Review

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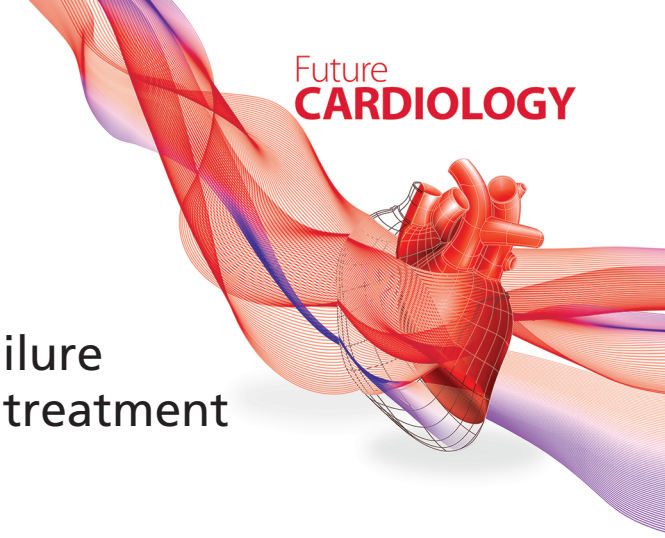

Temporal evolution of the heart failure phenotype in Barth syndrome and treatment with elamipretide

Hani N Sabbah*[,1], Carolyn Taylor[2] & Hilary J Vernon[3]

[1]Department of Medicine, Division of Cardiovascular Medicine, Henry Ford Hospital, Henry Ford Health, 2799 West Grand Boulevard, Detroit, MI 48202, USA

[2]Department of Pediatrics, Division of Cardiology, Medical University of South Carolina, Charleston, SC 29425, USA

[3]Department of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

*Author for correspondence: hsabbah1@hfhs.org

Barth syndrome (BTHS) is a rare genetic disorder caused by pathogenic variants in *TAFAZZIN* leading to reduced remodeled cardiolipin (CL), a phospholipid essential to mitochondrial function and structure. Cardiomyopathy presents in most patients with BTHS, typically appearing as dilated cardiomyopathy (DCM) in infancy and evolving to hypertrophic cardiomyopathy (HCM) resembling heart failure (HF) with preserved ejection fraction (HFpEF) in some patients ≥12 years. Elamipretide localizes to the inner mitochondrial membrane where it associates with CL, improving mitochondrial function, structure and bioenergetics, including ATP synthesis. Numerous preclinical and clinical studies in BTHS and other forms of HF have demonstrated that elamipretide improves left ventricular relaxation by ameliorating mitochondrial dysfunction, making it well suited for therapeutic use in adolescent and adult patients with BTHS.

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Barth syndrome (BTHS) is a rare, X-linked genetic disorder with an estimated incidence of 1 in 1 million live births [1]. This disorder is caused by pathogen variants in the *TAFAZZIN* gene, which is located on chromosome Xq28 and encodes a transacylase responsible for the final remodeling step in the synthesis of cardiolipin (CL), a phospholipid essential to mitochondrial structure and function [2]. The pathogenesis of BTHS is relatively unique as it is the only mendelian disorder of CL remodeling. Phenotypically, BTHS is characterized by cardiomyopathy, skeletal muscle weakness, neutropenia and growth abnormalities. BTHS is associated with significant morbidity and mortality, most often due to cardiac complications, with mortality highest in infancy [3,4]. The cardiomyopathic phenotype observed in BTHS patients through natural history control (NHC) studies is widely variable in presentation, morphology, response to therapy and long-term outcomes [5].

Current treatment modalities for patients with BTHS address the organ-specific symptoms and are not targeted to underlying pathophysiology of the disease; namely, CL abnormalities and resultant mitochondrial dysfunction. However, a recent phase II/III clinical trial and a natural history comparison study provided evidence for a clinical benefit of elamipretide in BTHS [6,7]. Elamipretide is a novel mitochondria-targeting peptide that has shown promising results across various preclinical and clinical models of heart failure (HF) and BTHS [8]. This review will address the pathophysiology of BTHS and explore the evolution of the cardiomyopathic phenotypes with advancing age in these patients. It will also detail the preclinical and clinical evidence supporting elamipretide as a therapeutic option for BTHS patients and discuss the clinical significance of the altered BTHS HF phenotype in the selection of treatment options.

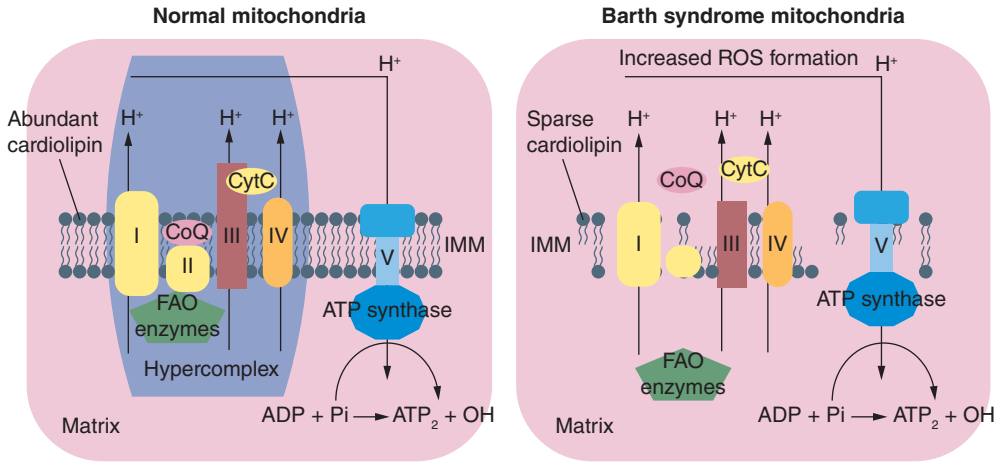

Figure 1. Proposed pathophysiological mechanism of Barth syndrome. Left panel: Normal mitochondria with abundant cardiolipin in the inner membrane. The respiratory complexes (I, II, III and IV) are assembled into supercomplexes and possibly form even hypercomplexes by interacting with fatty acid oxidation (FAO) enzymes. Hypercomplexes, are large metabolic units made up of FAO enzymes and electron transport chain complexes. **Right Panel:** Cardiolipin-depleted mitochondria as in Barth syndrome (BTHS) hearts. Supercomplexes are destabilized and reduced in quantity. The defects in the respiratory chain complexes likely result in overproduction of reactive oxygen species (ROS). Dissociation of FAO enzymes from the respiratory chain complexes can reduce the efficiency of metabolic channeling through this pathway. Defects in respiratory chains can lead to dissociation of cytochrome c (CytC) and loss of co-enzyme Q_{10} (CoQ) leading to further reduction of ATP generation through oxidative phosphorylation.
Adapted from Huang *et al.* with permission from [17].
IMM: Inner mitochondrial membrane; ROS: Reactive oxygen species.

