Recommendations for mitochondria transfer and transplantation nomenclature and characterization

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Abstract

Intercellular mitochondria transfer is an evolutionarily conserved process in which one cell delivers some of their mitochondria to another cell in the absence of cell division. This process has diverse functions depending on the cell types involved and physiologic or disease context. Although mitochondria transfer was first shown to provide metabolic support to acceptor cells, recent studies have revealed diverse functions of mitochondria transfer, including but not limited to the maintenance of mitochondria quality of the donor cell and the regulation of tissue homeostasis and remodeling. Many mitochondria transfer mechanisms have been described using a variety of names, generating confusion about mitochondria transfer biology. Furthermore, several therapeutic approaches involving mitochondria transfer biology have emerged, including mitochondria transplantation and cellular engineering using isolated mitochondria. In this consensus statement, we define relevant terminology and propose a nomenclature framework to describe mitochondria transfer and transplantation as a foundation for further development by the community as this dynamic field of research continues to evolve.

Introduction

Mitochondria are functionally and structurally diverse organelles that are essential for the health of nearly all eukaryotic cells.[1,2] These organelles are frequently associated with their roles in energy metabolism as the sites where the citric acid cycle, β-oxidation, electron transport chain, oxidative phosphorylation, and other metabolic pathways occur. However, mitochondria have many other important functions, including cell signalling, viral sensing, cellular calcium homeostasis, redox homeostasis, iron handling, haem and lipid synthesis, cell division, and cell death.[1] Phylogenetic and fossil record studies indicate that mitochondria evolved through an ancient endosymbiotic process in which α-proteobacteria capable of aerobic respiration were engulfed by an anaerobic archaeal cell. The transition from an endosymbiosed bacteria to an ancestral organelle that gave rise to modern respiring mitochondria involved the development of complex protein transport systems, massive loss of thousands of α-proteobacterial genes to the host nuclear genome, highly integrated organelle division processes, and bioenergetic flexibility and function.[3-5] This process enabled complex multicellular life to form, as evidenced by fossil records dating to approximately 1.6 billion years ago and the evolution of all extant eukaryotic organisms today.[6-9]

Over the past ~20 years, we have come to understand that mitochondria can be transferred from one cell to another, a process that is termed horizontal or intercellular mitochondria transfer. Although the origins of mitochondria transfer are unclear, it may be a preserved relic of their α-proteobacterial ancestry that confers a selective advantage to multicellular eukaryotes. Indeed, mitochondria transfer has been observed in evolutionarily diverse eukaryotes, including yeast[10-12], mollusks[13], fish[14], and rodents[15] as well as human cells[16], and mtDNA transfer has been reported between cells in plants[17-19]. We are just beginning to understand the physiologic functions of mitochondria transfer, how alterations in this process contribute to disease pathogenesis, and how to harness mitochondria transfer biology to develop new therapies.

ICMTTN process and scope of review

The rapid pace of research on mitochondria transfer has created the need for clarity about terminology used in this field. To address this, we organized an International Committee on Mitochondria Transfer and Transplantation Nomenclature (ICMTTN), comprised of an international team of 31 investigators (working in 13 countries) in the field to assimilate a

contemporary understanding of mitochondria transfer pathways and therapeutic approaches and provide guidance about recommended terminology and characterization standards (**Fig 1**). The ICMTTN convened as a full group twice and operated in two geographically defined groups working in parallel. Each group met three times, and each participant was afforded dedicated time for presentation of their ideas followed by group discussion. The Chair (JRB) and Co-Chair (KKS) participated in both working groups to provide continuity but presented their ideas once. An initial draft consensus document was created, and each participant was invited to provide comments and suggestions over a 4-week period. Following incorporation of these improvements, we held a 30-day public commenting period hosted by Mitochondria World (www.mitoworld.org) and considered suggestions and critiques before peer review.

We begin by providing an abbreviated history of the field, highlighting some of the important milestones that have advanced our understanding of mitochondria transfer and transplantation. We then establish definitions of basic terms and concepts regarding mitochondria transfer and transplantation (**Box 1**). We recommend nomenclature for cell contact-dependent and contact-independent mitochondria transfer processes and for therapeutic approaches involving mitochondria transplantation. We recognize that this field is evolving quickly and that these recommendations may not always fully conform to future insights and conceptual advances. Therefore, we present this nomenclature framework below as a guide and set of recommendations that will likely need to be revisited in future years.

A brief history of the field

Foundational early discoveries

The earliest discoveries about intercellular mitochondria transfer were based on foundational observations about the ability of cells to acquire mitochondria and their components from their environment (**Fig 2**). In 1977, it was reported that mitochondrial genotypes could be obtained by ρ^0 yeast protoplasts, which lacked mtDNA, by fusing them with protoplasts of wildtype cells[20]. These results were extended a few years later using isolated mitochondria or enucleated cells.[10-12] In 1982, it was shown that isolated mitochondria from chloramphenicol and efrapeptin resistant mouse tumor cells could be endocytosed by drug-sensitive tumour cells in culture and confer resistance, indicating that uptake of extracellular mitochondria could genetically transform drug-

sensitive cells.[21] These results were later recapitulated by microinjection of isolated mitochondria into human tumour cell lines.[22]

It was then shown that injection of myoblast mitochondria harbouring a pathogenic mtDNA $tRNA^{Lys}$ mutation into mtDNA-deficient cell lines formed cytoplasmic hybrid cells called “cybrids” and that the donor mtDNA conferred defects in mitochondrial protein translation and cytochrome *c* oxidase activity.[23] A few years later, it was shown that platelets[24] and sperm[25] from healthy individuals can deliver mtDNA to ρ^0 cells to form cybrids with rescued aerobic respiration, and later breast cancer cybrids harbouring the G10398A mtDNA variant were reported to have apoptotic resistance and enhanced metastatic potential.[26] Although horizontal mtDNA gene transfer was found to occur between cells in flowering plants[17,18], broadening the diversity of organisms known to exchange mitochondrial components, there is not yet direct evidence for the transfer of intact mitochondria between plant cells.

Recent studies on mitochondria transfer

In 2006, it was reported that human bone marrow-derived mesenchymal stem cells or fibroblasts could deliver mitochondria to neighbouring A549 ρ^0 lung carcinoma cells in culture to rescue cell-intrinsic defects in aerobic respiration[16], providing the first evidence that mitochondria transfer could confer metabolic properties to acceptor cells. This was confirmed shortly thereafter using isolated mouse mitochondria taken up by human ρ^0 cancer cells,[27] although we recognize that in healthy cells mouse mitochondria can fuse with human mitochondria but are not maintained over long periods of time due to mitonuclear conflict.[28]

A series of studies have since demonstrated that mitochondria transfer occurs *in vivo* in complex animal systems. In 2011, it was reported that canine transmissible venereal tumour cells obtain mitochondria from host cells,[29] and the next year it was shown that bone marrow stromal cells transfer mitochondria to pulmonary alveoli to dampen endotoxin-induced acute lung injury in mice.[15] Several years later it was shown that ρ^0 tumours obtain mtDNA[30] via intact mitochondria[31] from host cells in mice to support tumorigenesis by promoting *de novo* pyrimidine synthesis.[32] In 2016, it was also shown that mouse oocyte differentiation is regulated in part by mitochondria transfer from sister cyst germ cells.[33] That same year, retinal ganglion cells were shown to shed damaged mitochondria and deliver them to adjacent astrocytes for degradation, establishing the concept of transmitophagy or licensed mitophagy, where one cell type borrows the degradative

function of another.[34] Transmitophagy is now known to occur in several organ systems such as the heart[35,36], brown adipose tissue[37], and retina[14,34] to preserve mitochondrial homeostasis of the donor cells.[38] Furthermore, platelets were shown to release mitochondria upon activation[39], a process that was recently linked to wound healing.[40]

We now know that many cell types participate in mitochondria transfer in the central nervous system[41-44], peripheral nervous system[45], lung[15,46-48], heart[35,49], white adipose tissue[50,51], brown adipose tissue[37,51], bone[52-54], hematopoietic system[39,55-57], and the tumour microenvironment[30,31,58,59]. In some cases, respiration-competent mitochondria are released into the blood of mice and humans[39,60], serving as a conduit through which cells in one organ can transfer mitochondria to cells in another organ (i.e. interorgan mitochondria transfer). This was shown with the delivery of adipocyte-derived mitochondria to the heart in obesity[51,61], however it is likely that many cell types contribute the pool of circulating extracellular mitochondria (ex-Mito) in blood. Mitochondria transfer has been found to regulate a diverse set of physiologic processes, including regulation of adipose tissue homeostasis, cardiovascular health, wound healing, angiogenesis, haematopoiesis, and inflammation, topics that were recently reviewed in more detail elsewhere.[38,62-65]

Development of therapeutic approaches

There has been considerable effort to harness the biology of mitochondria transfer pathways for therapeutic purposes. These approaches involve some aspects of mitochondria transfer biology, such as cellular uptake mechanisms, but are procedures to deliver mitochondria to an intended organ and/or cell type. One of the earliest studies attempting to use isolated mitochondria for therapeutic purposes was the isolation and transplantation of cardiac mitochondria into the hearts of rabbits subjected to ischemia followed by reperfusion, an intervention that reduced ischemia-reperfusion injury and improved functional recovery.[66] A similar finding was later observed in pigs.[67] In subsequent clinical trials, skeletal muscle mitochondria were isolated from paediatric patients requiring extracorporeal membrane oxygenation (ECMO) for autologous transplantation, allowing most of the patients to be successfully separated from ECMO faster.[68,69] Mitochondria transplantation is now being developed for other therapeutic indications, including but not limited to ischemic stroke, ischemic limb injury, spinal cord injury, neurodegeneration, cardiac resuscitation, refractory dermatomyositis or polymyositis, and inherited mitochondrial diseases.[43,70-80]

Furthermore, isolated mitochondria can be modified or implanted in gel matrices to improve mitochondria uptake efficiencies or therapeutic benefits.[74,81,82] Other groups have used isolated mitochondria to metabolically engineer cells in culture. For example, it was shown that culturing hematopoietic stem cells (HSCs) with isolated mitochondria leads to improved engraftment in mice receiving bone marrow transplants.[83] This method was used in a clinical trial where HSCs from patients with large-scale mtDNA deletion syndromes were co-cultured with maternal mitochondria *in vitro* prior to autologous HSC transplantation.[84] These patients had reduced anaemia and improved quality of life. These encouraging studies suggest that therapies involving mitochondria transfer biology have the potential to treat a variety of diseases, calling for robust nomenclature that can empower and harmonize academic and industrial efforts to understand and leverage this biology to improve human health.

Basic concepts and terms

Mitochondria transfer

As cells differentiate and divide, their mitochondria are passed on to the two daughter cells. This process is referred to as "*vertical inheritance of mitochondria*," a term that specifically relates to mitochondria sorting during cell division, which can be asymmetric in some cases such as stem cell differentiation.[85,86] In contrast, horizontal or intercellular "*mitochondria transfer*" is a process in which one cell donates some of its mitochondria to another cell in the absence of cell division (**Fig 3a**). The "*donor cell"* is the cell from which the transferred mitochondria originate, and the cell that obtains the transferred mitochondria is referred to as an "*acceptor cell."* The term "acceptor cell" applies equally well when that cell retains, degrades, or re-exports the transferred mitochondria and is intended to be agnostic of fate or processing. The donor and acceptor cells are frequently but not necessarily developmentally distinct cell types.

A "*mitochondria transfer axis"* can be defined when both the donor and acceptor cell types are established *in vivo* (**Fig 3b**). As one of many possible examples, adipocytes transfer mitochondria to macrophages in white and brown adipose tissues in mice, establishing an adipocyte-to-macrophage mitochondria transfer axis.[37,50] Adipocytes also appear to transfer some of their mitochondria to several other cell types, evoking the concept of a "*mitochondria transfer network*"

to reflect that cells may participate in multiple mitochondria transfer axes that can occur in parallel or stacked in series (**Fig 3c**).[51]

Furthermore, adipocytes can release their mitochondria into the blood, allowing them to be transported to other organs, such as the heart.[51,61] These studies establish the concept of "*interorgan mitochondria transfer*," a process that is defined as long-distance mitochondria transfer axis between a donor cell in one organ and an acceptor cell in another organ (**Fig 3d**). In this case, the blood[39,51,60,61], lymph, or cerebrospinal fluid[87] may serve as a conduit to distribute "*extracellular mitochondria (ex-Mito)"* from the donor cell to the acceptor cell. The ex-Mito population is heterogeneous, containing multiple different types and from different cellular sources, a topic that is discussed in more detail below.

