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Current landscape of therapeutic interventions to restore mitochondrial function and integrity in primary mitochondrial diseases and in acute and chronic conditions with mitochondrial dysfunction in preclinical models: Protocol for a systematic review

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ADMINISTRATIVE INFORMATION

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Review Stage at time of this submission - Preliminary searches.

Conflicts of interest - Authors have filed a patent application for the utilization of CPP-based mtDNA-containing nanocomplexes in the study of mitochondrial biology/metabolism/genetics and treatment of mitochondrial-related diseases.

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 9 July 2025 and was last updated on 9 July 2025.

INTRODUCTION

Review question / Objective The objective of this systematic review is to comprehensively identify and categorize preclinical therapeutic strategies for managing mitochondrial dysfunction in human disease, including mitochondrial targeting and delivery technologies, functional modulation, genome correction, or cell-based approaches.
What therapeutic strategies have been developed to directly target mitochondrial dysfunction—via delivery systems, modulation of mitochondrial function, correction of mtDNA defects, or alternative approaches—and how are these strategies applied in preclinical models of primary mitochondrial diseases and complex conditions involving secondary mitochondrial impairment?

Rationale Despite significant advances in mitochondrial biology, no comprehensive systematic review currently synthesizes and integrates the full spectrum of therapeutic strategies targeting mitochondria in preclinical models. Existing reviews are siloed—focusing individually on gene therapies, classes of small molecules/manipulation of mitochondrial function, or mitochondrial transfer—without integrating the broader landscape. This review addresses that gap by categorizing and critically evaluating delivery platforms, mitochondrial function modulators, genome-targeting methods, cell-based, and alternative approaches across diverse preclinical models of human disease. While this review will not directly compare therapies or assess relative efficacy—given the heterogeneity of disease models and endpoints—it will highlight key

strengths, limitations, and translational considerations within each therapeutic category to support future development and clinical planning. Overall, it will provide researchers and translational scientists with a consolidated view of where the field of mitochondrial therapeutics stands and where major gaps remain along the path to clinical application.

Condition being studied This review focuses on mitochondrial dysfunction as a pathological mechanism underlying both primary mitochondrial diseases and a wide range of complex or degenerative conditions. Because mitochondrial dysfunction contributes to many distinct disease states, the specific conditions being studied are intentionally not restricted to a predefined list. Instead, the review will include any human disease modeled in preclinical systems (in vitro or in vivo) where mitochondrial dysfunction is a central feature of pathophysiology. The key factor of interest is the direct targeting or modulation of mitochondria by a therapeutic intervention—whether through delivery of a therapeutic agent to the mitochondria, modulation of mitochondrial function or dynamics, correction of mitochondrial DNA defects, or replacement or transfer of mitochondrial components. Interventions must demonstrate a mechanistic focus on mitochondria rather than indirect or systemic effects alone. A comprehensive understanding of these therapeutic strategies is essential to inform the development of clinically viable treatments for patients suffering from diseases driven by mitochondrial impairment.

METHODS

Participant or population This review will include preclinical in vitro and in vivo models that represent human diseases characterized by mitochondrial dysfunction. Eligible populations include animal models (e.g., rodents, zebrafish, or other laboratory organisms) used to mimic human mitochondrial diseases or conditions with secondary mitochondrial impairment, as well as cell-based models such as immortalized cell lines, primary cells, patient-derived cells, or induced pluripotent stem cells (iPSCs), provided they are used to model disease-relevant mitochondrial dysfunction. Included studies must explicitly model a disease or pathophysiological state that is relevant to human health, rather than investigating mitochondrial function in the context of healthy physiology alone. Mitochondrial dysfunction must be a central feature of the model, whether genetically induced (e.g., mtDNA mutations, nuclear-encoded mitochondrial gene defects) or acquired (e.g., through stressors, aging, or toxic exposures). Studies that include both eligible and ineligible populations will be included only if outcomes are clearly reported for the disease-relevant mitochondrial model. Studies focused solely on cancer cell lines will be excluded unless the model involves engineered immune or therapeutic cells intended for translational application in human disease.

