Emerging therapies for mitochondrial diseases

Michio Hirano, Valentina Emmanuele and Catarina M. Quinzii
Department of Neurology, Columbia University Medical Center, New York, NY, U.S.A.
Correspondence: Michio Hirano (mh29@columbia.edu)

For the vast majority of patients with mitochondrial diseases, only supportive and symptomatic therapies are available. However, in the last decade, due to extraordinary advances in defining the causes and pathomechanisms of these diverse disorders, new therapies are being developed in the laboratory and are entering human clinical trials. In this review, we highlight the current use of dietary supplement and exercise therapies as well as emerging therapies that may be broadly applicable across multiple mitochondrial diseases or tailored for specific disorders. Examples of non-tailored therapeutic targets include: activation of mitochondrial biogenesis, regulation of mitophagy and mitochondrial dynamics, bypass of biochemical defects, mitochondrial replacement therapy, and hypoxia. In contrast, tailored therapies are: scavenging of toxic compounds, deoxynucleoside and deoxynucleotide treatments, cell replacement therapies, gene therapy, shifting mitochondrial DNA mutation heteroplasmy, and stabilization of mutant mitochondrial transfer RNAs.

Introduction

There are disappointingly few therapies for mitochondrial diseases [1,2]. Over the last decade, for most mitochondrial disease patients, treatment has been largely restricted to exercise, dietary supplements of uncertain benefit. Only a handful of diseases respond to specific supplements such as coenzyme Q10 (CoQ10) for primary and secondary forms of CoQ10 deficiency [3–5]. Obstacles to the discovery of treatments for mitochondrial disorders include the disease: rarity, clinical diversity, pathogenic complexity, etiological heterogeneity, and insufficient clinical trials [6]. In contrast, there has been remarkable progress in our understanding of the molecular genetic causes, pathomechanisms, and clinical presentations of mitochondrial diseases [2,7]. Due to these advances and emerging clinical trials, new treatment modalities are on the horizon.

Existing therapeutic options

Pharmacological approaches

Multiple vitamins and cofactors are often used in patients with mitochondrial disorders, although these therapies are not yet standardized or definitively proven to be effective. The dietary supplements are used with different purposes, such as (1) increase respiratory chain flux (CoQ10, riboflavin), (2) serve as antioxidants (e.g. CoQ10, idebenone, α-lipoic acid, vitamin C and E), and/or act as cofactors (e.g. riboflavin, thiamine), or (3) function as mitochondrial substrates (L-carnitine).

In many mitochondrial diseases due to respiratory chain dysfunction, excessive toxic reactive oxygen species (ROS) may lead to pathogenic cellular damage. Moreover, a transgenic murine model overexpressing a catalase targeted to mitochondria extended life span [8]. Based on this rationale, antioxidants are frequently used in the treatment of mitochondrial patients. CoQ10 is the most commonly utilized and many clinical trials have been investigating its efficacy and that of its analogs, like idebenone and EPI-743, in multiple mitochondrial diseases (see "clinical trials"). Other antioxidants like vitamins C and E might also be beneficial in patients with mitochondrial diseases. An example is an analog of vitamin E, trolox ornithylamide hydrochloride, when applied to fibroblasts from patients with Leigh syndrome reduced ROS...
levels and increased activities of mitochondrial complexes I, IV, and citrate synthase [9]. Nevertheless, efficacy of antioxidants in patients with mitochondrial diseases remains controversial. A Cochrane review of mitochondrial therapies has found little evidence supporting the use of any vitamin or cofactor [1]. However, benefits of various agents (riboflavin, α-lipoic acid etc.) have been anecdotally reported.

Consensus recommendations from the Mitochondrial Medicine Society [5] aimed to standardize treatment options for mitochondrial patients. According to these recommendations, patients with primary mitochondrial disorders should be offered CoQ10 in its reduced form (ubiquinol), and plasma or leukocyte levels should be monitored to assess adherence to treatment. In addition, α-lipoic acid (ALA) and riboflavin are frequently offered to mitochondrial patients. L-carnitine should be administered when deficient. Folinic acid should be given to mitochondrial patients when deficient and the central nervous system is involved. Moreover, supplements should be given starting with one supplement at the time, avoiding “cocktails” initially.

In contrast with the recommendations of the Mitochondrial Medicine Society, a survey on the use of dietary supplements conducted by the North American Mitochondrial Disease Consortium (NAMDC) [10] revealed that in practice, the majority of patients take a cocktail of at least four dietary supplements. Patients reported no or minor side effects with overall subjective improvements; however, the economic burden to the families was considerable; 90% of patients purchased the supplements out-of-pocket. The authors conclude that this burden and the potential side-effects are not justifiable, considering the lack of evidence for using these “cocktails”. Importantly, this survey underscored the importance of considering the patients’ perception of their care and quality of life; this approach is critical in selecting reliable outcome measures for future randomized placebo-controlled double-blind clinical trials, which is still the unattained gold-standard for assessing dietary supplements as therapy for mitochondrial disease.

Effective treatment of acute stroke-like episodes or their prevention has not been established. Open-label studies suggest that treatment of acute mitochondrial stroke-like episodes with intravenous (IV) arginine hydrochloride, a precursor of nitric oxide, is beneficial for patient with the m.3243A>G mutation in MTTLI [11]. Open-label studies also suggest that daily oral arginine to prevent strokes should be considered in patients with mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes (MELAS) due to the m.3243A>G mutation [5]. Placebo-controlled randomized clinical trials are necessary before L-arginine can be definitively recommended to ameliorate or treat stroke-like episodes in MELAS.

Exercise

Exercise has been proven beneficial in some patients with mitochondrial diseases [12,13]. In particular, aerobic endurance training can increase mitochondrial mass, by stimulating mitochondrial biogenesis, and increase muscle mitochondrial enzyme activities and muscle strength. Endurance training has been proven beneficial and safe in trials of patients with mitochondrial DNA mutations [14,15]. A combination of progressive endurance with or without resistance exercise should be recommended to mitochondrial patients [5].