Some studies have used the term "mitochondria transfer," whereas others have used "mitochondrial transfer." The term "mitochondria transfer" uses mitochondria as a noun, reflecting that a cell can transfer mitochondria (noun) to another cell. The term "mitochondrial transfer" uses the adjective form "mitochondrial" to describe a property or characteristic of the transfer. From a practical perspective, these two terms are synonymous and have been used interchangeably. Therefore, we suggest that "mitochondria transfer" and "mitochondrial transfer" are both acceptable and that the choice of which term to use is an investigator-specific preference.

<u>Therapeutic approaches</u>

There are also several emerging therapeutic approaches that involve mitochondria transfer pathways or mechanisms. One is called "*mitochondria transplantation*," a procedure in which mitochondria are isolated from a cellular or tissue source and then administered directly to an animal with the intent of eliciting a therapeutic response. Many studies have alternatively used the term "mitochondrial transplant," which is also acceptable. In this article, we use the term "mitochondria transplant" to conform with well-established transplant terminology, where the noun form of the transplanted organ is used (e.g. heart transplant). As discussed in more detail later in this review, mitochondria transplantation has been employed in preclinical studies using model organisms[70,88-90] and in recent clinical trials with promising early results,[68,69,91,92] including ongoing clinical trials that have not yet been published.

Another therapeutic approach involves *in vitro* cell engineering using isolated mitochondria, where cells obtained from a patient are exposed to isolated mitochondria as part of the cell manufacturing process.[83] This approach has been used in a recent clinical trial with encouraging findings, as discussed below.[84] It has also been used to support the survival[93] and boost the efficacy of engineered cells, such as chimeric antigen receptor (CAR) T cells.[94]

Methods to define mitochondria transfer

Mitochondria reporter proteins

These basic terms raise a methodologic question about how mitochondria transfer can be defined at a practical level. This topic has been discussed recently[95] so is covered here only briefly. There are multiple methodologic approaches to detect mitochondria transfer. The most robust method is to utilize fluorescent reporter proteins attached to mitochondria localization signals, which are short polypeptide sequences that direct the fluorescent protein to the inner or outer mitochondrial membrane or the mitochondrial matrix. There are several mitochondria reporter protein constructs, which can be used under the control of a stop-flox system, allowing one to define a donor cell based on the cell type specificity of a Cre recombinase.[50,96,97] One can then define a mitochondria transfer axis or network by identifying the labelled mitochondria in other cell types.[50,51] Similarly, one can express other mitochondrially targeted proteins (e.g. Halo[98,99]) that allow for detection of mitochondria transfer.

An important limitation of this approach is it can detect mitochondria transfer only if the mitochondria reporter protein is contained within the transferred mitochondrial structure. This is not always the case. One example is that a fraction of mitochondria-derived vesicles (MDVs) contain highly selected cargo and are generated as either single, outer membrane vesicles or double membrane vesicles[100,101], therefore a fluorescent reporter protein targeted to the IMM would not report all MDV transfer events. MDVs are transported into multivesicular bodies and can be released as exosomes. There is indeed evidence of selective sorting of mitochondrial components in MDVs that are ejected from cells. For example, brown adipocytes transfer MDVs to macrophages, and those MDVs lack the quintessential brown adipocyte IMM protein Uncoupling protein 1.[37]

Mitochondrial dyes

An alternative fluorescence-based method used in many studies is to stain donor cells with fluorescent mitochondrial dyes and track the transferred mitochondria in another cell type *in vitro*. While this method may initially seem attractive due to the low effort and cost required to test an idea related to mitochondria transfer, these dyes are leaky and highly susceptible to producing false-positive results, even after extensive washing.[102,103] All mitochondrial dyes likely efflux from the donor cell *in situ* during co-culture, creating dye transfer artifacts that drastically over-estimate mitochondria transfer efficiency or suggest that mitochondria transfer occurs when in fact it does not. As one of many examples, mature red blood cells, which lack mitochondria, are able to transfer MitoTracker dyes to 293T cells.[102]

Therefore, we strongly recommend against the use of mitochondrial dyes to define mitochondria transfer. If dyes are used, it is critical that many controls are used to reduce concern about dye leak and titrating the dye to identify the lowest possible concentration with the goal of reducing off-target staining of other organelles. Great caution should be exercised when interpretating mitochondria transfer results obtained using dyes, and any such findings should be validated using a dye-independent method to avoid reporting false-positive results.

<u>Other methods</u>

Other approaches to define mitochondria transfer include using donor cell types with divergent mtDNA sequence variants[16,30-32,104,105], effectively serving as an unambiguous molecular barcode detectable with DNA sequencing or PCR. However, detection of transferred mtDNA on its own is not sufficient evidence of mitochondria transfer because the mitochondria could be stuck to the surface of the cell without internalization and because free mtDNA by itself can be transferred between cells. Alternatively, one may detect an acquired mitochondria-associated protein or property conferred to the acceptor cell, such as rescue of respiratory competence in cells that are deficient in oxidative phosphorylation.[16,21]

<u>Methods to enforce mitochondria transfer</u>

There are also several useful *in vitro* methods to study specific steps involved in mitochondria transfer (e.g., mechanisms of mitochondria uptake or release). Examples include the addition of isolated mitochondria to cells in culture or the detection of released mitochondria into media,

although in the latter case it is important to control for death that occurs in cell culture conditions. Some cell types are efficient at taking up ex-Mito, whereas others are not. There are several strategies to enhance the efficiency of uptake by acceptor cells, such as the addition of a centrifugation step (e.g., MitoCeption)[106,107], pressure (e.g., MitoPunch)[104,105,108,109], or conjugation with a cell penetrating peptide (e.g., Pep-1).[110] Cell culture methods can also be used with transwell systems to physically separate the donor and acceptor cells, allowing one to distinguish between contact-dependent and contact-independent transfer mechanisms.[59]

Fates of mitochondria after cell entry

Another important consideration when studying mitochondria transfer axes is to consider the fate of the transferred mitochondria. Are the transferred mitochondria quickly degraded and how? Do transferred mitochondria gain access to the cytoplasm and get incorporated into the acceptor cell's pool of endogenous mitochondria? Do the transferred mitochondria gain access to the nucleus and contribute to insertions of mtDNA fragments into the nuclear genome (i.e., numtogenesis[111])? Is there durable genetic transformation of the acceptor cell from the mtDNA contained within the transferred mitochondria? Are the transferred mitochondria repackaged and later ejected from the cell for delivery to another cell type?

The fates of internalized mitochondria likely vary by donor cell type, acceptor cell type, physiologic context, or disease state. We caution against assuming that the fate of transferred mitochondria in one cell type is the same as in another. We also suggest that determining the fate and processing of the transferred mitochondria is not required to establish whether mitochondria transfer occurs, though addressing questions about fate and processing are likely to reveal important insights about the functions of mitochondria transfer in the context under investigation. Similarly, in the context of mitochondria transplantation, understanding the biodistribution, fate, and processing of the transplanted mitochondria may shed light on the mechanisms of action of this therapeutic modality.

Mechanism-based nomenclature

Current findings suggest that cells utilize several distinct mechanisms to transfer mitochondria. These transfer mechanisms can be roughly grouped into 2 categories: *"contact-dependent mitochondria transfer"* and "*contact-independent mitochondria transfer*." The identification of one

transfer mechanism is not mutually exclusive of other mechanisms, as cells may participate in multiple mitochondria transfer mechanisms simultaneously. Several recent reviews have discussed the molecular mechanisms of these mitochondria transfer pathways[63-65], therefore this section focuses on nomenclature used to describe these pathways.

Contact-dependent mitochondria transfer

Tunnelling nanotubes

In the most general sense, contact-dependent mitochondria transfer occurs when the donor and acceptors are required to be in physical contact with each other for the transfer event to occur. The most frequently reported contact-dependent mechanism is via tunnelling nanotubes (TNTs), which are long, thin membranous structures that form between two cells with support of filamentous (F)-actin[112] (**Fig 3e**). Mitochondria are transported along a microtubular highway using motor/adaptor complexes that involve the mitochondrial Rho GTPase 1 (Miro1).[113,114]

TNT formation is a complex process but appears to be mediated in part by growth associated protein 43 (GAP43), a protein that neurons utilize at the axonal growth cone to facilitate outgrowth of axons,[115] or the M-Sec protein that triggers re-arrangement of plasma membrane protein prior to TNT formation.[30] Hallmark features of TNTs are their thin diameter, typically measuring 0.5-1.5 μm in diameter, the presence of F-actin, and the direct continuity of cytoplasm between cells. However, not all contact-dependent mitochondria transfer occurs via TNTs.

Dendritic structures

Cells can also make contact via dendritic structures to facilitate the mitochondria transfer, which we define as "*dendritic structure-mediated mitochondria transfer*" (**Fig 3e**). Three examples of this process involve the delivery of mitochondria from osteocytes to other osteocytes[52], from osteocytes to endothelial cells, and from astrocytes to neurons.[41] These dendritic structures tend to be 1.5-3 μm in diameter and form contact with other cells via end feet, which are widened termini of the dendritic structure. The end feet contact but do not fuse with the acceptor cell's plasma membrane. The models propose that the end feet structures spatially determine the site where ex-Mito are released in a CD38-, Mitofusin 2-, and/or MIRO-1-dependent manner for delivery to the acceptor cell[41,52,116], although other transfer mechanisms are also possible and

could involve the release of ex-Mito for delivery to the cell in contact with the end feet. At a practical level, it is difficult to distinguish between TNT- and dendrite-mediated mitochondria transfer. This can be addressed at least partially by demonstrating that there is a lack of continuity between the cytoplasm of one cell and that of another, determining the width of the tubular structure extending from the donor cell, and identifying end feet at the junction of the dendritic structure and the acceptor cell.

Adhesion via gap junctional channels

Another contact-dependent transfer mechanism involves Connexin 43 (Cx43), a process that we define as "*adhesion-mediated mitochondria transfer*" (**Fig 3e**).[15] This gap junction protein has a very small pore that is too small to permit the passage of mitochondria from one cell type to another. Rather, Cx43 mediates the adhesion of two cells, and the acceptor cell can then obtain mitochondria from the donor cell through the formation and internalization of annular gap junction vesicles.[117] This process is analogous to trogocytosis in the immune system.[118] Alternatively, Cx43-mediated gap junctional channels require that the two cells be directly adjacent to each other, which may enable mitochondria transfer via other mechanisms, including the transfer of ex-Mito. If a donor cell ejects ex-Mito, those mitochondria are statistically more likely to be taken up by their closest neighbours. TNTs or dendritic structures can also theoretically form at the Cx43 contact site. These possibilities should be distinguished if feasible when adhesion-mediated mitochondria transfer is observed and will be further clarified as the field evolves.

Cell fusion is not mitochondria transfer

A frequent question that the field has encountered is whether cell fusion leading to the formation of multinucleated cells is a type of mitochondria transfer. Giant cells and osteoclasts are examples of multinucleated cells that form by cell fusion.[119] We suggest that this process does not constitute mitochondria transfer because all cellular components fuse into a single cellular entity. Therefore, cell fusion has no definable donor or acceptor cell. This process is better characterized as a form of vertical inheritance of mitochondria occurring in reverse directionality.

<u>Contact-independent mitochondria transfer</u>

Release of extracellular mitochondria

Cells do not need to be in direct physical contact to engage in mitochondria transfer. Rather, donor cells can eject their mitochondria to produce ex-Mito that can be captured by another cell type. Contact-independent mitochondria transfer processes can be described based on the structural characteristics of the mitochondria that are transferred (**Fig 3f**). We recommend a tiered nomenclature framework. Ex-Mito is a general term that includes all forms of mitochondria or structurally intact mitochondrial fragments (e.g. MDVs) found in extracellular fluid. In contrast, EVs that contain free-floating, cytosolic mtDNA and that are devoid of mitochondria or MDVs are not ex-mito.

Extracellular vesicles with mitochondria

Some of these ex-mito are enclosed within extracellular vesicles (EVs), which can vary dramatically in terms of both size and cargo. EVs containing mitochondria (EV-Mito) can range in diameter from ~120 nm to 3-4 μm and can contain MDVs or intact mitochondria, with some EV-Mito containing multiple mitochondria.[35,37,41,60,61,120] There is a great deal of heterogeneity in these structures, and cells can release more than one type of EV-Mito.[51,61] Although we considered whether the types of mitochondrial cargos should be used to define EV-Mito, we felt there was insufficient information to make cargo-based classifications at this time. However, we do encourage investigators to characterize EV-Mito cargo when possible.