Pathophysiology of Barth syndrome

Mitochondrial bioenergetics

Mitochondria are double-membraned organelles considered to be the powerhouses of eukaryotic cells due to their role in regeneration of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) using macromolecular protein complexes that form the electron transport chain (ETC) within the inner mitochondrial membrane (IMM). These protein complexes include nicotinamide-adenine dinucleotide (NADH) dehydrogenase (complex I), succinate dehydrogenase (complex II), cytochrome bc1 (complex III) and cytochrome c oxidase (complex IV) [9]. While electrons flow through the ETC, protons are pumped from the matrix into the intercristae lumen establishing a proton gradient with a highly negative mitochondrial membrane potential. Protons re-enter the matrix through ATP synthase (complex V), providing energy to regenerate ATP from inorganic phosphate (Pi) and ADP [10–12]. Oxidative phosphorylation, the coupling of ATP formation with substrate oxidation in the mitochondria, is crucial to tissue and organ health [9].

Cardiolipin abnormalities

BTHS is caused by pathogenic variants in the *TAFAZZIN* gene which encodes for tafazzin, a transacylase essential for the final remodeling and maturation of CL [13–15]. CL, a phospholipid located almost exclusively on the IMM, plays an integral role in mitochondrial structure and function [16], including assembly and stability of the ETC (Figure 1), mitochondrial fusion and fission, regulation of cristae formation, mitochondrial DNA stability and segregation, protein import, and organization of the respiratory complexes into higher-ordered supercomplexes [17–20].

Approximately 90% of CL exists as tetralinoleoyl CL (L4CL) in cardiomyocytes and skeletal muscle [21]. The formation of L4CL depends on a series of phospholipid remodeling reactions, with tafazzin catalyzing the final remodeling step to generate mature CL [22]. Deficient tafazzin activity in BTHS results in the presence of immature CL species, accumulation of MLCL, and overall loss of mature CL species, leading to the elevated MLCL/CL ratio that is pathognomonic for BTHS [23] and observed in every patient.

Whereas tafazzin is the primary enzyme responsible for remodeling of CL in the IMM, other enzymes have been shown to have remodeling capabilities. CL remodeling can occur via the endoplasmic reticulum localized enzyme acyl-CoA:lysocardiolipin acyltransferase (ALCAT1) [24,25] and via mitochondrial monolysocardiolipin

acyltransferase (MLCAT) [26,27]; however, the precise role that ALCAT1- and MLCAT-mediated CL remodeling play in the final complement of mitochondrial CL is not known and the presence of these enzymes does not compensate for the absence of tafazzin function *in vivo* [16].

Role of mitochondrial dysfunction in heart failure in Barth syndrome

The heart is the most metabolically demanding organ in the body, subsequently containing the highest amount of mitochondria [28] and accounting for about 8% of total ATP consumption in the body [29]. The heart uses almost 90% of the cellular ATP generated within the myocardium for the ATP-dependent actions of cardiac muscle contraction and relaxation [30]. Accordingly, mitochondrial dysfunction plays a central role in a variety of cardiac diseases, ranging from common acquired HF to rare genetic cardiomyopathies such as BTHS [31,32].

As a biologic process, ATP synthesis must match the rate of ATP consumption for proper cell function because ATP cannot be stored. ATP synthesis occurs through glycolysis in the cytoplasm and oxidative phosphorylation within the mitochondrial ETC [33]. Mismatch between ATP supply and demand has been observed in most etiologies of HF and contributes to worsening of the HF state [29]. *In vivo* and *in vitro* studies in experimental models of *TAFAZZIN*-deficiency and clinical studies in patients with BTHS have demonstrated multiple facets of mitochondrial dysfunction in the heart. Studies in induced pluripotent stem cell (iPSC)-derived *TAFAZZIN*-deficient cardiac myocytes have revealed sarcomere disarray, impaired contractility and increased mitochondrial ROS production, suggesting that defective mitochondrial structure and function due to altered CL play central roles in BTHS cardiomyopathy [34]. In addition, increased LV wall thickness and decreased LV volumes resembling HF with preserved ejection fraction (HFpEF) have been observed in *TAFAZZIN*-knockdown mice, confirming the phenotype of hypertrophic cardiomyopathy (HCM) in BTHS [35]. Mitochondrial calcium (Ca^{2+}) uptake is defective in BTHS and contributes both to the inability to increase left ventricular stroke volume (LVSV) during exertion and vulnerability to life-threatening ventricular arrythmias [36].

Natural history of cardiomyopathy in Barth syndrome

Cardiomyopathy is the most frequently encountered clinical manifestation of BTHS, occurring in approximately 90% of BTHS patients with a wide variety of phenotypical manifestations and severities [31,37]. Given the clinical heterogeneity and rarity of BTHS, limited data exist on the temporal evolution of the cardiac phenotype and no known mechanism exists to explain the presence of different cardiomyopathic phenotypes in BTHS [3,38]. Natural history data and longitudinal studies have become useful tools in understanding the pathophysiology, phenotypic patterns and potential therapies in rare diseases [3,4,7,39].

A variety of cardiomyopathic phenotypes have been described in BTHS, including dilated cardiomyopathy (DCM), LV noncompaction (LVNC) either alone or in conjunction with other cardiomyopathic phenotypes, endocardial fibroelastosis and HCM. The most common presentation in infancy is DCM, characterized by decreased LV systolic function as well as increased LV mass, LV end-diastolic (LVED) dimension and LV end-systolic (LVES) dimension (Figure 2A) [37,38,40,41]. LVNC, alone or in conjunction with other phenotypes, occurs in approximately 50% of BTHS patients and consists of LV trabeculations with intertrabecular recesses in the ventricular myocardium [16]. An undulating phenotype, presenting with transition between distinct phenotypes in the setting of LVNC [42], as well as the endocardial fibroelastosis phenotype, are less commonly seen in patients with BTHS [43]. HCM, including an apical form of HCM [44], is typically defined by small LV volumes and poor LV relaxation (Figure 2B) [8,45]. Interestingly, hypertrophic remodeling has been reported to be a common form of cardiomyopathy in many mitochondrial disorders [46–49]. Independent of the severity of cardiomyopathy, a risk of ventricular arrythmias and sudden death exists for BTHS patients [23,43,50,51] with prolonged QTc $\geq$ 460 ms being more common in patients who progress to cardiac transplantation or death [39].

