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# Autologous mitochondrial transplant for acute cerebral ischemia: Phase 1 trial results and review

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## Abstract

The results of a Phase 1 trial of autologous mitochondrial transplantation for the treatment of acute ischemic stroke during mechanical thrombectomy are presented. Standardized methods were used to isolate viable autologous mitochondria in the acute clinical setting, allowing for timely transplantation within the ischemic window. No significant adverse events were observed with the endovascular approach during reperfusion therapy. Safety outcomes in study participants were comparable to those of matched controls who did not undergo transplantation. This study represents the first use of mitochondrial transplantation in the human brain, highlighting specific logistical challenges related to the acute clinical setting, such as limited tissue samples and constrained time for isolation and transplantation. We also review the opportunities and challenges associated with further clinical translation of mitochondrial transplantation in the context of acute cerebral ischemia and beyond.

## Keywords

Clinical, ischemia, mitochondria, stroke, transplant, trial

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## Introduction

Acute ischemic stroke (AIS) is a major cause of disability and death worldwide.[1] While prompt reperfusion can restore blood flow to ischemic brain tissue and prevent or reduce stroke severity, it may also paradoxically cause secondary ischemic/reperfusion injury (IRI).[2] The interruption of oxygen supply during AIS impairs mitochondrial oxidative phosphorylation, leading to adenosine triphosphate (ATP) depletion and the harmful accumulation of reactive oxygen species (ROS). Subsequent reperfusion exacerbates this condition by increasing ROS production and triggering inflammatory responses, further destabilizing mitochondrial function and contributing to neuronal injury and death.[3]

Extensive research on neuroprotective agents has sought to mitigate the detrimental effects of IRI including targeting NMDA receptors, free radical scavengers, and immune system modulators.[4–6] However, these

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strategies have failed to demonstrate consistent clinical efficacy due to a narrow therapeutic window, difficulties in drug delivery, and the complexity of the ischemic cascade, which involves multiple overlapping and interdependent pathways.[7]

Current AIS reperfusion strategies[8] including thrombolytic therapy and mechanical thrombectomy focus primarily on achieving arterial recanalization to restore blood flow, which does not mitigate cellular and molecular injury caused by the interruption of oxygen supply and subsequent IRI. Recently, mitochondrial transplantation has emerged as a potential adjunct to reperfusion to address this critical need. Successful trials in cardiac ischemia[9,10] provided the foundation for this approach, enabled by the FDA's Same Surgical Procedure Exception (21 CFR 1271.15(b)).

Several developments have supported the clinical translation of mitochondrial transplantation, as previously reviewed in detail.[11] Mitochondria can transfer from astrocytes to neurons following ischemic stroke,[12] and extracellular mitochondria can survive and function within the vascular compartment[13,14] without triggering significant immune or damage-associated molecular pattern responses.[15] Our preclinical studies[16,17] using a murine model of middle cerebral artery occlusion have demonstrated that intra-arterial delivery of mitochondria allows for their widespread distribution throughout the ischemic cerebral hemisphere, leading to integration into neural and glial cells. This method significantly elevated ATP concentrations in ischemic tissue, reduced infarct volume, and improved cell viability.

Together, these scientific and regulatory advancements enabled the current trial. Here, we present the safety outcomes and discuss the translational challenges that must be addressed to advance mitochondrial transplantation toward broader clinical application.

## Methods

### Trial design

This Phase 1, open-label, single-arm trial (NCT04998357[18]) evaluated the safety and feasibility of intra-arterial autologous mitochondrial transplantation in patients with AIS undergoing mechanical thrombectomy. Results are reported in accordance with the CONSORT Extension for Pilot and Feasibility Trials.[19]

### Participant selection and matching

The study enrolled adult patients with AIS from anterior circulation large-vessel occlusion who were eligible for endovascular thrombectomy and had sufficient time for informed consent. Exclusion criteria included contraindication to magnetic resonance imaging (MRI), known mitochondrial disease, and hemodynamic instability. A retrospective matching process, conducted by a blinded database administrator, identified appropriate controls from a comprehensive stroke center database between 2017–2024. Matching criteria included age (±6 years), sex, National Institutes of Health Stroke Scale (NIHSS) score (±4 points), Alberta Stroke Program Early CT (ASPECTS[20]) score (±2 points) and stroke laterality. Standardized mean differences (SMD) were calculated for each matched variable.

