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Towards a treatment for mitochondrial disease: current compounds in clinical development

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Summary

Primary mitochondrial diseases are a heterogeneous group of rare genetic disorders affecting approximately 125 persons per million. Mutations underlying these diseases give rise to biological changes (including decrease in cellular energy production and increase in reactive oxygen species), leading to organ failure, and commonly early morbidity. Mitochondrial diseases often present in early childhood and lead to the development of severe symptoms, with severe fatigue and myopathy being some of the most prevalent and debilitating clinical signs.

There are currently no cures for mitochondrial diseases, nor any approved pharmaceutical treatments for multisystemic disorders.

Current drug development in mitochondrial diseases focuses mainly on modulation of oxidative stress, regulation of the expression of genes involved in metabolic pathways, modulation of coenzymes, induction of mitochondrial biogenesis, and energy replacement.

In this short review, we present the current landscape of mitochondrial disease drug development, focusing on small molecules in clinical trials conducted by industrial sponsors.

1. Introduction

1.1. Primary mitochondrial diseases

Primary mitochondrial diseases are a heterogeneous group of rare genetic disorders, which stem from a defect in the cell's energy-producing organelles–the mitochondria. These diseases, often devastating, are caused by mutations in the nuclear DNA (nDNA) or mitochondrial DNA (mtDNA) which encode mitochondrial components such as subunits of the electron transfer system (ETS), mitochondrial assembly proteins, or factors regulating mtDNA translation [1] and give rise to biological changes (including decrease in energy production, deregulation of calcium signaling, and increase in reactive oxygen species) [2]. In affected individuals, these harmful cellular processes lead to cell death, tissue and organ failure, and in many cases early morbidity.

Affecting approximately 125 persons per 1 000 000 [3], mitochondrial diseases often present in early childhood and lead to severe symptoms and clinical signs such as fatigue, myopathy (muscle weakness and/or exercise intolerance), heart failure, intellectual disability, movement disorders, and epileptic seizures [4]. Among these, fatigue (typically together with myopathy) is one of the most prevalent and debilitating effects irrespective of sex, age or genotype [5]. In affected children, where Leigh syndrome is the most common mitochondrial disorder, regressive neurological symptoms are dominant [6, 7]. In adults, a spectrum of diseases caused by the mtDNA point mutation m.3243A>G, with clinical presentations such as mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS) and maternally inherited diabetes and deafness (MIDD), are one of the most common groups of primary mitochondrial diseases [8].

1.2. Diagnostics and current standard of care for patients with mitochondrial diseases

The effects of mitochondrial disease can arise in any organ, at any age, and with varying severity. In fact, in many cases the clinical phenotype is nonspecific [9], making these diseases difficult to diagnose. Together with the patient's family history, diagnostics have mainly relied on diagnostic criteria specifically for mitochondrial diseases, which take into account the clinical, biochemical and imaging findings and their importance [10], often involving the need of a muscle biopsy.

In recent years, great advances within diagnostics of mitochondrial diseases have been made with the increasing availability of Whole Genome/Exome Sequencing (WGS/WES) [11]. Moreover, the use of serum biomarkers as both predictors of mitochondrial dysfunction and diagnostic tools is an emerging field. Recently, fibroblast growth factor 21 (FGF21) and growth/differentiation factor 15 (GDF15) levels were correlated with disease severity in muscle [12]. In addition, a study confirmed the depletion of the co-enzyme and co-substrate nicotinamide adenine dinucleotide (NAD^+), a central molecule in cellular energy metabolism, including mitochondrial energetics, in mitochondrial disease patients suffering from myopathy [13].

There are currently no cures for primary mitochondrial diseases, nor any approved pharmaceutical treatments for multisystemic disorders. To date, the treatment strategies are mainly symptomatic, and limited to dietary supplementation with amino acids, antioxidants and other supplements (e.g. carnitine, creatine, riboflavin, coenzyme Q10

(CoQ_{10}), and vitamin K with ascorbate) [14, 15], the use of which is based on circumstantial evidence (no evidence of efficacy from properly controlled clinical studies). In some cases, therapy is palliative only [1].

To date, the synthetic antioxidant Raxone® (idebenone, a CoQ_{10} analog) is the only drug that has been approved, by the European Medicines Agency (EMA), for a mitochondrial disease, specifically for Leber's Hereditary Optic Neuropathy (LHON), a disorder causing degeneration of retinal ganglion cells, leading to progressive loss of central vision on both eyes. However, no evidence for the efficacy of either idebenone or other specific pharmaceutical compounds, in multisystemic mitochondrial diseases, have yet been demonstrated.