Dilated cardiomyopathic phenotype is characteristic in infancy

According to data from the Barth Syndrome Registry, 70% of BTHS patients present with cardiomyopathy in the first year of life and all BTHS patients who develop cardiomyopathy do so by 5 years of age [3]. Furthermore, an observational study found that 93% of patients have cardiomyopathy at initial presentation and 88% of those patients present within the first 6 months of life [52]. Initial presentation of cardiomyopathy in BTHS patients may mimic viral myocarditis or be precipitated by viral illness [2] and diagnosis is often delayed as evidenced in the largest longitudinal study in BTHS patients where diagnosis was delayed by an average of 3.3 years from onset [3].

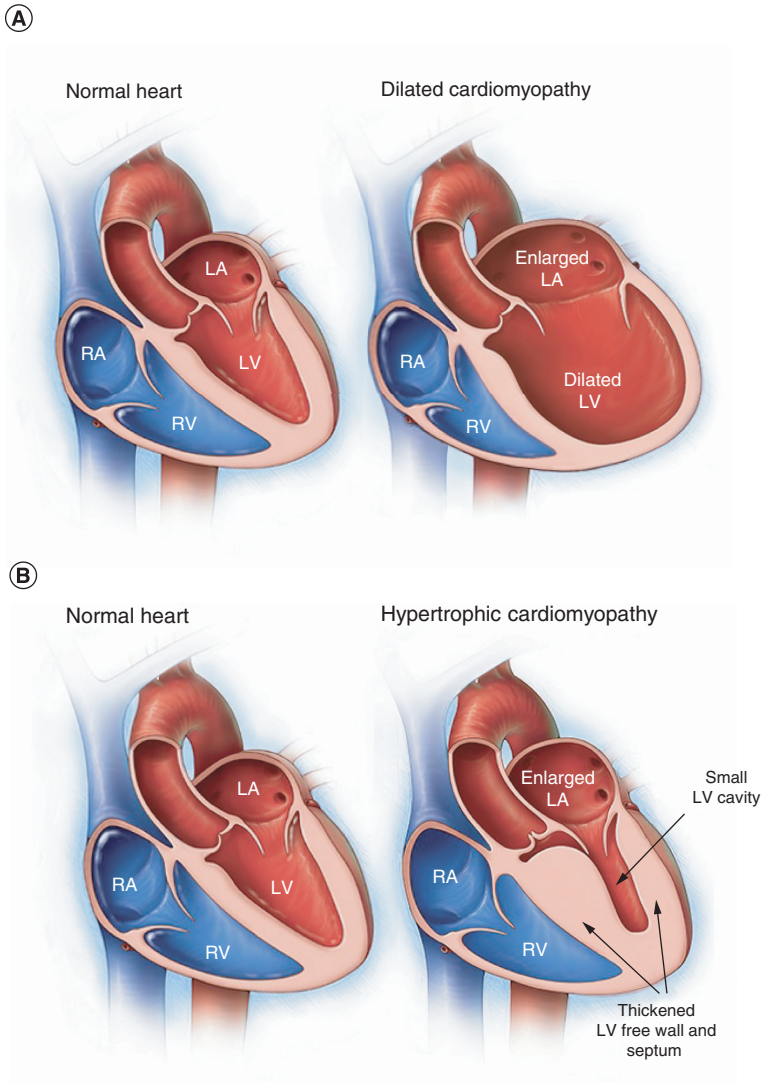

Figure 2. Cardiomyopathies in patients with Barth syndrome. (A) Dilated cardiomyopathy (DCM); **(B)** hypertrophic cardiomyopathy (HCM).
Adapted with permission from [41,45] © Mayo Clinic.
BTHS: Barth syndrome; LA: Left atrium; LV: Left ventricle; RA: Right atrium; RV: Right ventricle.

Characteristic cardiac abnormalities associated with BTHS, such as DCM and LVNC, typically present in infancy [37], a high-risk period for morbidity and mortality related to cardiac decompensation [3,38,39,52] and may stabilize thereafter [4]. In a French study of 22 BTHS patients, LV size, LV mass and LVED dimension were increased during the first 6 months of life, then decreased until 2 years of age when these measures stabilized [4]. The LV ejection fraction (LVEF) was altered in these patients in the first 6 months of life, but typically improved thereafter [4]. Similarly, a study in the UK on BTHS patients found reduction in LV size and normalization of systolic function by measure of fractional shortening (FS) after the first 3 years of life [52].

Rapid decompensation is often triggered by infections in infancy, prior to stabilization of heart function, making infancy and early childhood particularly vulnerable stages [53]. In the French cohort of BTHS patients, 50% of the patients died at a median age of 5.1 months with cardiac failure being the most common cause of death [4]. Although survival has improved in BTHS patients born after the year 2000, the median age at death has not changed [4], possibly due to the skewing of mortality toward infancy.

