Mitochondrial diseases largely stem from direct or indirect defects in oxidative phosphorylation - the synthesis of ATP using the chemical energy derived from oxidation of nutrients – and result in widely varying and tissue-specific manifestations. Being of genetic and inborn etiology, there are so far no curative therapies. Some of the strategies proposed, and to some extent tested in animal models and in patients, are pharmacological modulation of mitochondrial biogenesis and fission/fusion, and of central cellular pathogenesis-related processes such as apoptosis and autophagy, and metabolic bypass-supplementation therapies [1]. Intense research activities are ongoing to find and test treatment strategies for these devastating disorders. Valuable models for mechanistic studies and preclinical trials have been generated by introducing disease mutations into mice. In this issue, a study testing oral rapamycin in a mouse model (Coq9R239X) with compromised coenzyme Q (CoQ) biosynthesis, manifesting as encephalomyopathy, showed no beneficial effect [2]. This is contradictory to several previous reports that have shown beneficial effects in mitochondrial disease models [3–8].

Rapamycin is a natural bacterial macrolide metabolite first isolated from a soil sample from Easter Island (Rapa Nui) in 1972. Initially studied as an antifungal agent, its immunosuppressive and antiproliferative properties in mammalian cells were discovered in the 1990s. It (sirolimus) was approved in USA in 1999 for prophylaxis of rejection in renal transplantation patients. Rapamycin binds the 12 kDa FK506-binding protein (FKBP12), and this complex binds and inhibits mammalian target of rapamycin (mTOR, also known as mechanistic TOR) complex (mTORC) [9]. The mTOR nutrient and growth factor signaling pathway is a master regulator of cell growth and metabolism, and therefore implicated in numerous human diseases such as cancer, diabetes, obesity, neurological diseases, and genetic disorders [9]. In 2013, based on their earlier molecular studies in yeast, Johnson et al. first provided evidence that mTOR signaling can be involved in the pathogenesis of mitochondrial diseases [3]. They showed that pharmacological inhibition of the mTORC1 pathway with both oral [4] and intraperitoneally [3] injected rapamycin robustly alleviated encephalopathy and increased survival in Ndufs4−/− mice, a model of Leigh syndrome with respiratory chain complex I deficiency. Thus far, six preclinical studies on the effect and efficacy of rapamycin in mouse models of mitochondrial disease have been published ([3–8], Table 1). They show that high doses of rapamycin have beneficial effects in mitochondrial encephalopathy [4,6] and skeletal myopathy [5,8]. In a Tk2-H126N knock-in mouse model of mtDNA depletion syndrome with encephalopathy and myopathy, no convincing effect on tissue histopathology was observed upon mTOR1 inhibition with rapamycin, but the survival of the sick mice was extended [7]. The ‘deleter mouse’ (carrying a Twinkle helicase transgene with a dominant patient mutation) presenting with mitochondrial myopathy was reported to have changes in several pathways downstream of mTOR1 (such as folate cycle, serine biosynthesis, FGF21 secretion and fiber-specifically increased S6 phosphorylation) in the affected muscles, thereby indicating rapamycin as a potential treatment. Indeed, i.p. administration of 8 mg/kg resulted in robust improvement of muscle function and histology and normalization of mtDNA deletion load and several other molecular parameters [5]. Likewise, in muscle-specific Coq15 knockout mice with disrupted respiratory chain complex IV assembly, despite the lack of clear activation of mTOR1 downstream pathways, rapamycin stimulated autophagy and biogenesis of lysosomes, which the authors suggested resulted in improved muscle histology. However, if rapamycin affected the premature lethality of these mice was not reported [8].

The present study by Barriocanal-Casado et al. [2] investigated, in spite of no clear evidence of increased mTOR1 activity in their model, whether rapamycin has a general beneficial effect in mitochondrial encephalomyopathy. They utilized Coq9R239X mice with defective CoQ biosynthesis and the two oral dosages used, a low and a high (Table 1), corresponded to those previously shown efficacious in other models [4–6]. The outcome measures included survival, motor performance, brain imaging, tissue histology, and transcriptome and metabolite assessments. Apart from slight improvement in cerebral histopathology, rapamycin had no effect on any parameters of disease progression. The low dose resulted in changes of liver metabolites and midbrain transcriptomics, indicating an effect at molecular level. With the higher dose, all examined tissues responded to rapamycin as judged by S6 protein phosphorylation, a widely used and robust indicator of mTOR activity. Unexpectedly, the high dose resulted in an adverse effect on the mice, decreasing their survival. Interestingly, Barriocanal-Casado et al. did not observe induction of autophagy in any tissue analyzed (brain, heart, liver, and kidney) despite clear mTORC1 inhibition by the high...
Thus easier to adopt to clinical trials than novel molecules—should be animal studies using drugs already approved for human use. Evaluation and availability of unbiased evidence we suggest that preclinical intervention have been performed and left unpublished. To ensure accumulation and availability of unbiased evidence we suggest that preclinical animal studies using drugs already approved for human use— and thus easier to adopt to clinical trials than novel molecules—should be mandatorily registered in a preclinical database, as is the case for clinical trials.

**Disclosure**

The authors declared no conflicts of interest.

**References**