Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

Mitochondrial transplantation

Evidence Summary

This is an emerging set of techniques to address mitochondrial dysfunction. The preclinical and early clinical evidence is promising, but significant optimization, indication, and safety work is required.

Neuroprotective Benefit: Mitochondrial transfer may occur physiologically for neuroprotection, and some preclinical studies suggest a benefit for neurodegenerative diseases. No clinical work has yet been published.

Aging and related health concerns: It has been shown to have benefit in preclinical models, and preliminary clinical evidence points to potential benefit. The interaction of mitochondrial transplantation and cancer must be thoroughly explored.

Safety: Preclinical studies have largely not identified safety concerns, but the field is very new with many different approaches; the translational relevance is yet unclear. Initial small human trials found no safety concerns but were not randomized or blinded.

Availability: Under clinical development	**Dose**: Not yet optimized and/or published. Most advanced trial transplanted approximately 1 x 108 autologous mitochondria in total. Final dose may vary based on age of patient, source of mitochondria, and exact indication.
Half-life: Varies based on tissue, cell-type, and cell-compartment. Mitochondrial lifespan may range from 2-30 days. As mitochondrial DNA (mtDNA) is passed on to daughter mitochondria, effects of mtDNA can persist past the lifespan of the original mitochondrion.	**BBB**: Not yet known in humans. Preclinical studies have reported mixed results as to whether intravenously administered mitochondria reach neurons. Intranasal administration may be an alternative route of delivery for neurological indications.
Clinical trials: Mitochondrial transplantation trial data has been published on a total of 16 patients.	**Observational studies**: No observational trials of mitochondrial transplantation have been published.

What is it?

Mitochondria are cellular organelles that are responsible for ATP production through oxidative phosphorylation, a process that generates the bulk of most eukaryotic cell's energy needs. Mitochondria also perform a variety of other crucial cellular tasks, including production of reactive oxygen species (ROS), calcium buffering, and cell signaling in survival and apoptotic signaling cascades. It is thought that mitochondria were originally independent prokaryotic organisms that were taken up by other ancient cells and formed a mutually beneficial, permanent arrangement that ultimately resulted in most modern eukaryotic cells. Mitochondria still have their own DNA, typically denoted as mtDNA, which codes for some of the mitochondrial proteins; other mitochondrial genes are now found in nuclear DNA (Cooper, 2000; Spinelli & Haigis; 2018, Eisner et al., 2018).

Mitochondrial dysfunction, including alterations in mitochondrial genes coded by mtDNA, is characteristic of or contributes to many different health concerns, including aging itself (Moos et al., 2021; Lima et al., 2022). Modulating mitochondrial function is therefore an active area of research for many different health conditions. One emerging approach focuses not on modulating or improving the

function of existing mitochondria, but rather supplementing with new mitochondria, whether from another tissue in that patient's body or from a healthy donor. This technique may be most immediately applicable for diseases caused by genetic perturbations of mtDNA but has a wide range of potential applications, as functional mitochondria may improve a variety of cellular phenotypes by providing energy and other cellular functions needed to address cellular dysfunction.

There is accumulating evidence that mitochondria can and do transfer from cell to cell in vitro as well as in vivo, whether through tunneling nanotubules, cell-to-cell contact, vesicle-mediated transport such as through extracellular vesicles, or other, yet to be discovered routes. Free extracellular mitochondria have also been found, and at least some cell types appear to be able to take up these mitochondria. These transfers may occur for a variety of reasons: for instance, studies have suggested that astrocytes 'send' mitochondria to damaged neurons in ischemic situations or under toxic conditions such as chemotherapy treatment, and other studies have proposed that neurons treated with toxins may transfer damaged mitochondria to astrocytes to recycle the mitochondria, a process known as mitophagy (Hayakawa et al., 2016; Gao et al., 2019; Espino De la Fuente-Muñoz & Arias, 2020, English et al., 2020).