There are also multiple release mechanisms including exosomal release via the multivesicular body[49] and ectosomal release pathways through vesicle formation from the plasma membrane[39]. However, after EV-Mito are released from the cell, there is no reliable marker to distinguish between the exosomal and ectosomal release pathways, therefore these release mechanisms are not currently factored into EV-Mito nomenclature. In accordance with recent guidance by the International Society for Extracellular Vesicles (MISEV2023)[121], we discourage the use of the terms exosome and ectosome unless their subcellular origin can be clearly defined. Therefore, we recommend that EVs containing mitochondria may be called “EV-Mito” and that they should be characterized at least in terms of their size, as this is a physical characteristic that can be measured using a variety of methods. EV-Mito should otherwise be defined using the nomenclature conventions established by MISEV2023.[121]

Free mitochondria

In addition, cells can release ex-Mito that are not enclosed in an EV membrane. We recommend that these ex-Mito be referred to as "*free mitochondria*." While free mitochondria have been observed in tissues and in circulation[42,51,65,122,123], the mechanisms by which cells release free mitochondria are unclear and require further study. Technically, free mitochondria meet the MISEV2023 definition of being an EV because they are membrane-bound structures released by cells, but referring to free mitochondria as EVs is confusing and discouraged. Electron microscopy and small-particle flow cytometry suggest that free mitochondria are typically 0.5-1.1 μm in diameter.[39,42,51,60,124] If electron microscopy is not feasible, free mitochondria can be defined by immunoprecipitation using antibodies that bind outer mitochondrial membrane proteins (e.g., anti-TOM22) and that they lack EV markers (e.g., the tetraspannins CD9, CD63, and CD81).[51] However, there are also small free mitochondria about 100-200 nm in diameter found at least in cerebrospinal fluid[125,126], which may or may not be MDVs or small mitochondria generated by fission. Electron microscopy and the presence of monoamine oxidase (MAO)-B, which is an outer mitochondrial membrane protein, on the surface of these structures suggest that that they are free mitochondria with inner and outer mitochondrial membranes but that lack cristae.[125] We recommend that these structures be referred to as small free mitochondria.

Circulating extracellular mitochondria

Both EV-Mito and free mitochondria have been detected in plasma of mice and humans.[39,51,60,61] Indeed, many studies have reported the existence of cell-free mtDNA in plasma[127,128], and the vast majority is located within ex-Mito.[129] Evidence for this includes size-exclusion chromatography indicating that cell-free mtDNA can be removed from plasma using 0.45 μm filters.[130] Furthermore, centrifugation of plasma at 15,000-16,000 x *g* depletes plasma of the mtDNA signal and eliminates the ability of plasma to respire.[39,60,130] While detection of mtDNA in plasma may reflect the presence of circulating ex-Mito, measuring mtDNA is not sufficient on its own to identify this blood component, as mtDNA can also circulate without being contained in ex-Mito.[123,124,131,132] If the mtDNA is not contained within ex-Mito, then it will be susceptible to hydrolysis in a DNase I digestion assay. In contrast, mtDNA contained in ex-Mito should be susceptible to DNase I digestion after the addition of detergent.[124]

Circulating ex-Mito can be described in terms of their size, the proportions that are EV-Mito or free mitochondria, the donor cell type, morphology, or other relevant characteristics. These

circulating mitochondria can be delivered from cells in one organ to cells in another, as has been described between adipocytes and cells in the heart[51,61] and between platelets and cells in the vessel wall.[40] It is likely that many more donor cell types release mitochondria into blood, including immune cells. This raises the intriguing possibility that interorgan mitochondria transfer is a ubiquitous phenomenon involving numerous donor cell types and destinations. An exciting future direction for the field will be to determine the diversity, connectivity, and functions of interorgan mitochondria transfer networks.

Therapeutic approaches

Mitochondria transfer biology has inspired the development of some exciting therapeutic approaches. There are several companies that have been recently founded to develop therapies or technologies focused on mitochondria transfer or transplantation. These companies and academic investigators are spearheading efforts to develop and evaluate the safety and efficacy of these therapeutic approaches, including mitochondria transplantation and the use of isolated mitochondria during cell engineering or manufacturing (**Fig 4**). There is also an emerging concept of developing drugs that induce or inhibit mitochondria transfer to treat disease.

Mitochondria transplantation

Defining mitochondria transplants

As described above, mitochondria transplantation is a procedure in which mitochondria are isolated from a cellular source and delivered postnatally to an individual with the specific purpose of eliciting a therapeutic response.[133] The mitochondria can be administered via various routes, including but not limited to direct injection into a tissue or systemic delivery via intravascular routes. This procedure is fundamentally distinct from mitochondria replacement therapy (MRT), which is an *in vitro* fertilization procedure that is used to correct or reduce the heteroplasmy of pathogenic mtDNA mutations in the offspring and that involves microinjection to bypass the plasma membrane.[134] In contrast to MRT, mitochondria transplantation is a procedure performed after birth, and the mitochondria are not expected to genetically modify the germline. The mitochondria are not directly injected into cells like in MRT. Rather, they are taken up using similar mechanisms as contact-independent mitochondria transfer. Mitochondria transplantation can also be used to treat a wide variety of diseases (not just heritable diseases caused by mtDNA

mutations), can be autologous or allogeneic, and can be multiple times in each patient, unlike MRT.

Mitochondria transplantation has been reported to improve disease outcomes in many preclinical models, including ischemia-reperfusion injury in the heart[69,91,92], lung[88], brain[70,87,135], limbs[80], and kidney[136]; spinal cord injury[74,75,90]; cardiac resuscitation[77]; inherited mitochondrial diseases such as Leigh Syndrome[79], and others. The first clinical trial reporting autologous mitochondria transplantation was a case-series of neonates and infants requiring ECMO support due to cardiac ischemia-reperfusion injury, suggesting that the procedure was well tolerated and associated with improvements in ventricular function.[69] Subsequently, paediatric patients requiring ECMO were treated with or without autologous mitochondria transplants, with those who received mitochondria transplantation being more likely to be successfully separated from ECMO and less likely to experience another cardiovascular event compared to patients who did not receive mitochondria transplantation.[68] These and other studies have stimulated considerable interest in this therapeutic modality and inspired other clinical trials that are underway.

Types of mitochondria transplants

"*Autologous mitochondria transplants*" involve isolating mitochondria from the same individual who later receives the ex-Mito product as a therapy. This approach carries the least risk because all antigens and mtDNA in the mitochondria product are self-derived. *"Heterologous or allogeneic mitochondria transplants*" involve isolating mitochondria from another individual or a cell line for direct administration to a recipient. As the mtDNA genome is inherited via the maternal lineage in mammals, a variant of heterologous mitochondria transplantation involves preparing the ex-Mito product from an individual's biological mother or an individual from the same maternal lineage. Although there has not yet been a published heterologous mitochondria transplant clinical trial, at least one is currently in progress for refractory polymyositis or dermatomyositis (clinicaltrials.gov ID NCT04976140) based on encouraging preclinical data in mice.[137]

Some evidence suggests heterologous mitochondria transplants are likely to be well tolerated. Specifically, there are approximately 3-12 billion ex-Mito per unit of platelets[39], a heterologous blood product that is routinely and safely transfused to patients intravenously without significant adverse reactions, even without known mtDNA haplogroup matching. If there is persistence of mtDNA from the donor mitochondria, mtDNA haplogroups and the degree of achieved mtDNA

heteroplasmy should be taken into consideration.[138] Mice engineered to have high proportions of mtDNA heteroplasmy from two divergent but healthy mDNA genomes develop cardiopulmonary dysfunction and become frail,[139] although it is unlikely that this degree of mtDNA heteroplasmy could be achieved with mitochondria transplantation.[80,83] It will be important to consider the level of mtDNA heteroplasmy achieved in mitochondria transplant studies and to better understand the long-term stability and effects of any detected heteroplasmy on the recipient organism.

"*Xenogenic mitochondria transplants*" involve isolating mitochondria from one organism (e.g. human) and administering them to another (e.g. mouse). This scenario can be a useful experimental model but has unclear translational potential due to interspecies mito-nuclear conflict and the likely immunogenic response from exposure to foreign antigens in mitochondria from another species.

Durability of mitochondria transplants

The term "***transplant"*** implies that there is an intent for the administered mitochondria to be taken up by acceptor cells and to engraft the acceptor cells with those mitochondria. There are many examples in which cells in culture take up ex-Mito from media, use them for cellular respiration, and replicate the donor-derived mtDNA for propagation to daughter cells.[51,106,140-142] Metabolically compromised cells appear to be more efficient at retaining and utilizing ex-Mito in animal models, suggesting that the degree of engraftment might depend on the metabolic context of the acceptor cells.[51] Animal models involving ischemia-reperfusion injury suggest that ex-Mito can be detected for up to 28 days after administration, though longer time frames were not tested.[67] These studies support the concept that administered mitochondria can escape the endocytic compartment and engraft in or at least be used by acceptor cells.

On the other hand, recent studies suggest that endothelial cells quickly degrade ex-Mito, with split-green fluorescent protein experiments indicating that these mitochondria are not incorporated into the endothelial cell's endogenous pool of mitochondria.[80] This is consistent with other studies showing that healthy macrophages quickly engulf and degrade ex-Mito in tissues.[35,37] Taken together, these studies suggest that transplanted mitochondria can engraft, but this does not always occur even if a therapeutic response is observed. Indeed, it is possible that the therapeutic effects of mitochondria transplantation are due to the stimulation of mitophagy and mitochondrial biogenesis[80], not necessarily direct effects of the transplanted mitochondria.

The degree and durability of engraftment may depend on the acceptor cell type, source of the ex-Mito, route of administration, and context in which the transplant is performed. Although there is debate about the degree or durability of engraftment with mitochondria transplantation, we point out that there are many examples where transplanted material does not successfully engraft (e.g. rejection after a solid organ transplant). A transplant still occurred if the grafted material failed or was removed. For this reason, we contend that no accepted definitions of the term "*transplant*" require that engraftment occurs, though the term does imply the intent to engraft. Therefore, the term "mitochondria transplant" is consistent with established definitions of the word "transplant," as defined by other bodies such as the World Health Organization.

Mitochondria transplant heterogeneity

An important consideration is that not all mitochondria transplants are the same or should be expected to produce the same results. Mitochondria can differ dramatically depending on their cell-of-origin[143,144], and the methods of isolation and route of administration may differ from one study to another. Furthermore, isolated mitochondria can be complexed with other factors to alter their bioavailability and internalization by certain types of cells, as demonstrated by adding an asialoglycoprotein-based carrier to mitochondria for targeted delivery to the liver.[145] There is not yet enough information for us to provide guidance on mitochondria transplantation nomenclature that recognizes the heterogeneity of these factors.

Therefore, we suggest that mitochondria transplant studies should provide a transparent description of at least five factors: i) the source material, ii) isolation method, iii) size of the isolated mitochondria, iv) whether the ex-Mito product is free or Mito-EVs, and v) whether and how the ex-Mito were modified after isolation. When possible, we also encourage ex-Mito products to be characterized in terms of their inner and outer membrane integrity, capacity to respire, and enrichment. Purity of an ex-Mito product is challenging and may not be feasible to define because mitochondria are generally associated with other cellular components, including but not limited to endoplasmic reticulum, peroxisomes, and lysosomal membranes. We recommend that enrichment is the relevant term and can be defined as the proportion of particles that contain ex-Mito using small-particle flow cytometry[51] or other techniques, such as the degree of enrichment for mitochondrial proteins or mtDNA using immunoblots or quantitative PCR, respectively. Additional considerations that need to be evaluated are the unique pharmacokinetics and

pharmacodynamics of transplanted mitochondria, which are fundamentally different from that of small or large molecule therapies.

Cell engineering using extracellular mitochondria

As described above, many cell types can obtain ex-Mito from culture media or other cells. Some of the earliest observations in the field demonstrated that ex-Mito can endow cells with chloramphenicol and efrapeptin resistance, establishing the concept that cells can be engineered using ex-Mito. Although many cell types are highly efficient at importing and using ex-Mito, several methods have been developed to further enhance this process. One of these methods has been referred to as MitoCeption[106,107], a technique in which the mitochondria capture process is enhanced through centrifugation at 4°C followed by incubation at 37°C. This method is analogous to "spinfection" or "spinoculation," in which centrifugation enhances viral infection of cells in culture.[146,147] Pressure has also been used to enhance mitochondria uptake and associated mtDNA genetic transformation of ρ^0 cells *in vitro*, a process called MitoPunch.[104,105,108,109] These and other methods allow the introduction of mitochondria from one source into cells in culture.