Clinical trials

Few clinical trials have been conducted in mitochondrial diseases. In 2012, a comprehensive Cochrane review evaluated 1,335 studies comparing pharmacological and nonpharmacological treatments for mitochondrial diseases [1]. Only 12 studies were selected for inclusion in the review, with the most common reason for exclusion being lack of randomization/blinding and presence of methodological biases. The primary outcome measures included any change in muscle strength or neurological features. Secondary outcome measures included quality life evaluation, biochemical biomarkers (e.g. lactic acidosis), and negative outcomes. The 12 studies investigated the effects of CoQ10, dichloroacetate (DCA), creatinine, dimethylglycine, whey-based cysteine and combination therapy of creatine, α-lipoic acid and CoQ10. Dramatic effects were not observed in any of these studies, and one trial assessing the effects of DCA in MELAS patients had to be terminated because of toxicity (NCT00068913). Several clinical trials are currently underway or have been recently completed, but the results were not published for most of them and the outcomes are still unclear. The majority of the studies focused on patients with MELAS and LHON, which could be studied in relatively large cohorts. Many other trials analyzed less homogeneous cohort of patients, including, for instance, patients with similar phenotype (i.e. mitochondrial myopathy), but different genetic cause. A summary of the studies is reported in Table 1.

MELAS

MELAS is one of the most frequent maternally inherited mitochondrial disorders. The pathogenesis of this disorder is not completely understood and results from different factor. Energy failure due to faulty mitochondria is a common
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease</th>
<th>Design</th>
<th>Mechanism</th>
<th>Status</th>
<th>Outcome</th>
<th>Trial number</th>
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<tr>
<td><strong>Active studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPI-743</td>
<td>Mitochondrial diseases</td>
<td>Phase 2, Emergency use protocol in acutely ill patients (90 days eol care)</td>
<td>Antioxidant</td>
<td>Active/not recruiting</td>
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<td>EPI-743</td>
<td>Children (2–11 years of age) with MDs or metabolic diseases</td>
<td>Phase 2, randomized, double blind, placebo-controlled, crossover</td>
<td>Antioxidant</td>
<td>Active/not recruiting</td>
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<td>KH176</td>
<td>MELAS, MIDD, mitochondrial myopathies, mitochondrial diseases</td>
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<td>Recruiting</td>
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<td>RTA 408</td>
<td>Mitochondrial myopathy</td>
<td>Phase 2 randomized, double blind, placebo-controlled, dose-escalating</td>
<td>Antioxidant, NRF2 activator, NFκB inhibitor</td>
<td>Completed</td>
<td>na</td>
<td>NCT02255422</td>
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<tr>
<td>MTP-131 (Elamipretide)</td>
<td>Mitochondrial myopathy</td>
<td>Phase 2, randomized, double blind, placebo-controlled, crossover</td>
<td>Cardiolipin stabilization</td>
<td>Active/not recruiting</td>
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<tr>
<td>MTP-131 (Elamipretide)</td>
<td>Mitochondrial myopathy</td>
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<td>Cardiolipin stabilization</td>
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<td>Active/not recruiting</td>
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<tr>
<td>DCA (Dichloroacetate)</td>
<td>PDC deficiency</td>
<td>Phase 3, randomized, placebo-controlled, crossover</td>
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<td>Recruiting</td>
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<td>NCT02616484</td>
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<tr>
<td>Resistance exercise</td>
<td>Barth syndrome</td>
<td>Phase 2, open label</td>
<td>Increase glycolytic type 1 muscle fibers</td>
<td>Recruiting</td>
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<tr>
<td>scAAV2-P1ND4v2</td>
<td>LHON m.11778G&gt;A</td>
<td>Phase 1, open label, dose-escalating</td>
<td>Gene therapy</td>
<td>Recruiting</td>
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<td>rAAV2/2-ND4 (GS010)</td>
<td>LHON</td>
<td>Phase 1/2 safety, open label, dose escalating</td>
<td>Gene therapy</td>
<td>Active/not recruiting</td>
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<td>Allogenic HSCT</td>
<td>MNGIE</td>
<td>Phase 1 safety study</td>
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<td>Recruiting</td>
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<td><strong>Completed studies</strong></td>
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<td>CoQ10</td>
<td>Children with mitochondrial diseases with mtDNA mutations or specific OXPHOS complexes defects</td>
<td>Phase 3, randomized, double blind</td>
<td>OXPHOS/ROS</td>
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<td>NCT00432744</td>
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<td>Idebenone</td>
<td>LHON</td>
<td>Phase 2, randomized, double blind, placebo-controlled</td>
<td>Antioxidant</td>
<td>Completed</td>
<td>Primary endpoint did not reach statistical significance; secondary outcomes significantly differ in a subgroup of patients with discordant acuity at baseline</td>
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<td>Idebenone</td>
<td>MELAS</td>
<td>Phase 2, randomized, double blind, placebo-controlled, dose-finding</td>
<td>Antioxidant</td>
<td>Completed</td>
<td>na</td>
<td>NCT00887562</td>
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<td>EPI-743</td>
<td>Leigh syndrome</td>
<td>Phase 2b, randomized, double blind, placebo-controlled</td>
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<td>Completed</td>
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<td>EPI-743</td>
<td>Pearson syndrome</td>
<td>Phase 2, open label</td>
<td>Antioxidant</td>
<td>Terminated</td>
<td>Results from other studies did not support continuation of this trial</td>
<td>NCT02104336</td>
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<td>KH176</td>
<td>MELAS, LHON, Leigh, and other mitochondrial diseases</td>
<td>Phase 1, randomized, double blind, placebo-controlled, crossover</td>
<td>Antioxidant</td>
<td>Completed</td>
<td>Well tolerated with promising pharmacokinetic profile</td>
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Continued over
### Table 1 Recent clinical trials in mitochondrial disorders (Continued)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease</th>
<th>Design</th>
<th>Mechanism</th>
<th>Status</th>
<th>Outcome</th>
<th>Trial number</th>
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<td>Bezafibrate</td>
<td>Mitochondrial myopathy (m.3243A&gt;G)</td>
<td>Phase 2, open label</td>
<td>Mitochondrial biogenesis</td>
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<td>na</td>
<td>NCT02398201</td>
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<td>Curcumin</td>
<td>LHON</td>
<td>Phase 3, randomized, double blind, placebo-controlled</td>
<td>Antioxidant</td>
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<td>NCT00528151</td>
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<td>MTP-131</td>
<td>Mitochondrial myopathy</td>
<td>Phase 1/2 randomized, double blind, placebo-controlled, dose-escalating</td>
<td>Cardiolipin stabilization</td>
<td>Completed</td>
<td>na</td>
<td>NCT02367014</td>
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<tr>
<td>RP103 (Cysteamine bitartrate delayed-release)</td>
<td>Childhood mitochondrial diseases including Leigh syndrome</td>
<td>Phase 2 open-label, dose-escalating</td>
<td>Cysteine-depleting agent</td>
<td>Completed</td>
<td>Primary endpoint did not reach statistical significance; high percentage of serious adverse events (90.6%)</td>
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<tr>
<td>RP103 (Cysteamine bitartrate delayed-release)</td>
<td>Childhood MDs including Leigh syndrome</td>
<td>Phase 2, long term open-label extension study</td>
<td>Cysteine-depleting agent</td>
<td>Completed</td>
<td>na</td>
<td>NCT02473445</td>
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<td>Medium chain triglycerides</td>
<td>MELAS</td>
<td>Phase 1, open label</td>
<td>Shift heteroplasmy</td>
<td>Completed</td>
<td>na</td>
<td>NCT01252979L</td>
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<td>L-Arginine</td>
<td>MELAS</td>
<td>Phase 2, open label</td>
<td>NO precursor</td>
<td>Completed</td>
<td>Improvement in aerobic capacity and muscle metabolism</td>
<td>NCT01603446</td>
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<td>L-Arginine (IV)</td>
<td>MELAS</td>
<td>Phase 3, open label</td>
<td>NO precursor</td>
<td>Completed</td>
<td>Improvement of stroke-like symptoms</td>
<td>JMA-R-00023</td>
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<tr>
<td>L-Arginine (PO)</td>
<td>MELAS</td>
<td>Phase 3, open-label</td>
<td>NO precursor</td>
<td>Completed</td>
<td>Improved endothelial dysfunction</td>
<td>JMA-R-00025</td>
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<tr>
<td>Arginine and citrulline</td>
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<td>Phase 1, open-label</td>
<td>NO precursor</td>
<td>Completed</td>
<td>na</td>
<td>NCT01339494</td>
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<td>Lipic acid</td>
<td>Mitochondrial myopathy</td>
<td>Pilot compassionate use study</td>
<td>–</td>
<td>Completed</td>
<td>na</td>
<td>NCT00004770</td>
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<tr>
<td>RG2133 (2′,3′,5′-tri-o-acetyluridine)</td>
<td>Mitochondrial diseases</td>
<td>Phase 1, open label, dose escalated</td>
<td>–</td>
<td>Completed</td>
<td>na</td>
<td>NCT0060515</td>
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<tr>
<td>SPP-004 (S-Ala and SFC)</td>
<td>Mitochondrial diseases, mainly cranial nerve symptoms</td>
<td>Phase 2, randomized, placebo-controlled</td>
<td>–</td>
<td>Completed</td>
<td>na</td>
<td>JMA-R-000200</td>
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<tr>
<td>Taurine</td>
<td>MELAS</td>
<td>Phase 2/3 open-label</td>
<td>Taurine modification</td>
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<td>na</td>
<td>UMIN000011908</td>
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<td>Pyruvate</td>
<td>MELAS</td>
<td>Phase 2, randomized, placebo-controlled</td>
<td>NAD donor</td>
<td>Unknown</td>
<td>na</td>
<td>JMA-R-00093</td>
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<tr>
<td>DCA (Dichloroacetate)</td>
<td>MELAS</td>
<td>Phase 2, randomized, double blind, crossover</td>
<td>Lowering lactate levels</td>
<td>Terminated</td>
<td>Terminated because of peripheral nerve toxicity</td>
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<td>Cyclosporine</td>
<td>LHON acute phase</td>
<td>Phase 2, open label</td>
<td>Inhibition of mitochondrial PTP</td>
<td>Unknown</td>
<td>na</td>
<td>NCT02176733</td>
</tr>
<tr>
<td>rAAV2-ND4</td>
<td>LHON m.11778G&gt;A</td>
<td>Open label</td>
<td>Gene therapy</td>
<td>Completed</td>
<td>Improvement of visual acuity and enlargement of visual field</td>
<td>NCT01267422</td>
</tr>
</tbody>
</table>