As reviewed by Liu et al., 2022 and Clemente-Suarez et al., 2023, among others, there is a similar diversity in approaches to mitochondrial transplantation. Three broad categories of mitochondrial transplantation approaches, as well as their subtypes, are described below:

Autologous mitochondrial transplantation

In this form of mitochondria transplantation, mitochondria from one tissue in a patient are extracted and then returned to the same patient, typically to a different tissue. There are subtypes of this technique, including:

1. Isolating mitochondria from a different, mitochondria-rich tissue such as skeletal muscle and re-infusing or injecting the mitochondria back into the bloodstream or different tissue, typically during the same procedure.
2. Taking patient tissue and either isolating or converting to stem cells (called induced pluripotent stem cells, or iPSCS), and then using the stem cells to grow mitochondria. Eventually, the mitochondria are isolated and returned to the patient, whether directly as isolated mitochondria or in other cells such as certain blood cells that are then re-infused into the

patient. The stem cells may then transfer the healthy mitochondria to other tissues in the body, among other potential beneficial actions.

Donor mitochondrial transplantation:

In this form of mitochondrial transplantation, mitochondria from a donor are isolated and given to a patient. There are currently two main subtypes to this technique:

1. The mitochondria from the donor can be isolated and administered directly to the patient.
2. Donor mitochondria are isolated and then used to enrich patient's own cells ex vivo; the patient's cells are returned, now enriched with hopefully healthier mitochondria.

Sources of donor mitochondria can range from placental tissue from healthy birth to umbilical cord stem cells and beyond.

Mitochondrial replacement technology (MRT):

This term and approach is typically used in IVF contexts. Mitochondria are generally inherited only from the mother via mitochondria in the oocyte; thus, MRT represents a way to stop transmission of mitochondrial disease. MRT may also be a treatment for some forms of age-related infertility, as mitochondria are essential for early development and improving mitochondrial quality may help improve oocyte quality. This technique typically involves one of two processes.

I. Cytoplasm that contains mitochondria or isolated mitochondria from either donor oocytes or other tissue from patient themselves is transferred into the patient's oocyte. This technique does not fully replace the original mitochondrial population but rather supplements it with healthier mitochondria. This approach is fairly straightforward to implement alongside other common IVF procedures.
2. The second technique is more complex: the nucleus from a donor oocyte is removed and the nucleus from the patient's oocyte is transferred into the de-nucleated patient's oocyte. This approach should result in a mitochondrial population that is almost completely or completely descended from the donor's mitochondria.

MRT has been tested in a number of trials and has resulted in live birth, though the technology is not without legal considerations regarding the parentage of any resulting children and the ethical considerations as this technique can be considered a genetic modification that will be passed down to any subsequent generations through any female live births (Saxena et al., 2018, Rodriguez-Varela & Labarta, 2022).

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Preclinical evidence has suggested potential efficacy of mitochondrial transplantation in conditions ranging from acute kidney injury to acute respiratory distress syndrome to ischemic reperfusion injury to neurodegenerative disease. The exact mechanism of action is still debated, in part because there are so many potential avenues through which addition of healthy mitochondria could be a benefit (reviewed by Zhang & Miao, 2023, among others). While this is still an emerging technology with many questions to be answered, early small clinical trial results indicate potential benefit for cardiovascular disease (see "Safety" section for details) and more research in this area is underway.

Neuroprotective Benefit: Mitochondrial transfer may occur physiologically for neuroprotection, and some preclinical studies suggest a benefit for neurodegenerative diseases. No clinical work has yet been published.

Types of evidence:

- 6 reviews
- 13 laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:

No completed or published studies have examined the effects of mitochondrial transplantation on prevention of dementia, prevention of decline, or improved cognitive function.

Human research to suggest benefits to patients with dementia:

No completed or published studies have examined the effects of mitochondrial transplantation in patients with dementia.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

Mitochondria are absolutely essential for neuronal function. The brain is the most energy-demanding tissue in the body, and neurons - specifically, the synapse - is the most energy-demanding compartment of the brain. The ATP produced by mitochondria is required for neurotransmission; the process of synaptic vesicle cycling is extremely energy intensive, as are other necessary features of neuronal communication such as maintenance of ion gradients and calcium buffering. Mitochondrial deficits can underlie synaptic degeneration, which often precedes neuronal loss (Faria-Pereira & Morais, 2022). As mitochondrial dysfunction is implicated in numerous conditions, including cognitive decline and neurodegenerative diseases, different groups have hypothesized that perhaps one could replace or augment the mitochondrial population (reviewed by Espino De la Fuente-Muñoz & Arias, 2020).

