickness (40%), depression (30%), headaches (30%), sweating (30%), sleep quality (30%), and incontinence (20%). See **Figure 23**. These functional changes were reported to improve the daily life of most subjects, including with respect to daily life activities (90%), physical activities (90%), quality of life (80%), work/school (70%), and independence (50%). See **Figure 24**.

[20] Draft Guidance for Industry, FDA Staff and Other Stakeholders, Patient-Focused Drug Development: Methods to Identify What Is Important to Patients, February, 2022.

Figure 23 Functional Improvement Reported in PCPC Protocol

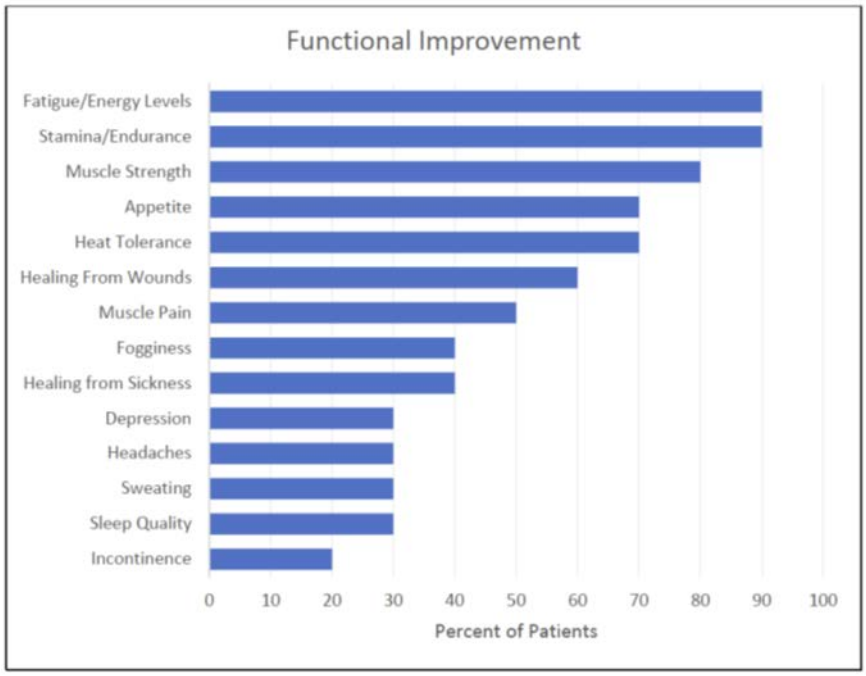
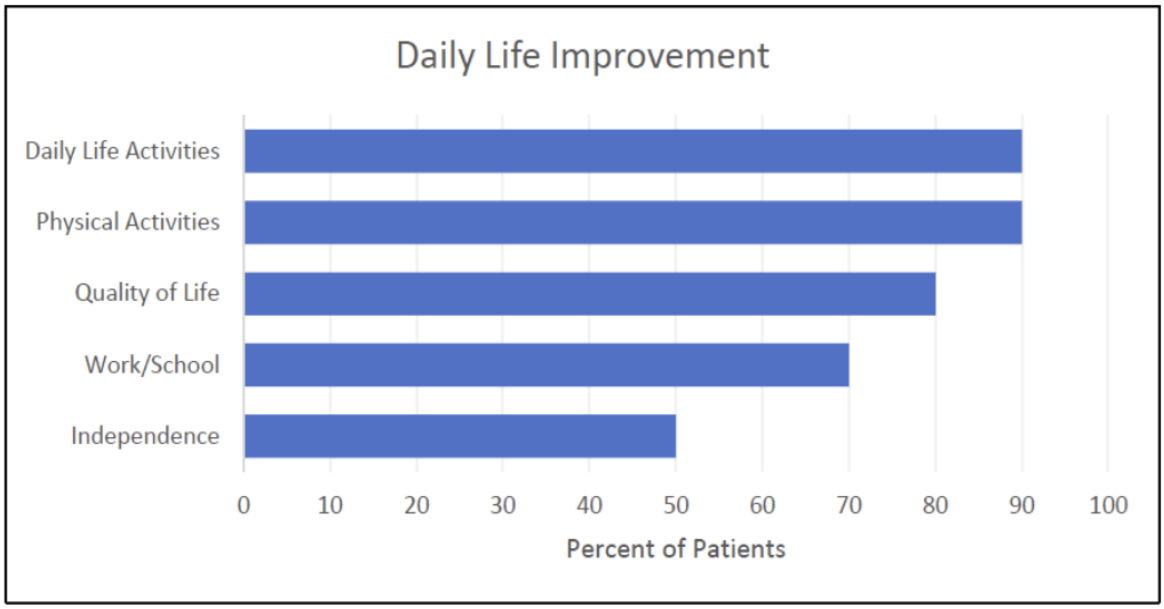


Figure 24 Daily Life Improvement Reported in PCPC Protocol



As shown in **Figure 23**, stamina/endurance was included as a coding category. The following responses by patients and/or their caregivers reflect a characterization of changes in

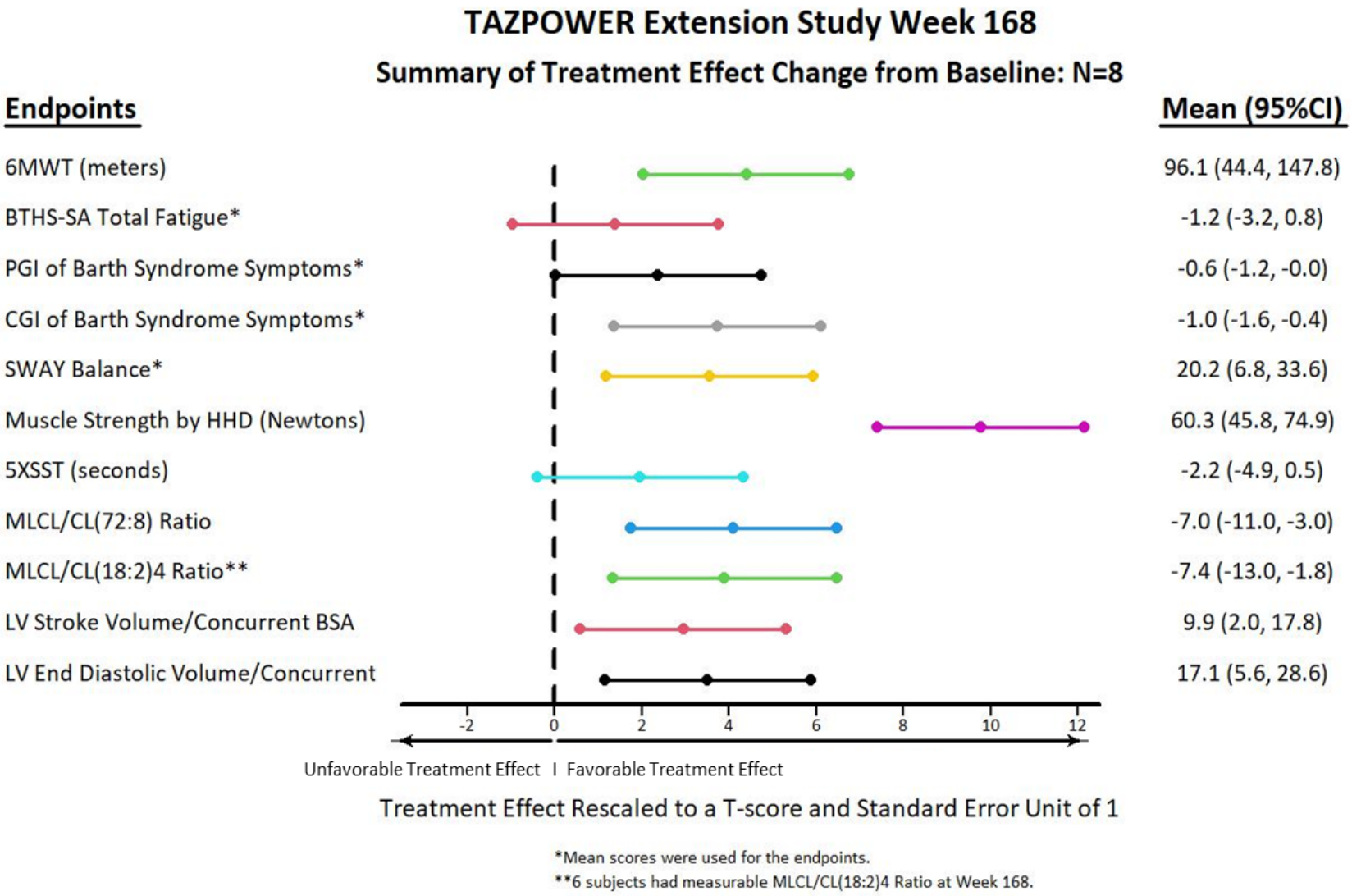
stamina/endurance for each PCPC participant: (i) Subject (b) (6) "My movement and mobility are much, much better; " (ii) Subject (b) (6) "I don't notice any change;" (iii) Caregiver for Subject (b) (6) "We can make it through the airport a lot faster when he was on the shots;" (iv) Subject (b) (6) "Now, I make it to the stop sign and back without taking any breaks. And I recover a lot faster from it, too;" (v) Subject (b) (6) "I'm able to walk a lot further without taking a break;" (vi) Subject (b) (6) "I still have to take breaks, but I think I can walk longer without taking breaks, but I still have to take breaks;" (vii) Subject (b) (6) "I can now go from one side of the school to the other side of the school without getting very tired, like I used to;" (viii) Subject (b) (6) "I am able to walk farther;" (ix) Subject (b) (6) "I can walk downtown, for example, for like two hours straight before I have to sit down. It's definitely gotten easier;" (x) Caregiver of Subject (b) (6) "He's able to take the dogs out on a normal walk which he didn't have the strength or the endurance to do before."

For further context, a short clip of the interviews of patients and caregivers who reported improvements can be viewed by clicking or typing www.stealthbt.com/poc or scanning the following QR code.

2.4.3.4 TAZPOWER Extension Provides Evidence of a Treatment Effect

The totality of the evidence from TAZPOWER Extension study demonstrates that elamipretide has a statistically significant and clinically meaningful treatment effect considering the primary and secondary endpoints assessed, as well as on pharmacodynamic endpoints assessing LV function (Section 2.6) and the pathognomonic MLCL:CL ratio (Section 2.4.3.1), as exhibited by the consistency of findings across a wide array of endpoints as shown in the Forest Plot (Figure 25). To compare all endpoints on a common scale on the Forest Plot, the treatment effect was rescaled to a T-score with a standard error unit of 1. The dot in the middle of each horizontal line represents the point estimate of the effect on the endpoint, and the dots at the ends of each line represent the confidence interval. Any horizontal line that crosses the vertical line, which signifies a null effect, represents an insignificant finding; any horizontal line wholly to the right of the null effect vertical line represents a significant finding. The Forest Plot demonstrates that a treatment effect or trend toward a treatment effect was observed on all endpoints at Week 168.

Figure 25 TAZPOWER Extension Study Efficacy Summary



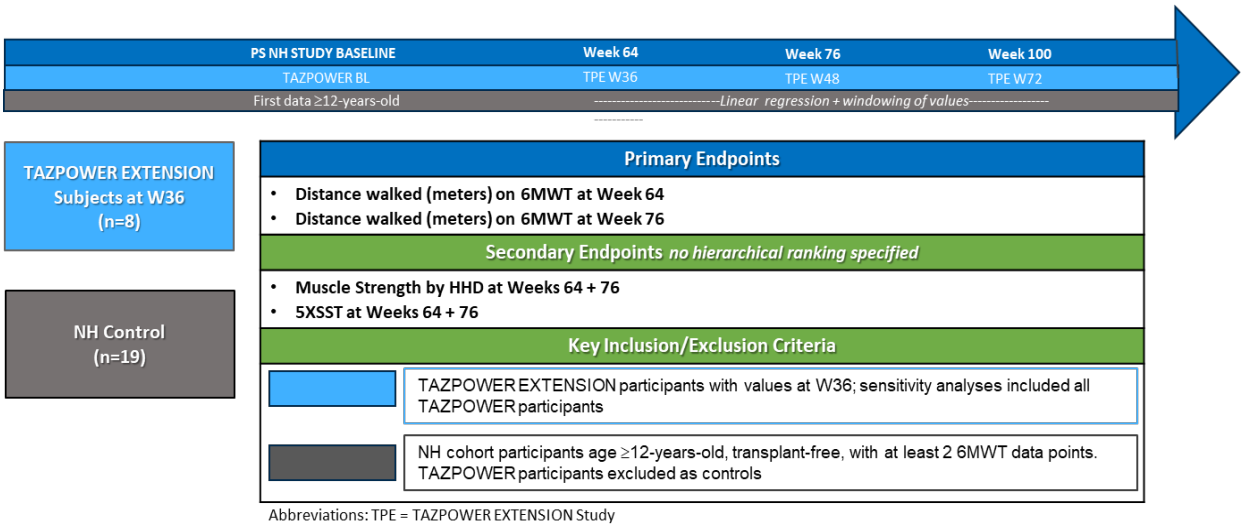
2.5 NH Control Study

To further enhance the interpretability of the efficacy observations in the TAZPOWER Extension study, Stealth identified the need to establish a control group to discriminate effects on patient outcomes between elamipretide and other influences (such as spontaneous change in the course of disease, placebo effect, or biased observations). The use of a longitudinal historical control was deemed warranted because of the persistent, progressive, and predictable nature of Barth syndrome and the infeasibility of conducting a second placebo-controlled trial in this ultra-rare disease. In accordance with FDA guidance, Stealth conducted the NH Control study to establish another external control.