Cell engineering using ex-Mito has been used therapeutically in a recent clinical trial. It was reported that HSCs exposed to isolated mitochondria from peripheral blood mononuclear cells engraft better in lethally irradiated mice compared to unmanipulated HSCs and lead to improved hematopoietic reconstitution.[83] Based on this study, HSCs were isolated from primary mitochondrial disease patients with large-scale mtDNA deletions and then cultured *in vitro* with isolated mitochondria from the patient's biological mother.[84] The mitochondria-engineered HSCs were then autologously transplanted back to the patient. This trial demonstrated that the HSCs loaded with maternal mitochondria led to reduced anaemia and improved quality of life. It remains unknown, however, how long the donor mitochondria and mtDNA persist following the autologous HSC transplant. Furthermore, other studies have demonstrated that chimeric antigen receptor (CAR) T cells exposed to isolated mitochondria exhibit increased proliferation and enhanced capacity to kill tumour cells *in vitro* and *in vivo*.[94,148] These studies suggest that adding ex-Mito to cells during manufacturing processes can improve therapeutic responses to cell therapies.

Again, there is a lack of information to recommend specific terminology about methods to engineer cells using ex-Mito. Like for mitochondria transplantation, we recommend that studies provide detailed methods to clarify the procedures used to engineer cells using ex-Mito and to

characterize those mitochondria in terms of source material, isolation method, size of the isolated mitochondria, whether the ex-Mito are free or Mito-EVs, and enrichment for mitochondria. If the ex-Mito product has itself been engineered through expression of a mitochondria-targeted protein or other modification[149], as was recently described, these features should also be clearly defined.

Drugs affecting mitochondria transfer

Several studies suggest that eliciting or inhibiting mitochondria transfer pathways with small molecules can treat a wide range of diseases. As one example, cancer cells obtain mitochondria from macrophages[59], T cells[58], fibroblasts[150], and other cell types[115,151,152] in the tumour microenvironment to support their own metabolic requirements, stimulate cell proliferation, suppress the anti-tumour immune response, and resist chemotherapeutic agents. In the future, it may be possible to use pharmacologic agents inhibit mitochondria transfer to cancer cells, which might slow cancer progression and/or make tumours more susceptible to other therapies. On the other hand, mitochondria transfer pathways also support the maintenance of mitochondrial homeostasis in several tissues, and these pathways are impaired in cardiometabolic diseases such as obesity and heart failure.[35,50] Restoring these pathways might reduce metabolic disease severity. As another example, a recent study suggested that pharmacologic induction of mitochondria transfer can slow the progression of pulmonary fibrosis.[46] Further research is needed to understand how to precisely modify relevant mitochondria transfer axes for therapeutic purposes.

Outlook

In this article, we propose a nomenclature framework to describe mitochondria transfer and transplantation. We have categorized mitochondria transfer processes based on broad mechanisms of mitochondria transfer and the structural characteristics of ex-Mito. We also provide guidance regarding the minimal criteria needed to define mitochondria transfer axes or networks. Therapeutic approaches, such as mitochondria transplantation and cell engineering using ex-Mito, should be described in terms of the procedure being performed and characteristics of the ex-Mito used. The goal of this proposed nomenclature is to reduce the confusion that can be caused by the introduction of different names for similar processes or ex-Mito subsets as this field has evolved. We recognize that mitochondria transfer and transplantation are very active areas of research and that it is possible that new findings and insights may necessitate updates

to the proposed nomenclature. For this reason, this article should be viewed as a work in progress, but we encourage researchers in the field to utilize the terms defined here.

Acknowledgements

The authors completed this work as the International Committee on Mitochondria Transfer and Transplantation Nomenclature (ICMTTN). We extend our thanks and appreciation to Ms. Dionne Brierton (Washington University School of Medicine) for her exceptional administrative support of this international effort. We also thank Gordon Freedman (Mitochondria World and the National Laboratory for Education Transformation) for hosting the public commenting period on www.mitoworld.org and Zachary Kirshner (Kirshner Design, kirshnerdesign.com) for website design and support. We thank the individuals and groups who submitted comments during the public commenting period. Costs associated with this publication were paid for by a grant from the Burroughs Wellcome Fund (CAMS #1019648) to JRB.

Author Contributions

JRB conceived of the article, organized the ICMTTN, chaired all meetings, wrote the initial draft of the manuscript, and led revisions. KKS contributed to the organization process, served as co-chair for all meetings, and assisted with writing the first draft and revisions. All authors participated in the introductory, working group, and summation meetings; contributed to the conceptual content and organization of this article; contributed to writing, editing, and/or revising this manuscript; and approved the final version.

Conflict of Interest

Conflicts of interest (COI) were obtained and shared with all coauthors prior to any deliberations. JRB is a member of the Scientific Advisory Board of LUCA Science, Inc.; receives research support from LUCA Science and Edgewise Therapeutics; is a consultant for Columbus Instruments, Inc.; has consulted for DeciBio within the past 12 months; receives royalties from Springer Nature Group; is an inventor on technology licensed to Columbus Instruments, Inc. with royalty rights; and is an inventor on pending patent applications related to the treatment of metabolic diseases (63/625,555) and allergic diseases (US20210128689A1) and mitochondria transfer (018984/US). KKS is a co-founder, holds equity in, and serves on the Scientific Advisory Board for YUVA Biosciences. EB holds patents on detection of extracellular mitochondria in inflammatory conditions (WO2015051466A1) and on mitochondrial autoantibodies in autoimmune diseases (WO2022/246565A1), and is a principal investigator and scientific advisor to MitrixBio. LB receives grant support from Philips Medical Systems, ZOLL Medical Corp, Nihon Kohden, PCORI, BrainCool, and United Therapeutics; is on the Scientific Advisory Board for Nihon Kohden, HP, and Philips; holds 7 issued patents and several pending patent applications

involving the use of medical slurries as human coolant devices, the creation of slurries, reperfusion cocktails, and measurement of respiratory quotient; and serves on committees for the American Heart Association (AHA) that has a financial interest in the outcome of resuscitation studies being conducted and that sells training materials worldwide on resuscitation techniques. AC is a founder of and scientific advisor to Dragon Biomed and a scientific advisor to Luvigix. JAE has collaborated with Minova Therapeutics. ÅBG is a consultant for Lexeo Therapeutics. JDM has pending patents for the isolation and use of isolated mitochondria and is a founder and member of the Scientific Advisory Board and Board of Directors for cellvie. MK is the chief scientific officer of Cells for Cells, EVast Bio, and Regenero (Chilean consortium for regenerative medicine) with Corporación de Fomento de la Producción (CORFO) support; has received research support from ANID (National Agency for Research and Development) basal #FB210024 and Cells for Cells; is an inventor on patents related to mesenchymal stem cells (pending patents WO2014135924A1, WO20170646770A2, WO2017064672A1, and WO2019051623). RN receives research support from LUCA Science, Inc. AJS is an employee of Mitochondria World. MAT is a co-founder and shareholder for NanoCav, LLC, a private start-up company with licenses for mitochondria transfer techniques and applications. ART is an inventor of a pending patent related to the diagnostic detection of cf-mtDNA (WO 2016/063122 A1). RT is a scientific advisor for Cytokinetics, Inc. and GenKardia, Inc. MW has a pending patent (US20210085713A1) related to compositions and methods for treating stroke. The other authors declare they have no conflicts of interest.

Box 1. Definitions of key terms

Donor cell: a cell from which transferred mitochondria originate

Acceptor cell: a cell to which mitochondria are transferred

Intercellular or horizontal mitochondria transfer: a process in which one or more mitochondria are transferred from a donor cell to an acceptor cell. This term implies that the acceptor cell has taken up the donor cell-derived mitochondria but does not imply the fate of those mitochondria.

Mitochondria transfer axis: A mitochondria transfer axis can be defined when both a donor and acceptor cell are known. This term is best used in the context of an endogenous mitochondria transfer process, rather than an *in vitro* process.

Mitochondria transfer network: The transfer of mitochondria from a defined donor cell to multiple acceptor cell types. Alternatively, a mitochondria transfer network can be understood as multiple connected mitochondria transfer axes in parallel or series.

Interorgan mitochondria transfer: The transfer of mitochondria from a donor cell in one organ to an acceptor cell in another organ, usually via the circulatory system or other biofluid.

Contact-dependent mitochondria transfer: a mitochondria transfer process that requires direct cell-to-cell contact to occur, often via tunnelling nanotubes, dendritic structures, or adhesion.

Contact-independent mitochondria transfer: a mitochondria transfer process that can occur without direct cell-to-cell contract via the release of extracellular mitochondria that are taken up by an acceptor cell.

Extracellular mitochondria (ex-Mito): mitochondria detected in an extracellular environment, including interstitial fluid, plasma, cerebrospinal fluid, saliva, or cell culture media.

Free mitochondria: ex-Mito that are not enveloped by an extracellular vesicle (EV)

EV-mitochondria (EV-Mito): ex-Mito that are enveloped by an EV, which can be derived from either exosomal or ectosomal release pathways.

Mitochondria transplantation: a procedure in which mitochondria are isolated and then administered *in vivo* to an organism with the intent to engraft and confer a therapeutic benefit.

Autologous mitochondria transplant: a mitochondria transplant in which in the recipient is also the source of the mitochondria.

Heterologous mitochondria transplant: a mitochondria transplant in which the source of the mitochondria is another individual of the same species.

Xenogenic mitochondria transplant: a mitochondria transplant in which the source of the mitochondria is different species from the recipient.

FIGURE LEGENDS

Figure 1. Process used for consensus statement construction. In Phase I, an international committee was formed and divided into two groups based on geography. After an introductory meeting with all members, Groups A and B met three times. Each co-author held the floor to present nomenclature considerations, followed by group discussions. In Phase II, an outline was presented to all co-authors for discussion prior to preparing the article. In Phase III, a preliminary version of the article was posted on Mitochondria World's website for a public commenting period. In Phase IV, the committee finalized the manuscript prior to formal peer review. Rep., Republic; USA, United States of America; N.Z., New Zealand. Created with BioRender.com.

Figure 2. Partial timeline of milestones in mitochondria transfer and transplantation. Milestones associated with isolated mitochondria and therapeutic approaches such as mitochondria transplantation and cell engineering are shaded in grey. Milestones associated with mitochondria transfer between cells are shaded purple. HSC, hematopoietic stem cell. CAR, chimeric antigen receptor. Created with BioRender.com.

Figure 3. Terminology to describe mitochondria transfer. a, Vertical inheritance of mitochondria occurs during cell division to pass on mitochondria to daughter cells. Horizontal or intercellular mitochondria transfer occurs in the absence of cell division and may occur between developmentally distinct cell types. **b**, The cell-of-origin is known as the donor cell, and the cells that obtain the transferred mitochondria are known as acceptor cells. When the donor and acceptor cells are defined *in vivo*, this is referred to as a mitochondria transfer axis. **c**, A mitochondria transfer network is the co-occurrence of multiple mitochondria transfer axes in parallel or in series. **d**, Interorgan mitochondria transfer occurs when cells in one organ release their mitochondria into a circulating body fluid, such as blood, for delivery to cells in another organ. **e**, Contact-dependent mechanisms of mitochondria transfer. Tunnelling nanotube-mediated mitochondria transfer occurs when two or more cells form connections that permit direct delivery of mitochondria from the cytoplasm of one cell to that of another. Dendritic structure-mediated mitochondria transfer is a process in which one cell extends a tubular structure that makes contact to acceptor cell via end feet. The cytoplasms of the two cells are not directly connected, as is the case with tunnelling nanotubes. Adhesion-mediated mitochondria transfer occurs when two cells make direct physical contact, sometimes mediated by a gap junctional channel such as Connexin 43. The mitochondria are transferred to the acceptor cell not via the channel pore, which is too

small to permit passage of large cargo, but by an endocytic mechanism at the cell-to-cell interface. Created with BioRender.com. **f**, Contact-independent mechanisms of mitochondria transfer. Donor cells can release mitochondria into the extracellular space in various forms, and these can then be imported by acceptor cells. All these forms can be referred to as extracellular mitochondria (ex-Mito). One subset of ex-Mito is known as free mitochondria, which are not enclosed within an extracellular vesicle (EV). There are several subsets of EVs that contain mitochondria (EV-Mito) that differ based on their size, type of mitochondrial cargo, and mechanisms of release. Cargo can include intact mitochondria or mitochondria-derived vesicles (MDVs). The mechanisms that regulate the release and uptake of ex-Mito remain the subject of ongoing investigation. Created with BioRender.com.