**Abbreviations:** AHSCT, allogeneic hematopoietic stem cell transplantation; IV, intravenous; LHON, Leber hereditary optic neuropathy; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MIDD, maternally inherited diabetes-deafness syndrome; MNGIE, mitochondrial neurogastrointestinal encephalomyopathy; na, not available; PDC, pyruvate dehydrogenase complex; PO, per oral; PTP, permeability transition pore.

Feature of mitochondrial diseases as well as the overproduction of ROS. Different approaches have been studied in order to reduce the oxidative stress in mitochondrial diseases and in particular in MELAS patients. Idebenone is a short-tail ubiquinone synthetic analog, which is more water-soluble compared with CoQ10 and acts as and antioxidant. Several studies have been conducted to assess the efficacy and safety of idebenone in Friedreich ataxia (FA), and proven that it is in fact well-tolerated but not clinically effective [16–20]. A phase 2a, randomized, double blind, placebo-control, dose-finding study in patients with MELAS syndrome has recently been completed and showed that the primary endpoint did not reach statistical significance (NCT00887562). KH176 is a small molecule vitamin E
derivative and a potent ROS scavenger that enhances the antioxidant thioredoxin/peroxiredoxin system [21]. After the completion of a dose escalating clinical trial with KH176 in healthy individuals that has demonstrated good tolerability and a promising pharmacokinetic profile [22], a double-blind randomized, placebo-controlled two-way crossover phase II study of mildly affected patients with the m.3243A>G mutation revealed no significant improvement in the primary outcome, gait analysis, but showed positive effects on attention performance and measures of depression and anxiety [21]. Moreover, bezafibrate is an activator of the transcription factor peroxisomal proliferator-activated receptors (PPARs), which, in turn, when activated promote transcription of nuclear-encoded mitochondrial genes. Its efficacy is being evaluated in patients with m.3243A>G mutation (NCT02398201) and evidence of myopathy, but results are not available.