### Interventional procedures

All steps in the mitochondrial isolation and transplantation process adhered to good manufacturing practice (cGMP) standards,[21–23] ensuring a sterile environment and quality control. This included the preparation of cGMP-compliant reagents, documentation of lot numbers, and maintenance of sterility logs. Due to the acute clinical setting and the limited timeframe for intervention, these protocols were optimized to provide high-quality, viable mitochondrial isolates within the constraints of rapid preparation and transplantation. All solutions and tissue samples were kept on ice to maintain mitochondrial viability during the isolation process. Trained personnel conducted sterility testing, purity assessment, and viability assays, with detailed documentation maintained throughout to ensure traceability.

During femoral arterial access for mechanical thrombectomy, blunt dissection was performed at the vascular access site to collect approximately 0.1 grams of skeletal muscle tissue.[22,24] The sample was processed in the procedure suite within a sterile workstation. Mitochondria were then isolated per validated protocol.[9,22,25–27] Briefly, a 1 M K-HEPES stock solution (pH 7.2, adjusted with KOH), a 0.5 M K-EGTA stock solution (pH 8.0, adjusted with KOH), a 1 M $KH_2PO_4$ stock solution, and a 1 M $MgCl_2$ stock solution were made. The Homogenizing Buffer contained 300 mM sucrose, 10 mM K-HEPES (pH 7.2), and 1 mM K-EGTA, while the Respiration Buffer consisted of 250 mM sucrose, 2 mM $KH_2PO_4$, 10 mM $MgCl_2$, 20 mM K-HEPES (pH 7.2), and 0.5 mM K-EGTA (pH 8.0). A 10× PBS stock solution was created by dissolving 80 g of NaCl, 2 g of KCl, 14.4 g of $Na_2HPO_4$, and 2.4 g of $KH_2PO_4$ in 1 L of double-distilled $H_2O$ (pH 7.4), then diluted to 1× PBS as needed. Subtilisin A and BSA were prepared in 4 mg and 20 mg aliquots, respectively, stored at −20°C, and dissolved in 1 ml of Homogenizing Buffer prior to use. All buffers were sterilized by filtration through a 22-μm

filter[24] and stored at 4°C until use. Fresh tissue samples were collected and stored in 1x PBS on ice, then homogenized in 5 ml of ice-cold Homogenizing Buffer (300 mM sucrose, 10 mM K-HEPES at pH 7.2, and 1 mM K-EGTA) using a gentleMACS$^{TM}$ Dissociator (Miltenyi Biotec, Bergisch Gladbach, Germany) for 60 seconds. Next, 250 µl of the dissolved Subtilisin A solution was added to the homogenate, which was mixed by inversion and incubated on ice for 10 minutes to aid in tissue dissociation. The homogenate was filtered sequentially through 40 µm and 10 µm mesh filters pre-wet with Homogenizing Buffer to remove debris and ensure purity. The filtrate was then centrifuged at 9,000 × g for 10 minutes at 4°C, and the supernatant was discarded. A portion of the mitochondrial pellet was reserved for quality assurance testing, including mitochondrial number estimation using hemocytometry, with counting performed under a sterile-draped light microscope at 400× magnification within the sterile workstation. The remainder of the mitochondrial pellet was resuspended in 1 ml of Respiration Buffer and kept on ice until transplantation (within 60 minutes) to preserve bioenergetic function.[28,29] Specimens were not frozen to avoid potential damage during the freeze-thaw cycle.[30,31]

Mechanical thrombectomy was performed at the site of large-vessel occlusion per standard of care[8,32] using a microcatheter inserted through an 8-French guide catheter positioned in the internal carotid artery ipsilateral to the occlusion. During thrombectomy, the delivery microcatheter was advanced distal to the occlusion in the M2 segment of the middle cerebral artery (MCA), which supplies approximately 70 g of brain tissue at risk for ischemia.[33–45] Based on the optimal dose of $2 \times 10^5$ mitochondria per gram of tissue wet weight established in cardiac studies,[24,25,46,47] the calculated effective dose would be equivalent to $1.4 \times 10^7$ mitochondria per participant. For this first-in-human brain study, safety considerations led to an IRB-imposed maximum dose of $7 \times 10^6$ mitochondria, half of the calculated effective dose based on prior studies. The IRB also stipulated that the total suspension volume must not exceed 8 mL. The volume of the transplant infused was therefore kept consistent for all participants, comprising approximately 0.1 mg of mitochondrial protein, suspended in equal parts of Respiration Buffer and non-ionic contrast solution.

Prior to transplant, a microinjection of 3 mL of non-ionic, iso-osmotic contrast solution[48–53] (iodixanol) was infused into the MCA branch distal to the occlusion over 2 seconds, followed by a 3 mL saline flush. Mitochondria were then injected via the microcatheter, followed by a 3 mL saline flush. Continuous real-time angiographic imaging of the cerebral vasculature was performed throughout the infusion process to ensure vessel patency. A follow-up angiogram through the microcatheter was also performed to evaluate delayed adverse effects.