There are numerous potential mechanisms that might underlie benefit of mitochondrial transplantation, from producing energy needed to rescue or protect cells to restoring cellular signaling that promotes survival to providing mtDNA to replacing damaged mtDNA. Many of the mechanisms are not mutually exclusive. Preclinical evidence from one group points to a specific effect of functional mitochondria, at least in some cell types; they found that transplantation of fresh, functional mitochondria provided cardioprotection while injection of non-viable or previously frozen mitochondria, as well as mitochondrial components, free ATP, or mtDNA or mtRNA did not provide any benefit (Guariento et al., 2021). However, these results do need to be replicated and rigorously studied in a neurological context.

Given the lack of clinical data on mitochondrial transplantation in clinical settings, this report will briefly cover preclinical work in this area. Nitzan et al., 2019 details the effects of mitochondrial transplantation into an AD mouse model. Mitochondria were freshly isolated from a human cancer cell line and administered intravenously to mice who had previously received injections of amyloid beta directly into their brains. In the two weeks after mitochondrial transfer, the mice underwent behavioral studies to assess learning and memory. The authors reported that the mice injected with amyloid beta and did not receive mitochondrial transfer showed deficits in some behavioral tasks that may reflect cognition, learning, and/or memory. These mice also had neurological changes such as neuronal loss and increased neuroinflammation. In comparison, mice who were injected with amyloid beta and received mitochondrial transfers had improved cognitive performance, less cell loss, and less neuroinflammation. The authors reported no signs of immune reaction or safety concerns. Interestingly, it did not appear that the mitochondria crossed the blood-brain barrier in this study. In a follow-up paper, Sweetat et al., 2023 also injected fresh mitochondria isolated from a human cancer cell line, but this time used a more

typical genetic model of AD and gave multiple doses of mitochondria over time. They similarly report some improvements in cognitive function, decreased neuronal loss in certain brain regions, and increased mitochondrial enzyme activity. These two papers did have several methodological concerns, and studies using different sources of mitochondria or different models of AD would be of interest.

Chang et al., 2016, Chang et al., 2021, and Shi et al., 2017 all report on mitochondrial transplantation in animal models of Parkinson's disease (PD). In the 2016 report, Chang and colleagues isolated mitochondria from cell culture lines. They added an additional tag called Pep-1 that helps deliver cargo into cells to some mitochondria, and injected both the Pep-1 modified mitochondria and unlabeled mitochondria directly into the brain of a chemically-induced rat model of PD. They found that rats injected with Pep-1-labeled mitochondria had improved locomotor activity, less cell loss of PD-relevant neurons, and had improved mitochondrial dynamics as compared to mice injected with unlabeled mitochondria or vehicle treatment. Conversely, in their 2021 paper, the group found that intranasal administration of either labeled or unlabeled mitochondria into chemically-induced PD rats resulted in improved motor performance, mitigated cell loss, and reduced oxidative stress.

Shi and colleagues isolated mitochondria from a cancer cell line and administered them via intravenous injection to a chemically-induced mouse model of PD. This PD mouse model has motor impairments and mitochondrial deficits; mice who received mitochondria had mitigated motor impairments and mitochondrial dysfunction as compared to their vehicle-treated PD model counterparts, though the mitochondrial treatment did not restore the mice fully to baseline. This group also reported that the injected mitochondria were found in the brain.

APOE4 interactions:

No differential effects of mitochondrial transplantation of any method have been reported based on APOE status.