In addition to energy failure and ROS accumulation, there has been growing evidence that nitric oxide (NO) deficiency plays a central role in the pathogenesis of the stroke-like episodes [23]. Arginine is the substrate of nitric oxide synthase, which produces NO, therefore arginine is a promising treatment for MELAS patients. Multiple open-label trials have been conducted (NCT01603446, JMA-IIA00023, and JMA-IIA00025) and have suggested efficacy of chronic oral administration [24–26] and acute intravenous administration [24,27] of arginine in patients with MELAS syndrome; however, a placebo-controlled randomized clinical trial is required to demonstrate convincing evidence of efficacy. Further preliminary evidence has suggested more potent effect of citrulline than L-arginine in stroke-like episodes in MELAS patients [23]. A Phase 1, open-label dose-escalation study is being conducted (NCT01339494). Other molecules investigated in clinical trials for patients with MELAS include pyruvate (JMA-IIA00093), taurine (UMIN000011908), and supplemental medium chain triglycerides (NCT01252979); however, those results of trials are still pending.

**Leber hereditary optic neuropathy (LHON)**

LHON is a mitochondrial disorder characterized by painless, subacute visual loss affecting the central visual field in one eye, followed by similar symptoms in the other eye typically within 2 to 3 months of delay. Three mtDNA mutations are commonly associated with LHON: m.3460G>A in MT-ND1, m.11778G>A in MT-ND4, or m.14484T>C in MT-ND6 [28]. Many therapeutic approaches have been tested in patients with LHON, the majority of which are focused on the use of antioxidants. In particular, idebenone has been evaluated in a clinical trial of LHON; although primary endpoints did not reach statistical significance, overall, almost one in three patients achieved clinically relevant recovery (30.2% versus 10.3% of placebo patients, \( P = 0.056 \)) and a possible beneficial effect was observed in a subgroup of patients with discordant visual acuity at baseline [29,30] (NCT00747487). Idebenone has been recently approved for the treatment of LHON in Europe.

MTP-131 (elamipretide) is a small molecule targeting inner mitochondrial membrane that has been proven able to correct excessive ROS and increase ATP synthesis in preclinical studies [31]; this molecule has entered clinical trials for LHON (NCT02693119), as well as for mitochondrial myopathies and mitochondrial diseases (NCT02367014, NCT02976038, NCT02805790) and has shown promising preliminary results. Curcumin, a derivative of the spice turmeric (Curcuma longa), has also displayed antioxidant properties and a trial has been completed in patients with LHON, but no results are available (NCT00528151). At last, aiming at mitochondrial delivery of a normal mtDNA-encoded ND4 protein, allotopic expression has been attempted in patients with LHON, despite controversial preclinical results. One trial (NCT01267422) has been recently completed and has shown significant improvement of visual acuity and enlargement of visual field in the treated group [32]. Two additional trials are underway (NCT02161380; NCT02064569).

**Mitochondrial neurogastrointestinal encephalomyopathy**

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive mitochondrial disorder characterized by severe gastrointestinal dysmotility, cachexia, progressive external ophthalmoplegia, myopathy, and peripheral demyelinating neuropathy [33]. It is caused by autosomal recessive mutations in TYMP gene, encoding thymidine phosphorylase (TP). TP is a cytosolic enzyme that catalyzes the first step of thymidine and deoxyuridine catabolism. When the enzyme is deficient, thymidine and deoxyuridine accumulate and become toxic, leading to mtDNA instability. A retrospective analysis of 24 MNGIE patients who underwent allogeneic hematopoietic stem cell transplant (AHSC) as a mean to replace TP enzyme, for MNGIE patients, revealed that only 7 (37.5%) were alive at last follow-up with an unacceptable high mortality attributed to the transplant (9 patients 37.5%); however, survival was associated with human leukocyte antigen match (10/10) and absence of liver disease and gastrointestinal pseudo-obstruction [34]. After successful AHSTC, patients showed normalization of buffy coat TP activity and plasma thymidine and deoxyuridine levels as well as clinical improvements more than 2 years after transplant. Two
trials at Columbia University, USA, are currently recruiting to define natural history of the disease (NCT01694953) and to assess the safety of AHSC (NCT01694953). Liver transplantation has been proposed as a safer alternative to AHSC due to absence of a stressful conditioning regimen prior to this procedure. One patient who underwent a successful hepatic transplant showed normalization of plasma thymidine and deoxyuridine with clinical stabilization for over 1 year; however, data from additional patients are needed before this therapy can be recommended. Preclinical studies indicate that gene therapy is also a potentially effective means to restore TP activity in MNGIE [35–37]. Erythrocyte-encapsulated thymidine phosphorylase has also been demonstrated to be effective at delivering TP to MNGIE patients and may be useful as a bridge therapy to more permanent treatment [38].

**Heterogeneous cohort of patients with mitochondrial diseases**

Many of the therapeutic interventions acting on common pathogenic pathways of mitochondrial diseases have been tested in nonhomogeneous cohort of patients or in different disorders. One example is EPI-743, a para-benzoquinone analog modified to exert a higher antioxidant effect compared with CoQ10 and idebenone [39]. This molecule is supposed to enhance the biosynthesis of glutathione (GSH), which is an important cellular antioxidant. Two open label studies conducted independently in North America with patients with various mitochondrial diseases and in Italy with a cohort of Leigh syndrome patients showed promising results [40,41]. As a result, a randomized clinical trial has started in patients with Leigh syndrome (NCT01721733) as well as in patients with FA (NCT01728064), and in acutely ill patients (90 days of end of life care) (NCT01370447). A clinical trial of Pearson syndrome, a disorder caused by single large-scale mtDNA deletions, has been terminated because results of other studies have not supported continuation (NCT02104336). EPI-743 has also been studied in an open-label trial of patients with LHON with favorable outcome [42].