2.5.1 Design

Key design features are depicted in Figure 26 and summarized in Table 5.

Figure 26 Schema of NH Control Study



Abbreviations: TPE = TAZPOWER EXTENSION Study

Table 5 Key Design Features of NH Control Study

Study Objectives
The primary objective was to assess the effectiveness of single daily subcutaneous (SC) doses of 40 mg elamipretide as a treatment for subjects ≥12-years-old with Barth syndrome compared to historical controls. The secondary objectives were to assess "Patients-As-Their-Own-Control" and describe the clinical presentation of affected patients and the course of disease.
Site Selection
The NH Control study was conducted at Johns Hopkins, which had collected and maintained the natural history database utilized in NH Control study to establish an external control.
Patient Enrollment and Inclusion Criteria
NH controls were derived from the Johns Hopkins natural history database in which there were 19 subjects who (i) were not enrolled in TAZPOWER, (ii) had 6MWT, age and height data on or after age 12, and (iii) had at least one additional 6MWT datapoint (all prespecified to be considered evaluable for inclusion into the NH analysis). For reference, the 12 TAZPOWER subjects and 19 NH subjects evaluable for the NH Control study represent >45% of the potentially eligible (≥12-years-old and transplant-free) Barth syndrome patients in the US. Baseline characteristics for an additional 20 subjects ≥12-years-old enrolled in the Johns Hopkins database but lacking longitudinal data were also evaluated to ensure lack of selection bias, bringing the total percentage of subjects characterized to ~75% of eligible affected individuals in the US. See **Figure 3**.
Design
This was a Phase 3, single center, control arm trial to prospectively evaluate the long-term efficacy of subcutaneous injections of elamipretide compared to a retrospective historical control cohort in subjects with genetically confirmed BTHS (21 CFR 314.126(b)(2)(v)). NH Control study utilized interventional data from TAZPOWER Extension study and long-term NH data from Johns Hopkins. The NH data for 79 subjects (comprising >50% of the US Barth syndrome patient population) was collected contemporaneous to TAZPOWER (between 2012 and 2019), primarily at the Johns Hopkins annual outpatient clinic or the BSF bi-annual advocacy conferences, by the same Johns Hopkins team involved in the TAZPOWER clinical trial, using substantially identical procedures with respect to the same efficacy endpoints (all important factors to minimize bias, per FDA guidance). The study employed broad and inclusive entry criteria and valid epidemiological approaches to reduce selection bias (e.g., inclusion and exclusion criteria, prespecified statistical analysis plans). Multiple, rigorous safeguards were built into the protocol to ensure that there was no bias in matching historical controls to the TAZPOWER Extension study subjects, including utilization of two statistical teams such that the team developing the prognostic match criteria remained blinded to longitudinal natural history data.
Choice of Control
Historical controls were considered appropriate because the NH of Barth syndrome is well-characterized. The NH data derives from comparable subjects who were healthy enough to travel to bi-annual BSF conferences in Florida in 2016 and 2018[21] or the annual Barth syndrome outpatient clinic conducted at Johns Hopkins for routine follow-up, where data collection occurred. Safeguards were embedded in the prognostic match methodology to ensure comparability of historical controls to TAZPOWER Extension study subjects. Subjects in the two cohorts were compared using a propensity score model for the primary and secondary efficacy endpoints. The propensity score method was used to derive the propensity scores and compute stabilized weights based on the propensity scores to balance the two cohorts and minimize the impact of selection bias on estimates of treatment differences. Logistic regression was used to compute propensity scores for eligible subjects in both cohorts using age, height, and BL 6MWT distance as BL prognostic covariates as these factors were available for most subjects in both cohorts and were considered the

[21] Subjects in attendance characterized as "healthier than the average" since they can travel (VOP 2019).

most impactful. The final propensity score model including age at baseline was selected as being the model with the best fit.

Although concomitant medications were not assessed during the propensity score model development due to limited available data in the NH database, since (i) it was the same clinical team following and evaluating these patients, (ii) both datasets were collected contemporaneously, and (iii) there are no approved or known therapies for Barth syndrome that could influence the functional testing utilized in this study, the likelihood of different underlying therapies was believed to be minimal and any impact of such differences would be expected to be negligible. All subjects in the interventional cohort (from the TAZPOWER Extension study) received at least 1 concomitant medication; in general, cardiac medications listed with a reason for use as "cardiomyopathy" or "heart failure" were initiated years before enrolling in the trial with no dose increases occurring during the trial.

Multiple sensitivity analyses were included to test for potential bias (i.e., inclusion of TAZPOWER study patients who did not participate in TAZPOWER Extension study OLE or had early termination visits, jackknife resampling, additional timepoints, "runner up" propensity model, unweighted results for analyses). In addition, for the 20 NH subjects ≥12-years-old ineligible to serve as controls due to lack of longitudinal data, baseline characteristics were evaluated to confirm lack of selection bias.

Blinding

The NH Control study was conducted following FDA Guidance to minimize potential bias (robust prognostic matching and blinding methodologies to reduce selection bias, consistency of investigators and assessment protocols and procedures, contemporaneous data collection for both historical control and interventional cohorts). To ensure the propensity scores were determined in an unbiased manner, the long-term interventional data (from the TAZPOWER Extension study) and long-term NH data and outcome reports were not available to the independent statistical team performing the propensity score modeling.

Study Endpoints

The NH Control study utilized all endpoints overlapping with TAZPOWER to minimize any perceived bias in endpoint selection. In addition, a multi-domain responder index (MDRI) was developed for this study.

- 6MWT was retained as the primary endpoint for this study to minimize any perceived bias in primary endpoint selection.
  - FDA has commented that the modest placebo effect (mean 30-meter) observed on 6MWT during the TAZPOWER study complicates interpretability of the NH Control study 6MWT finding.
  - Stealth has noted that the 30-meter placebo effect observed is well within the recognized variability of the assessment as reported in the placebo cohort of multiple heart failure trials (Olsson 2005).
- Muscle strength by HHD, 5XSST, and SWAY balance were assessed as secondary endpoints.
  - FDA has expressed no explicit concern regarding interpretability of these changes since no notable placebo or treatment effect was observed on any of these secondary endpoints during the TAZPOWER study.
  - For example, muscle strength by HHD improved by only 4.7 newtons (1 pound) with elamipretide treatment and 6.15 newtons (1.4 pounds) with placebo treatment. Time to complete the 5XSST increased (worsened) for both groups (by one second with elamipretide treatment and a half-second with placebo treatment).
- In addition, a MDRI was developed for first-time use in this study. The MDRI utilized a Minimally Clinically Important Difference (MCID) "Responder Definition" which defined responses based on a clinically meaningful change in the individual domain (10% for each domain) scored +1 (-1) for a best (worst) score of +4 (-4).
- The Division of Rare Disease and Medical Genetics recommended a post-hoc analysis of LV volumes in this study (Type C Meeting, March 31, 2020). Accordingly for week 100 (corresponding to week 72 of TAZPOWER Extension study), two-dimensional (2 D) and three-dimensional (3 D) echocardiographic measurements of LV-SVI, LV-EDVI and LV-ESVI were assessed.

Statistical Methods

BL for subjects in the NH cohort was the first record available at age ≥12 years. BL for subjects in the elamipretide-treated cohort was the TAZPOWER pre-dose (Visit 2) value or, if missing, the Screening (Visit 1)

value. To capture the "change from BL" at week 36, week 48 and week 72 (post-hoc sensitivity analysis) in the TAZPOWER Extension study OLE, the corresponding timeframe of interest in the NH cohort of week 64, week 76 and week 100 were used for the evaluation, respectively. With respect to the testing of the secondary endpoints, although no hierarchical testing strategy was specified, utilizing the Bonferroni adjustment would result in a finding of significance for the 6MWT, Muscle strength by HHD and the MDRI at the two timepoints of primary interest (week 64 and week 76).

Sensitivity Analyses

Sensitivity analyses were performed to assess the robustness of the observed results. These included (i) analysis of additional time points at including weeks 40, 52 and 100 (corresponding to weeks 12, 24 and 72 of TAZPOWER Extension study OLE); (ii) analysis using a second-best or "runner-up" propensity model as determined by an independent statistical team; (iii) using unweighted results; (iv) evaluation of treatment effects for discontinued treated subjects (n=4) using observed rather than interpolated values using the best-fit line of regression and with and without the subjects who elected not to participate in (n=2) or discontinued early from (n=2) TAZPOWER Extension study; and (v) a jackknife resampling analysis, conducted to confirm the impact of variance and ensure overall response was not driven by any "super-responders". All these analyses were supportive of the primary findings. In addition, during NDA review, an analysis of baseline characteristics for the 20 ≥12-year-old subjects without longitudinal data from the Johns Hopkins dataset was conducted to confirm that no inherent differences in baseline characteristics might contribute to potential selection bias.

Duration

The primary timepoint of interest was 64 and 76 weeks of elamipretide exposure, in the elamipretide-treated group (corresponding to weeks 36 and 48, respectively, of the TAZPOWER Extension study, see Table 6), versus the same course of time in the historical control group. A post-hoc sensitivity analysis conducted at FDA's request included an additional timepoint at 100 weeks of elamipretide exposure (corresponding to week 72 of the TAZPOWER Extension study); in addition, week 40 and 52 (corresponding to weeks 12 and 24 of the TAZPOWER Extension study) were assessed.

Table 6 Guide to Timepoints in the TAZPOWER Extension and NH Control Studies

NH Control study	W 40	W 52	W 64	W 76	W 100
TAZPOWER Extension study	W 12	W 24	W 36	W 48	W 72

2.5.2 NH Control Study Selection of Controls and Comparability of BL Demographics

The NH Control study was a Phase 3 externally controlled clinical investigation conducted to establish a control for the TAZPOWER Extension study data. The interventional dataset was drawn from the TAZPOWER Extension study. The NH controls (n=19) were prognostically matched from the Johns Hopkins NH dataset. The 31 total evaluable subjects (12 from TAZPOWER; 19 from NH) together represented a majority (>45%) of the 66 potentially eligible (≥12-years-old and transplant-free) Barth syndrome patients in the US.

An important consideration when using an external control is assuring comparability of patients receiving the investigational drug to those in the control group. The 19 NH controls derived from 39 eligible subjects ≥12-years-old with data entries in a 79-subject Johns Hopkins NH dataset

(**Figure 3**). Although 20 of these 39 subjects were ineligible to serve as NH controls due to lack of longitudinal data, the independent statistical teams and reviewing statistician, Dr. Janet Wittes, evaluated the overall NH dataset, including all subjects ≥12-years-old, to confirm that the patients excluded as potential controls did not have systematically different characteristics from those included. We further characterized the comparability of demographic and functional assessments for all subjects ≥12-years-old during the NDA review (Table 7).

Table 7 Baseline Demographics of Elam, NH, and Excluded NH Subjects

Covariate	Treatment Cohort		Excluded NH subjects ≥12-years-old (N=22**) with only 1 datapoint
	Elamipretide-treated TRTS (N = 8)	Historical control UNTS (N = 19*)	
Age at BL Mean (SD) Median Min, Max	18.3 (5.02) 17.4 12.9, 28.7	21.0 (5.46) 21.2 12.0, 32.6	16.55 (4.48) 14.9 12.2, 31.1
Height at BL (cm) Mean (SD) Median Min, Max	166.6 (12.43) 167.1 152.5, 180.8	168.6 (14.25) 168.8 121.3, 186.0	156.5 (14.56) 156.25 137.0, 187.0
Weight at BL (kg) **Mean (SD)** **Median** **Min, Max**	49.4 (16.28) 43.8 33.0, 74.5	57.0 (17.82) 58.4 21.1, 92.2	46.71 (16.8) 41.6 25.7, 80.0
BMI at BL (kg/m^2) **Mean (SD)** **Median** **Min, Max**	17.4 (3.57) 15.6 13.8, 23.0	19.42 (4.41) 18.41 13.8, 28.1	18.6 (4.34) 17.26 12.6, 26.7
BSA at BL **Mean (SD)** **Median** **Min, Max**	1.5 (0.30) 1.4 1.2, 1.9	1.60 (0.31) 1.7 0.8, 2.2	1.41 (0.31) 1.34 1.0, 2.0
BL 6MWT Mean (SD) Median Min, Max	381.88 (64.18) 359.50 313.0, 495.0	394.9 (75.22) 403.6 267, 536	412.3 (66.51) 405.0 299, 532
BL HHD **Mean (SD)** **Median** **Min, Max**	132.1 (26.5) 132.89 79.62, 159.24	151.8 (52.3) 152.4 53.4, 241.8	127.27 (54.66) 113.76 58.5, 291.5
BL 5XSST Mean (SD)	12.89 (2.86)	11.18 (3.03)	8.32 (2.52)

Covariate	Treatment Cohort		Excluded NH subjects ≥12-years-old (N=22**) with only 1 datapoint
	Elamipretide-treated TRTS (N = 8)	Historical control UNTS (N = 19*)	
Median Min, Max	13.18 9.18, 16.65	11.8 6.6, 12.3	7.22 5.3, 15.0
BL SWAY Mean (SD) Median Min, Max	 70.9 (19.51) 76.01 45.4, 90.3	 68.7 (20.7) 73 17, 89	 73.1 (19.54) 76.8 42, 99