Hypertrophic cardiomyopathy in adolescent & adult patients with Barth syndrome

Another high-risk time period in patients with BTHS appears to be prepubescence into early adulthood when BTHS patients often experience rapid catch-up growth, placing more strain on the compromised heart [53]. An unpublished subgroup analysis from a recently published cardiac natural history study in BTHS patients [39] demonstrated that patients ≥12 years of age had negative LVSV z-scores, indicating impaired LV function (similar to the baseline observations noted in the TAZPOWER clinical trial [6]). In addition, longitudinal echocardiographic

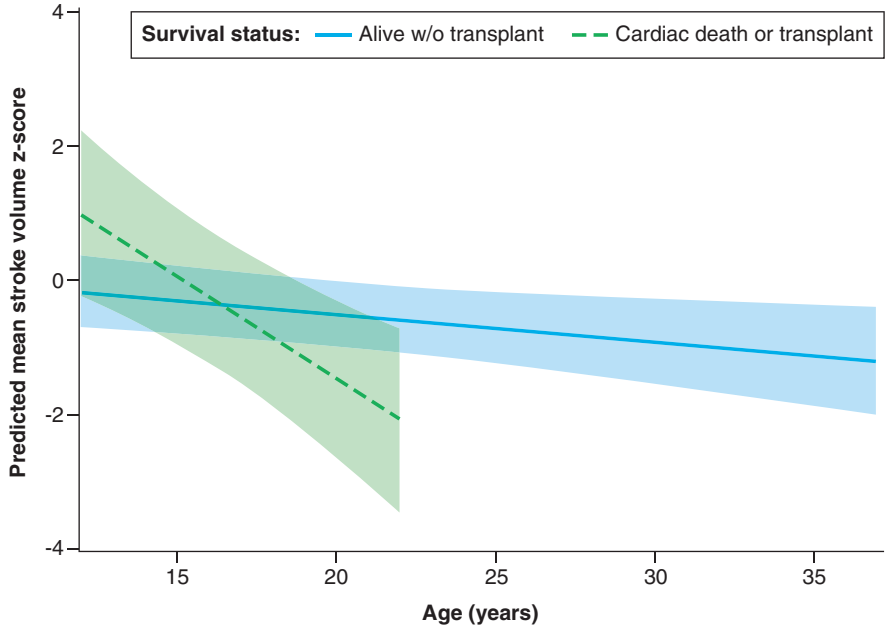

Figure 3. Left ventricular stroke volume relationship to morbidity and mortality in patients ≥12-years-old with ≥2 echocardiograms from the Cardiac Natural History Study.
w/o: Without.

data, available for six of the eight ≥12-year-old patients with cardiac-related mortalities in the cardiac natural history study, may support a relationship between LVSV, morbidity and mortality. The decline in LVSV for these patients is greater than in patients who are alive without transplant (p = 0.004) (Figure 3) [39]. The majority of patients experiencing cardiac morbidity or mortality were between 12 and 20 years of age with a mean age of 16.78 years. It should be noted that this was an older cohort of the original dataset of patients (median age of 7 years at enrollment) and does not represent BTHS mortality data as a whole.

Data from the Barth Syndrome Registry has shown that for each 5-year increase in age, the ejection fraction (EF) z-score decreases by 0.6 [3]. Natural history cohorts have shown a gradual decline in LVSV, and therefore in cardiac output [7]. In a recent longitudinal study of BTHS patients, a decline in LV size with advancing age, as demonstrated by reduction in LVED volume (LVEDV) and LVED internal diameter, was noted (Figure 4) [39]. Although LVEF and LV fractional shortening (LVFS) did not change over time in this longitudinal study, a significant decline in LVSV and significant worsening of LV global longitudinal strain (GLS), right ventricular (RV) fractional area of change (FAC) and septal E:e' were observed, suggestive of cardiac remodeling with advancing age [39].

Despite evidence of decline in cardiac function over time in BTHS patients, the majority (75%) of patients experience relative stabilization or even cardiac reverse remodeling leading to improved or normalized LV size and function [3,4,38,39,52]. Specifically, LV dilation by volume improves over time [39] with preserved LVEF that remains stable [38] or only mildly deteriorates with time [3]. Echocardiograms of BTHS patients have consistently demonstrated that LVFS and LVEF are only mildly abnormal outside of infancy [3]. In this regard, BTHS cardiomyopathy has similarities to HFpEF [36], where LVEF is preserved at rest and does not increase during exercise [37], while diastolic function is compromised [54–56].

Clinical features of TAZPOWER clinical trial patients

TAZPOWER was a phase II, randomized, double-blind, placebo-controlled trial that evaluated the efficacy and safety of elamipretide in 12 patients (mean age 19.5 years, range 12–35 years) with genetically confirmed BTHS, followed by a long-term, open-label extension (OLE) of elamipretide treatment [6]. The cardiac phenotype seen in these adolescent and adult subjects was characterized by small LVEDV, small LVES volume (LVESV), normal LVEF and poor LV active and passive filling leading to reduced LVSV, aligning with the HFpEF type of LV failure [8]. Specifically, abnormal echocardiographic findings included a low cardiac index (CI) (mean 2.3), low LVESV and LVEDV (both below the 8th percentile z-scores), and negative LVSV z-scores. These findings support