Figure 4. Therapeutic approaches using extracellular mitochondria. Mitochondria can be isolated from a cellular source or biofluid and used for therapeutic purposes. In mitochondria transplantation, the mitochondria are directly administered to a patient to elicit a therapeutic response. This procedure is distinct from mitochondria replacement therapy, which is an *in vitro* fertilization procedure that modifies the germline during reproduction. Isolated mitochondria can also be administered to cultured cells during cell manufacturing or processing in preparation for subsequent cellular therapies. In this scenario, the excess or unincorporated mitochondria are removed prior to infusion of the cells to the recipient. Created with BioRender.com.

REFERENCES

1 Monzel, A. S., Enríquez, J. A. & Picard, M. Multifaceted mitochondria: moving mitochondrial science beyond function and dysfunction. *Nature Metabolism* **5**, 546-562, doi:10.1038/s42255-023-00783-1 (2023).

2 McBride, H. M., Neuspiel, M. & Wasiak, S. Mitochondria: More Than Just a Powerhouse. *Current Biology* **16**, R551-R560, doi:10.1016/j.cub.2006.06.054 (2006).

3 Friedman, J. R. & Nunnari, J. Mitochondrial form and function. *Nature* **505**, 335-343, doi:10.1038/nature12985 (2014).

4 Roger, A. J., Muñoz-Gómez, S. A. & Kamikawa, R. The Origin and Diversification of Mitochondria. *Curr Biol* **27**, R1177-r1192, doi:10.1016/j.cub.2017.09.015 (2017).

5 Srinivasainagendra, V. *et al.* Migration of mitochondrial DNA in the nuclear genome of colorectal adenocarcinoma. *Genome Med* **9**, 31, doi:10.1186/s13073-017-0420-6 (2017).

6 Geiger, O., Sanchez-Flores, A., Padilla-Gomez, J. & Degli Esposti, M. Multiple approaches of cellular metabolism define the bacterial ancestry of mitochondria. *Sci Adv* **9**, eadh0066, doi:10.1126/sciadv.adh0066 (2023).

7 Miao, L., Yin, Z., Knoll, A. H., Qu, Y. & Zhu, M. 1.63-billion-year-old multicellular eukaryotes from the Chuanlinggou Formation in North China. *Sci Adv* **10**, eadk3208, doi:10.1126/sciadv.adk3208 (2024).

8 Pennisi, E. The power of many. *Science* **360**, 1388-1391, doi:10.1126/science.360.6396.1388 (2018).

9 Pennisi, E. Tiny fossils upend timeline of multicellular life. *Science* **383**, 352-353, doi:10.1126/science.ado2396 (2024).

10 Fukuda, H. & Kimura, A. Transfer of mitochondria into protoplasts of saccharomyces cerevisiae by mini-protoplast fusion. *FEBS Letters* **113**, 58-60, doi:https://doi.org/10.1016/0014-5793(80)80494-7 (1980).

11 Gunge, N. & Sakaguchi, K. Fusion of mitochondria with protoplasts in Saccharomyces cerevisiae. *Mol Gen Genet* **170**, 243-247, doi:10.1007/bf00267057 (1979).

12 Yoshida, K. Interspecific and intraspecific mitochondria-induced cytoplasmic transformation in yeasts1. *Plant and Cell Physiology* **20**, 851-856, doi:10.1093/oxfordjournals.pcp.a075878 (1979).

13 Luo, Y. & Wang, W.-X. Increasing Intercellular Communication and Directional Organelle Transfer in Oyster Hemocytes under Copper Stress. *Environmental Science & Technology Letters* **10**, 831-837, doi:10.1021/acs.estlett.3c00625 (2023).

14 Hutto, R. A. *et al.* Cone photoreceptors transfer damaged mitochondria to Müller glia. *Cell Rep* **42**, 112115, doi:10.1016/j.celrep.2023.112115 (2023).

15 Islam, M. N. *et al.* Mitochondrial transfer from bone-marrow–derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nat Med* **18**, 759-765, doi:10.1038/nm.2736 (2012).

16 Spees, J. L., Olson, S. D., Whitney, M. J. & Prockop, D. J. Mitochondrial transfer between cells can rescue aerobic respiration. *Proceedings of the National Academy of Sciences* **103**, 1283-1288, doi:10.1073/pnas.0510511103 (2006).

17 Bergthorsson, U., Adams, K. L., Thomason, B. & Palmer, J. D. Widespread horizontal transfer of mitochondrial genes in flowering plants. *Nature* **424**, 197-201, doi:10.1038/nature01743 (2003).

18 Won, H. & Renner, S. S. Horizontal gene transfer from flowering plants to Gnetum. *Proc Natl Acad Sci U S A* **100**, 10824-10829, doi:10.1073/pnas.1833775100 (2003).

19 Gurdon, C., Svab, Z., Feng, Y., Kumar, D. & Maliga, P. Cell-to-cell movement of mitochondria in plants. *Proc Natl Acad Sci U S A* **113**, 3395-3400, doi:10.1073/pnas.1518644113 (2016).
20 Ferenczy, L. & MarÁZ, A. Transfer of mitochondria by protoplast fusion in Saccharomyces cerevisiae. *Nature* **268**, 524-525, doi:10.1038/268524a0 (1977).
21 Clark, M. A. & Shay, J. W. Mitochondrial transformation of mammalian cells. *Nature* **295**, 605-607, doi:10.1038/295605a0 (1982).
22 King, M. P. & Attardi, G. Injection of mitochondria into human cells leads to a rapid replacement of the endogenous mitochondrial DNA. *Cell* **52**, 811-819, doi:10.1016/0092-8674(88)90423-0 (1988).
23 Chomyn, A. *et al.* In Vitro Genetic Transfer of Protein Synthesis and Respiration Defects to Mitochondrial DNA-Less Cells with Myopathy-Patient Mitochondria. *Molecular and Cellular Biology* **11**, 2236-2244, doi:10.1128/mcb.11.4.2236-2244.1991 (1991).
24 Chomyn, A. *et al.* Platelet-mediated transformation of mtDNA-less human cells: analysis of phenotypic variability among clones from normal individuals--and complementation behavior of the tRNALys mutation causing myoclonic epilepsy and ragged red fibers. *Am J Hum Genet* **54**, 966-974 (1994).
25 Manfredi, G., Thyagarajan, D., Papadopoulou, L. C., Pallotti, F. & Schon, E. A. The fate of human sperm-derived mtDNA in somatic cells. *Am J Hum Genet* **61**, 953-960, doi:10.1086/514887 (1997).
26 Kulawiec, M., Owens, K. M. & Singh, K. K. mtDNA G10398A variant in African-American women with breast cancer provides resistance to apoptosis and promotes metastasis in mice. *Journal of Human Genetics* **54**, 647-654, doi:10.1038/jhg.2009.89 (2009).
27 Katrangi, E. *et al.* Xenogenic transfer of isolated murine mitochondria into human rho0 cells can improve respiratory function. *Rejuvenation Res* **10**, 561-570, doi:10.1089/rej.2007.0575 (2007).
28 Yoon, Y. G., Haug, C. L. & Koob, M. D. Interspecies mitochondrial fusion between mouse and human mitochondria is rapid and efficient. *Mitochondrion* **7**, 223-229, doi:10.1016/j.mito.2006.11.022 (2007).
29 Rebbeck, C. A., Leroi, A. M. & Burt, A. Mitochondrial capture by a transmissible cancer. *Science* **331**, 303, doi:10.1126/science.1197696 (2011).
30 Tan, A. S. *et al.* Mitochondrial genome acquisition restores respiratory function and tumorigenic potential of cancer cells without mitochondrial DNA. *Cell Metab* **21**, 81-94, doi:10.1016/j.cmet.2014.12.003 (2015).
31 Dong, L. F. *et al.* Horizontal transfer of whole mitochondria restores tumorigenic potential in mitochondrial DNA-deficient cancer cells. *Elife* **6**, doi:10.7554/eLife.22187 (2017).
32 Bajzikova, M. *et al.* Reactivation of Dihydroorotate Dehydrogenase-Driven Pyrimidine Biosynthesis Restores Tumor Growth of Respiration-Deficient Cancer Cells. *Cell Metab* **29**, 399-416.e310, doi:10.1016/j.cmet.2018.10.014 (2019).
33 Lei, L. & Spradling, A. C. Mouse oocytes differentiate through organelle enrichment from sister cyst germ cells. *Science* **352**, 95-99, doi:10.1126/science.aad2156 (2016).
34 Davis, C. H. *et al.* Transcellular degradation of axonal mitochondria. *Proc Natl Acad Sci U S A* **111**, 9633-9638, doi:10.1073/pnas.1404651111 (2014).
35 Nicolás-Ávila, J. A. *et al.* A Network of Macrophages Supports Mitochondrial Homeostasis in the Heart. *Cell* **183**, 94-109.e123, doi:10.1016/j.cell.2020.08.031 (2020).
36 Zhang, K. *et al.* TREM2hi resident macrophages protect the septic heart by maintaining cardiomyocyte homeostasis. *Nature Metabolism* **5**, 129-146, doi:10.1038/s42255-022-00715-5 (2023).
37 Rosina, M. *et al.* Ejection of damaged mitochondria and their removal by macrophages ensure efficient thermogenesis in brown adipose tissue. *Cell Metabolism* **34**, 533-548.e512, doi:10.1016/j.cmet.2022.02.016 (2022).

38 Nicolás-Ávila, J. A., Pena-Couso, L., Muñoz-Cánoves, P. & Hidalgo, A. Macrophages, Metabolism and Heterophagy in the Heart. *Circ Res* **130**, 418-431, doi:10.1161/circresaha.121.319812 (2022).
39 Boudreau, L. H. *et al.* Platelets release mitochondria serving as substrate for bactericidal group IIA-secreted phospholipase A2 to promote inflammation. *Blood* **124**, 2173-2183, doi:10.1182/blood-2014-05-573543 (2014).
40 Levoux, J. *et al.* Platelets Facilitate the Wound-Healing Capability of Mesenchymal Stem Cells by Mitochondrial Transfer and Metabolic Reprogramming. *Cell Metabolism* **33**, 283-299.e289, doi:10.1016/j.cmet.2020.12.006 (2021).
41 Hayakawa, K. *et al.* Transfer of mitochondria from astrocytes to neurons after stroke. *Nature* **535**, 551-555, doi:10.1038/nature18928 (2016).
42 Joshi, A. U. *et al.* Fragmented mitochondria released from microglia trigger A1 astrocytic response and propagate inflammatory neurodegeneration. *Nat Neurosci* **22**, 1635-1648, doi:10.1038/s41593-019-0486-0 (2019).
43 Zhou, J. *et al.* Astrocytic LRP1 enables mitochondria transfer to neurons and mitigates brain ischemic stroke by suppressing ARF1 lactylation. *Cell Metabolism*, doi:https://doi.org/10.1016/j.cmet.2024.05.016 (2024).
44 Liu, D. *et al.* Regulation of blood-brain barrier integrity by Dmp1-expressing astrocytes through mitochondrial transfer. *Science Advances* **10**, eadk2913, doi:doi:10.1126/sciadv.adk2913 (2024).
45 van der Vlist, M. *et al.* Macrophages transfer mitochondria to sensory neurons to resolve inflammatory pain. *Neuron* **110**, 613-626.e619, doi:10.1016/j.neuron.2021.11.020 (2022).
46 Huang, T. *et al.* Efficient intervention for pulmonary fibrosis via mitochondrial transfer promoted by mitochondrial biogenesis. *Nature Communications* **14**, 5781, doi:10.1038/s41467-023-41529-7 (2023).
47 Scozzi, D. *et al.* Mitochondrial damage–associated molecular patterns released by lung transplants are associated with primary graft dysfunction. *American Journal of Transplantation* **19**, 1464-1477, doi:10.1111/ajt.15232 (2019).
48 Huang, T. *et al.* Iron oxide nanoparticles augment the intercellular mitochondrial transfer-mediated therapy. *Sci Adv* **7**, eabj0534, doi:10.1126/sciadv.abj0534 (2021).
49 Liang, W. *et al.* Mitochondria are secreted in extracellular vesicles when lysosomal function is impaired. *Nature Communications* **14**, 5031, doi:10.1038/s41467-023-40680-5 (2023).
50 Brestoff, J. R. *et al.* Intercellular Mitochondria Transfer to Macrophages Regulates White Adipose Tissue Homeostasis and Is Impaired in Obesity. *Cell Metabolism* **33**, 270-282.e278, doi:10.1016/j.cmet.2020.11.008 (2021).
51 Borcherding, N. *et al.* Dietary lipids inhibit mitochondria transfer to macrophages to divert adipocyte-derived mitochondria into blood. *Cell Metabolism* **34**, 1499-1513.e1498 (2022).
52 Gao, J. *et al.* Endoplasmic reticulum mediates mitochondrial transfer within the osteocyte dendritic network. *Science Advances* **5**, eaaw7215, doi:doi:10.1126/sciadv.aaw7215 (2019).
53 Suh, J. *et al.* Mitochondrial fragmentation and donut formation enhance mitochondrial secretion to promote osteogenesis. *Cell Metab* **35**, 345-360.e347, doi:10.1016/j.cmet.2023.01.003 (2023).
54 Ding, P. *et al.* Mitochondria from osteolineage cells regulate myeloid cell-mediated bone resorption. *Nature Communications* **15**, 5094, doi:10.1038/s41467-024-49159-3 (2024).
55 Yang, C. *et al.* Mitochondria transfer mediates stress erythropoiesis by altering the bioenergetic profiles of early erythroblasts through CD47. *J Exp Med* **219**, doi:10.1084/jem.20220685 (2022).