**Emerging therapies**

In the past few years, many potential treatments have been proposed for mitochondrial disorders. These approaches act on different disease mechanisms and can be broadly divided in “non-tailored strategies”, acting on common pathways thus in theory relevant to different mitochondrial diseases, and “disease-tailored” strategies [43]. Examples of these strategies are summarized in Table 2. The first group includes strategies aimed at: (1) activation of mitochondrial biogenesis; (2) regulation of mitophagy and mitochondrial dynamics; (3) bypass of OXPHOS defects; (4) mitochondrial replacement therapy (MRT); and (5) chronic hypoxia. The second group includes: (1) scavenging of specific toxic compounds; (2) supplementation of nucleosides and nucleotides; (3) cell replacement therapies; (4) gene therapy; (5) shifting mutant mtDNA heteroplasmy; and (6) stabilizing mutant tRNAs. Some of these approaches have been proven effective only in preclinical models while others have already been successfully applied anecdotally to patients with mitochondrial diseases (Table 2).

**Activation of mitochondrial biogenesis**

Energy failure is a hallmark of mitochondrial diseases and various therapeutic interventions have been used to stimulate mitochondrial biogenesis. Although these interventions do not rectify the underlying cause of the disease, increasing mitochondrial mass may increase energy production, thus ameliorating the phenotype. There is increasing evidence in in vitro studies and animal models that increased mitochondrial biogenesis might be beneficial in many mitochondrial diseases. Interestingly, a recent observation suggested that increased mitochondrial content influences incomplete penetrance in LHON patients and that mitochondrial biogenesis could be used as a therapeutic strategy in this group of patients [44]. The biological pathway that controls mitochondrial biogenesis is complex and relies mostly on the PPARα coactivator 1α (PGC1α). PGC1α interacts with several transcription factors, including nuclear respiratory factors 1 and 2 (NRF1 and NRF2) and PPAR α, β, and γ. Once activated, NRFs increases the transcription of OXPHOS genes and PPARs increase the expression of genes related to fatty acid oxidation (FAO) [45]. Besides, PGC1α activity is increased by deacetylation and phosphorylation. Importantly, two enzymes responsible for these modifications, deacetylation by Sirt1 and phosphorylation by AMPK, can be modulated by drugs [46] and have been tested in preclinical models. Different agents used with this purpose are listed below.

Sirt1 is a nuclear deacetylase that utilizes oxidized nicotinamide adenine dinucleotide (NAD+) to deacetylate residues of acetyl-lysine in proteins. Notably, Sirt1 is activated by increased cellular levels of NAD+. This increase can be achieved by providing NAD precursor, such as nicotinamide riboside, or inhibiting NAD consuming enzymes, such as poly(ADP) ribosylpolymerase 1 (PARP1). These approaches have shown beneficial effects in animal models of mitochondrial myopathies [47,48].
Table 2: Examples of preclinical therapies in mitochondrial disorders

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<tr>
<th>Strategy</th>
<th>Method</th>
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<td><strong>Nontailored</strong></td>
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<tr>
<td>Activation of mitochondrial biogenesis</td>
<td>Nicotinamide riboside and PARP1 [47,48]</td>
<td>Sco2 knockout/knockin mouse, Deletor mouse model</td>
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<td></td>
<td>AICAR [49]</td>
<td>Patients fibroblasts and mouse models of COX deficiency</td>
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<td></td>
<td>Bezafibrate [49–54]</td>
<td>Patients fibroblasts, cybrids, mouse models of COX</td>
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<td>Resveratrol [55–57]</td>
<td>deficiency, Deletor mouse model</td>
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<td></td>
<td>Retinoic acid [58]</td>
<td>In vitro models, Drosophila models, human fibroblasts</td>
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<td>Endurance exercise [59–62]</td>
<td>Cybrids (m.3243A&gt;G)</td>
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<td>Mouse models of COX deficiency, mtDNA mutator mice, patients with MDs</td>
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<td>Regulating mitophagy and mitochondrial dynamics</td>
<td>Rapamycin [65,66]</td>
<td>In vitro models and Ndufs4&lt;sup&gt;−/−&lt;/sup&gt; mouse model of Leigh syndrome;</td>
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<td>7K2&lt;sup&gt;H1236V/H1236P&lt;/sup&gt; mouse model</td>
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<td>Bypassing OXPHOS blocks</td>
<td>Ndi1 (Complex I defect) [67,68] and AOX (Complex III and IV defects)</td>
<td>In vitro models and Drosophila models</td>
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<td>Mitochondrial replacement therapy</td>
<td>Oocyte nuclear genetic material transfer [71]</td>
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<td>Chronic hypoxia</td>
<td>Genetic or small molecule activation of the hypoxia response [72]</td>
<td>Nonhuman primates; healthy subjects and patients with mitochondrial</td>
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<td></td>
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<td>diseases</td>
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<td></td>
<td>Moderate environmental hypoxia [72]</td>
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<td><strong>Disease-tailored</strong></td>
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<td>Scavenging of specific toxic compounds</td>
<td>N-acetyl cysteine and metronidazole [73]</td>
<td>Efe1&lt;sup&gt;−/−&lt;/sup&gt; mouse model of EE; patients with EE</td>
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<td>Hemioliadase [54]</td>
<td>Patients with MNGIE</td>
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<td>Supplementation of nucleotides/nucleosides</td>
<td>Deoxyctydine and deoxythymidine monophosphates [75];</td>
<td>7K2&lt;sup&gt;H1236V/H1236P&lt;/sup&gt; mouse model; patients with TK2</td>
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<td>deoxyctydine and deoxythymidine [76];</td>
<td>deficiency</td>
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<td>Deoxyctydine or tetrahaduridone [77]</td>
<td>thymidine-induced mtDNA depleted cells and Tup1</td>
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<td>Deoxguanosine [77]</td>
<td>knockout murine model of MNGIE</td>
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<td>Cell replacement therapies</td>
<td>Erythrocyte-encapsulated thymidine phospholysylase [38]</td>
<td>dGK deficient human fibroblasts</td>
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<td>Allogenic HSCT [54]</td>
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<td>Liver transplantation [78]</td>
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<td>Gene therapy</td>
<td>AAV-mediated gene therapy [35,79–82]</td>
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<td>CRIPR/Cas9 [84]</td>
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<td>Alotopic expression of mtDNA encoded proteins [85–88]</td>
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<td>Shifting heteroplasmy</td>
<td>Mitochondrial-targeted restriction endonucleases [89,92–97,99]</td>
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<td></td>
<td>mZFNs [90,98]</td>
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<td>TALENS [91,99]</td>
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<td>Stabilizing mutant tRNAs</td>
<td>Overexpressing cognate and noncognate aminoacyl</td>
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<td>mt-tRNA synthetase [100–105]</td>
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Abbreviations: CRISPR, clustered regularly interspaced palindromic repeat; EE, ethylmalonic encephalopathy; HSCT, hematopoietic stem cell transplantation; IPS, induced pluripotent stem; MNGIE, mitochondrial neurogastrointestinal encephalomyopathy; TALEN, transcription activator-like effectors nucleases; ZFN, zinc finger endonuclease.