The APOE4 isoform has been associated with mitochondrial dysfunction in multiple ways, with some studies pointing to APOE4 exacerbating mitochondrial deficits (Orr et al., 2019; Yin et al., 2020), and at least one preclinical paper finding that mitochondrial dysfunction increased expression of APOE (Wynne et al., 2023). If mitochondrial transplantation were shown to improve mitochondrial phenotypes in AD, it is theoretically possible that these effects could be particularly beneficial for APOE4 carriers. However, this is speculation based on early preclinical evidence rather than human data.

Aging and related health concerns: It has been shown to have benefit in preclinical models, and preliminary clinical evidence points to potential benefit. The interaction of mitochondrial transplantation and cancer must be thoroughly explored.

Types of evidence:

- 1 open-label study published in 2 papers
- 4 reviews

Preclinical models have suggested potential benefit of mitochondrial transplantation in several age-related conditions, such as diabetes and stroke (Clemente-Suarez et al., 2023). The data in clinical settings, however, is sparse. There is an ongoing trial utilizing mitochondrial transplantation in stoke (see "Research Underway" section for more details).

It is possible that mitochondrial transplantation would not be advised in certain conditions. For instance, some preclinical work indicates that mitochondrial transfer can promote cancer cell proliferation and invasion, and that mitochondrial transfer may contribute to chemoresistance. Inhibition of mitochondrial transfer may be of more interest to neoplasmic conditions (reviewed by Zampieri et al., 2021).

Cardiovascular Disease: POTENTIAL BENEFIT FOR CERTAIN CONDITIONS

A group at Boston's Children's Hospital has evaluated mitochondrial transplantation in pediatric patients who had cardiac surgery and then experienced a serious cardiac event that necessitated life support from extracorporeal membrane oxygenation (ECMO). ECMO takes over the work of the heart and lungs to give those organs a chance to recover. A 2021 retrospective paper compared 14 patients who received standard of care to 10 patients who received standard of care plus mitochondrial transplantation (MT). This was not a randomized or blinded trial, though there were no significant differences in baseline clinical characteristics.

The mitochondrial transplantation was performed as described in the group's earlier preliminary report. Mitochondria were isolated from skeletal muscle (~ 1 x 10^8 mitochondria) and then injected directly into the heart muscle affected by the cardiac event (Emani et al., 2017).

The authors found that rates of durable separation from ECMO were significantly higher in the MT group than in the control group, with 8 of the 10 (80%) MT patients still off ECMO 1 week after separation, whereas 4 of the 14 (29%) of the control patients were still off ECMO 1 week after separation (p=0.02). The median time to functional recovery was significantly shorter in the MT group as compared to the control group (2 days; range, 2 to 3 days vs 9 days; range, 6 to 13 days, respectively, p=0.02). The occurrence of cardiovascular events was lower in the MT patients as compared to control patients (2 patients (20%) vs 11 patients (79%), respectively, p<0.01). Median time to ECMO separation, overall mortality, and length of hospital stay did not differ between the two groups (Guariento et al., 2021).

This work is in very ill pediatric patients and is not a randomized controlled trial. It is worth noting that while these early clinical trials in humans have suggested a benefit in acute injury conditions, it is not clear whether there would be a benefit in chronic conditions like neurodegeneration; studies in neurodegenerative contexts may require long-term dosing, for instance.

Infertility: POTENTIAL BENEFIT

Infertility is defined as the inability to conceive a pregnancy in less than 12 months. Fertility is known to decline with age, particularly in women, as all oocytes that a woman has were formed when she herself was in utero. Mitochondria are necessary for oocyte function, and improving mitochondrial quality such as through mitochondrial replacement during the IVF process may also improve oocyte quality and thus increase chance of successful live birth. Mitochondrial replacement technology was largely designed to prevent genetic transmission of mitochondrial diseases. However, it has also been explored for patients who have oocyte quality concerns, such as those of advanced maternal age or have had repeated IVF failures. Some of these trials have resulted in live birth (reviewed in Saxena et al., 2018 and Rodriguez-Varela & Labarta, 2022; see "What is it" section for more detail).

Safety: Preclinical studies have largely not identified safety concerns, but the field is very new with many different approaches; the translational relevance is yet unclear. Initial small human trials found no safety concerns but were not randomized or blinded.