56 Court, A. C. *et al.* Mitochondrial transfer from MSCs to T cells induces Treg differentiation and restricts inflammatory response. *EMBO Rep* **21**, e48052, doi:10.15252/embr.201948052 (2020).

57 Luz-Crawford, P. *et al.* Mesenchymal stem cell repression of Th17 cells is triggered by mitochondrial transfer. *Stem Cell Research & Therapy* **10**, 232, doi:10.1186/s13287-019-1307-9 (2019).

58 Saha, T. *et al.* Intercellular nanotubes mediate mitochondrial trafficking between cancer and immune cells. *Nature Nanotechnology* **17**, 98-106, doi:10.1038/s41565-021-01000-4 (2022).

59 Kidwell, C. U. *et al.* Transferred mitochondria accumulate reactive oxygen species, promoting proliferation. *eLife* **12**, e85494, doi:10.7554/eLife.85494 (2023).

60 Dache, Z. A. A. *et al.* Blood contains circulating cell-free respiratory competent mitochondria. *The FASEB Journal* **34**, 3616-3630, doi:10.1096/fj.201901917RR (2020).

61 Crewe, C. *et al.* Extracellular vesicle-based interorgan transport of mitochondria from energetically stressed adipocytes. *Cell Metabolism* **33**, 1853-1868.e1811, doi:10.1016/j.cmet.2021.08.002 (2021).

62 Dong, L. F. *et al.* Mitochondria on the move: Horizontal mitochondrial transfer in disease and health. *J Cell Biol* **222**, doi:10.1083/jcb.202211044 (2023).

63 Borcherding, N. & Brestoff, J. R. The power and potential of mitochondria transfer. *Nature* **623**, 283-291, doi:10.1038/s41586-023-06537-z (2023).

64 Liu, D. *et al.* Intercellular mitochondrial transfer as a means of tissue revitalization. *Signal Transduct Target Ther* **6**, 65, doi:10.1038/s41392-020-00440-z (2021).

65 Al Amir Dache, Z. & Thierry, A. R. Mitochondria-derived cell-to-cell communication. *Cell Rep* **42**, 112728, doi:10.1016/j.celrep.2023.112728 (2023).

66 McCully, J. D. *et al.* Injection of isolated mitochondria during early reperfusion for cardioprotection. *Am J Physiol Heart Circ Physiol* **296**, H94-h105, doi:10.1152/ajpheart.00567.2008 (2009).

67 Kaza, A. K. *et al.* Myocardial rescue with autologous mitochondrial transplantation in a porcine model of ischemia/reperfusion. *The Journal of Thoracic and Cardiovascular Surgery* **153**, 934-943, doi:https://doi.org/10.1016/j.jtcvs.2016.10.077 (2017).

68 Guariento, A. *et al.* Autologous mitochondrial transplantation for cardiogenic shock in pediatric patients following ischemia-reperfusion injury. *The Journal of Thoracic and Cardiovascular Surgery* **162**, 992-1001, doi:10.1016/j.jtcvs.2020.10.151 (2021).

69 Emani, S. M., Piekarski, B. L., Harrild, D., del Nido, P. J. & McCully, J. D. Autologous mitochondrial transplantation for dysfunction after ischemia-reperfusion injury. *The Journal of Thoracic and Cardiovascular Surgery* **154**, 286-289, doi:10.1016/j.jtcvs.2017.02.018 (2017).

70 Norat, P. *et al.* Intraarterial Transplantation of Mitochondria After Ischemic Stroke Reduces Cerebral Infarction. *Stroke: Vascular and Interventional Neurology* **3**, e000644, doi:doi:10.1161/SVIN.122.000644 (2023).

71 McCully, J. D., del Nido, P. J. & Emani, S. M. Mitochondrial transplantation: the advance to therapeutic application and molecular modulation. *Frontiers in Cardiovascular Medicine* **10**, doi:10.3389/fcvm.2023.1268814 (2023).

72 Liu, Z., Sun, Y., Qi, Z., Cao, L. & Ding, S. Mitochondrial transfer/transplantation: an emerging therapeutic approach for multiple diseases. *Cell & Bioscience* **12**, 66, doi:10.1186/s13578-022-00805-7 (2022).

73 Gollihue, J. L. & Rabchevsky, A. G. Prospects for therapeutic mitochondrial transplantation. *Mitochondrion* **35**, 70-79, doi:10.1016/j.mito.2017.05.007 (2017).

74 Patel, S. P. *et al.* Delivery of mitoceuticals or respiratory competent mitochondria to sites of neurotrauma. *Mitochondrion* **68**, 10-14, doi:https://doi.org/10.1016/j.mito.2022.11.001 (2023).

75 Xu, J. *et al.* Targeted transplantation of engineered mitochondrial compound promotes functional recovery after spinal cord injury by enhancing macrophage phagocytosis. *Bioact Mater* **32**, 427-444, doi:10.1016/j.bioactmat.2023.10.016 (2024).
76 Nakamura, Y., Park, J.-H. & Hayakawa, K. Therapeutic use of extracellular mitochondria in CNS injury and disease. *Experimental Neurology* **324**, 113114, doi:10.1016/j.expneurol.2019.113114 (2020).
77 Hayashida, K. *et al.* Exogenous mitochondrial transplantation improves survival and neurological outcomes after resuscitation from cardiac arrest. *BMC Med* **21**, 56, doi:10.1186/s12916-023-02759-0 (2023).
78 Walker, M., Federico, E., Sancak, Y. & Levitt, M. R. Mitochondrial Transplantation in Ischemic Stroke: Insights from a First-in-Human Brain Trial. *Current Transplantation Reports*, doi:10.1007/s40472-024-00428-6 (2024).
79 Nakai, R. *et al.* Mitochondria transfer-based therapies reduce the morbidity and mortality of Leigh syndrome. *Nature Metabolism*, doi:10.1038/s42255-024-01125-5 (2024).
80 Lin, R. Z. *et al.* Mitochondrial transfer mediates endothelial cell engraftment through mitophagy. *Nature* **629**, 660-668, doi:10.1038/s41586-024-07340-0 (2024).
81 Nakano, T., Nakamura, Y., Park, J. H., Tanaka, M. & Hayakawa, K. Mitochondrial surface coating with artificial lipid membrane improves the transfer efficacy. *Commun Biol* **5**, 745, doi:10.1038/s42003-022-03719-9 (2022).
82 Park, J. H. *et al.* O-GlcNAcylation is essential for therapeutic mitochondrial transplantation. *Commun Med (Lond)* **3**, 169, doi:10.1038/s43856-023-00402-w (2023).
83 Jacoby, E. *et al.* Mitochondrial augmentation of CD34+ cells from healthy donors and patients with mitochondrial DNA disorders confers functional benefit. *npj Regenerative Medicine* **6**, 58, doi:10.1038/s41536-021-00167-7 (2021).
84 Jacoby, E. *et al.* Mitochondrial augmentation of hematopoietic stem cells in children with single large-scale mitochondrial DNA deletion syndromes. *Science Translational Medicine* **14**, eabo3724 (2022).
85 Hinge, A. *et al.* Asymmetrically Segregated Mitochondria Provide Cellular Memory of Hematopoietic Stem Cell Replicative History and Drive HSC Attrition. *Cell Stem Cell* **26**, 420-430.e426, doi:10.1016/j.stem.2020.01.016 (2020).
86 Döhla, J. *et al.* Metabolic determination of cell fate through selective inheritance of mitochondria. *Nat Cell Biol* **24**, 148-154, doi:10.1038/s41556-021-00837-0 (2022).
87 Chou, S. H.-Y. *et al.* Extracellular mitochondria in cerebrospinal fluid and neurological recovery after subarachnoid hemorrhage. *Stroke* **48**, 2231-2237, doi:10.1161/STROKEAHA.117.017758 (2017).
88 Cloer, C. M. *et al.* Mitochondrial transplant after ischemia reperfusion promotes cellular salvage and improves lung function during ex-vivo lung perfusion. *J Heart Lung Transplant* **42**, 575-584, doi:10.1016/j.healun.2023.01.002 (2023).
89 Alway, S. E. *et al.* Mitochondria transplant therapy improves regeneration and restoration of injured skeletal muscle. *J Cachexia Sarcopenia Muscle* **14**, 493-507, doi:10.1002/jcsm.13153 (2023).
90 Zhu, Z. *et al.* Photobiomodulation augments the effects of mitochondrial transplantation in the treatment of spinal cord injury in rats by facilitating mitochondrial transfer to neurons via Connexin 36. *Bioeng Transl Med* **8**, e10473, doi:10.1002/btm2.10473 (2023).
91 Masuzawa, A. *et al.* Transplantation of autologously derived mitochondria protects the heart from ischemia-reperfusion injury. *American Journal of Physiology-Heart and Circulatory Physiology* **304**, H966-H982, doi:10.1152/ajpheart.00883.2012 (2013).
92 McCully, J. D. *et al.* Injection of isolated mitochondria during early reperfusion for cardioprotection. *American Journal of Physiology-Heart and Circulatory Physiology* **296**, H94-H105, doi:10.1152/ajpheart.00567.2008 (2009).

93 Court, A. C. *et al.* Survival advantage of native and engineered T cells is acquired by mitochondrial transfer from mesenchymal stem cells. *Journal of Translational Medicine* **22**, 868, doi:10.1186/s12967-024-05627-4 (2024).

94 Baldwin, J. G. *et al.* Intercellular nanotube-mediated mitochondrial transfer enhances T cell metabolic fitness and antitumor efficacy. *Cell* **187**, 1-17, doi:10.1016/j.cell.2024.08.029 (2024).

95 Tiash, S., Brestoff, J. R. & Crewe, C. A guide to studying mitochondria transfer. *Nat Cell Biol* **25**, 1551-1553, doi:10.1038/s41556-023-01246-1 (2023).

96 Hutto, R. A. *et al.* Cone photoreceptors transfer damaged mitochondria to Müller glia. *Cell Reports* **42**, doi:10.1016/j.celrep.2023.112115 (2023).

97 Pham, A. H., McCaffery, J. M. & Chan, D. C. Mouse lines with photo-activatable mitochondria to study mitochondrial dynamics. *Genesis* **50**, 833-843, doi:10.1002/dvg.22050 (2012).

98 Los, G. V. *et al.* HaloTag: A Novel Protein Labeling Technology for Cell Imaging and Protein Analysis. *ACS Chemical Biology* **3**, 373-382, doi:10.1021/cb800025k (2008).

99 López-Andarias, J. *et al.* Cell-Penetrating Streptavidin: A General Tool for Bifunctional Delivery with Spatiotemporal Control, Mediated by Transport Systems Such as Adaptive Benzopolysulfane Networks. *J Am Chem Soc* **142**, 4784-4792, doi:10.1021/jacs.9b13621 (2020).

100 König, T. *et al.* MIROs and DRP1 drive mitochondrial-derived vesicle biogenesis and promote quality control. *Nature Cell Biology* **23**, 1271-1286, doi:10.1038/s41556-021-00798-4 (2021).

101 König, T. & McBride, H. M. Mitochondrial-derived vesicles in metabolism, disease, and aging. *Cell Metab* **36**, 21-35, doi:10.1016/j.cmet.2023.11.014 (2024).

102 Chen, C., Li, H., Zhang, J. & Cheng, S. C. Exploring the limitations of mitochondrial dye as a genuine horizontal mitochondrial transfer surrogate. *Commun Biol* **7**, 281, doi:10.1038/s42003-024-05964-6 (2024).