Intervention This review will include interventions explicitly designed to directly target mitochondria in the context of disease-relevant preclinical models. Eligible interventions may fall into one or more of four broad and expected categories: (1) delivery strategies designed to reach the mitochondria, including nanoparticles, liposomes, DQAsomes, MitoPorter systems, mitochondrial targeting peptides, and other technologies enabling subcellular or organelle-specific delivery; (2) therapeutic agents that modulate mitochondrial function, such as small molecules, peptides, antioxidants, or biologics that affect processes like oxidative phosphorylation, reactive oxygen species balance, mitophagy, or mitochondrial biogenesis; (3) genome-targeted strategies, including mtDNA supplementation, allotopic expression, mitochondrial DNA editing (e.g., TALENs, ZFNs, DdCBEs), or stabilization of mitochondrial RNA or tRNA; and (4) alternative strategies, including but not limited to mitochondrial transplantation, extracellular vesicle-mediated transfer of mitochondrial components, synthetic mitochondrial constructs, or other emerging approaches. These categories are intended to provide a useful structure for synthesis and analysis but are not intended to be exclusionary. Therapeutic strategies that do not neatly fit within these groups will not be excluded solely on that basis, provided they involve a direct mechanistic focus on mitochondria. Interventions may be pharmacological, genetic, biologic, or bioengineered, but must demonstrate engagement with mitochondrial structure, function, genome, or subcellular targeting.

Comparator N/A - The purpose of this review is to identify, describe, and categorize therapeutics directly targeting the mitochondria. Additionally, given the variability of preclinical outcome measures, data synthesis will not be performed.

Study designs to be included Peer-reviewed, primary research studies using preclinical experimental designs.

Eligibility criteria Studies will be included if they meet the following criteria: (1) they are primary, peer-reviewed research articles published in

English on or after the year 1999; (2) they are conducted in preclinical models—either in vitro or in vivo—that are used to model a human disease or pathological condition involving mitochondrial dysfunction; and (3) they evaluate a therapeutic intervention that directly targets mitochondria through a defined mechanism, such as delivery to mitochondria, modulation of mitochondrial function or dynamics, correction of mitochondrial genome defects, or replacement/transfer of mitochondrial components. Studies must include at least one outcome that reflects a mitochondrial-specific endpoint, such as ATP production, mitochondrial membrane potential, ROS levels, mtDNA integrity or expression, heteroplasmy, mitochondrial localization, or persistence of therapeutic effect.

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Studies will be excluded if mitochondrial engagement is not a central or primary focus of the intervention. Specifically, interventions that improve cellular health or metabolism more generally, without direct or mechanistically linked mitochondrial analysis, will not be included. Studies where mitochondrial outcomes are incidental or secondary observations, or where mitochondrial effects are inferred rather than directly measured, will also be excluded from full analysis—though they may be noted for future relevance. Interventions focused solely on cancer cell lines will be excluded due to the distinct and often inconsistent metabolic features of cancer mitochondria, unless the study involves engineered immune or therapeutic cells (e.g., T cells) intended for treatment of human disease. In silico-only models will be excluded unless validated with biological data. Clinical trials and human studies will be excluded, as no FDA-approved mitochondrial therapies currently exist and recent literature has already reviewed the clinical trial landscape.

Information sources PubMed/MEDLINE, Embase, Web of Science, Scopus.

Main outcome(s) Primary outcomes of interest are those that directly reflect mitochondrial engagement, modulation, or targeting in response to a therapeutic intervention. When available, persistence of effect will be collected. For studies investigating functional impact, key readouts include ATP production, mitochondrial membrane potential, oxygen consumption rate, and reactive oxygen species levels. For therapies targeting the mitochondrial genome, main outcomes include mtDNA copy number, heteroplasmy, and gene expression. Studies investigating mitochondrial delivery strategies will be parsed for outcomes related to localization via microscopy, fractionation, and subcellular tracking. General metabolic or cellular measures, including broad metabolite changes or redox ratios, but which are not clearly linked to mitochondrial mechanisms will not be considered sufficient.