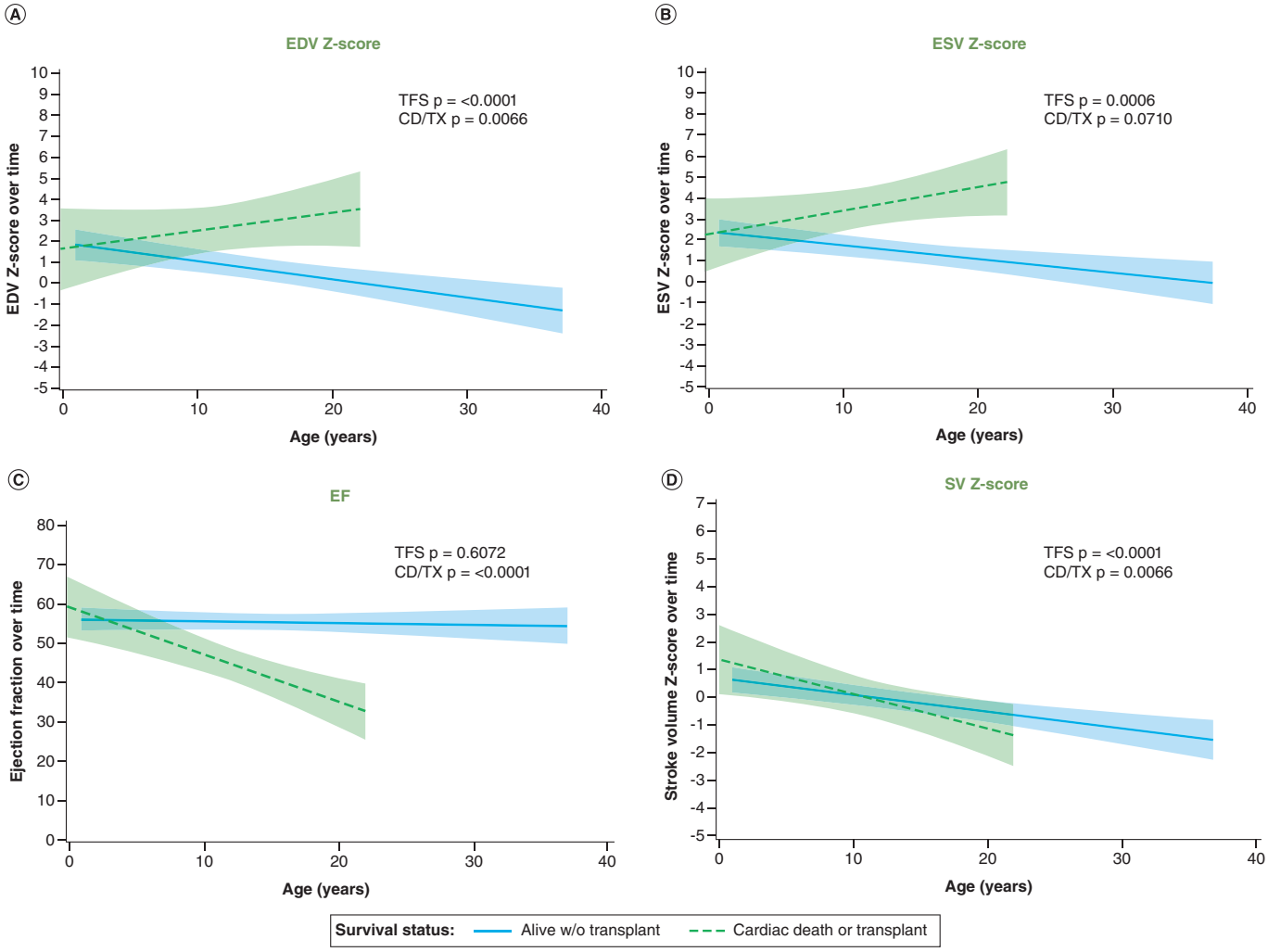

Figure 4. Changes in cardiac parameters with advancing age in patients (age range 0–22 years) with Barth syndrome grouped by those who underwent cardiac transplant or died and transplant-free survivors. (A) End-diastolic volume z-scores; **(B)** end-systolic volume z-scores; **(C)** ejection fraction and **(D)** stroke volume z-scores.
CD/TX: Cardiac death or transplant; EDV: End-diastolic volume; EF: Ejection fraction; ESV: End-systolic volume; SV: Stroke volume; TFS: Transplant-free survival; w/o: Without.
Adapted from Chowdhury *et al.* with permission from [39].

data published in multiple natural history and longitudinal studies in patients with BTHS and support that BTHS patients ≥12 years of age portray a HCM phenotype with features similar to HFpEF [7].

Treatment of cardiomyopathy in patients with Barth syndrome

Current treatment of cardiomyopathy in patients with BTHS consists of symptomatic treatment, and is not targeted to the underlying pathophysiology of the disease. Medical therapies are typically directed at the specific cardiac phenotype being treated and include angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) for the treatment of DCM [57]. Other commonly used medications to treat cardiomyopathy in BTHS include beta-blockers, diuretics and anticoagulants [44,58,59]. For patients with worsening HF, more aggressive therapies may be required, such as intravenous inotropes, left ventricular assist devices and/or cardiac transplantation [60–62].

Targeting cardiolipin with elamipretide

Given that the primary defect underlying BTHS is altered CL content, CL is a logical therapeutic target. Elamipretide is an aromatic–cationic tetrapeptide that readily penetrates the outer mitochondrial membrane and transiently

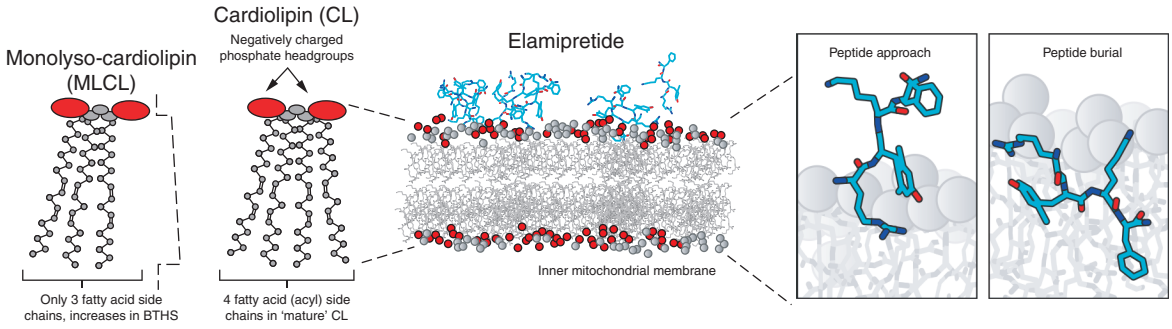

Figure 5. Elamipretide interaction with cardiolipin in the inner mitochondrial membrane. The peptide, elamipretide, diffuses across the outer mitochondrial membrane and to the inner mitochondrial membrane where its positively charged residues interact electrostatically with the anionic headgroups of cardiolipin, increasing local concentration levels, while the nonpolar side of its chains interact hydrophobically with the acyl chains.
Adapted from Mitchell *et al.* with permission from [69].
BTHS: Barth syndrome.

localizes to the IMM where it associates with CL (Figure 5) [63–69]. This association improves IMM stability; enhances ATP synthesis in several organs including the heart, kidney, neurons and skeletal muscle; and reduces reactive oxygen species (ROS) production [63–68]. At the molecular level, the positively charged residues of elamipretide bind electrostatically with the anionic head groups of CL while its nonpolar side chains penetrate the IMM to interact hydrophobically with the acyl chains of CL [69]. This interaction leads to increases in lipid packing, membrane curvature and membrane surface area central to cristae formation, supercomplex association and efficient oxidative phosphorylation [69]. Since the interaction between elamipretide and the membrane bilayer is not dependent on the content or composition of CL side chains, it can interact with MLCL in a similar ratio, making elamipretide well suited for BTHS [69].