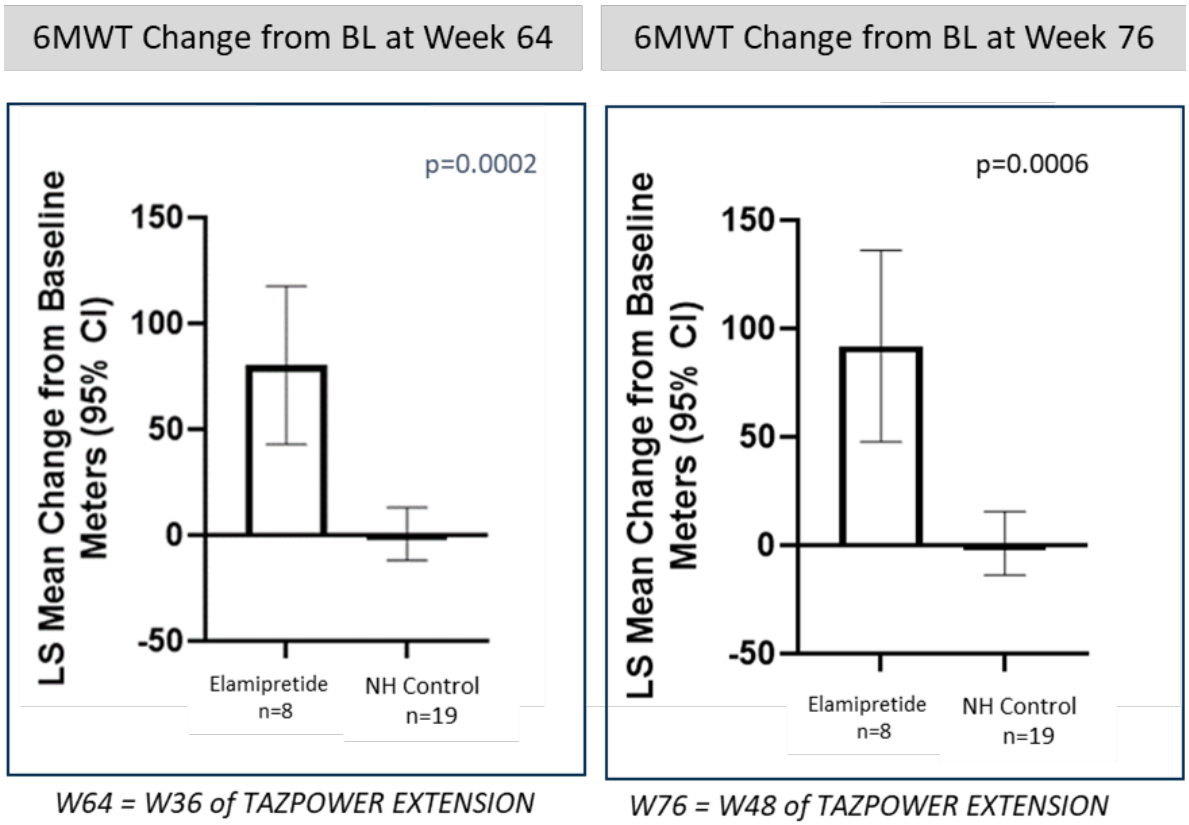
* n=18 for SWAY,

** Includes 3 TAZPOWER subjects; n=20 for HHD, n=13 for 5XSST, n=12 for SWAY

2.5.3 Efficacy Observed on Primary Endpoint

The NH Control study met its primary endpoint, distance walked on the 6MWT, at both timepoints of primary interest (weeks 64 and 76, corresponding to weeks 36 and 48 of the TAZPOWER Extension study) (Figure 27). An FDA-requested post-hoc analysis of week 100, corresponding to week 72 of the TAZPOWER Extension study, also showed benefit (116.92-meter increase from baseline for elamipretide-treated subjects vs. 1.73-meter increase for NH-control subjects, p=0.0003).

Figure 27 Elamipretide Improved Distance Walked on 6MWT Relative to NH Controls

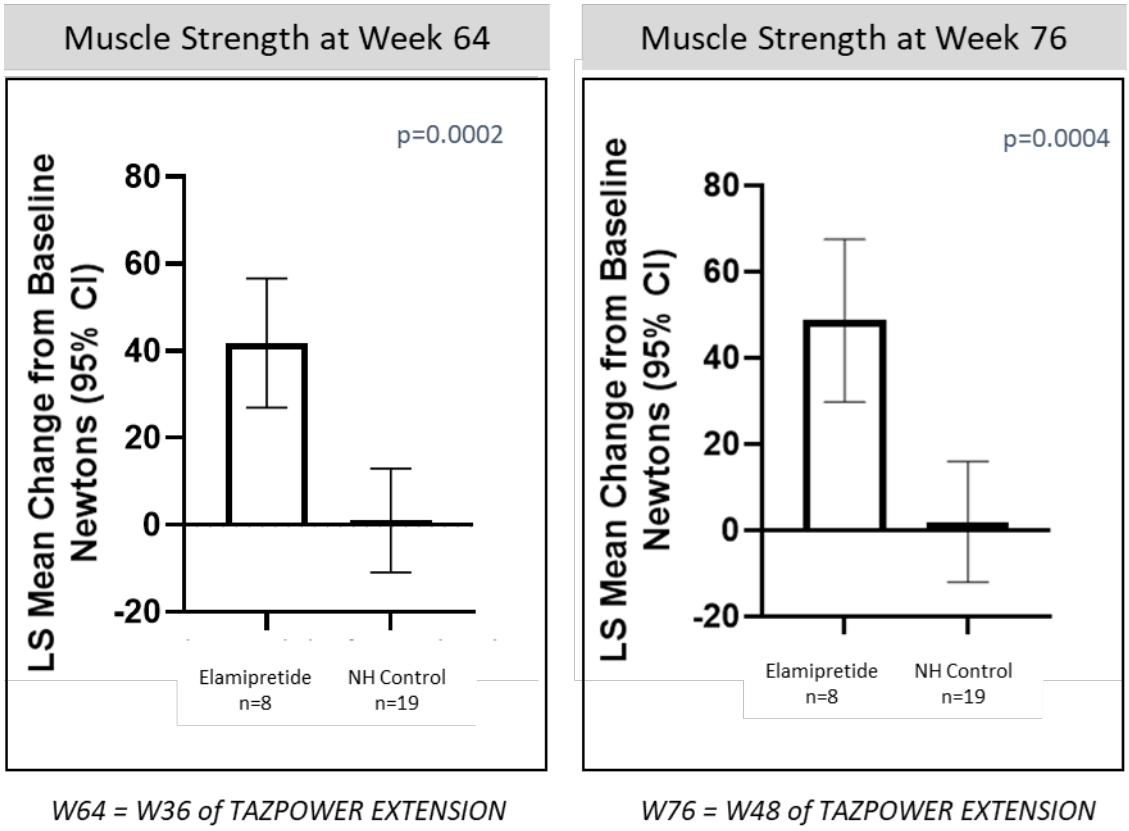


2.5.4 Efficacy Observed on Most Secondary Endpoints

Most secondary endpoints were also met at multiple timepoints assessed. As no hierarchical testing of secondary endpoints was specified, we applied a Bonferroni analysis which protects endpoints achieving significance at $p \leq 0.005$. This resulted in a finding of significance for 6MWT, Muscle Strength and the MDRI at the timepoints of primary interest (week 64 and week 76).

Muscle strength assessed by HHD improved at both timepoints of primary interest (Figure 28). An FDA-requested post-hoc analysis of week 100, corresponding to week 72 of the TAZPOWER Extension study, also showed benefit (change from baseline 62.07 newtons for elamipretide-treated subjects vs. 3.89 newtons for NH-control subjects, p=0.0002).

Figure 28 Muscle Strength Improved Relative to NH Controls



W64 = W36 of TAZPOWER EXTENSION

W76 = W48 of TAZPOWER EXTENSION

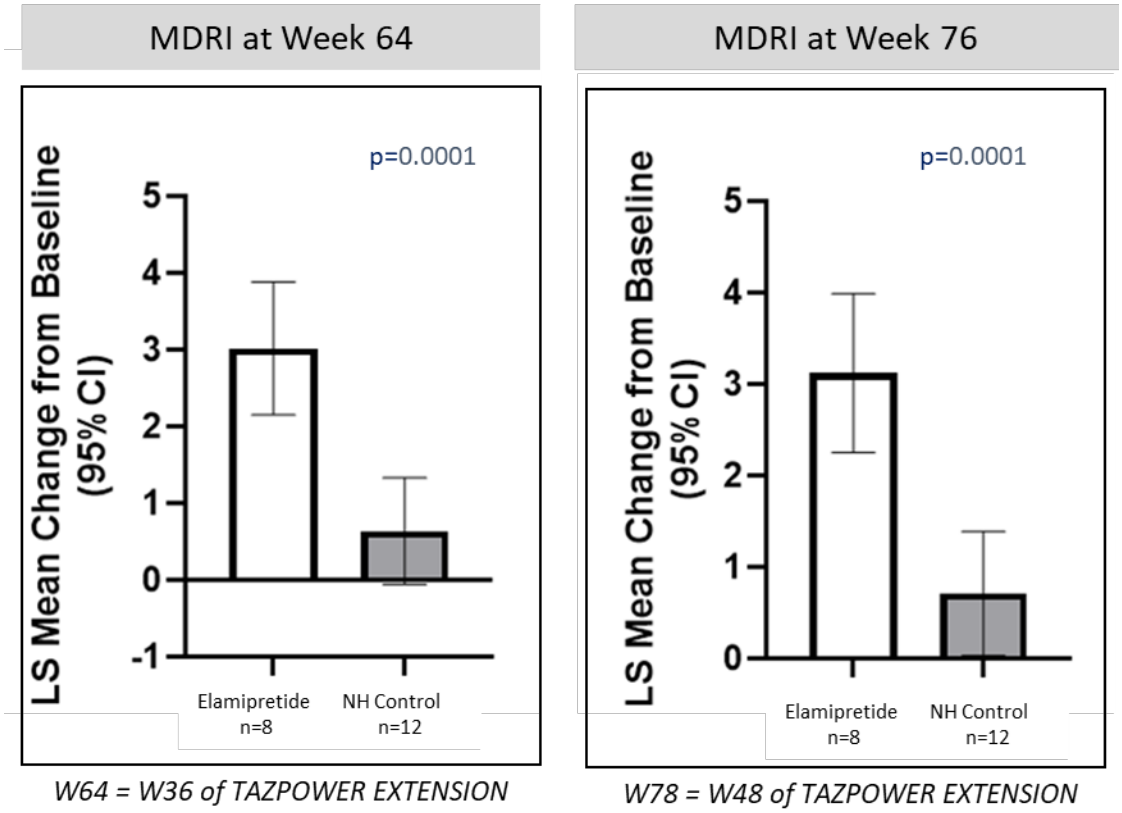
Like Barth syndrome, many diseases have multiple sequelae, and an effect demonstrated on any one of these aspects (such as 6MWT or Muscle strength) could support a conclusion of effectiveness. FDA also recognizes the utility of multi-component endpoints as a within-subject combination of two or more components under which an individual subject's evaluation is dependent upon observation of all the specified components in that subject. FDA recognizes that the use of within-subject multi-component endpoints may be efficient if the treatment effects on the different components are generally trending in the same direction within a subject.[22]

[22] FDA Guidance for Industry: Multiple Endpoints in Clinical Trials, October 2022.

The Multidomain Responder Index (MDRI) was designed to assess subjects' health across multiple disease-relevant assessments. The MDRI prespecified a minimally clinically important difference (MCID) as 10% for each of 4 domains (6MWT, muscle strength, 5XSST and balance). This was based primarily on literature references for 6MWT, where an MCID of ~30-meters has been established across multiple disease settings (Holland 2009, Puhan 2008, Shoemaker 2013, McDonald 2013). Although other endpoints utilized historically in monitoring the progression of Barth syndrome (Thompson 2016; Hornby 2019) have been less well characterized, across multiple studies with different outcome measures a universally applicable rule of thumb that the MCID is equal to 0.5 of the standard deviation has been reported (Draak 2019), which translated to 10% of elamipretide-treated subjects' BL values. The possible score ranged from -4 (worst – subjects declined by ≥10% on all 4 domains) to 4 (best – subjects improved by ≥10% on all domains).