103 de Almeida, M. J., Luchsinger, L. L., Corrigan, D. J., Williams, L. J. & Snoeck, H. W. Dye-Independent Methods Reveal Elevated Mitochondrial Mass in Hematopoietic Stem Cells. *Cell Stem Cell* **21**, 725-729.e724, doi:10.1016/j.stem.2017.11.002 (2017).

104 Patananan, A. N. *et al.* Pressure-Driven Mitochondrial Transfer Pipeline Generates Mammalian Cells of Desired Genetic Combinations and Fates. *Cell Reports* **33**, 108562, doi:https://doi.org/10.1016/j.celrep.2020.108562 (2020).

105 Sercel, A. J. *et al.* Generating stable isolated mitochondrial recipient clones in mammalian cells using MitoPunch mitochondrial transfer. *STAR Protoc* **2**, 100850, doi:10.1016/j.xpro.2021.100850 (2021).

106 Caicedo, A. *et al.* MitoCeption as a new tool to assess the effects of mesenchymal stem/stromal cell mitochondria on cancer cell metabolism and function. *Sci Rep* **5**, 9073, doi:10.1038/srep09073 (2015).

107 Nzigou Mombo, B. *et al.* MitoCeption: Transferring Isolated Human MSC Mitochondria to Glioblastoma Stem Cells. *J Vis Exp*, doi:10.3791/55245 (2017).

108 Sercel, A. J. *et al.* Stable transplantation of human mitochondrial DNA by high-throughput, pressurized isolated mitochondrial delivery. *Elife* **10**, doi:10.7554/eLife.63102 (2021).

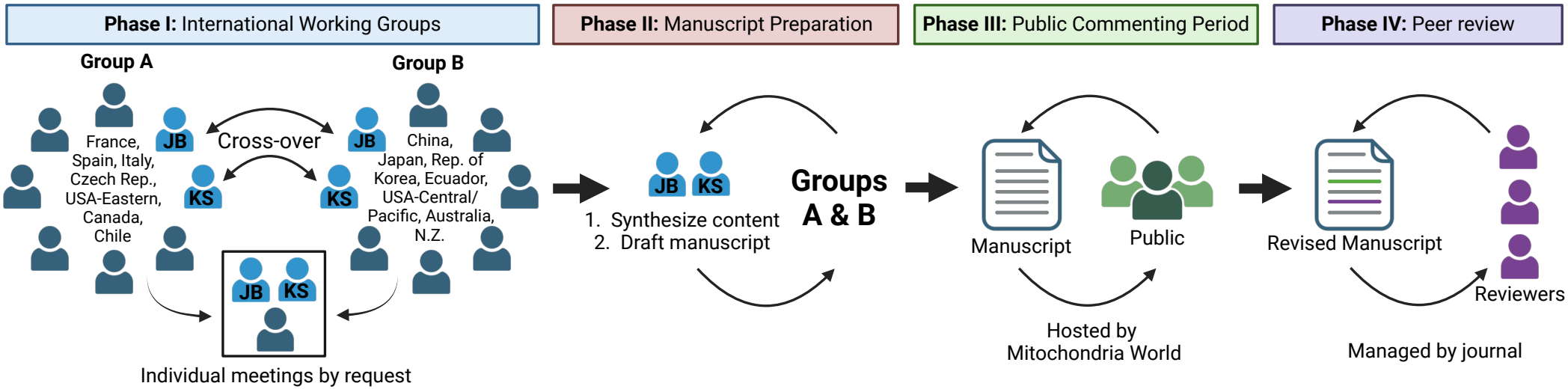
109 Dawson, E. R., Patananan, A. N., Sercel, A. J. & Teitell, M. A. Stable retention of chloramphenicol-resistant mtDNA to rescue metabolically impaired cells. *Sci Rep* **10**, 14328, doi:10.1038/s41598-020-71199-0 (2020).

110 Chang, J. C. *et al.* Treatment of human cells derived from MERRF syndrome by peptide-mediated mitochondrial delivery. *Cytotherapy* **15**, 1580-1596, doi:10.1016/j.jcyt.2013.06.008 (2013).

111 Singh, K. K., Choudhury, A. R. & Tiwari, H. K. Numtogenesis as a mechanism for development of cancer. *Semin Cancer Biol* **47**, 101-109, doi:10.1016/j.semcancer.2017.05.003 (2017).
112 Ljubojevic, N., Henderson, J. M. & Zurzolo, C. The Ways of Actin: Why Tunneling Nanotubes Are Unique Cell Protrusions. *Trends in Cell Biology* **31**, 130-142, doi:https://doi.org/10.1016/j.tcb.2020.11.008 (2021).
113 Ahmad, T. *et al.* Miro1 regulates intercellular mitochondrial transport & enhances mesenchymal stem cell rescue efficacy. *EMBO J* **33**, 994-1010, doi:10.1002/embj.201386030 (2014).
114 Zhang, Y. *et al.* iPSC-MSCs with High Intrinsic MIRO1 and Sensitivity to TNF-α Yield Efficacious Mitochondrial Transfer to Rescue Anthracycline-Induced Cardiomyopathy. *Stem Cell Reports* **7**, 749-763, doi:10.1016/j.stemcr.2016.08.009 (2016).
115 Watson, D. C. *et al.* GAP43-dependent mitochondria transfer from astrocytes enhances glioblastoma tumorigenicity. *Nature Cancer* **4**, 648-664, doi:10.1038/s43018-023-00556-5 (2023).
116 Liao, P. *et al.* Osteocyte mitochondria regulate angiogenesis of transcortical vessels. *Nature Communications* **15**, 2529, doi:10.1038/s41467-024-46095-0 (2024).
117 Norris, R. P. Transfer of mitochondria and endosomes between cells by gap junction internalization. *Traffic* **22**, 174-179, doi:https://doi.org/10.1111/tra.12786 (2021).
118 Joly, E. & Hudrisier, D. What is trogocytosis and what is its purpose? *Nature Immunology* **4**, 815-815, doi:10.1038/ni0903-815 (2003).
119 Brukman, N. G., Uygur, B., Podbilewicz, B. & Chernomordik, L. V. How cells fuse. *Journal of Cell Biology* **218**, 1436-1451, doi:10.1083/jcb.201901017 (2019).
120 Huang, Y. *et al.* TP53/p53 Facilitates Stress-Induced Exosome and Protein Secretion by Adipocytes. *Diabetes* **72**, 1560-1573, doi:10.2337/db22-1027 (2023).
121 Welsh, J. A. *et al.* Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. *J Extracell Vesicles* **13**, e12404, doi:10.1002/jev2.12404 (2024).
122 Stephens, O. R. *et al.* Characterization and origins of cell-free mitochondria in healthy murine and human blood. *Mitochondrion* **54**, 102-112, doi:10.1016/j.mito.2020.08.002 (2020).
123 Caicedo, A. *et al.* The diversity and coexistence of extracellular mitochondria in circulation: A friend or foe of the immune system. *Mitochondrion* **58**, 270-284, doi:https://doi.org/10.1016/j.mito.2021.02.014 (2021).
124 Melki, I. *et al.* Platelets release mitochondrial antigens in systemic lupus erythematosus. *Sci Transl Med* **13**, doi:10.1126/scitranslmed.aav5928 (2021).
125 D'Acunzo, P. *et al.* Mitovesicles secreted into the extracellular space of brains with mitochondrial dysfunction impair synaptic plasticity. *Molecular Neurodegeneration* **19**, 34, doi:10.1186/s13024-024-00721-z (2024).
126 D'Acunzo, P. *et al.* Mitovesicles are a novel population of extracellular vesicles of mitochondrial origin altered in Down syndrome. *Science Advances* **7**, eabe5085, doi:doi:10.1126/sciadv.abe5085 (2021).
127 Trumpff, C. *et al.* Stress and circulating cell-free mitochondrial DNA: A systematic review of human studies, physiological considerations, and technical recommendations. *Mitochondrion* **59**, 225-245, doi:https://doi.org/10.1016/j.mito.2021.04.002 (2021).
128 Knez, J. *et al.* Correlates of Peripheral Blood Mitochondrial DNA Content in a General Population. *American Journal of Epidemiology* **183**, 138-146, doi:10.1093/aje/kwv175 (2015).
129 Roch, B. *et al.* Plasma derived cell-free mitochondrial DNA originates mainly from circulating cell-free mitochondria. *bioRxiv*, 2021.2009.2003.458846, doi:10.1101/2021.09.03.458846 (2021).

130 Chiu, R. W. K. *et al.* Quantitative Analysis of Circulating Mitochondrial DNA in Plasma. *Clinical Chemistry* **49**, 719-726, doi:10.1373/49.5.719 (2003).
131 Caicedo, A. *et al.* Decoding the nature and complexity of extracellular mtDNA: Types and implications for health and disease. *Mitochondrion* **75**, 101848, doi:10.1016/j.mito.2024.101848 (2024).
132 McArthur, K. *et al.* BAK/BAX macropores facilitate mitochondrial herniation and mtDNA efflux during apoptosis. *Science* **359**, doi:10.1126/science.aao6047 (2018).
133 McCully, J. D., Levitsky, S., Del Nido, P. J. & Cowan, D. B. Mitochondrial transplantation for therapeutic use. *Clin Transl Med* **5**, 16, doi:10.1186/s40169-016-0095-4 (2016).
134 Herbert, M. & Turnbull, D. Progress in mitochondrial replacement therapies. *Nat Rev Mol Cell Biol* **19**, 71-72, doi:10.1038/nrm.2018.3 (2018).
135 Huang, P.-J. *et al.* Transferring Xenogenic Mitochondria Provides Neural Protection Against Ischemic Stress in Ischemic Rat Brains. *Cell Transplantation* **25**, 913-927, doi:10.3727/096368915X689785 (2016).
136 Rossi, A. *et al.* Mitochondria Transplantation Mitigates Damage in an In Vitro Model of Renal Tubular Injury and in an Ex Vivo Model of DCD Renal Transplantation. *Ann Surg* **278**, e1313-e1326, doi:10.1097/sla.0000000000006005 (2023).
137 Yu, S. H. *et al.* Human umbilical cord mesenchymal stem cell-derived mitochondria (PN-101) attenuate LPS-induced inflammatory responses by inhibiting NFκB signaling pathway. *BMB Rep* **55**, 136-141, doi:10.5483/BMBRep.2022.55.3.083 (2022).
138 Lechuga-Vieco, A. V., Justo-Méndez, R. & Enríquez, J. A. Not all mitochondrial DNAs are made equal and the nucleus knows it. *IUBMB Life* **73**, 511-529, doi:10.1002/iub.2434 (2021).
139 Lechuga-Vieco, A. V. *et al.* Heteroplasmy of Wild-Type Mitochondrial DNA Variants in Mice Causes Metabolic Heart Disease With Pulmonary Hypertension and Frailty. *Circulation* **145**, 1084-1101, doi:doi:10.1161/CIRCULATIONAHA.121.056286 (2022).
140 Gäbelein, C. G. *et al.* Mitochondria transplantation between living cells. *PLOS Biology* **20**, e3001576, doi:10.1371/journal.pbio.3001576 (2022).
141 Kim, M. J., Hwang, J. W., Yun, C.-K., Lee, Y. & Choi, Y.-S. Delivery of exogenous mitochondria via centrifugation enhances cellular metabolic function. *Sci Rep* **8**, 3330, doi:10.1038/s41598-018-21539-y (2018).
142 Coon, H. G. & Ho, C. Transformation of cultured cells to chloramphenicol resistance by purified mammalian mitochondrial DNA. *Brookhaven Symp Biol*, 166-177 (1977).
143 Fecher, C. *et al.* Cell-type-specific profiling of brain mitochondria reveals functional and molecular diversity. *Nature Neuroscience* **22**, 1731-1742, doi:10.1038/s41593-019-0479-z (2019).
144 Kuznetsov, A. V. & Margreiter, R. Heterogeneity of mitochondria and mitochondrial function within cells as another level of mitochondrial complexity. *Int J Mol Sci* **10**, 1911-1929, doi:10.3390/ijms10041911 (2009).
145 Liu, X., Khouri-Farah, N., Wu, C. H. & Wu, G. Y. Targeted delivery of mitochondria to the liver in rats. *J Gastroenterol Hepatol* **35**, 2241-2247, doi:10.1111/jgh.15091 (2020).
146 Ye, L. *et al.* Centrifugal enhancement of hepatitis C virus infection of human hepatocytes. *J Virol Methods* **148**, 161-165, doi:10.1016/j.jviromet.2007.11.002 (2008).
147 Guo, J., Wang, W., Yu, D. & Wu, Y. Spinoculation triggers dynamic actin and cofilin activity that facilitates HIV-1 infection of transformed and resting CD4 T cells. *J Virol* **85**, 9824-9833, doi:10.1128/jvi.05170-11 (2011).
148 Harada, S. *et al.* Intercellular Mitochondrial Transfer Enhances Metabolic Fitness and Anti-Tumor Effects of CAR T Cells. *Blood* **140**, 2356-2357, doi:10.1182/blood-2022-162535 (2022).
149 Suh, J. & Lee, Y.-S. Mitochondria as secretory organelles and therapeutic cargos. *Experimental & Molecular Medicine* **56**, 66-85, doi:10.1038/s12276-023-01141-7 (2024).