Impact of elamipretide on mitochondrial dysfunction in preclinical studies

Impact on pathways critical to the pathogenesis of BTHS have been observed in studies evaluating the role of elamipretide in other experimental models of HF [14]. Importantly, elamipretide has been shown to ameliorate abnormally expressed genes and proteins involved in CL biosynthesis and remodeling in a canine model of HF [70], as well as to ameliorate abnormally peroxidated CL species and depleted CL and L4CL levels in the myocardium [63]. Furthermore, in the canine HF model, long-term elamipretide therapy not only improved the maximum rate of ATP synthesis and mitochondrial respiratory complex activities (most notably complexes I and IV), but also normalized mitochondrial respiration, sarco/endoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a) activity, mitochondrial permeability transition pore (MPTP) opening, and mitochondrial membrane potential as well as reduced cytosolic cytochrome c release and ROS formation [63]. A similar range of improvements were seen after three months of elamipretide therapy in a porcine model with a HFpEF phenotype [71]. In explanted LV tissue from humans with HF, improvements in the activities of supercomplex-associated complexes I, III and IV were observed after treatment with elamipretide [72]. In cells engineered using CRISPR to have a *TAFAZZIN* mutation, the decrease in several ETC subunits and defective mitochondrial quality control pathways were reversed after 7 days of elamipretide administration [70]. Furthermore, elamipretide preserved supercomplex-dependent mitochondrial function in the LV myocardium of rats after ischemia [73]. Similarly, *TAFAZZIN*-knockdown mice treated with elamipretide demonstrated improvement of mitochondrial respiration as well as increased assembly and stability of supercomplexes, without afftecting the MLCL:CL ratio [74].

CL is also essential in mitochondrial biogenesis, cristae structure, fission and fusion and mitophagy, processes which rely on several proteins, including peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α), fission proteins DRP1 and fission 1 (FIS1), fusion proteins OPA1 and mitofusin2 (MFN2) and mitofilin [75,76]. Abnormalities in all of these proteins were observed in the failing LV myocardium of dogs as well as in LV myocardium from explanted failed human hearts [75]. Chronic elamipretide therapy in dogs with experimental HF reversed the dysregulation of the fusion and fission proteins, PGC-1α and mitofilin [75,77].

The beneficial effects of elamipretide on the mitochondrial structure have been demonstrated in a number of different models. In biomimetic membranes modeling the IMM, elamipretide showed improvement in IMM

biophysical properties by aggregating CL [78]. This effect was maintained even when the CL levels were lowered to levels consistent with BTHS [78]. Electron microscopy of ischemia reperfusion rat hearts demonstrated improvement in disease-induced fragmentation of the mitochondrial cristae networks following elamipretide treatment [78]. In induced pluripotent stem cells (iPSCs) from BTHS and closely related pediatric cardiomyopathies, elamipretide improved mitochondrial morphology and bioenergetics rapidly (within hours or days) [34,79]. In another study involving BTHS patient-derived cell lines and iPSC-derived cardiomyocytes from pediatric patients with cardiomyopathy, elamipretide restored proper mitochondria structure [69]. A similar observation was made in rats whereby ultrastructure was restored in defective mitochondria following elamipretide therapy [78].

Beneficial effect of elamipretide on cardiac function in preclinical studies

Considering the similarities of adolescent and adult cardiac phenotypes in patients with BTHS to HFpEF, benefits of elamipretide in other models of HFpEF may support its use in BTHS. Elamipretide therapy has demonstrated improvement in cardiac parameters in a number of animal HF models. A porcine model with a HFpEF phenotype, as evidenced by preserved LVEF, LV hypertrophy, and poor LV relaxation, demonstrated improved LV relaxation and amelioration of LV cardiac hypertrophy without affecting blood pressure or systolic LV function upon three months of elamipretide therapy [71]. In dogs with HF with reduced ejection fraction (HFrEF), three months of elamipretide treatment resulted in improved LV systolic function and prevented progressive LV enlargement without affecting heart rate, blood pressure or systemic vascular resistance [63]. Specifically, elamipretide significantly increased LVEF and LVFS, while significantly reducing LVES volume and n-terminal pro-brain natriuretic peptide (NT-proBNP) plasma concentration [63]. The magnitude of improvement was comparable to that seen after chronic treatment with ACE inhibitors, ARBs and/or beta-blockers [80]. Hypertrophic HF models have demonstrated promising results with elamipretide therapy. For example, elamipretide reduced LV cardiac hypertrophy and improved LV diastolic function in mice with hypertensive cardiomyopathy [81]. Similarly, in rats with HF secondary to transverse aortic constriction (TAC) as evidenced by increases in LV mass, LV dilation, and myocardial fibrosis, elamipretide reduced LV hypertrophy, improved LV systolic dysfunction and limited LV myocardial fibrosis [67]. In old mice with LV diastolic dysfunction, 8 weeks of elamipretide therapy normalized the diastolic functional deficit, increased ratio of early-to-late diastolic mitral annulus velocities (Ea/Aa), improved exercise tolerance with regression of cardiac hypertrophy and normalized mitochondrial proton leak and ROS [82]. This study also demonstrated that the beneficial effects of elamipretide last well beyond its half-life and are not likely to immediately subside upon withdrawal of the drug [8,82].

A randomized clinical trial in patients with HFrEF examined the efficacy, safety and tolerability of ascending single doses of intravenous infusions of elamipretide when added to guideline-directed HF medical therapy [83]. The highest dose (0.25 mg/kg/h) of elamipretide resulted in significant reduction (improvement) in LVEDV (-18 ml; $p = 0.009$) and LVESV (-14 ml; $p = 0.005$), while not impacting cardiac hemodynamics [83]. Elamipretide was generally safe and well tolerated in this study [83].

Elamipretide treatment in patients with Barth syndrome: TAZPOWER trial

The TAZPOWER clinical trial was designed to evaluate the safety, tolerability and efficacy of 12 weeks of treatment with daily subcutaneous elamipretide in patients ≥12 years of age with BTHS, followed by a 168-week open label extension (OLE) on elamipretide [6]. At baseline, these patients presented with low LVEDV and normal LVEF, consistent with the phenotype of HCM [14]. In the first treatment phase (12 weeks) of the trial, small improvements in cardiac function, including LVSV, were observed for most subjects, but were not considered significant [84].