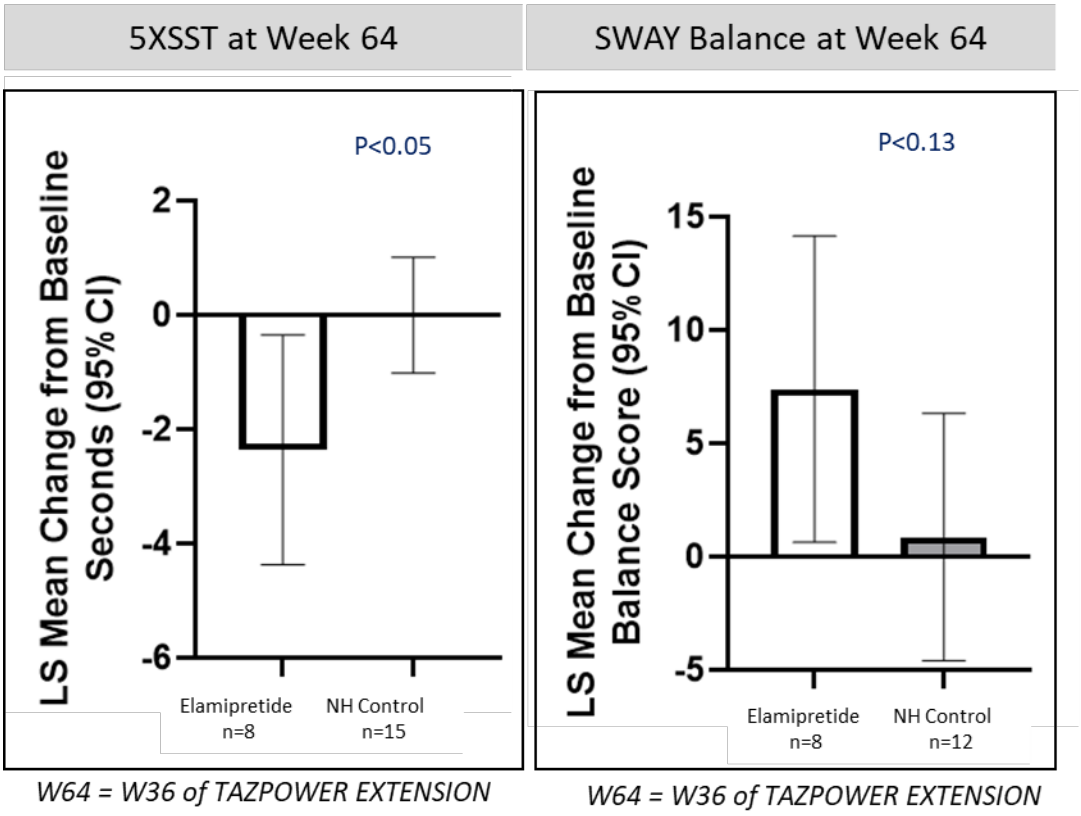
The MDRI improved at both timepoints of primary interest (Figure 29). The FDA-requested post-hoc analysis of week 100, corresponding to week 72 of TAZPOWER extension, was also significant with a between-group least squares mean difference of 2.41 favoring elamipretide (p=0.0001).

Figure 29 Multidomain Responder Index Improved Relative to NH Controls



Improvements were observed on the 5XSST and SWAY balance at the timepoints of primary interest (Figure 30 shows week 64), though these did not reach significance at p≤0.005. A favorable change was also observed on 5XSST (i) at week 78, with a -2.76 second reduction on elamipretide versus -0.004 for NH controls (p=0.04) and (ii) at week 100 (post-hoc), with a -3.6 second reduction on elamipretide versus -0.37 on NH controls (p=0.008). A favorable change was also observed on SWAY balance (i) at week 78, with a 8.69 improvement on elamipretide versus 1.11 for NH controls (p=0.12) and (ii) at week 100 (post-hoc), with a 12.16 improvement on elamipretide versus 0.24 for NH controls (p=0.03).

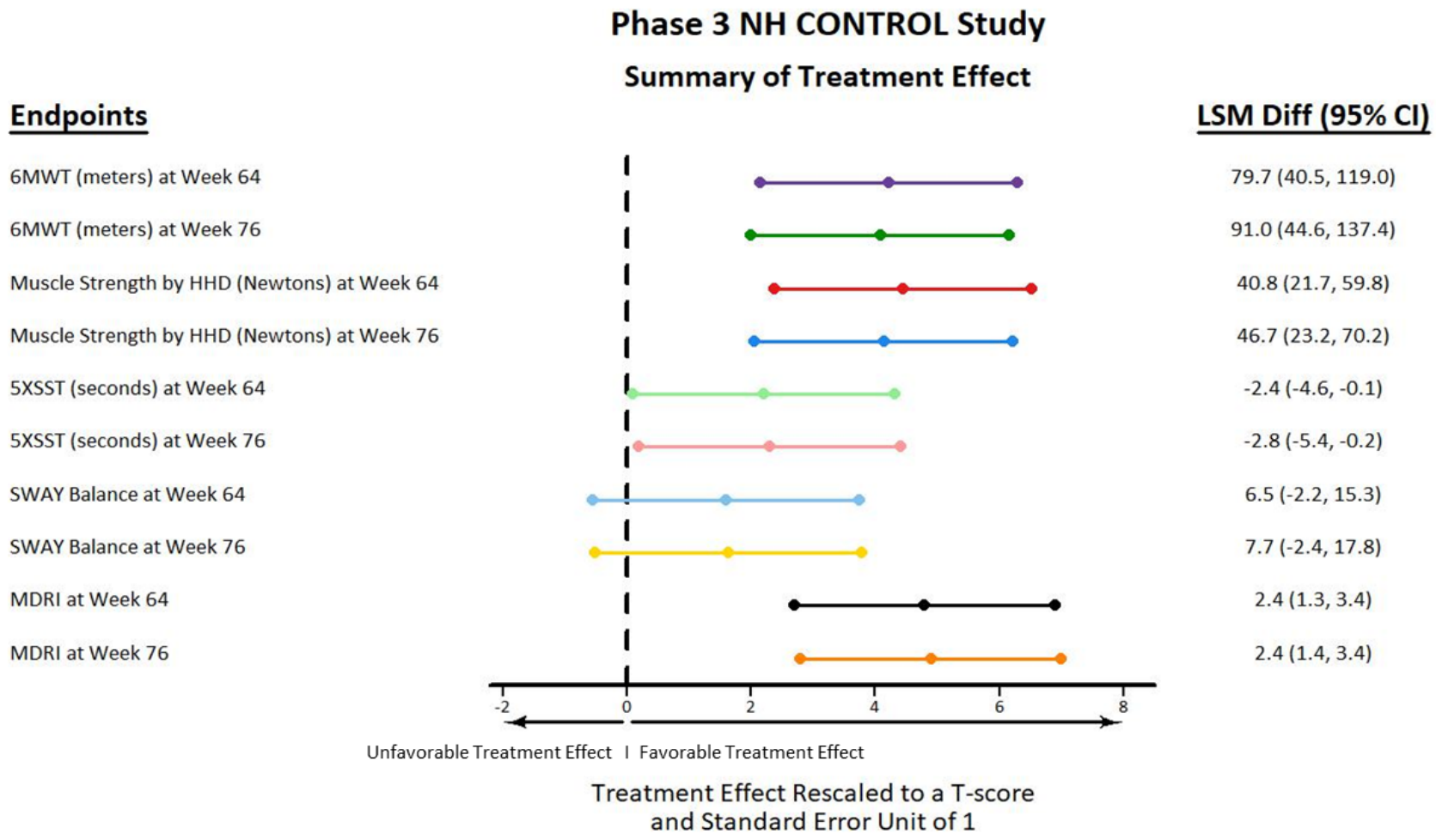
Figure 30 Trends Toward Improvement in 5XSST and Balance



2.5.5 NH Control Study Provides Evidence of a Treatment Effect

The NH Control study demonstrates that elamipretide has a clinically meaningful and statistically significant treatment effect on all primary and most secondary endpoints assessed. This is underscored by the consistency of findings shown in the Forest Plot below (Figure 31).

Figure 31 NH Control Study Efficacy Summary



2.6 Cardiac Physiological Findings

FDA recently announced its establishment of the Rare Disease Innovation Hub ("Rare Hub") to enhance and advance outcomes for patients living with ultra-rare diseases such as Barth syndrome. In doing so, FDA acknowledged that the natural history can be variable and not fully understood for certain ultra-rare diseases, such that development of therapies for these conditions can be particularly challenging. Our discovery of an intermediate cardiac phenotype in Barth syndrome, as subsequently confirmed by an in-depth review of the cardiac NH data, illustrates that the cardiac NH for the middle years of Barth syndrome was not previously well-understood.

Prior to the conduct of the TAZPOWER study, the cardiac NH of Barth syndrome was informed by studies of infant mortality in the disease, thus describing the predominantly dilated pediatric phenotype that is associated with an astonishingly high rate of cardiac mortality between birth and age 5. This high-risk period was reported to be followed by an inexplicable cardiac "honeymoon period" between age 5 and age 12 (pre-pubescence), during which cardiac function "normalized." Heart failure was known to "re-emerge" shortly after, during the teenage years, likely exacerbated by the metabolic stress of growth. Most young adult deaths are also cardiac-related, with reported reemergence of dilated cardiomyopathy or development of conduction defects.

TAZPOWER shed an important light on an intermediate cardiac phenotype that may more accurately characterize the middle years of Barth syndrome. Although most TAZPOWER study participants presented with diastolic dysfunction in infancy,[23] upon enrollment in TAZPOWER, at the mean age of 19.5-years-old, these subjects demonstrated a non-obstructive hypertrophic-like phenotype characteristic of most mitochondrial cardiomyopathies (Arbustini 2018; Braunwald 2023). This finding is supported by an in-depth review of external cardiac NH data.

[23] Nine of the twelve TAZPOWER subjects were diagnosed with cardiomyopathy in their first 18-months of life, six have a history of heart failure, and all twelve have a history of cardiac abnormalities, in addition to clinical symptoms typically associated with heart failure, including skeletal muscle issues (hypotonia, fatigue, muscle weakness, nocturnal enuresis), feeding and nutrition issues (failure to thrive, malnutrition, reduced appetite, gastritis, dyspepsia, pyloric stenosis, G-tube), growth issues (growth hormone deficiency, short stature, headaches or migraines, and neutropenia.

Cardiac assessments of LV volumes were followed mainly for safety during the TAZPOWER study, and were collected via echocardiogram rather than cardiac MRI so that the ~28% of the teenage and young adult affected population with implantable cardioverter-defibrillators would not be excluded. These assessments were, however, of particular interest in the TAZPOWER Extension study, as discussed with FDA as early as 2019 (although the statistical analysis plan was not formally amended), because LV volumes assessed by 3-D echo improved for 10 out of 12 subjects following elamipretide treatment in TAZPOWER, and these improvements did not appear to decrease following withdrawal of therapy. This observation provoked an interrogation of baseline values which revealed that LV-SVI and LV-EDV were extremely low in all trial participants relative to age-matched published standards/z-scores.

At the TAZPOWER study screening and BL visits, the treating cardiologist assessed heart function as within normal range for all study participants based on normal EF. After observing small LV volume changes for 10 out of 12 TAZPOWER study participants after exposure to elamipretide (most notably, small increases in LV EDV), Stealth ascertained that 3-D echocardiographic z-scores[24] for LV volumes, in particular LV EDV, were extremely low for most subjects. This resulted in impaired cardiac function, with LV-SVI z-scores at least two standard deviations from normal in most subjects and a mean BL cardiac index (echo derived) of 2.3L/min/m^2 (approximately 45% below normal for that age group) (Table 8).

Table 8 TAZPOWER BL 3D Cardiac Parameters: Normal EF + Reduced LV Volumes Resulted in Low LV SVI

Subject (b) (6)	Age	Ejection Fraction	LV SV Indexed	LV SVI z-score*	Z-score percentile
	17.0	60.0	36.7	0.32	63rd
	35.0	64.0	21.4	-4.81	< 2nd
	16.0	57.0	28.4	-1.02	15th
	17.0	61.0	15.3	-2.25	2nd
	28.0	68.0	27.2	-2.80	2nd
	13.0	66.0	30.0	-2.59	< 2nd
	12.0	66.0	33.3	-0.42	34th
	31.0	60.0	21.7	-3.74	< 2nd
	16.0	61.0	30.0	-0.41	34th
	22.0	56.0	31.8	-2.23	2nd

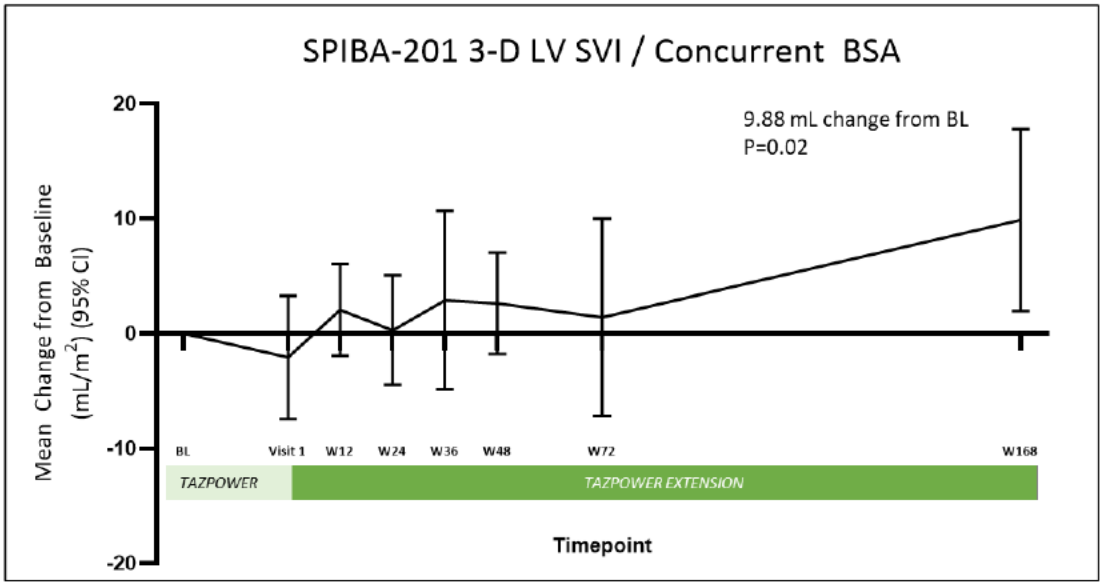
[24] Z-scores scores play a crucial role in pediatric practice, where due to somatic growth: a single reference range cannot be applied across patients of vastly different sizes and ages. Z scores express how many SD above (positive values) or below (negative values) a given measurement lies with respect to the size-specific mean.

(b) (6)					
	14.0	67.0	30.8	-0.73	23rd
	14.0	63.0	25.8	-2.51	2nd

Subsequent work to characterize the natural history of cardiac progression in older (≥12-years-old) subjects with Barth syndrome led to characterization of a hypertrophic-like intermediate cardiac phenotype (see Section 2.1.2.2).