5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), an adenosine monophosphate analog, is an agonist of adenosine monophosphate-activated protein kinase (AMPK) that has been used to increase the respiratory chain complex activities in three mouse models of cytochrome c oxidase (COX) deficiency (Surf<sup>−/−</sup>, Sco2<sup>−/−</sup>, and ACTA-Cox15<sup>−/−</sup>) with striking improvement of motor performances only in the Sco2 model [49].

Bezafibrate, a pan-PPAR activator, was tested in fibroblasts of patients with heterogeneous mitochondrial diseases and stimulated PGC1α and improved mitochondrial respiratory chain defects [50]. These findings were subsequently buttressed by in vivo studies in mouse models of COX-deficiency [51,52]. However, studies on other mouse models did not show induction of mitochondrial biogenesis or increased mitochondrial respiratory chain enzyme activities [49,53,54].

Resveratrol has also been described as an activator of mitochondrial biogenesis in animal models and in human fibroblasts [55,56], although its mechanism of action is still unknown and it did not appear to increase OXPHOS activities in another study on human fibroblasts [57].
Retinoic acid has been used to stimulate the retinoid X receptor-α (RXRα) in hybrid cells containing the m.3243A>G mutation, ameliorating the respiratory chain defect [58].

Endurance training has also been used as an activator of mitochondrial biogenesis and has been reported to be beneficial and safe in the mitochondrial DNA (mtDNA) mutator mice [59], and in patients with mitochondrial diseases [60,61]. Endurance training seems able to regulate not only PGC1α but also PGC1β, AMPK, and the hypoxia inducible factors (HIFs) [62].

Regulating mitophagy and mitochondrial dynamics
Mitophagy is the selective elimination of dysfunctional mitochondria, a physiological process fundamental for maintaining normal mitochondrial function [63,64]. This process is under the regulation of various pathways. One way of targeting mitophagy is via mTOR inhibition, which can be achieved by rapamycin. This approach has been investigated in a mouse model of Leigh syndrome (Ndufs4−/−) [65] and in a knockin mouse model of mtDNA depletion syndrome [66] and appeared to ameliorate the clinical phenotype and life span of the treated mice, although the oxidative phosphorylation (OXPHOS) defects were not rescued and mechanism of action remains uncertain.

Bypassing OXPHOS defects
The use of single-peptide enzymes derived from yeast or low eukaryotes to bypass mitochondrial respiratory chain defects has been tested in in vitro and in vivo models. In particular, Ndi1 substitutes complex I in yeast and transfers electron to CoQ, without pumping protons across the membrane. AOX is present in lower eukaryotes and bypasses complex III and IV by accepting electrons from CoQ. Expression of these enzymes has been used to bypass complex I deficiency [67,68] and complex III–IV deficiencies in human cells and Drosophila [69,70], but not in mammals in vivo.

Mitochondrial replacement therapy
As discussed in the accompanying Essays in Biochemistry article “Advances in methods for reducing mitochondrial DNA disease by replacing or manipulating the mitochondrial genome” by Rai et al. [71], replacement of mutant mtDNA in oocytes or single-cell embryos by mitochondrial replacement therapy is a novel method to prevent maternal transmission of mtDNA mutations (for further details, please the Rai et al. review [71]).

Hypoxia
An innovative approach to treatment of mitochondrial diseases is activation of the hypoxic response pathway [72]. Through a genome-wide Cas9-mediated screen of pharmacologically induced complex III deficiency, inhibition of the Von Hippel–Lindau (VHL) factor was identified as the most effector suppressor of the mitochondrial dysfunction. Because VHL negatively regulates HIFs, down-regulation of VHL activates the HIF transcriptional response, which, in part, shifts cellular bioenergetic reliance on mitochondrial OXPHOS. Genetic and pharmacological activation of the HIF pathway in zebrafish models as well as hypoxic treatment in the Ndusf4 knockout mouse model of Leigh syndrome provided further in vivo support for this therapeutic strategy.

Scavenging of specific toxic compounds
Ethylmalonic encephalopathy (EE) is a devastating disorder of infancy due to ETHE1 mutations. ETHE1 encodes a mitochondrial sulfur dioxygenase (SDO) involved in the elimination of H2S. Accumulation of H2S, produced by the catabolism of amino acids and by the anaerobic flora of the intestine, is toxic and leads to inhibition of COX activity and to endothelial damage. N-acetyl cysteine (NAC) is a precursor of glutathione and can be used to buffer intracellular H2S. Metronidazole is an intestinal antibiotic active against anaerobic bacteria that produce H2S. The use of metronidazole and NAC in a mouse model of ethylmalonic encephalopathy (Ethe1−/−) prolonged the life span and ameliorated the clinical phenotype of this model. Moreover, the administration of these compounds in a cohort of patients with EE was able to improve some of the clinical features of the disease [73]. This treatment has not been tested in clinical trials yet.