Although no significant effect of elamipretide was observed during the 12-week phase for the primary or secondary study end points, elamipretide treatment was associated with significant improvements in functional and cardiac outcomes compared with baseline during the OLE part of the study [6]. Treatment with elamipretide led to a 16% improvement (30.5 ml/m^2 to 35.3 ml/m^2) in LVSV indexed to body surface area (BSA) at 36 weeks of the OLE [6], and a greater than 45% improvement (mean 14.4 ml; $p = 0.007$) in this same parameter at 168 weeks (Figure 6) [85,86]. LVEDV and LVESV, both indexed to baseline BSA, significantly improved at all time points in the OLE. Specifically, mean improvements of 24.42 ml ($p = 0.003$) for LVEDV and 10.04 ml ($p = 0.0008$) for LVESV were observed overall [87], suggesting the occurrence of cardiac remodeling with improvement/reduction in myocardial stiffness due to elamipretide. The QTc prolongation decreased by 8.5% (mean 452.4 ms) at 168 weeks of the OLE [87], while heart rate, blood pressure and EF remained unchanged, suggesting that the cardiac work did

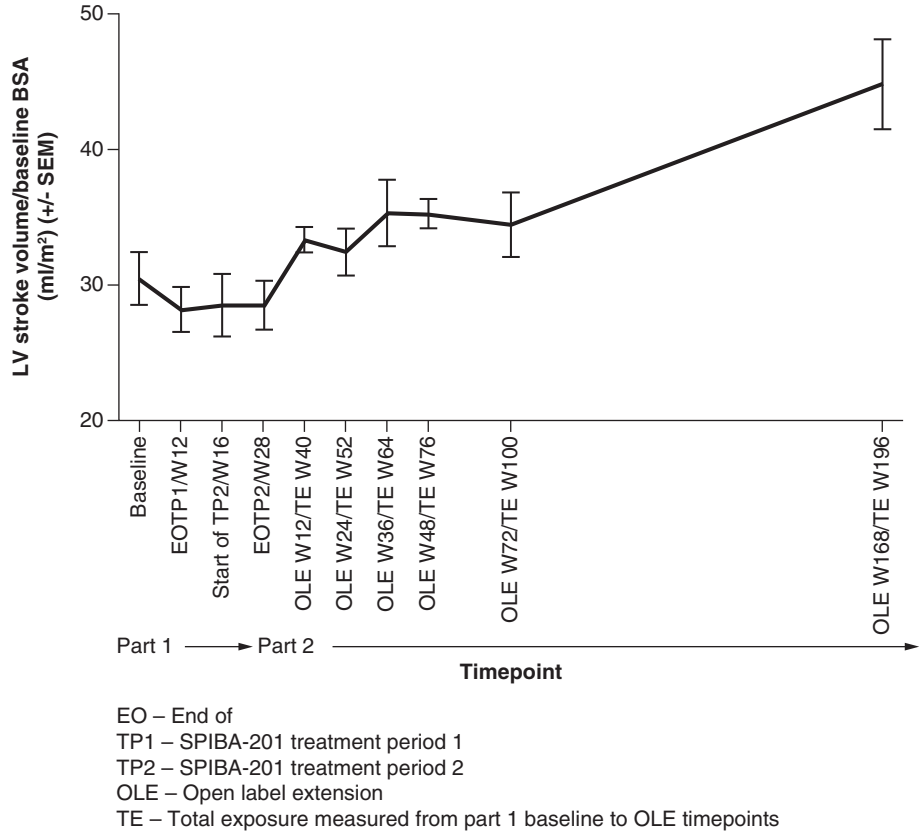

Figure 6. Mean (± standard error of the mean) left ventricular stroke volume indexed to the baseline body surface area in the TAZPOWER trial and open label extension.
BSA: Body surface area; LV: Left ventricular; OLE: Open label extension; SEM: Standard error of the mean.

not increase during elamipretide treatment [87]. Collectively, these results support a trend toward normalization of cardiac performance [87].

Significant improvement was observed in the primary end point of the TAZPOWER trial, the six-minute walk test (6MWT), such that by the 36-week mark in the OLE, a 25% improvement (95.5 meters) in the 6MWT was observed [6]. At the end of the OLE treatment period, a 60% improvement (122.7 meters) in 6MWT and cumulative reduction of 2.5 in total fatigue score were noted compared with baseline [87]. Spearman correlation analysis of cardiac volumes revealed a positive association between improvements in functional measures (6MWT and muscle strength) and LV volume changes observed over time with elamipretide treatment [87]. Improvements in LVEDV and LVSV are major determinants of peak exercise capacity in patients with HCM and, therefore, these findings support that improved cardiac function and possibly improved skeletal muscle energetics, likely contributed to improved exercise tolerance and strength in these patients [8]. The delayed treatment effect seen in these subjects may be due to the length of time needed for skeletal and cardiac muscle remodeling to occur [6].

In comparing the TAZPOWER and OLE elamipretide-treated subjects to BTHS patients in a natural history cohort, significant improvements in several cardiac parameters were observed [7]. LVSV index increased significantly in elamipretide-treated patients compared with natural history controls (Figure 7). Furthermore, improvements in LVEDV index and LVESV index were observed in elamipretide-treated patients, but these changes were not significant [7]. Elamipretide was generally well tolerated by the TAZPOWER subjects with injection site reactions being the most frequently reported adverse event [6]. Overall, the promising results from the TAZPOWER trial support the conclusion that long-term treatment with elamipretide may improve overall cardiac function, quality of life and the long-term disease progression trajectory of BTHS patients.

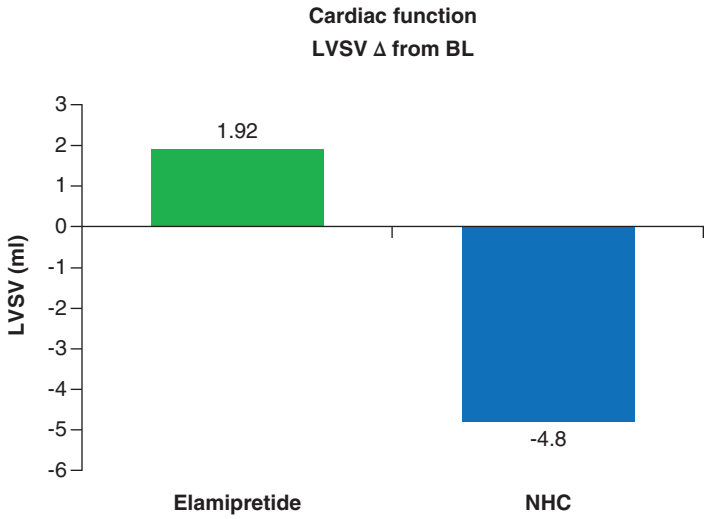

Figure 7. Change in left ventricular stroke volume in subjects treated with elamipretide versus natural history control subjects. A mixed model repeated measures analysis of the change in LVSV indexed to baseline body surface area from baseline demonstrated a least squares mean improvement from BL in elamipretide-treated subjects versus an observed decline in NHC subjects (least squares means 6.72; $p = 0.002$).
Data taken from [7].
BL: Baseline; LVSV: Left ventricular stroke volume; NHC: Natural history control.