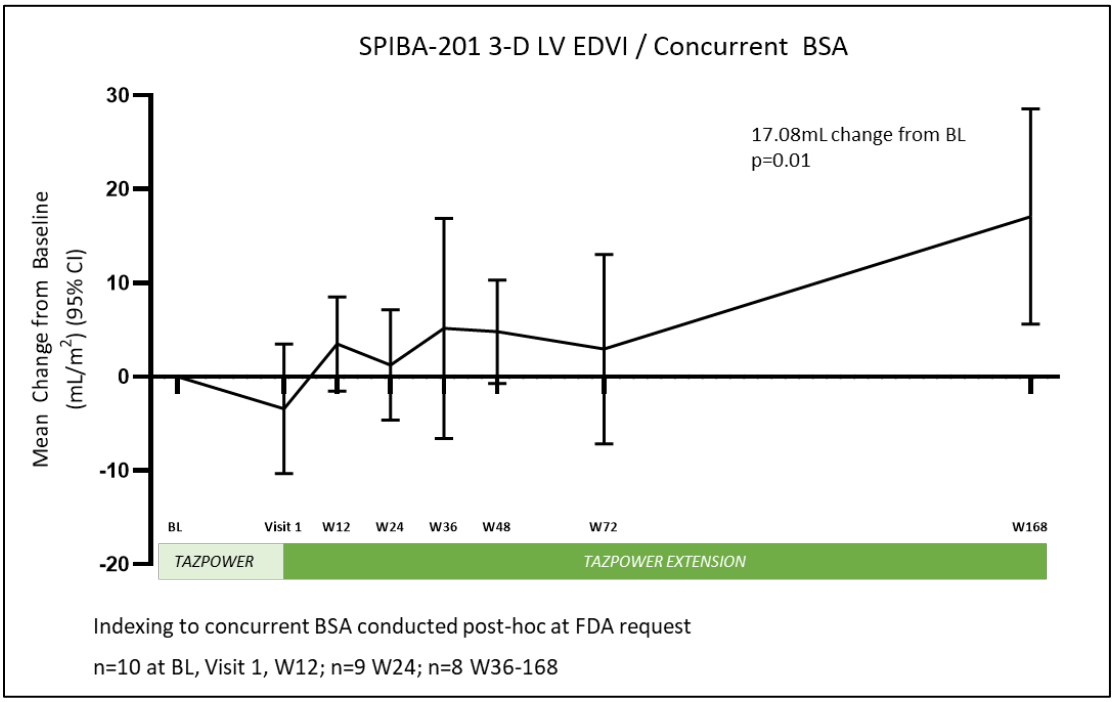
Incremental changes were observed over time during the TAZPOWER Extension study, culminating in a mean >45% improvement from BL (the prespecified analysis) in LV-EDVI and LV-SVI, which were nominally significant. Sensitivity analyses assessing LV z-scores based on concurrent BSA (conducted at FDA request) demonstrate normalization of mean LV-SV (from -1.38 at BL to -0.26 at week 168) (Figure 32) and LV-EDV (from -1.59 at BL to –0.46 at week 168) by week 168 (Figure 33). Notably, all subjects received at least 1 concomitant medication; however, cardiac medications listed with a reason for use as "cardiomyopathy" or "heart failure" were initiated years before enrolling in the trial with no dose increases occurring during the trial

Figure 32 TAZPOWER Extension Study 3D LV-SVI Concurrent BSA



Indexed to concurrent BSA post-hoc at FDA request; pre-specified analysis indexed to BL BSA
n=10 BL, Visit 1, W12; n=9 W24; n=8 W36-168

Figure 33 TAZPOWER Extension Study 3D LV-EDVI Concurrent BSA



A weak association between improvements in LV-SVI and LV-EDVI, on the one hand, and 6MWT and Muscle strength by HHD, on the other, appeared to peak at week 72 of the TAZPOWER Extension (LV- SVI:6MWT r = 0.52, p=0.18; LV-SVI:HHD r=0.48, p=0.23; LV-EDVI:6MWT r=0.64, p=0.09; LV-EDVI:HHD r=0.62, p=0.10). Stealth's evaluation of the CARDIOMAN dataset corroborated the relationship between LV volumes and exercise capacity, with changes in LV-SVI and LV-EDVI correlated with achieved work rate (r=0.77 and 0.76; p=<0.05 for both) (all p values nominal). It is noted that cardiac dysfunction is believed to contribute only partially to exercise intolerance in Barth syndrome, since mitochondria in the muscle system are also dysfunctional (Spencer 2011).

No other parameters of cardiac function appeared abnormal at study BL, and no other meaningful changes in cardiac parameters were observed during TAZPOWER or TAZPOWER Extension (Table 9**).**

Table 9 Overview of Cardiac Parameters Assessed During TAZPOWER Extension

Parameter*	Unit	TAZPOWER BL Mean (SD)	TAZPOWER Extension W168 Mean (SD)
(3D) LV EDV z-score		-2.07 (1.36)	-1.16 (2.16)
(3D) LV ESV z-score		-1.84 (0.97)	-1.20 (1.74)
(3D) LV SV z-score		-1.93 (1.52)	-0.86 (0.53)
(2D) LV mass z-score		-0.37 (0.90)	-0.10 (0.59)
(2D) FS z-score		-0.09 (1.25)	-0.90 (0.78)
(3D) LV EF	%	61.12 (4.19)	61.9 (3.40)
(2D) GLS triplane	%	-19.82 (1.69)	-18.99 (2.03)
HS-Troponin 1	ug/L	0.01 (0.007)	0.009 (0.00)
NT pro-BNP	pmol/L	12.18 (6.27)	15.96 (15.28)

* NOTE: LV volume analysis n=12 at BL, n=2 at TAZPOWER final visit, n=2 at early termination visit, n=8 at TAZPOWER Extension W168; all other parameters shown are n=10 at BL, n=8 at W168

To further interrogate the robustness of the cardiac findings, we engaged Cardiovascular Clinical Sciences (Tufts) to perform an independent overread to evaluate the reproducibility of echocardiographic measurements of left ventricular parameters. Overall, 77 echocardiograms were evaluated across all visits for the 12 patients. Inter-observer variability and correlations were calculated for LVEDV, LVESV, LVSV, and LVEF.

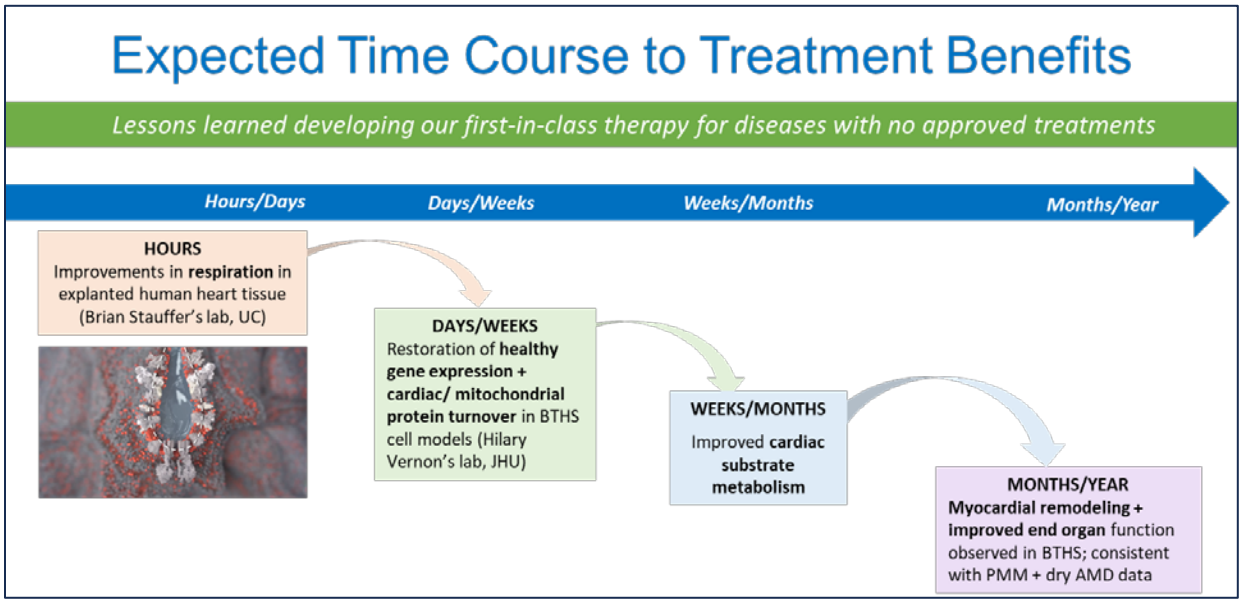
The correlation between the echocardiographic measurements for LVEDV, LVESV, and LVSV was strong and statistically significant, with Pearson correlation coefficients of 0.8193, 0.8171, and 0.8088, respectively, and p-values less than 0.0001 for each measure. This indicates a high degree of correlation between the two core labs in measuring these parameters. The correlation for LVEF was weaker, with a Pearson correlation coefficient of 0.0513 and a p-value of 0.6580. Although the weaker correlation coefficient suggests less agreement between the two LVEF measurements, it was noted that the LVEF results are all within the normal range of that parameter, thus the range of values is much narrower than the other parameters such that a correlation would be more challenging to detect. Overall, the findings indicate that there is a small difference between the two core labs for all parameters, suggesting good agreement and reproducibility between readers.

FDA recommended conducting a post-hoc analysis as part of the NH Control study comparing TAZPOWER Extension study cardiac findings to NH controls at week 100 (corresponding to week 72 of TAZPOWER Extension). The analysis demonstrated a decline in LV SV in the historical control subjects (n=12 with longitudinal data) versus an improvement observed in elamipretide-treated subjects. Given the sparse longitudinal NH cardiac data, mixed availability of 2-D and 3-D echocardiograms in the NH data, and noting that matching parameters were non-cardiac in nature, the utility of this analysis is limited. It is, however, consistent with the literature which describes an expected decline in LV volumes (LV EDV and ESV) and associated decline in LV SV with age (Chowdhury 2022; Sabbah 2023).

Overall, the data points to improvements in LV function, which was substantially impaired at BL, consistent with a progressive decline expected in older patients with the disease. These improvements are consistent with nonclinical effects of elamipretide observed in models of Barth syndrome and other diseases of cardiac dysfunction (see Section 2.2.2). These improvements in cardiac function likely contributed to functional recovery in elamipretide-treated patients.

As previously described, we now know that the cardiac phenotype for older boys and young men with Barth syndrome is a hypertrophic or HFpEF-like phenotype without overt diastolic dysfunction, consistent with most mitochondrial myopathies (Braunwald 2023; Arbustini 2018). Much of the current scientific understanding of the cardiac phenotype in this age range derived from our findings in TAZPOWER, as subsequently supported by extensive evaluation of the cardiac NH of the disease and related mitochondrial cardiomyopathies. Moreover, these findings in TAZPOWER and TAZPOWER Extension have critically informed our perspective on the expected time course to elamipretide treatment benefits in patients with mitochondrial cardiomyopathies (Sabbah 2022) (Figure 34).

Figure 34 Expected Time Course to Elamipretide Treatment Benefits



2.7 Expanded Access Case Study

Expanded access (compassionate use) is a pathway (EAP) recognized by FDA for a patient with a serious or immediately life-threatening disease or condition to gain access to an investigational medical product outside of clinical trials when no comparable or satisfactory alternative therapy options are available and provided that the FDA is satisfied that the potential patient benefit justifies the potential risks of treatment.

Stealth opened an intermediate expanded access protocol (IEAP) in 2021 to ensure continued provision of therapy to the TAZPOWER Extension study subjects following study close-out. In addition to the TAZPOWER Extension subjects, other patients with Barth syndrome ≥12-years-old are eligible for inclusion. Seven of the eight long-term TAZPOWER Extension subjects enrolled in the IEAP in 2021, and six remain currently enrolled.

Stealth also receives requests for access to elamipretide therapy for younger Barth syndrome patients ineligible for inclusion in the IEAP. For these patients, individual INDs or, in the case of an emergency, emergency INDs may be opened if FDA agrees. The burden of paperwork and oversight to pursue expanded access in these situations is significant for both medical professionals, families, and Stealth and comes with significant cost to the sponsor (Goldstein 2023).

Since 2021, Stealth has received requests for compassionate use for 44 affected individuals from 10 countries worldwide, including requests for 30 affected individuals in the US; 22 affected individuals have received elamipretide under this program including 11 children <12-years-old (10 of whom were <2-years-old). The volume of requests has increased dramatically since we announced our plan to resubmit the NDA, with more than half of the requests received since early 2023. Many recent requests were for infants in acute cardiac distress, which is surprising because we had previously understood that only a few babies are born and diagnosed with Barth syndrome annually in the US. FDA has approved every US-based request submitted, usually within 24-hours, including one asking for delivery of elamipretide to (b) (6) (b) (6) before the child was born.[25]

There have been reports of improvements observed in the EAP including with respect to cardiac function, where elamipretide has been added to standard of care therapies, but it is important to contextualize that children with Barth syndrome can recover from acute heart failure in infancy, including with standard of care heart failure medical management (Yester 2021). No suspected unexpected serious adverse reaction has been reported in the EAP program, and the safety profile reported has been consistent with the experience across our development programs.

Although cardiac dysfunction can be stabilized with standard of care therapy, once instrumentation such as a ventricular assist device, or Berlin heart (VAD), is introduced, such recovery is reportedly unprecedented in the setting of Barth syndrome (Goldstein 2024) and is rare (~5%) in other pediatric settings (Blume 2006). One notable EAP case reported by Goldstein et al., below, occurred in the setting of a VAD intended to bridge to transplant.