1.3. Aim and methods

Current drug development in mitochondrial diseases focuses on specific mechanisms of action, including modulation of oxidative stress, regulation of the expression of genes involved in metabolic pathways, modulation of coenzymes, induction of mitochondrial biogenesis, energy replacement, and specific cell- or gene therapy. In this short review, we present the current landscape of systemic mitochondrial disease drug development, focusing on small molecules in ongoing clinical trials conducted by industrial sponsors. In addition to primary literature, sources have included www.clinicaltrials.gov and the targeted drug development companies' websites.

2. Compounds for the treatment of primary mitochondrial diseases in ongoing clinical trials

A large percentage of the planned or ongoing clinical studies of mitochondrial disease are being conducted by pharmaceutical companies testing small molecules in healthy volunteers and/or patients with systemic mitochondrial diseases (Table 1).

2.1. Metabolic reprogramming and mitochondrial biogenesis

NADH:quinone oxidoreductase (NQO1) is a cytoplasmic antioxidant flavoprotein that transfers two electrons from NADH to reduce quinones into hydroquinones, some of which can then donate their electrons to the ETS [16, 17].

KL1333 is an orally bioavailable synthetic ortho-quinone, in clinical development by Abliva AB in collaboration with Yungjin Pharmaceuticals. Seo et al [18] demonstrated in a cell-free system that KL1333 is reduced by NQO1 using NADH as electron donor and re-oxidized by transferring its electrons to cytochrome *c*, undergoing a redox cycle more potently and rapidly than that of other quinones such as idebenone and CoQ10. SIRT1, a NAD^+-dependent protein deacetylase from the family of silent information regulators [19], has been shown to regulate several metabolic pathways such as mitochondrial biogenesis, gluconeogenesis, lipolysis and fatty acid oxidation [19-22]. *In vitro* studies in MELAS patient cells showed that KL1333 was able to restore NAD^+/NADH levels and activate the SIRT1/AMPK/PGC-1α pathway, increasing mitochondrial biogenesis [18]. KL1333 has been clinically evaluated in a combined Phase Ia/b study in healthy volunteers and patients with primary mitochondrial diseases confirming its safety and tolerability as well as showing promising trends of improvement of fatigue and functional muscle strength in patients treated with active drug compared to placebo [23]. A registrational Phase II/III study is planned to start in 2022.

Table 1. Ongoing* clinical trials of primary mitochondrial diseases

Molecule: company	MoA	Clinical trials				
		Indication	Study acronym	Phase	ClinicalTrials.gov Identifier	Efficacy: Primary endpoint
Bocidelpar (ASP0367): Astellas	PPARδ agonist	Primary Mitochondrial Myopathy	MOUNTAINSIDE	II and III	NCT04641962	6-minute walk test
Elamipretide (MTP-131): Stealth Biotherapeutics	Cardiolipin-binding peptide	Primary mitochondrial myopathy with nuclear DNA mutations	NuPower	III	NCT05162768	6-minute walk test
IW-6463 (CY6463): Cyclerion Therapeutics	Guanylate cyclase stimulator	MELAS with neurological features	-	IIa	NCT04475549	-
KL1333: Abliva AB	NAD^+/NADH modulator	Primary mitochondrial disease (mtDNA)	FALCON	II and III	-	Fatigue and 30 s sit-to-stand
MT1621: Modis Therapeutics/ Zogenix (UCP)	Pyrimidine nucleosides	TK2 deficiency	-	IIIb	NCT04581733	Proportion of subjects acquiring a motor milestone
REN001: Reneo Pharmaceuticals	PPARδ agonist	Primary Mitochondrial Myopathy	STRIDE	II	NCT04535609	6-minute walk test
			STRIDE AHEAD	II and III	NCT05267574	-
Sonlicromanol (KH176): Khondrion BV	ROS-Redox modulator	m.3243A>G-associated disease with attentional dysfunction	KHENERGYZE	IIb	NCT04165239	Cognitive functioning: Attention
			KHENEREXT	IIb	NCT04604548	-
		Children with mitochondrial disease and motor symptoms	KHENERGYC	II	NCT04846036	Motor Symptom Severity
Vatiquinone (PTC743 and EPI-743): PTC Therapeutics	Oxidative stress modulator	Mitochondrial Disease with Refractory Epilepsy	MIT-E	II and III	NCT04378075	Number of motor seizures

* Recruiting, not yet recruiting, or active (not recruiting) trials investigating small molecules.

Peroxisome proliferator-activated receptor delta (PPARδ), a nuclear receptor and transcription factor, is part of the steroid hormone receptor superfamily [24]. Endogenous ligands of PPARδ are mainly lipids [25] and induce an increase in the mitochondrial capacity to oxidize fatty acids [26]. PPARδ has been proposed as a therapeutic target in metabolic syndrome through its regulation of metabolism in skeletal muscle, the heart, the liver, and adipose tissue [27].