Types of evidence:

- 2 open label studies published in 3 papers

- 1 book chapter
- 5 reviews
- 1 commentary paper
- 6 laboratory studies

Mitochondrial transplantation is still a largely preclinical treatment, and safety concerns are still being explored. It is worth noting that some studies have found that extracellular mitochondria can be pro-inflammatory or otherwise modulate immune responses (Boudreau et al., 2014), though other preclinical and clinical studies have not reported immune reactions to mitochondrial transfer as measured by cytokine levels (Chang et al., 2021; Guariento et al., 2021). The effects of extracellular mitochondria may be specific to source, target tissue, and administration route. More research will be needed to fully understand the full benefits and risks of this approach.

As discussed in the "Aging and related health conditions" section, endogenous mitochondrial transfer may contribute to cancer cell proliferation, invasion, and chemoresistance. Careful studies will be needed to clarify this risk.

Guariento et al., 2021 describes the outcomes of a retrospective study comparing pediatric patients on ECMO who received mitochondrial transplantation to those who did not receive mitochondrial transplantation. The study included 24 patients overall: 10 who received mitochondrial transplantation and 14 who did not. While this study was not blinded or randomized, the authors did not identify any safety concerns of the procedure. They did not detect any new onset cardiac issues, including hematomas from the injection. There were no observed changes in any inflammatory marker that the group looked at; the transplant was not associated with any immune, autoimmune, or inflammatory response in this study. There were no significant differences in other measures such as hospital stay length or overall mortality.

Jacoby et al., 2022 describes the results of a compassionate-use, open-label trial of mitochondrial augmentation treatment in 6 patients with single large-scale mitochondrial DNA deletion syndrome (SLSMDs). SLSMDs are rare diseases where patients do not have a full mitochondrial DNA genome. In this study, hematopoietic stem and progenitor cells (HSPCs) were pharmacologically mobilized and collected from the patient, and a certain subpopulation known as CD34+ cells were isolated. These CD34+ cells were then incubated with previously cryopreserved mitochondria isolated from the patient's mother's peripheral blood mononuclear cells. The mitochondrial-enriched CD34+ cells were

then intravenously re-infused into the patient. Most adverse events were considered unrelated to the mitochondrial augmentation itself; some were related to the mobilization and collection of HSPCs but were expected in this population of patients. No serious adverse events were considered related to the mitochondrial augmentation. The authors also assessed the presence of anti-mitochondrial antibodies in the serum of patients at 6 months and 12 months post-treatment; they did not observe any evidence of increased antimitochondrial antibodies. No safety concerns were noted in the follow up period, and all patients lived for up to 4.5 years after treatment.

Drug interactions:

It is not yet known what drug or conditions might be contraindicated with mitochondrial transplantation.

Research underway:

There are five ongoing clinical trials investigating the safety and efficacy of mitochondrial transplantation that are registered on clinicaltrials.gov, and one additional study investigating a stem cell treatment that the group claims has superior mitochondrial function than other stem cell treatments. Only one of these trials focuses on a neurodegenerative disease, but all may be potentially relevant as they may shed light on important aspects of safety, practicability, and feasibility for this novel treatment approach. The details of each are discussed briefly below.

NCT02851758 is currently enrolling up to 16 pediatric patients who are being placed on extracorporeal membrane oxygenation (ECMO), an intervention that essentially acts as a heart and lungs for patients whose own hearts and lungs are unable to oxygenate their blood or perfuse the body on their own. During the clinically indicated ECMO procedure or catheterization, small biopsies will be taken of already-exposed skeletal muscle from the chest. The mitochondria from the tissue will be extracted and then injected back into the heart and/or aorta, termed an 'autologous mitochondrial transfer' as it is re-injecting the patient's own mitochondria. All patients in the study will receive the treatment.

This procedure may be repeated if the patient does not have a marked improvement and also has another needed procedure that would expose the heart. The primary outcome is incidence of severe adverse events in the first week following the procedure. The secondary outcome measures are improvements in heart function and the patient no longer requiring ECMO support because their own

heart and lungs show signs of being able to function on their own. Both of these secondary outcomes will be assessed in the month following the mitochondrial transfer. The study started in 2017 and is projected to reach primary completion in 2024. Initial results have been published and are discussed in both the "Aging and related health conditions" section and the "Safety" section of this report.