An 11-month-old child presented unresponsive and in cardiogenic shock and was diagnosed genetically with Barth syndrome. The child's clinical status was quickly declining, and he was placed on extracorporeal membrane oxygenation (ECMO). Elamipretide therapy was initiated under an emergency access request approved within 24-hours by FDA. The child was placed on a ventricular assist device (VAD) and listed for heart transplant. The treating physician consulted with cardiologists familiar with Barth syndrome who said that severe cardiomyopathy may be controlled with medication and time, which may lead to a spontaneous recovery as noted above. The experts opined that once a VAD is required, there has been no known case of successful explant with the native heart intact. Conversely, all known cases with a VAD in Barth syndrome have led to transplant or death. The patient stabilized, and by 14.5-months-old, his cardiac function had improved. At 18-months, the VAD was explanted and the child was discharged with

[25] Supra note**s 4** and **5**.

his native heart intact. He is reported at 23-months to be doing well (Dubuque 2024; Goldstein 2024).

The EAP experience, while uncontrolled, suggests the potential therapeutic utility of elamipretide for children <12-years-old affected by Barth syndrome. Importantly, FDA's consistent approval of these requests is further evidence that the potential benefits of elamipretide outweigh the potential risks for this serious disease.

The recent uptick in volume of requests is unprecedented in a disease this rare. We can only assume that improved disease awareness is leading to beneficial earlier diagnosis and treatment.

3 Safety

3.1 Overall Safety

Elamipretide is a first-in-class mitochondrial protective agent. No known adverse event characteristics are associated with this pharmacologic class. The safety of elamipretide has been well characterized in a comprehensive battery of nonclinical studies, and the overall nonclinical safety data are supportive of the clinical use of elamipretide in the treatment of Barth syndrome in children and adults. Over 1,000 individuals have been exposed to elamipretide over a combined >300 patient years of exposure. Clinical studies with elamipretide from several indications and phases have been included in the integrated analysis of safety, including PMM, heart failure, reperfusion injury, age-related macular degeneration, age-related mitochondrial function decline, and healthy subjects.

In subjects administered SC elamipretide, as in Barth syndrome, commonly reported treatment-emergent adverse events were mild or moderate injection site reactions (ISRs) characterized by erythema, induration, bruising, pruritus, pain, and/or urticaria. Mild eosinophilia (based on laboratory data) has been reported beginning at approximately 30 days after initiation of elamipretide treatment in a majority of subjects but (i) has not been associated with any systemic clinical manifestations of eosinophilia and (ii) in general, these elevations appeared to trend downward after longer duration of treatment (16 weeks) and have returned to within normal range or baseline levels over time without intervention or withdrawal of elamipretide. No other identified safety concern emerged in elamipretide trials with respect to other clinical laboratory results, physical examinations, vital signs, or electrocardiogram (ECG) data or concerns with respect to special populations between elamipretide and placebo. Likewise, there do not appear to be differences in safety based on age, disease severity, or concomitant medicines. See Table 10.

Table 10 TAZPOWER Adverse Events

System Organ Class Preferred Term	ELAM 40 mg n (%)	Placebo n (%)
At least one TEAE	12 (100.0)	10 (83.3)
General disorders and administration site conditions	12 (100.0)	8 (66.7)
Injection site erythema	12 (100.0)	3 (25.0)
Injection site pain	9 (75.0)	5 (41.7)
Injection site induration	8 (66.7)	2 (16.7)
Injection site pruritus	8 (66.7)	2 (16.7)
Injection site bruising	3 (25.0)	0
Injection site urticaria	3 (25.0)	0
Medical device site irritation	2 (16.7)	1 (8.3)
Nervous system disorders	3 (25.0)	5 (41.7)
Headache	1 (8.3)	3 (25.0)
Gastrointestinal disorders	1 (8.3)	4 (33.3)
Aphthous ulcer	0	2 (16.7)
Infections and infestations	4 (33.3)	3 (25.0)
Bronchitis	2 (16.7)	1 (8.3)
Injury, poisoning, and procedural complications	2 (16.7)	3 (25.0)
Ligament sprain	2 (16.7)	1 (8.3)

Abbreviations: AE = Adverse event; SOC = System Organ Class; TEAE = Treatment-emergent adverse event; TAZPOWER = SPIBA-201 Part 1. An AE was considered treatment-emergent if the date of onset is on or after the date of the first dose of IMP and was associated with the treatment most recently received by the patient at the time of onset or worsening. Values are n (%) of patients with at least 1 event within the studied population. Table includes TEAEs where preferred term was reported in ≥2 patients and were more frequent than placebo.

In general, the safety profile from individual studies using systemic elamipretide was consistent with the pre-existing, comorbid medical conditions.

3.2 Barth Syndrome-Specific Safety Considerations

During the TAZPOWER and TAZPOWER Extension studies, 10 of the 12 subjects gained height from baseline until last visit recorded, with 7 subjects growing 5 cm or more. Weight and BMI also increased during the trial; at week 168, mean height, weight, and BMI changes from baseline of 9.74 cm, 8.71 kg, and 1.10 kg/m^2, respectively, were observed in the 8 subjects remaining in the trial. Growth is expected for the younger age group studied (≤21 years of age); however, the literature suggests that periods of growth during puberty are often accompanied by worsening of disease, including cardiac decompensation, recurrent cardiac problems, increased fatigue, increased eating/digestive problems, increased sleep issues, and increased pain. This growth-associated symptomatic decline was not observed during the TAZPOWER and TAZPOWER Extension studies.

At the time of NDA submission and through the 120-day safety update, subjects with Barth syndrome had been exposed to elamipretide 40 mg SC in clinical trials for up to 1,291 days (about 3 and a half years), and many have remained on therapy through expanded access for up to about 7-years (mid-2017 TAZPOWER initiation to mid-2024). There have been no significant trends in treatment-emergent adverse events observed with increased duration of exposure except for the relationship between duration of treatment and increased eosinophils which is self-limiting and resolves without intervention with continuing treatment. Likewise, no suspected unexpected serious adverse reactions occurred.

No death was reported in the Barth syndrome safety population. Three treatment-emergent serious adverse events were reported in the Barth syndrome safety population after administration of elamipretide; all were deemed unrelated to study medication. Two subjects experienced treatment-emergent adverse events of urticaria; one of whom also had drug eruption. Both were withdrawn from TAZPOWER Extension study because of these events and symptoms resolved.

3.3 Methods to Prevent, Mitigate, or Manage ISRs

Some subjects have been successfully treated with topical and systemic antihistamines and/or topical corticosteroids to manage the signs and symptoms of the injection site reactions with SC elamipretide (Sullivan 2023).

4 Post-Marketing Evaluation

4.1 Registry

Stealth recognizes that additional study may be needed to understand more fully the longitudinal course of Barth syndrome, both to inform the potential of elamipretide to affect long-term outcomes and to support future development of new therapies. The only feasible and ethical way to achieve this goal is through a post-marketing evaluation using a registry enrolling all willing patients, including those on and naïve to treatment.

Stealth has committed in its NDA to institute a post-marketing registry that will enroll patients with Barth syndrome receiving elamipretide therapy and patients with Barth syndrome who are not receiving elamipretide therapy to monitor long-term safety and assess long term outcomes. The registry will collect standard of care assessments and certain additional assessments (including MLCL:CL ratio), a patient-reported outcome assessment, records of hospitalization and death and other electronic medical record data.

4.2 Phase 4 study to Better Inform Pediatric Dosing

Based on the presentation and natural history of Barth syndrome there is a pressing need for therapy for younger children (<12-years-old) with Barth syndrome. While we have proposed an indication that does not discriminate by age, we acknowledge the limited experience dosing younger children, largely coming from the >34 children <12-years-old (including 16 children <2-years-old) with Barth syndrome and other diseases of mitochondrial dysfunction who have received elamipretide under our expanded access program. This includes 11 children <12-years-old with Barth syndrome, 10 of whom were <2-years-old.

Daily dosing of children >12-years-old in our EAP varied from 0.25 mg/kg to 0.5 mg/kg. For these cases, we relied on modelling and simulation work conducted using Gastroplus to predict target doses for pediatric subjects ages 2-12 years old, which resulted in similar mg/kg doses predicted to match the target adult AUC0-inf across ages. This was confirmed in a single pediatric EAP case of an infant with Senger's syndrome, a disease of CL-pathology related to Barth syndrome (Koenig 2023).

While we do not expect age related differences in safety and effectiveness, we are cognizant that pediatric dosing could be better informed and optimized following a formal evaluation of the safety, tolerability, and pharmacokinetics of subcutaneous elamipretide in children aged 2-11-years. If elamipretide is approved for the treatment of Barth syndrome, we plan to conduct this study to refine dosing parameters for younger children.

5 Regulatory Discussions Pertinent to Clinical Conclusions on Efficacy

5.1 NH Control Study

Although concurrent placebo-controlled trials are generally recognized as the "gold-standard" for clinical research, the regulations provide, and regulatory precedent and regulatory guidance both recognize, that externally controlled clinical investigations at times are necessary and can meet the requirements for a controlled clinical investigation (21 CFR 314.126:10).[26] Particularly in

[26] "Adequate and well-controlled clinical investigations provide the primary basis for determining whether there is substantial evidence to support the claims of effectiveness. FDA regulation at 21 CFR 314.126(b) describes characteristics of an adequate and well-controlled clinical investigation, including choice of control, method of patient assignment to treatment (e.g., randomization), adequate measures to minimize bias (e.g., blinding), well-defined and reliable assessment of individuals' response (i.e., efficacy endpoint), and adequate analysis of the clinical investigation's results to assess the effects of the drug (i.e., statistical methods). Although randomized

situations in which an intervention is necessary, but randomization is considered unethical, such as in very rare disease settings with small patient populations like Barth syndrome, a comparison group from a patient registry or natural history study may be selected in order to assess the impact of the intervention on the study subjects. FDA has identified strategies for mitigating the bias inherent in all externally controlled studies, which are most effective in situations where the disease course is predictable and the treatment effect dramatic, all of which were followed and achieved in the design, execution, and results of NH Control study.

Specific design limitations of the NH Control study have been discussed with the FDA and addressed as summarized in Table 11.

Table 11 NH Control Study Design Characteristics Discussed with FDA

Design limitations	Discussion[27]
NH Control study did not prespecify a hierarchy to control multiplicity among the secondary endpoints and multiple timepoints assessed.	Weeks 64 and 76 were the prespecified primary timepoints of interest. Application of a Bonferroni analysis allocating alpha at p=0.05 across all primary and secondary endpoints for both timepoints protects endpoints that achieved significance at p≤0.005. This results in significance of 6MWT, Muscle strength by HHD and MDRI at both primary timepoints of interest. DCN previously indicated in discussions regarding new trial designs that, for this ultra-rare disease, a p value of >0.05 could be acceptable.
The number and timing of assessments in the NH dataset, which was derived from annual visits to the Johns Hopkins outpatient clinic and/or biennial patient advocacy conferences, do not match the number and timing of assessments in the TAZPOWER Extension study during which scheduled visits were conducted every three months up to week 72.	When using external controls, data collection intervals typically vary from interventional study intervals because real world data are not expected to have endpoints that match a trial. The use of linear regression to impute data for specific timepoints between two observed measurements is a reasonable and inherently unbiased approach in this setting. From a clinical perspective, the NH dataset demonstrates that

double-blinded, concurrently controlled superiority trials are usually regarded as the most rigorous design…five types of controls are described in section 314.126:10 placebo concurrent control, dose-comparison concurrent control, no treatment concurrent control, active treatment concurrent control, and historical control (a type of external control). Of note, when the first version of the rule was published in 1970, historical controls and active treatment controls were included. Thus, from its earliest description of adequate and well-controlled trials, FDA included trial designs…that may be more difficult to interpret, which reflected FDA's recognition that different trial designs (including choice of control) may be appropriate in different disease settings. (Draft Guidance for Industry, Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products, December 2019, emphasis added).

[27] An expert assessment of the rigor of the NH Control study was submitted to FDA and is attached at Appendix B Figure 1.

Design limitations	Discussion[27]
	during a mean data collection period of 74.9 weeks (SWAY balance) to 195.9 weeks (6MWT), no change was observed on any functional assessments for the NH control subject, whereas the TAZPOWER Extension study demonstrates that during a data collection period of 196 weeks, sustained improvement was observed at multiple timepoints on most functional assessments.
The pre-specified inclusion criteria for evaluable subjects (≥12-years-old with 2 longitudinal 6MWT datapoints and not a TAZPOWER participant) excluded 60/79 NH subjects. An understanding of whether these excluded individuals had systematically different disease characteristics than the 19 NH controls was considered relevant for analysis.	FDA guidance for externally controlled trials emphasizes the importance of ensuring balance in attributes of patients likely to influence outcomes in an external control arm, with age as the foremost example of baseline attributes of concern.[28] In compliance with the guidance, the NH Control study protocol and SAP specified inclusion of subjects ≥12-years-old with at least 2 longitudinal datapoints for appropriate comparison. 19 subjects in the JHU NH dataset met the prespecified parameters. These 19 NH control subjects, together with the 12 TAZPOWER subjects, represent >45% of the evaluable US patient population in this age range(see **note 2**). Stealth also characterized baseline characteristics of 20 additional NH control subjects who did not have longitudinal data to confirm relative baseline comparability with NH Control study participants. Overall, the analyses reflect comparable functional impairment among >75% of the eligible US patient population (i.e., n=66 eligible affected individuals ≥12-years-old).
The propensity score method to minimize potential selection bias only considered age, height, and baseline 6MWD. FDA asked to understand why only three baseline factors were considered for the propensity score model and noted that results indicated an imbalance in covariates even after weighting with the conventional standardized mean difference greater than 0.1.	Published best practices for use of external controls specify that "it is important to ensure that the patients selected actually have the disease and to consider that patient treatment status may depend on factors such as disease severity, overall health status, or age." [29] The statistical team charged with constructing the prognostic match, which was blinded to long-term values, assessed the factors present within the dataset with the most relatedness to the endpoint of primary interest. In such a small dataset, inclusion of additional criteria would have further narrowed potential matches.