Clinical significance of altered Barth syndrome cardiomyopathy phenotype: approach to therapy & expected signs of beneficial outcomes

As previously discussed, while DCM is predominant in infants with BTHS, the cardiomyopathy observed in adolescence and beyond is often characterized by LV hypertrophy and small LV volume, resembling the HFpEF phenotype rather than HFrEF [8]. DCM causes a phenotype similar to HFrEF, resulting in a weakened LV myocardium incapable of properly contracting, whereas HCM leads to a phenotype of HFpEF, characterized by a stiffened LV incapable of normal LV relaxation and filling. ACE inhibitors, ARBs, beta-blockers and diuretics are the most common treatment modalities for DCM and act through energy-sparing mechanisms, such as reductions in heart rate, preload and afterload, that help decrease overall cardiac workload [88]. Treating HCM with therapies indicated for DCM will not properly address the underlying pathology and are less likely to improve symptoms. Instead, treatment of BTHS adolescents and adults, as clinically indicated, should be approached as if the phenotype may be that of HFpEF as indicated by imaging/echocardiographic parameters.

In BTHS, a small LV volume and subsequent poor LV filling may lead to reduced stroke volume, a key component of both cardiac output and LVEF [8]. Stroke volume, the volume of blood ejected from the LV with each systolic contraction, can only increase in the setting of HFpEF if the LV enlarges through improved LV relaxation and, hence, LV filling. Therefore, expected benefits of a targeted treatment for HCM of BTHS should include increased LV volumes and LVSV. In preclinical and clinical studies in HF and BTHS models, elamipretide has been shown to increase LVSV, LVEDV and LVESV as well as increase LV mass in patients with BTHS [63,67,71,81,87]. The LV enlargement in this scenario is due to improved LV relaxation, mediated by improved mitochondrial function through increased availability of ATP, as a direct effect of therapy with elamipretide. These findings support elamipretide as a therapeutic option for adolescent and adult BTHS patients with a HCM phenotype similar to HFpEF.

Conclusion

As demonstrated in multiple longitudinal and natural history studies, the HF phenotype in patients with BTHS evolves from infancy to adulthood [7,39,87]. The phenotype during infancy is primarily that of DCM with reduced LVEF, whereas the phenotype in adolescence and adulthood may evolve to HCM with preserved LVEF resembling that of HFpEF. Given the distinct HCM phenotype, therapeutic measures that improve LV relaxation can be targeted for use in adolescent and adult patients with BTHS. As shown through increased LV volumes and LVSV in multiple preclinical and clinical models, elamipretide improves LV relaxation through its role in ameliorating mitochondrial dysfunction. We hypothesize that since myocardial relaxation is an ATP-dependent process [89], the ATP deficiency in BTHS caused by CL abnormalities and subsequent mitochondrial dysfunction must be overcome to improve LV relaxation. As demonstrated in several studies, through its association with CL, elamipretide enhances ATP synthesis and in turn improves LV relaxation and subsequent LVSV. A fuller appreciation for the temporal evolution of the cardiomyopathic phenotype in patients with BTHS will allow for the introduction of targeted

therapies at various stages during the natural history of disease progression that better address relief of disease symptomatology and are more effective in achieving long-term improvement of disease trajectory.

Future perspective

We expect that over the next 5 to 10 years, drugs that target the mitochondria and CL, such as elamipretide, will likely be approved for the treatment of BTHS patients. Approval of this class of drugs is expected to improve the quality of life and possibly, life expectancy, of BTHS patients. During this time period and beyond, we expect that additional research will be conducted to further support the use of gene therapy for the treatment of many human genetic disorders, including BTHS, with the likelihood of clinical application sometime over the next two decades.

Executive summary

Pathophysiology of Barth syndrome

- Pathogenic variants in *TAFAZZIN* underlie Barth syndrome (BTHS) and lead to abnormal cardiolipin (CL) composition, a phospholipid of the inner mitochondrial membrane (IMM) crucial for mitochondrial structure and function, including ATP production.
- Given that cardiac contraction and relaxation are ATP-dependent processes, mitochondrial dysfunction likely plays a central role in a variety of cardiac pathologies, including common idiopathic forms of heart failure (HF) and rare genetic forms, such as BTHS.

Nautral history of cardiomyopathy in Barth syndrome

- Cardiomyopathy manifests in nearly every patient with BTHS, most commonly presenting as dilated cardiomyopathy (DCM) in infancy and evolving in adolescence and adulthood to a hypertrophic cardiomyopathic (HCM) phenotype resembling HF with preserved ejection fraction (HFpEF) in many patients.

Treatment of cardiomyopathy in patients with Barth syndrome

- Elamipretide is a mitochondria-targeting peptide that localizes to the IMM where it associates with CL and improves IMM stability, enhances ATP synthesis, and reduces reactive oxygen species production.
- Preclinical and clinical studies have consistently demonstrated improvement in left ventricular (LV) stroke volume as well as LV end-diastolic and end-systolic volumes with elamipretide therapy.
- Treating HCM in BTHS with therapies indicated for DCM will not properly address the underlying pathology and instead, treatment of BTHS adolescents and adults should be approached as if the phenotype is that of HFpEF.
- Data from the TAZPOWER trial showed that patients with BTHS manifest small LV volumes and poor LV relaxation and, hence, LV filling, all of which lead to reduced stroke volume. Achieving a therapeutic increase in stroke volume would require improved LV relaxation and filling, manifested by increased LV end-diastolic volume.
- Since myocardial relaxation is an ATP-dependent process, the ATP deficiency in BTHS caused by CL abnormalities and subsequent mitochondrial dysfunction must be overcome to improve LV relaxation, making elamipretide a well-suited therapeutic candidate for adolescent and adult patients with BTHS.

Author contributions

All authors contributed equally to production of this review. All authors read and approved the final manuscript.

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