NCT05669144 is a currently enrolling study aiming to enroll approximately 20 patients ages 35 – 80 who are a candidate for coronary artery bypass grafting (CABG) surgery. The study will have 4 groups that patients will be randomized to: one group that will receive mitochondria-containing exosomes isolated from mesenchymal stem cells from human umbilical cords; one group that will receive autologous mitochondria from muscle biopsy during the CABG surgery; one group that will receive both mitochondria-containing-exosomes and autologous mitochondria; and one placebo group. All groups will receive injections of placebo or organelles directly into the heart. Outcome measures include measures of cardiac function and safety, including immune system reaction.

NCT04998357 aims to enroll 20 patients ages 18 to 85 experiencing a cerebral ischemic event to assess the effects of autologous mitochondrial transfer during standard-of-care treatment for cerebral ischemia. A muscle tissue biopsy will be taken and mitochondria will be extracted. The mitochondria will then be infused into the relevant brain artery during standard reperfusion treatment. All patients will receive the intervention. The primary outcome measures all assess incidence of severe adverse events during and after the procedure. The secondary outcome is the reduction of infarct volume. The study is currently enrolling and projects a 2024 completion date.

NCT06017869 is from Minovia Therapeutics. Minovia is a biotech company that produces cell therapies, including one called MNV-201 that they term a mitochondrial augmentation technology. MNV-201 is a treatment where certain hematopoietic stem and progenitor cells (HSPCs) from a participant are enriched with placental-derived mitochondria. The HSPCs are then infused back into the participant. These healthy donor mitochondria can then improve different hematopoietic phenotypes and potential other tissue phenotypes, as mitochondria may transfer to other cell types. The company published on an earlier compassionate use trial, detailed in the "Safety" section. This study seeks to assess the safety and efficacy of MNV-201 in pediatric patients with Pearson syndrome, a mitochondrial disorder caused by genetic changes in mitochondrial DNA. The study aims to enroll 5 patients. HSPCs from each patient will be collected, enriched with donor mitochondria, and then infused back into the patient. The primary outcome measure will be incidence of treatment-related adverse events. Secondary outcomes will include improvements in blood phenotypes such as improvement in anemia or frequency of blood

transfusions, quality of life scales, and frequency and length of hospitalization. The study began in the summer of 2023 and estimates that they will reach primary competition in 2026.

NCT04976140 is an ongoing study by Paean Biotechnology that aims to enroll 18 adult patients with refractory polymyositis or dermatomyositis, two inflammatory diseases that result in muscle weakness. This open-label, dose escalation study involves treating patients with PN-101, which appears to be an intravenous administration of mitochondria extracted from umbilical cord mesenchymal stem cells. While the clinical trial registration states that there will be a low, middle, and high dose group assuming no safety concerns, they do not detail what the doses are. The outcome measures are incidence of adverse events and a variety of scales to assess polymyositis or dermatomyositis symptoms and progression.

NCT05094011 is a not-yet-recruiting study from Taiwan Mitochondrion Applied Technology Co. that aims to enroll 9 patients 45 to 70 years of age with Parkinson's disease. The company reports that their stem cell manufacturing process utilizes unique methods and culture reagents that result in stem cells that are more efficient and have better mitochondrial function than other stem cells. They call these stem cells 'MitoCells'. Published studies on MitoCells were not identified in a literature search. The patients in the study will all receive injections of MitoCells directly into PD-relevant brain region in both hemispheres. The study outcomes include safety, a variety of health exams such as blood work and physical exams as well as neurodegenerative disease measures including the MMSE and PD-specific rating scales.

Search terms:

Pubmed, Google: mitochondrial transplantation, mitochondrial transfer, autologous mitochondria

- Dementia, Alzheimer's diseaese, cancer, cardiovascular

Websites visited for mitochondrial transplantion:

- Clinicaltrials.gov

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