[28] Draft Guidance for Industry: Considerations for the Design and Conduct of Externally Controlled Trials for Drug and Biological Products, February 2023

[29] Understanding the Need for Non-Interventional Studies Using Secondary Data to Generate Real-World Evidence for Regulatory Decision Making, and Demonstrating Their Credibility, Duke-Margolis Center White Paper, November 25, 2019 Link

Design limitations	**Discussion**[27]
Potential selection bias/residual confounding due to systematic differences between the elamipretide treated patients and those in the NH control not addressed by the propensity score weighting.	The study accounted for "[i]mportant factors for appropriate study design...utilizing a control group with as close to the same characteristics as the intervention group as possible and the roles of time and temporality."[30] The NH control group comprised >25% of the potentially eligible patients in the US (see **note 2**). It is improbable to perceive meaningful selection bias given the significant percentage of possible controls included.
Lack of blinding and randomization in NH Control study could introduce potential bias in the assessment of 6MWT, an effort-dependent endpoint on which a 30.1-meter mean placebo effect was observed in TAZPOWER (consistent with placebo effect characterized across multiple heart failure trials (Olsson 2005).	FDA Guidance states that a large effect size, as observed here, can help overcome a presumption of bias. The effect size is large relative to baseline controls (>25% mean improvement) and relative to changes observed in the NH (>99% improvement over NH controls) and far outstrips (>2.5X) the 30.1-meter placebo effect observed in TAZPOWER. The effects size (>80-meters at all timepoints) is well-above the ~30-meter MCID as characterized in multiple relevant disease indications (see Section 2.4.3.2.1). Best practices were followed in constructing these external controls. "In retrospective studies using secondary data, blinding of patients and providers is outside the control of study investigators, since patients and prescribers make treatment decisions together…Pre-specification of the study protocol and statistical analysis plan and blinding of data analysts are important techniques for minimizing information bias on the part of data analysts. Blinding data analysts can reduce potential for analyst bias, but it also restricts the ability of analysts to perform unplanned tests or study modifications based on preliminary results. Data aggregators and preparers should use detailed, prespecified methods for selecting and assessing eligible patients."[31]

[30] Id.

[31] Id.

5.2 TAZPOWER Extension Study

Baseline controls as utilized in the TAZPOWER Extension study are a form of external control that are regularly used and can meet the requirements for a controlled clinical investigation under 21 CFR 314.126:10. For context, of the 45 non-oncology products approved by FDA between 2010 and 2019 based on externally controlled studies, 33% of these utilized baseline controls (Jahanshahi 2021).

We understand the importance of controlling for the potential effect of hope bias when utilizing baseline controls for effort dependent functional endpoints such as 6MWT. In the TAZPOWER Extension study, the evidence demonstrates that patients were not simply trying harder; in fact, the BORG post-exertional fatigue scale (Figure 16) showed a >25% mean decrease in effort from BL. The science tells us that it is highly improbable that Barth syndrome patients could sustain such a large and durable improvement in physiology, feel and function, based on both data demonstrating that their mitochondria cannot respire more under maximal stress conditions (Wang 2016; Gang 2016) and clinical data in the context of endurance exercise training, where they fail to improve (Cade 2017). The pharmacodynamic data support this conclusion, demonstrating improvements in LV volumes and the pathognomonic MLCL:CL ratio that are neither effort-dependent nor inherently subject to bias, in both cases contrary to the established natural history of the disease. These pharmacodynamic changes, which were associated (albeit weakly) with the functional improvements, support the interpretability of the totality of the evidence which overwhelmingly favors elamipretide. In short, it is wholly unprecedented in the setting of Barth syndrome and other progressive diseases of mitochondrial dysfunction to observe this durable reversal of the progressive decline in feel, function, and physiology characterizing the otherwise inexorable disease trajectory.

6 Overall Conclusion

Barth syndrome, a progressive and ultimately fatal disease of mitochondrial dysfunction, affects an estimated 130 US individuals. There are no approved therapies and no other therapies in clinical development.

The evidence demonstrates that elamipretide is safe and effective in the treatment of Barth syndrome. The main objective of the elamipretide development efforts was to demonstrate the clinical benefit of elamipretide to improve feel and function in individuals living with this devastating disease. This objective was met across multiple endpoints and relative to two forms of external control in the TAZPOWER Extension study and the NH Control study. Durable clinical improvements of the magnitude observed across functional, patient- and clinician-reported, and pharmacodynamic endpoints have never been seen in the setting of Barth

syndrome; these improvements are completely inconsistent with the progressive decline expected in the natural history of the disease as extensively characterized in >50% of the US affected patient population. Likewise, the totality of the evidence confirms that improvements in key determinants of effectiveness all favor treatment over control. Importantly, these benefits accrued with little safety risk.

The well-understood pathophysiology of Barth syndrome – a disease of severe cardiolipin deficiency - and the mechanism of action of elamipretide – which targets and compensates for the loss of cardiolipin - strengthen the biological plausibility of the meaningful treatment effects observed. The temporal relationship between biological effects and clinical effects further demonstrates that elamipretide has a clinically relevant and durable impact on disease progression in Barth syndrome. Taken together, these observations show that elamipretide improves signs and symptoms of Barth syndrome and support full approval for the indication being sought – treatment of Barth syndrome.

This conclusion regarding the benefit of elamipretide for the treatment of Barth syndrome is supported by a risk benefit analysis of the well-characterized (and generally benign) safety profile of elamipretide as well as patients' willingness to accept some uncertainty with respect to benefit. Indeed, the FDA has regularly reached this conclusion in the setting of the expanded access program for this drug by promptly approving every request submitted, including those for acutely ill infants with Barth syndrome.

Should any residual uncertainty regarding the degree of benefit remain, the only ethical and feasible way to address that uncertainty is in the post-marketing setting. To that end, Stealth is committed to continued evaluation of the safety and appropriate pediatric dosing of elamipretide which can only be achieved via the proposed post marketing registry and pediatric study. We appreciate the chance to present this program to the Advisory Committee and look forward to your input and guidance. We thank the individuals living with this disease, their families and caregivers and their physicians who have participated in our studies and allowed their data to be used for the benefit of others.

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Appendix A Regulatory Interactions

Appendix A Table 1 Regulatory Interactions

Date/IND/Division Summary of Interaction
Pre-2017-2018/123,553/ DNP Barth syndrome development efforts including CMC discussions conducted under umbrella of larger development effort in primary mitochondrial myopathy.
2018-2019/137,429/ DNP • Opened new IND for elamipretide for the treatment of Barth syndrome (per DNP request). • Granted Fast Track Designation and Orphan Drug designation, recognizing urgency of unmet need, severity of disease, and plausibility of elamipretide development efforts. • Carcinogenicity studies deferred to post-approval. • In April, 2019 Type C meeting to discuss TAZPOWER study and Part 2 through week 24 data. DNP noted that the nonclinical package appeared acceptable. DNP expressed concern that TAZPOWER Extension study data was uninterpretable absent a control arm and suggested a randomized withdrawal of the 8 remaining Part 2 subjects until they experience a functional decline (i.e., on 6MWT or a PRO) to establish efficacy, suggesting that "trends" would suffice in response to Stealth's powering concerns. DNP advised intent to transfer to DGIEP; Stealth understood that the transfer was prompted by DGIEP's recent approval of Brineura for an ultra-rare lysosomal storage disorder based on a natural history control for single-arm subjective endpoint data.
2019/137,429/DGIEP • DGIEP commented on the proposed matching methodology in the NH Control study protocol; these concerns were addressed in the revised protocol. • DGIEP cited the minutes of the April 25, 2019 meeting recommending a randomized withdrawal trial enrolling the subjects remaining in TAZPOWER Extension study.
2020/137,429/ DRDMG • Due to FDA organization the IND was transferred to from DGIEP to DRDMG. • Rare pediatric designation was granted, recognizing the seriousness of the disease, the urgency of the unmet need, and the pediatric focus of the development program. • In March, 2020, at a Type C meeting to discuss positive NH Control study Phase 3 data, TAZPOWER Extension study echocardiographic data, and regulatory pathway, DRDMG requested additional data from later timepoints during TAZPOWER Extension study and NH Control study and cardiac natural history data to provide further support for NDA review. DRDMG agreed that the echo data was inherently objective and that objective biomarker findings could aid in interpretability of data package. DRDMG also suggested the randomized withdrawal of the remaining OLE subjects. • In July, 2020, DRDMG again suggested the randomized withdrawal trial.
2020 - 2023/ PIND 153,135/137,429/DCN • In November, 2020, DCN granted a Type B pre-IND meeting in response to Stealth's request for a consultation to discuss the emerging cardiac signal. DRDMG also attended the meeting. DCN was intrigued by the data, but shared its concerns about Subpart H due to the Agency's historic sparse use of Subpart H withdrawal procedures post-approval. Several pre- and post-marketing trial designs were discussed, including proposals by Stealth and by DCN. DCN requested additional data regarding mechanism of action of elamipretide to support the reasonable likelihood of a post-marketing trial providing confirmatory evidence to support Subpart H. • In February, 2021, a meeting was held to discuss a potential NDA submission. DCN commented favorably on various manufacturing questions. With respect to NDA submission, DCN agreed that an NDA could be

submitted which it would file and review for full approval, noting that further pre- or post-marketing studies (several designs for which were discussed) seem unfeasible because of ultra-rare nature of disease. DCN also recommended that Stealth conduct additional analyses to improve the interpretability of the TAZPOWER Extension study and NH Control study data. Stealth and DCN discussed approaches for clarifying this further in the NDA submission. DCN agreed to respond to Stealth's pre-NDA questions and to provide an NDA checklist for Stealth.

- In March, 2021, DCN and OCHEN advised that despite the agreement regarding NDA filing, a Medical Policy Review Committee had determined that the data package was weak to support an NDA and Stealth should collect additional data from a controlled trial.
- From March through June, 2021, Stealth, DCN and OCHEN had several telephone meetings and advice correspondence in which several alternative protocol designs, including the time-to-treatment-failure randomized withdrawal protocol originally recommended by DNP, were proposed and discussed. Ultimately, DCN and OCHEN were uncomfortable with the powering considerations and potential for unblinding of the randomized withdrawal protocol and the powering considerations and/or other design features of other protocols proposed. In June, 2021, OCHEN agreed to Stealth's establishment of an Expanded Access Protocol to ensure continued access to drug for patients transitioning off TAZPOWER Extension study, and acknowledged that there appeared to be no feasible path forward for generation of new data.
- In August, 2021 Stealth submitted the NDA. In October, 2021, FDA refused to file (RTF) the NDA. NH Control study was not referenced in the RTF notification.
- In November, 2021, a Type A meeting was held to discuss a potential path forward. A new trial design was discussed, but powering concerns continued to be raised. DCN recommended consideration of approval under the animal rule, stating that "it is every bit as difficult to establish effectiveness in a very rare disease as it is for drugs to combat threats such as anthrax," i.e., due to powering concerns. In subsequent correspondence, DCN continued to discuss powering concerns, the potential to add younger children (which Stealth cautioned would add to variability and complicate endpoint selection and recruitment), and revisited the utility of a time-to-treatment-failure randomized withdrawal trial.
- Meanwhile, analysis of the final data from TAZPOWER Extension study demonstrated durability of functional and patient and clinician reported improvements, a large (>40%) improvement in LV SVI and LV EDVI, and improvement in the abnormal MLCL:CL ratios. Stealth requested and was granted a Type B pre-NDA meeting in August, 2022. DCN agreed in principle that the changes in LV SVI and LV EDVI could support accelerated approval, subject to demonstration of the reasonable likelihood of clinical benefit through establishment of a modeled relationship which can be proved within a few years from approval in a post-marketing study. Subsequent informal meetings in Fall, 2022, confirmed that observational data could be used to establish the modeled relationship and to better define the effect size. A 0.1 p value was deemed acceptable for the post-marketing study.
- In June 2023, at a Type A meeting, DCN agreed data from TAZPOWER Extension study showing change from BL in LV-SVI and its weak, statistically nonsignificant correlation to change from BL in 6MWT provide weak evidence (i) to support LV-SVI as a surrogate for 6MWT and (ii) that elamipretide affects LV-SVI. Stealth shared a model developed with reference to datasets from the CARDIOMAN trial; DCN agreed the CARDIOMAN data provide evidence of a relationship between change in LV-SVI and peak work rate. However, DCN expressed concerns that the proposed post-marketing trial target enrollment of 35 patients would not provide adequate power and that the treatment duration may be too long to retain patients. DCN acknowledged that further development of elamipretide for Barth syndrome faces multiple constraints-legal/regulatory framework, availability/willingness of patients or caregivers, and investment.
- In November 2023, DCN met with Dr Rachel Sherman, former Deputy Commissioner of FDA, who had reviewed Stealth's data package. It emerged from meetings and correspondence with Dr Sherman that a main concern about NH Control study was a misassumption that patients being followed vigorously outside of a development program are apt to be doing worse than average, thus needing close follow-up.
- Stealth was able to correct this misassumption in a December 2023 Type A meeting, noting that the natural history patients were in fact thought to be doing "better from average" since they were healthy enough to travel to outpatient clinics for routine care and monitoring and/or attend advocacy conferences. Stealth and

DCN also discussed the statutory basis for approval based on externally controlled trials such as NH Control study and TAZPOWER Extension study.