Additional outcome(s) Additional outcomes of interest include features that may not be consistently reported but are important for evaluating the strengths, weaknesses, and translational potential of each therapeutic approach. These include mitochondrial uptake efficiency, subcellular targeting specificity, and indicators of mitochondrial toxicity or off-target effects. Toxicity-related outcomes may include loss of membrane potential, excessive ROS generation, or structural disruption of mitochondria. While these measures are not required for inclusion, they will be extracted when available to support interpretation of the data and to highlight feasibility, limitations, and future development needs across therapeutic categories.

Data management Two authors will independently conduct the systematic review, including title/abstract and full-text screening in the Covidence platform (Veritas Health Innovation Ltd, Melbourne, Australia. Available at http://www.covidence.org). Prior to formal screening, a pilot phase will be conducted using a small subset of articles to ensure consistency in interpretation and application of inclusion/exclusion criteria. Disagreements between reviewers will first be discussed directly between Authors 1 and 2. If consensus is not reached, the issue will be referred to Author 3, a senior mentor to both reviewers, who will adjudicate after reviewing the rationale provided by both parties. Data will be exported and updated consistently in the LabArchives electronic lab notebook.

Quality assessment / Risk of bias analysis Risk of bias assessment will be conducted using established tools appropriate to the study design. For in vivo studies, the CRIME-Q framework (1) will be used. CRIME-Q incorporates validated elements from SYRCLE, CAMARADES, and ARRIVE, and is well-suited for assessing internal validity in animal studies, including randomization, blinding, exposure clarity, and completeness of outcome reporting.
For in vitro studies, a modified approach will be used based extended to accommodate methodological studies, as reported by Rooney et al (2). Two reviewers will apply the appropriate tool to each included study independently. Any disagreements will be discussed collaboratively, and unresolved conflicts will be referred to a third reviewer. Studies with serious concerns regarding internal validity or methodological quality will be flagged and noted in the narrative synthesis.

Strategy of data synthesis As this review will focus on a diverse range of therapeutic strategies studied in heterogeneous preclinical models, no quantitative data synthesis will be performed. Instead, findings will be synthesized qualitatively. Included studies will be categorized according to the primary therapeutic modality. Within each category, extracted data will be presented in structured summary tables alongside narrative descriptions to highlight mechanisms of action, delivery methods, persistence, relevant mitochondrial outcomes and translational relevance.

Subgroup analysis While no quantitative subgroup analyses will be performed, the review will categorize therapeutic approaches and compare outcomes within each group. These findings will be descriptive based on the nature of preclinical outcome measures.

Sensitivity analysis To ensure comprehensive coverage of the literature, the search strategy will be validated against a pre-identified set of key studies known to meet inclusion criteria. If any of these studies are not retrieved by the search, the strategy will be revised to improve sensitivity. Search string terms will be iteratively applied to assess the effect of each term on article retrieval. Forward and backward citation searching of included studies will be conducted to identify relevant articles that may have been missed by database queries. Any modifications to eligibility criteria or screening decisions made after the piloting phase will be transparently documented.

Language restriction English.

Country(ies) involved USA.

Keywords Mitochondrial dysfunction; Mitochondria therapeutics; mitochondrial gene therapy; cellular bioenergetics; precision medicine; comprehensive literature review; systematic review.

Dissemination plans Findings from this review will be submitted for publication in a peer-reviewed journal and shared with a panel of experts in mitochondrial therapeutics for feedback prior to submission. The goal is to identify gaps in the therapeutic development pipeline and inform future research. Results will also be shared with patient advocacy groups and clinician networks to support translation and guide development priorities. Key findings may be presented at relevant scientific conferences.

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