In MNGIE, hemodialysis has been used to attempt to remove toxic deoxynucleosides but was not effective in decreasing thymidine or deoxyuridine levels [74].

Nucleoside substrate enhancement and nucleotide bypass therapies
Supplementation of deoxyribonucleotides and deoxyribonucleosides has been exploited in in vitro and in vivo models of mitochondrial deoxynucleotide triphosphate (dNTP) pool unbalance. Mitochondrial dNTP pool unbalance
causes mtDNA instability and consequent mtDNA depletion, multiple deletions, and point mutations. Different enzymes are involved in the maintenance of dNTP pools, such as thymidine kinase 2 (TK2), deoxyguanosine kinase (dGK), and thymidine phosphorylase (TP).

TK2 is a mitochondrial matrix protein that phosphorylates thymidine and deoxycytidine nucleosides to generate deoxythymidine and deoxycytidine monophosphate (dTMP, dCMP), which are then converted into dNTPs, fundamental for mtDNA synthesis. Recessive mutations in TK2 gene cause dNTP pool unbalance and mtDNA instability. The consequent clinical phenotypes range from a severe infantile neuromuscular form to adult-onset chronic progressive external ophthalmoplegia. Promising results were obtained in a Tk2 knockin mouse model (Tk2<sup>H126N/H126N</sup>) with oral administration of deoxycytidine and deoxythymidine monophosphates and subsequently deoxycytidine and deoxythymidine; both treatments increased mtDNA levels and mitochondrial respiratory chain enzyme activities, and prolonged the life span of the homozygous mutant mice [75,76].

Depletion of mtDNA has been corrected in vivo in a TppUpp double knockout mouse model of MNGIE disease by administrating deoxythymidine or tetrahydrouridine [77]. In the same study, the addition of deoxycytidine and tetrahydrouridine to a cell model of MNGIE disease (dThd-induced mtDNA depleted fibroblasts) was also able to prevent mtDNA depletion. Depletion of mtDNA was also corrected in dGK deficient human fibroblasts by adding deoxyguanosine [77].

Cell replacement therapies
Cell replacement therapies such as erythrocyte-encapsulated thymidine phosphorylase [38] as well as AHSCT and liver transplantation to restore TP activity in MNGIE are described above.

Liver transplantation has been recently performed in an infant with EE due to ETHE1 mutations [78]. The patient showed progressive improvement of the neurological function and normalization of the biochemical abnormalities. Liver transplantation can replace the deficient enzyme and clear the toxic compounds that accumulate in this disorder, constituting a feasible therapeutic option in patients with EE.

Gene therapy
Nuclear DNA defects
Adeno-associated viruses (AAVs) are good candidates as viral vectors for gene therapy, given their low risk of insertional mutagenesis, and a long-term persistence in cells. AAVs are currently the most widely used in preclinical approaches. AAV-mediated gene therapy has been performed in different mouse models of nuclear-encoded mitochondrial diseases. The first animal model treated with muscle injections of AAV2 was an Ant1<sup>−/−</sup> mouse [79]. In another approach, AAV2 vector targeted to retina was used to express AIF1 in the eye of the Harlequin mouse and restore complex I deficiency [80]. A liver specific AAV2/8 serotype with <code>ETHE1</code> was applied to a mouse model of EE and dramatically improve the clinical course and the biochemical abnormalities of mutant mice [81]. This study demonstrated that restoring ETEN1 activity selectively in the liver was sufficient to correct the enzymatic defect and led to the hypothesis that liver transplant could be used in patients with EE. Similarly, the same hepatotropic vector was used in a mouse model of MNGIE and was proven successful as noted previously [77]. AAV2,8 vector was also used to express the wild-type MPV17 protein in a Mpv17 knockout mouse model of mtDNA depletion and hepatocerebral syndrome [82]. The vector was able to rescue the mtDNA depletion and prevent liver steatosis induced by ketogenic diet in this model.

Another approach using the CRISPR/Cas9 system, an endonuclease-based system, has been reported to rescue mitochondrial and skeletal muscle impairment in an iPS cell model of CoQ<sub>10</sub> deficiency due to a mutation in the COQ4 gene [83,84].

mtDNA defects
Even more challenging is delivering gene therapy into mitochondria. One attempted approach is to allotopically express recombinant mtDNA encoded proteins containing a mitochondrial targeting sequence (MTS) in the nucleus. This approach has been tried in fibroblasts carrying mutations in ND1, ND4, and ATP6 genes [85-87] and in an animal model of LHON [88]. Despite the controversial preclinical results, clinical trials have started in patients with LHON (NCT01267422, NCT02064569, NCT02161380) (see “clinical trials”).
Shifting mtDNA mutation heteroplasmy
Pathogenic mtDNA mutations are usually heteroplasmic, requiring a minimum critical mutation load to cause mitochondrial dysfunction. Shifting heteroplasmic levels in order to reduce the amount of mutated DNA below this threshold, therefore, has been used as a therapeutic approach. This can be achieved with different techniques: mitochondrial-targeted restriction endonucleases [89], zinc finger endonucleases (ZFNs) [90], transcription activator-like effectors nuclease (TALENs) [91], and CRISPR (clustered regularly interspaced palindromic repeat)/Cas9. Restriction endonuclease SmaI has been used in cybrids carrying the m.8399T>G mutation and was able to reduce the mutation load and increase ATP levels [92,93]. Restriction endonucleases have been exploited also in heteroplasmic mouse models, using AAV vectors, with promising results [94–97]. The major limitation of this approach is the uncommon generation of a suitable restriction site by the mtDNA mutation. The introduction of programmable nucleases such as ZFNs and TALENs overcomes this limitation. ZFNs are engineered mitochondrially targeted heterodimeric zinc finger peptides conjugated to the nucleolytic domain of the type IIs restriction enzyme FokI. Each zinc finger domain recognizes three nucleotides, so arranging zinc finger modules appropriately allow for recognition of virtually any DNA sequence. Expression of mtZFNs in cybrids was able to reduce the mutant mtDNA and restore mitochondrial function [98]. TALENs also work as heterodimers, requiring two monomers to bind close DNA sequence in order to allow the FokI nuclease to dimerize and cleave DNA, as for the mtZFNs. Reengineered TALENs targeted to different mtDNA point mutations and deletion (MitoTALENs) were able to permanently reduce the mutation load in patient-derived cells [91]. A possible limitation to the use of MitoTALENs in clinical practice is the small packaging capacity (generally <5 kb) of AAV vectors. Moreover, the risk of a rapid reduction in mtDNA copy numbers of inducing a potential mtDNA depletion syndrome remains a limitation for the potential clinical application of these approaches. Mitochondria-targeted restriction endonucleases and TALENs have also been used in the selective elimination of mtDNA mutations in the germline of the heteroplasmic mouse model and artificial mammalian oocytes as a potential approach for preventing transmission of mtDNA mutations [99].