Appendix BTriNetX Critical Analysis of NH Control Study

Appendix B Figure 1 Critical Analysis of NH Control Study

TriNetX

125 Cambridgepark Drive, Suite 500
Cambridge, MA 02140 USA
+1 (857) 285-6037 | join@trinetx.com

January 6, 2024

CRITICAL ANALYSIS OF SPIBA-001

BY E-MAIL

TO: James Carr, PharmD, Chief Clinical Development Officer, Stealth BioTherapeutics

FROM: Jeffrey Stuart Brown, PhD, Chief Scientific Officer, TriNetX

SUBJECT: Assessment of SPIBA-001 as an Adequate and Well-Controlled Clinical Investigation

You asked me to summarize my views regarding the rigor of the design and conduct of SPIBA- 001 as an adequate and well-controlled clinical investigation (AWC) under 21 CFR 314.126(b). My review of this matter is based upon my extensive experience with real world data including, without limitation, in the context of my decades of work implementing real world data studies for FDA's Sentinel project and other FDA-mandated studies for industry.

As shared with the FDA during the December 21, 2023 Type A meeting, my conclusion following my critical analysis of the clinical study report is that SPIBA-001, on its face, meets the requirements of an AWC. The rigor of the design and execution of the study controlled for potential bias to the maximum extent possible in accordance with FDA regulations and guidance. The study was conducted consistent with good real-world data research practices as espoused by FDA and relevant professional societies.

Externally controlled clinical investigations are recognized as meeting the requirements for a controlled clinical investigation under 21 CFR 314.126:10. Particularly in situations in which an intervention is necessary but randomization is considered unethical, such as in very rare disease settings with small patient populations, a comparison group from a patient registry or natural history study

may be selected in order to assess the impact of the intervention on the study subjects[1]. FDA acknowledges, however, that inability to control for certain biases can limit the ability of externally controlled trials to demonstrate substantial evidence of effectiveness. Consequently, FDA has identified strategies for mitigating bias which are most suitable in situations where the disease course is predictable and the treatment effect dramatic.[2] Both of these conditions appear to be met in the setting of Barth syndrome and SPIBA-001. I have concluded that SPIBA-001 complied in all material ways with the strategies set forth in the NH Guidance to minimize bias, as set forth in Table 1 (Appendix).

The considerations for minimizing bias in the conduct of externally controlled trials fall within the larger framework of the defining characteristics of an adequate and well controlled clinical investigation. A Duke-Margolis working group convened to explore this topic identified the seminal characteristics of adequate and well controlled investigations.[3] I have concluded that SPIBA-001 meets these seminal characteristics of an AWC, as set forth in Table 2 (Appendix).

A review of Table 24 located at 2.7.3.3.3.2.6 of the application, provides further assurance that the NH controls are behaving in a manner similar to what would be expected in a controlled study. Although there was a modest placebo response on 6MWT in SPIBA-201 Part 1, which is within the known variability of that assessment (Olsson et al., 2005), the placebo group otherwise performed

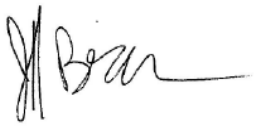
[1] A Framework for Regulatory Use of Real World Evidence, Duke-Margolis Center White Paper, September 13, 2017, Link)

[2] Draft Guidance for Industry, Rare Diseases: Natural History Studies for Drug Development, March 2019 ("NH Guidance").

[3] Understanding the Need for Non-Interventional Studies Using Secondary Data to Generate Real-World Evidence for Regulatory Decision Making, and Demonstrating Their Credibility, Duke-Margolis Center White Paper, November 25, 2019 Link

similarly to the NH controls on all other functional assessments (Muscle strength by HHD, 5XSST, SWAY balance).

In conclusion, the design and conduct of SPIBA-001 were both rigorous and compliant with regulatory requirements of an AWC. All reasonable and appropriate safeguards to minimize and protect against potential bias were adopted and implemented. As recommended by generally accepted good research practices, multiple sensitivity analyses were employed to test the assumptions utilized to minimize bias, all of which supported the primary findings. Accordingly, I conclude that SPIBA-001 is a positive AWC which, on its face, supports a review of the application for elamipretide for the treatment of Barth syndrome.



Jeffrey S. Brown, PhD
Chief Scientific Officer, TriNetX, LLC

APPENDIX

Table 1 NH Guidance Relative to SPIBA-001

NH Guidance	SPIBA-001
The external control group needs to be very similar to the treated group in all respects, including disease severity, duration of illness, prior treatments, and any other aspects of the disease that could affect outcomes and the timing of outcomes.	The patients included in the NH control cohort (n=79, comprising >50% of the US Barth syndrome patient population) from which the prognostically matched NH controls were derived were patients healthy enough to travel to biennial patient advocacy meetings and/or annual outpatient routine follow-up clinic visits. The prognostic matching criteria was designed to further ensure similarity between groups by matching patients on the basis of age, height, and distance walked on 6MWT.
The availability of patient level data can help provide support for comparison between the control group and the group receiving the investigational drug.	Patient level data is available for the NH control cohort in SPIBA-001.
Use of valid epidemiological approaches can reduce selection bias (e.g., inclusion/exclusion criteria, prespecified statistical analysis plan)	Inclusion/exclusion criteria were broad and inclusive and were comparable for treated and NH control cohorts, except that for NH control availability of longitudinal data was required. The statistical analyses plans were prespecified and two statistical teams were utilized such that the team developing the prognostic match criteria remained blinded to longitudinal NH data.
Critical patient disease characteristics may not have been assessed or may have been assessed differently based on	The NH control cohort was evaluated on the same functional efficacy endpoints (6MWT, Muscle strength by HHD, 5XSST and SWAY balance) as the treated cohort by the same

historical approaches, resulting in a lack of comparability (e.g., disease definitions, diagnostic techniques, and approaches to safety monitoring may have evolved).	team of clinicians at Johns Hopkins University using substantively identical procedures during the same time period (NH data collected between 2012 and 2019; SPIBA-201 conducted between 2016 and 2020) within which disease definitions, diagnostic techniques and approaches to safety monitoring remained unchanged.
Aspects of standard of care may have changed.	The NH data collection (2012-2019) overlapped with the conduct of SPIBA-201 (2016-2020). Standard of care remained unchanged.
Data collection intervals and quality may lack consistency and not be comparable.	Comparability of data collection quality was ensured by use of the same team of clinicians with expertise in Barth Syndrome at Johns Hopkins University using substantively identical procedures on the same assessments. Data collection intervals will typically vary from interventional study interval when using external controls – real world data are not expected to have endpoints that match a trial, so using a linear regression is reasonable to impute a value to match the trial measurement. The general approach to missing data is appropriate. Using linear regression to impute data for specific time points between two observed measurements should be acceptable. I do not believe that the specified approach to imputing endpoints for specific evaluation time points (weeks) can be found to have biased the findings towards treatment.

Use of an external control group is especially challenging if the outcome assessments used in the external control group are not well defined and reliable and, therefore, not suitable for regulatory use.	The endpoints utilized were well-established in the disease natural history. When external controls are utilized for effort dependent endpoints, a large effect size and confirmatory pharmacodynamic data can be supportive (e.g., burosumab pediatric approval).
An external control is most interpretable when a treatment effect is large in comparison to potential biases and the known variability in progression.	The treatment effect observed is objectively large, with >90% difference between treated subjects and NH controls on 6MWT and Muscle strength and >80% differences on the multi-domain responder index. The large size of the NH cohort (>50% of the US patient population) supports that the variability in progression is well-established.

Table 2 SPIBA-001 as an Adequate and Well-Controlled Investigation

Summary of Adequate and well Controlled Characteristics (21 CFR § 314.126)[4]	**SPIBA-201 CSR**
There is a clear statement of the objectives of the investigation and a summary of the proposed or actual methods of analysis in the protocol for the study and in the report of its results.	The objectives of the study were clearly stated (CSR §8). The investigational plan and methods of analysis were prospectively defined (CSR §9).

[4] Understanding the Need for Non-Interventional Studies Using Secondary Data to Generate Real-World Evidence for Regulatory Decision Making, and Demonstrating Their Credibility, Duke-Margolis Center White Paper, November 25, 2019 Link

The study uses a design that permits a valid comparison with a control to provide a quantitative assessment of drug effect.[5]	NH patients were derived from patients attending an advocacy conference and/or routine out-patient monitoring visits rather than in a critical care setting (CSR §9.1.3). All patients were confirmed to have a genetic diagnosis of BTHS (CSR §9.1.4; §9.3.1.1; §9.3.1.2). Data included disease characteristics, medical history, genetic and metabolic/biomarker characteristics, and functional assessments (CSR §9.1; 9.1.4) allowing detailed comparison. Contemporaneous datasets were utilized for comparison during a period in which there were no approved therapies for BTHS and no significant change in the standard of care (CSR §9.2), addressing concerns of time or temporality. The requirement that subjects chosen for the NH control cohort have longitudinal assessment data also help address this consideration (CSR §9.7.1.3).
The method of selection of subjects provides adequate assurance that they have the disease or condition being studied.	Genetic confirmation of BTHS was an inclusion criterion for both the NH control (for which data extraction parameters included genetic testing (TAZ variant) and the diagnostic MLCL:4L-CL ratio) (CSR §9.1.4; §9.3.1.1) and interventional arms (CSR §9.3.1.2).

[5] Important factors for appropriate study design are utilizing a control group with as close to the same characteristics as the intervention group as possible (Id.) and the roles of time and temporality (Id.). If researchers use a no-treatment group, it is important to ensure that the patients selected actually have the disease and to consider that patient treatment status may depend on factors such as disease severity, overall health status, or age.

The method of assigning patients to treatment and control groups minimizes bias and is intended to assure comparability of the groups with respect to pertinent variables.[6]	There are no approved therapies for BTHS and no other therapies currently in clinical development. Subjects in the long-term NH control arm received standard of care treatment for BTHS symptoms (CSR §9.4.7). Subjects in the long-term interventional arm were on stable (unchanged and constant) medications (CSR §9.3.1.2). Propensity score methods were used to balance the two treatment cohorts (CSR § 9.7.1.1). Logistic regression was used to compute propensity scores for eligible subjects in both cohorts with terms for age and height at baseline and baseline 6MWT distance walked as baseline prognostic covariates, as these factors were available for most subjects in both cohorts and were considered the most impactful (CSR § 9.7.1.1).
Adequate measures are taken to minimize bias on the part of the	In SPIBA-001, the study protocol and statistical analysis plan were prespecified (CSR §9). To maintain the integrity of the study, and to minimize bias in the planned analysis methods, the data remained blinded to those who were responsible for defining the analyses in this

[6] "The fourth characteristic is related to how patients are assigned to treatment versus control/comparator group and minimizing selection bias. [I]t is necessary to ensure balanced covariates (both known and unknown) across both groups to help isolate the effect of the drug. While this is ideally done through randomization, there are methods to reduce the selection bias that can occur when treatment cannot be assigned as in non-interventional studies using secondary data." Id. Common concerns regarding selection bias include whether different interventions may be prescribed based on level of disease severity. Id.

subjects, observers, and analysts of the data (e.g., blinding).[7]	study (CSR §9.1.1; CSR §9.4.6). A propensity-score model team was identified to take on the responsibility of generating and finalizing the propensity-score model to ensure finalization of the propensity scores in an unbiased manner (CSR § 9.7.1.1).
The methods of assessment of subjects' response are well-defined and reliable. The protocol for the study and the report of results should explain the variables measured, the method of observation, and criteria used to assess response.	For both the long-term NH control arm and the long-term interventional arm, measures used to assess efficacy were chosen because they are used in the routine course of care of BTHS patients at the Kennedy Krieger Multidisciplinary Clinic at Johns Hopkins and have been referenced in the literature as useful endpoints for therapeutic evaluation (CSR § 9.5.3). Since 6MWT, muscle strength by HHD, 5XSST, and SWAY balance have been regularly used in the care and treatment of BTHS to assess functional exercise capacity and strength, these were considered to be reliable, accurate, and relevant for this purpose (CSR § 9.5.3). All efficacy assessments in the long term NH dataset were captured by expert clinicians

[7] "In retrospective studies using secondary data, blinding of patients and providers is outside the control of study investigators, since patients and prescribers make treatment decisions together. One advantage of retrospective studies is that participants do not know that they are being studied for a particular research question when data are collected, so there is reduced potential for responder bias. However, study analysts can be masked to patient status for some non-interventional studies. Prespecification of the study protocol and statistical analysis plan and blinding of data analysts are important techniques for minimizing information bias on the part of data analysts. Blinding data analysts can reduce potential for analyst bias, but it also restricts the ability of analysts to perform unplanned tests or study modifications based on preliminary results. Data aggregators and preparers should use detailed, prespecified methods for selecting and assessing eligible patients." Id.

	using standard protocols in a manner similar to the SPIBA 201 clinical trial; consequently, the long-term interventional data and the long-term NH data were highly consistent with respect to assessment methodology and conduct (CSR § 9.5.1).
There is an analysis of the results of the study adequate to assess the effects of the drug. The report of the study should describe the results and the analytic methods used to evaluate them, including any appropriate statistical methods.	The CSR provides a comprehensive analysis of the study results and the analytic methods used to evaluate them.