Synthetic agonists of PPARδ have been shown to modulate PPARδ activity [27, 28]. The small molecule **bocidelpar** (**ASP0367**), developed by Mitobridge, a subsidiary of Japanese Astellas Pharma, has been tested in a Phase I clinical trial in healthy volunteers. Results showed no harmful effects, and the upregulation of PPARδ target genes was observed [29]. The company is currently recruiting primary mitochondrial disease patients with myopathy in a Phase II/III study, assessing the effect of bocidelpar on functional improvements and fatigue.

US-based Reneo Pharmaceuticals is similarly developing a PPARδ agonist, **REN001**. Results from a concluded proof-of-concept Phase I study in patients with mitochondrial fatigue showed, according to the company, promising improvements in a walk test and in symptoms questionnaires. Reneo is currently recruiting patients in a Phase II/III study in mitochondrial myopathy patients, primarily investigating the effect of REN001 on improvements in the distance walked in a walk test.

US-based Cyclerion Therapeutics' **CY6463**, described as a CNS-penetrant soluble guanylate cyclase stimulator, is in development for the treatment of a subset of neurological conditions, including MELAS. Guanylate cyclase is an important enzyme of the nitric oxide signaling pathway and catalyzes synthesis of the second messenger cGMP [30]. cGMP, in turn, drives the activation of protein kinases, ion channels, and phosphodiesterases [31], and has been shown to induce mitochondrial biogenesis as well as ATP formation [32]. In preclinical studies, CY6463 has been shown to improve neuronal activity, mediate neuroprotection, and increase cognitive performance [33]. The company is currently investigating the safety, tolerability, pharmacokinetics, and pharmacodynamics of the compound in a Phase IIa study in MELAS patients with neurological manifestations. In addition, Cyclerion will assess near-term impact on disease-specific biomarkers, brain perfusion, neurodegeneration, and cognition.

2.2. Energy replacement and substrate enhancement

Complex I (CI) dysfunction, one of the most common mitochondrial impairments, can lead to a decrease in the NAD^+/NADH ratio, a decrease in mitochondrial membrane potential, and increased succinate utilization by Complex II (CII)/succinate dehydrogenase [7, 34-37]. Patients presenting with primary mitochondrial disorders with CI dysfunction could benefit from replenishment of succinate and additional energetic inputs to the ETS through CII, bypassing the dysfunctional CI. Due to the lack of passive transport of succinate in most cellular membranes, extracellular succinate treatment would result in limited bioenergetic effects. Abliva AB has designed and tested permeable succinate prodrugs. NV189, the first generation of permeable succinate prodrugs, has been shown to ameliorate the increase of lactate production in chemically CI-inhibited human platelets and increase the spare respiratory capacity of Leigh syndrome patient-derived fibroblasts [38]. **NV354,** the second generation of cell membrane permeable succinate, has been designed as an oral chronic treatment of Leigh syndrome. Due to its high brain exposure, NV354 might potentially be tested for other

mitochondrial diseases with neurological symptoms such as MELAS or LHON. A clinical Phase I trial is planned to start in 2022.

Similar to Leigh syndrome patients, children suffering from the ultra-rare mtDNA depletion syndrome thymidine kinase 2 (TK2) deficiency have a short life expectancy. TK2 serves a role in the supply of deoxynucleotides for mtDNA synthesis, and patients with TK2 deficiency exhibit severe myopathy and ultimately respiratory failure. The drug candidate **MT1621**, containing pyrimidine nucleosides, has been developed by US Modis Therapeutics (subsidiary of Zogenix, which was recently acquired by Belgian UCP), for the treatment of TK2 deficiency. Results from a retrospective Phase II observational study with MT1621 in TK2 deficiency patients showed an improved survival and scores in predefined response thresholds, and some patients regained functions that they had previously lost. MT1621 will be evaluated in a Phase III single-arm study in children and adolescents, evaluating the proportion of subjects acquiring a motor milestone, and the time to acquisition of a motor milestone, not present before treatment, as well as survival.

2.3. Regulation of reactive oxygen species

Mitochondria are the main producers of reactive oxygen species (ROS) such as superoxide anion (O_2-), produced mainly in CI and CIII and converted to H_2O_2 by superoxide dismutase (SOD) [39]. Oxidative stress is caused by the imbalance between ROS production and antioxidant cellular capacity leading to cellular damage of macromolecules [40, 41].

Sonlicromanol (KH176) is an orally bioavailable hydrophilic vitamin E-based compound from Dutch Khondrion BV. Sonlicromanol has a multi-modal mechanism acting both as ROS and redox modulator with the latter caused by activation of thioredoxin/peroxiredoxin activity [42]. Furthermore, sonlicromanol presents anti-inflammatory properties due to the inhibition of mPGES-1 [43]. Safety and tolerability of sonlicromanol were confirmed in mitochondrial disease patients with m.3243A>G mutation [44]. Its efficacy for cognitive function is currently being evaluated in a Phase IIb trial and will be further evaluated for long-term effects for patients that have completed the previous study. In addition, sonlicromanol will also be tested in children (<17 years old) with confirmed mitochondrial disorder with oxidative phosphorylation defects suffering from motor symptoms (Phase II).