150 Ippolito, L. *et al.* Cancer-associated fibroblasts promote prostate cancer malignancy via metabolic rewiring and mitochondrial transfer. *Oncogene* **38**, 5339-5355, doi:10.1038/s41388-019-0805-7 (2019).
151 Marlein, C. R. *et al.* NADPH oxidase-2 derived superoxide drives mitochondrial transfer from bone marrow stromal cells to leukemic blasts. *Blood* **130**, 1649-1660, doi:10.1182/blood-2017-03-772939 (2017).
152 Moschoi, R. *et al.* Protective mitochondrial transfer from bone marrow stromal cells to acute myeloid leukemic cells during chemotherapy. *Blood* **128**, 253-264, doi:10.1182/blood-2015-07-655860 (2016).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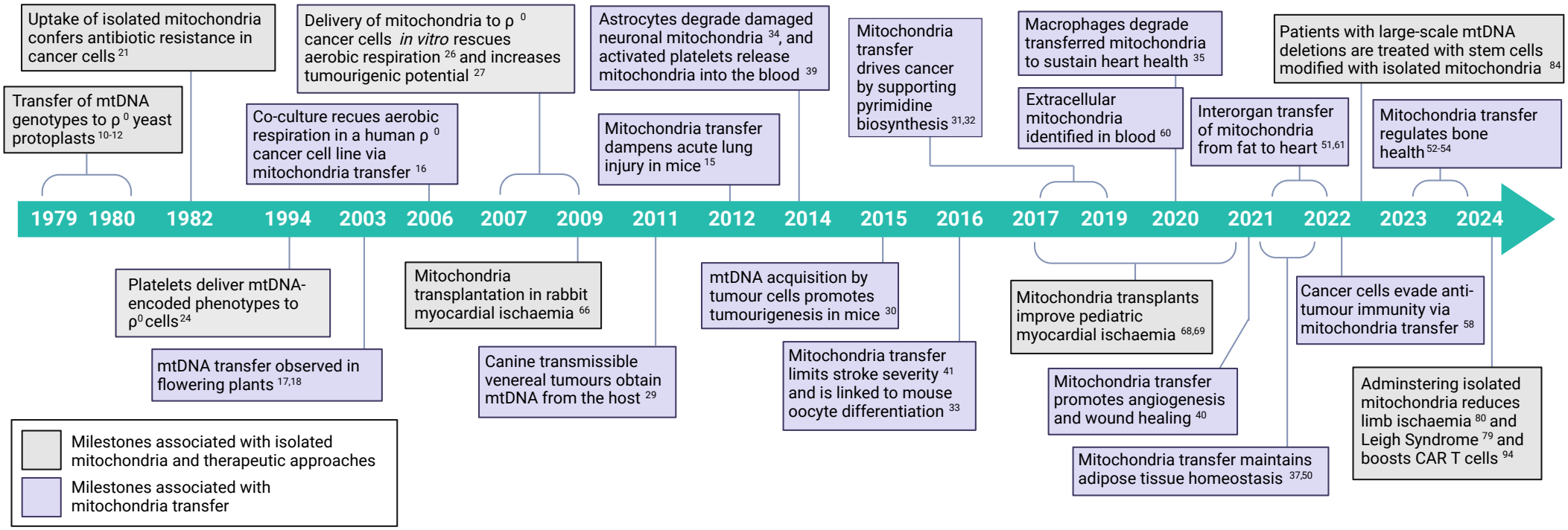
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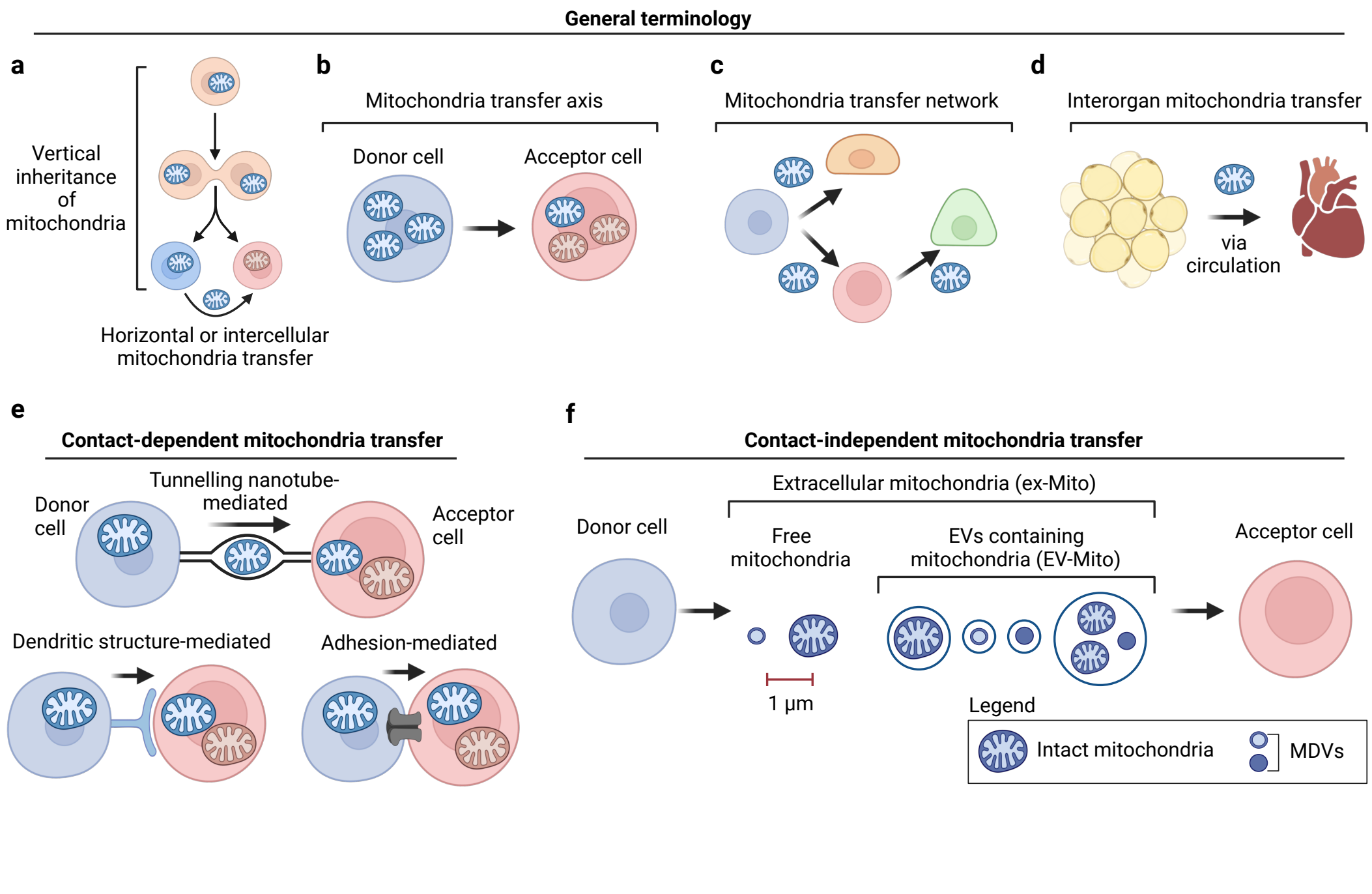
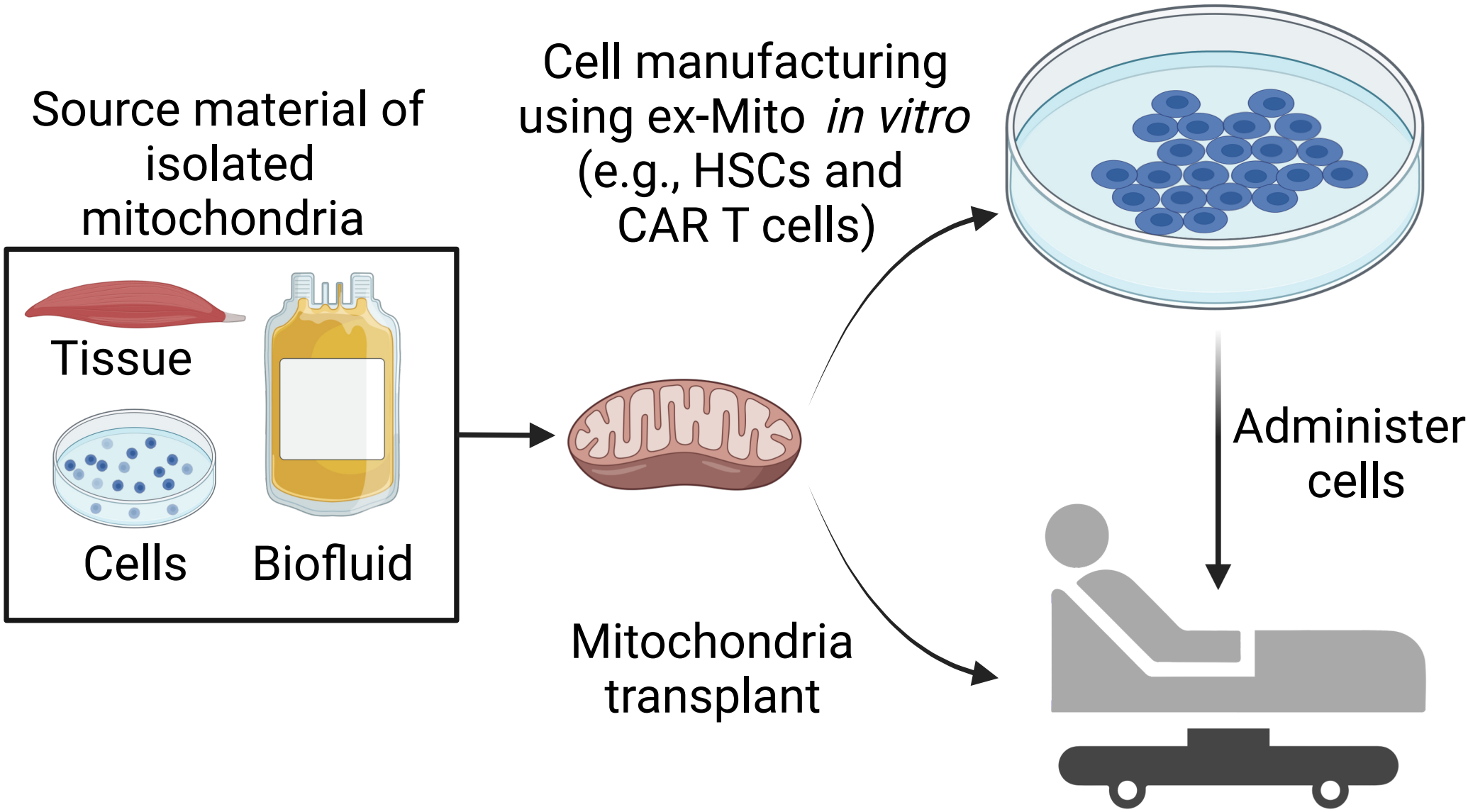
Milestones associated with mitochondria transfer


General terminology
a
Vertical inheritance of mitochondria
Horizontal or intercellular mitochondria transfer
b
Mitochondria transfer axis
Donor cell
Acceptor cell
c
Mitochondria transfer network
d
Interorgan mitochondria transfer
via circulation
e
Contact-dependent mitochondria transfer
Tunnelling nanotube-mediated
Donor cell
Acceptor cell
Dendritic structure-mediated
Adhesion-mediated
f
Contact-independent mitochondria transfer
Extracellular mitochondria (ex-Mito)
Donor cell
Free mitochondria
EVs containing mitochondria (EV-Mito)
Acceptor cell
1 µm
Legend
Intact mitochondria
MDVs


Source material of isolated mitochondria
Tissue
Cells
Biofluid
Cell manufacturing using ex-Mito *in vitro* (e.g., HSCs and CAR T cells)
Mitochondria transplant
Administer cells