Stabilizing mutant mitochondrial tRNAs
The majority of the mtDNA mutations are localized to tRNA genes. It is not surprising therefore that several therapies have been targeting mt-tRNAs. In particular, tRNA synthetases are enzymes that catalyze the attachment of amino acids to their cognate tRNA during protein synthesis. Many studies indicated that overexpressing cognate and noncognate aminoacyl mt-tRNA synthetases, or their fragments, could stabilize mt-tRNAs and attenuate the detrimental effect of the mutation in yeast and human cell lines [100–105].

Conclusions
Remarkable progress has been achieved in the three decades of mitochondrial medicine since the identification of the first mtDNA mutations. Many potential therapeutic approaches for mitochondrial diseases have been proposed and are now at different stages of development. Translating preclinical studies to bedside remains challenging and well-controlled clinical trials of high quality are necessary to define the efficacy of potential therapies already in use and to develop novel drugs [6]. Based on the knowledge acquired with the previous studies, these future trials may overcome the challenges posed by this heterogeneous group of disorders in the context of multicenter collaborations, by selecting numerous subgroups of homogeneous patients and by selecting outcome measures that are objective and relevant to patient care and quality of life. Clearly, there are important unmet needs for evidence-based guidelines in the treatment of mitochondrial patients and the development of more effective therapies. The emerging therapies provide exciting promise for clinically meaningful treatments for mitochondrial diseases.

Summary
- Although dietary supplements are frequently used by patients with mitochondrial disease, efficacy in most individuals is uncertain.
- Exercise therapy has been demonstrated to be beneficial for mitochondrial diseases.
- Emerging therapies for mitochondrial disease include nontailored therapies that can be applied across multiple mitochondrial diseases: (1) activation of mitochondrial biogenesis; (2) regulation of
mitophagy and mitochondrial dynamics; (3) bypass of OXPHOS defects; (4) mitochondrial replacement therapy (MRT); and (5) hypoxia.

- New tailored therapeutic strategies for mitochondrial diseases include: (1) scavenging of specific toxic compounds; (2) supplementation of deoxynucleosides and deoxyribonucleotides; (3) cell replacement therapies; (4) gene therapy; (5) shifting mtDNA mutation heteroplasmy; and (6) stabilizing mutant mitochondrial tRNAs.

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**Competing Interests**

M.H. is a co-inventor on patent applications and holds Orphan Drug Designation (ODD) from the Food and Drug Administration and Rare Pediatric Disease Designation for deoxynucleoside therapy for mitochondrial DNA depletion syndrome including TK2 deficiency. The patent, RDD, and OPDs have been licensed via the Columbia Technology Ventures office to Modis Pharmaceuticals. M.H. and Columbia University Medical Center (CUMC) have filed patent applications covering the potential use of deoxynucleoside treatment for TK2 deficiency in humans. CUMC has licensed pending patent applications related to the technology to Modis Pharmaceuticals, Inc. and CUMC may be eligible to receive payments related to the development and commercialization of the technology. Any potential licensing fees earned will be paid to CUMC and are shared with MH through CUMC distribution policy. M.H. is a paid consultant to Modis Pharmaceutical, Inc. The other authors declare no conflicts of interest.

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The authors thank the patients and their families who have collaborated with the clinical therapy studies on mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) and thymidine kinase 2 (TK2) deficiency. With special thanks to JJMP and AE and their families for their contributions to TK2 deficiency research.

**Abbreviations**

AAV, adeno-associated virus; AHSCT, allogeneic hematopoietic stem cell transplantation; AICAR, 5-aminimidazole-4-carboxamide ribonucleotide; AMPK, adenosine monophosphate-activated protein kinase; CoQ10, coenzyme Q10; COX, cytochrome c oxidase; CRISPR, clustered regularly interspaced palindromic repeat; DCA, dichloroacetate; dCMP, deoxycytidine monophosphate; DGK, deoxyguanosine kinase; dNTP, deoxynucleotide triphosphate; dTMP, deoxythymidine monophosphate; EE, ethylmalonic encephalopathy; FA, friedreich ataxia; HIF, hypoxia inducible factor; IV, intravenous; LHON, Leber hereditary optic neuropathy; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MIDD, maternally inherited diabetes-deafness syndrome; MNGIE, mitochondrial neurogastrointestinal encephalomyopathy; mtDNA, mitochondrial DNA; MTS, mitochondrial targeting sequence; NAC, N-acetyl cysteine; NAD, nicotinamide adenine dinucleotide; NAMDC, North American Mitochondrial Disease Consortium; NO, nitric oxide; NRF1, nuclear respiratory factors 1; NRF2, nuclear respiratory factors 2; OXPHOS, oxidative phosphorylation; PARP1, poly(ADP) ribose/polymerase 1; PDC, pyruvate dehydrogenase complex; PO, per oral; PPAR, peroxisomal proliferator-activated receptors; PTP, permeability transition pore; ROS, reactive oxygen species; SDO, sulfur dioxygenase; TALEN, transcription activator-like effectors nuclease; TK2, thymidine kinase 2; TP, thymidine phosphorylase; VHL, Von Hippel–Lindau; ZFN, zinc finger endonuclease.

**References**