Elamipretide (SS-31, MTP-131, Bendavia) developed by US Stealth Biotherapeutics, is a permeable tetrapeptide that reaches and localizes to the mitochondrial inner membrane. Elamipretide targets cardiolipin, a phospholipid situated in the mitochondrial inner membrane important for mitochondrial morphology [45], improving the cristae architecture, decreasing pathogenic ROS production and increasing ATP generation [46, 47]. The tolerability and efficacy of elamipretide has been clinically tested in genetically confirmed primary mitochondrial myopathy (PMM) patients. The results indicated that elamipretide was well-tolerated, and the drug showed a positive trend towards improvement in the six-minute walk test (6MWT) (primary endpoint) in a phase II trial [46]. During Phase III, the evaluation of efficacy did not meet the primary endpoints which included 6MWT and total fatigue score on the Primary Mitochondrial Myopathy Symptom Assessment (PMMS assessment). Post hoc analysis, however, revealed a significant difference in the primary endpoint on nDNA-related PMM. Therefore, a Phase III clinical trial will be performed to evaluate the efficacy of elamipretide in PMM patients with replisome-related nDNA mutations.

Barth syndrome is a primary mitochondrial disorder involving defects in the acyltransferase tafazzin, reducing acylation of monolysocardiolipin, which leads to decreased production of mature cardiolipin [48]. This, in turn, leads to altered mitochondrial morphology, increased ROS levels, and alterations in the ETS [48]. The disease gives rise to symptoms such as skeletal muscle weakness and cardiomyopathy.

Clinical Phase II and III trials have been performed for the evaluation of safety, tolerability, and efficacy (6MWT, Barth Syndrome-Symptom Assessment) in genetically confirmed Barth syndrome patients. In part 1 of the study, no significant improvements were observed after 12 weeks of elamipretide exposure in a placebo-controlled crossover trial. The evaluation of long-term exposure in an open label extension (no control group, part 2) supported long-term safety and tolerability of elamipretide (primary endpoints) with significant improvements over time in secondary endpoints such as 6MWT and total fatigue score on the Barth Syndrome system assessment [49].

Vatiquinone (EPI-743, ATQ3) is a para-benzoquinone from PTC Therapeutics (former BioElectron and Edison Pharmaceuticals) derived from the hydrolysis of vitamin E [50]. Vatiquinone, when compared to other evaluated antioxidants, demonstrated a higher efficacy and potency in protecting cells against oxidative stress, which led to increased cell viability in glutathione-depleted Friedreich ataxia and Leigh syndrome patient-derived fibroblasts [50, 51]. Interestingly, vatiquinone had the capacity to replenish the reduced form of the glutathione pool [51, 52]. Moreover, vatiquinone inhibited 15-lipoxygenase (15-LO) which decreased lipid oxidation and protected Leigh syndrome patient-derived fibroblasts against ferroptosis, a type of cell death that can be activated due to an imbalance in glutathione peroxidase 4 and 15-LO activity [53]. Vatiquinone's safety, oral bioavailability and capacity to penetrate the blood-brain barrier have also been shown [50, 51]. Vatiquinone is currently being tested in a Phase II/III study in mitochondrial disease patients presenting refractory epilepsy to primarily evaluate the number of observable motor seizures per 28 days.

3. Conclusions

Historically, drug development within primary mitochondrial diseases, and other rare diseases, has fallen behind, due in large part to the complexity of identifying, recruiting, and treating patients with these rare conditions. The growing understanding of mitochondrial disease complexity, heterogeneity, and the underlying genetics, as well as the shift towards patient involvement in drug development, have gradually changed this. Moreover, going back to 1983, the US Congress enacted a new law, the Orphan Drug Act, to reduce the cost and provide financial incentive for developing drugs for rare conditions, so-called orphan drugs, by offering tax credits, a waiver of the Prescription Drug User Fee, and extended market exclusivity options [54]. Today, due to its success, orphan drug legislation also exists in the EU, Singapore, Japan, Australia, South Korea, and Taiwan [55].

Currently, there are more than 100 ongoing or planned clinical interventional studies listed in www.clinicaltrials.gov investigating the safety and/or efficacy of potential treatments for patients with primary mitochondrial diseases. These clinical trials are focused on dietary supplements, medical devices, gene therapy, mitochondrial supplementation, mitochondrial donation *in vitro* fertilization, new treatment approaches, and small molecules. The interest in mitochondrial medicine is clearly

increasing, and we anticipate and hope that new treatments will become available to primary mitochondrial disease patients within the next few years.

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