Following transplantation, the thrombectomy procedure was completed according to the standard of care, and all catheters were removed. The vascular access site was closed percutaneously using a vascular closure device and patients were given 2 mg IV cefazolin. All patients underwent immediate post-procedure head CT and admission to the neurological intensive care unit. Routine laboratory evaluations were conducted daily for the first 48 hours and up to one week, as part of both standard clinical monitoring and systemic adverse event monitoring.

### *Safety endpoints and monitoring*

Four safety endpoints were monitored: procedure-related adverse events (AEs), vascular AEs, systemic AEs, and access site AEs. Procedure-related AEs, such as vessel occlusion, vasospasm, arterial dissection, or embolization into a new territory, were detected via real-time angiographic monitoring during thrombectomy. Vascular AEs, including vessel re-occlusion, hemorrhagic transformation, or infarct growth, were identified through cross-sectional imaging (computed tomography (CT) or MRI) performed within three hours post-procedure. Systemic AEs were tracked through routine laboratory testing of peripheral blood samples. Physical examinations were conducted up to six hours post-procedure to monitor for bleeding and vascular access site AEs, including hematoma and pseudoaneurysm formation.

Laboratory parameters—platelet count, white blood cell (WBC) count, calcium, creatinine, and glucose—were measured pre-procedure and at 24 and 48 hours, consistent with standard of care for ischemic stroke patients. When assessed at baseline, these values not only assist in monitoring but also offer procedural safety and predictive value for stroke patients who undergo thrombectomy.[54] Platelet count was measured to assess the risk of hemorrhagic complications, with thrombocytopenia increasing the likelihood of hemorrhagic transformation and poor procedural outcomes.[55–58] Elevated WBC count (leukocytosis) and hyperglycemia both reflect systemic inflammation and metabolic stress, which are associated with worsened 3-month functional outcomes, larger infarct sizes, and increased risk of reperfusion injury.[59–62] Total serum calcium, measured rather than ionized or albumin-corrected calcium due to its greater prognostic relevance in ischemic stroke,[63] was tracked for its role in neuronal signaling and vascular function, where low levels destabilize vascular integrity and can exacerbate ischemic injury.[64–66] Creatinine was measured to detect

kidney dysfunction, particularly in relation to contrast-induced nephropathy.[67–70]

Additionally, in patients with certain comorbidities such as type 2 diabetes, combinations of these laboratory values, such as elevated WBC count and renal dysfunction, have been associated with worse outcomes and increased mortality after thrombectomy.[71,72] Although markers such as C-reactive protein (CRP) and lactate dehydrogenase (LDH) are known to reflect systemic inflammation and tissue damage post-thrombectomy, their levels can vary significantly in the context of acute[73–75] and post-reperfusion[76,77] injury. Monitoring these markers was not included due to their limited specificity for indicating unique adverse events in this setting.

### *Mitochondrial quality assessment*

Specimens were collected from AIS subjects as well as from neurosurgery patients who underwent cerebrovascular procedures but did not undergo transplant. The protein concentration of each mitochondrial aliquot was determined using the Bradford Assay[78] (Bio-Rad Laboratories, Hercules, CA, USA; 5000205) in a sterile setting.[79,80] One microliter (μL) of mitochondrial aliquot was mixed with 1 mL of Bradford reagent. The absorbance of the Bradford reagent solution was measured using a VWR V-1200 Spectrophotometer (VWR, Radnor, PA, USA). If the absorbance fell outside the linear range of 0.1–0.9 optical density (OD) units, the sample was diluted or more sample was added until the absorbance fell within the linear range. Protein concentration was calculated based on the known absorbance of 5 μg of bovine serum albumin (0.3 OD).[78]

Mitochondrial purity was assessed via transmission electron microscopy (TEM).[10,81–84] For ultrastructural analysis, specimens were fixed in glutaraldehyde, post-fixed in osmium tetroxide, and embedded in resin. Ultrathin sections (~70 nm) were mounted on copper grids and stained with uranyl acetate and lead citrate. Images were acquired at 11,000×, 18,500× and 68,000× magnification using a FEI Tecnai G2 Spirit BioTWIN electron microscope operating at 80 kV. Given the time-sensitive nature of the clinical workflow, rapid assessment methods were employed, with TEM samples preserved for future analysis.

Mitochondrial viability and function were evaluated using the Resazurin Cell Viability Assay Kit (alamarBlue™) (Biotium, Fremont, CA, USA; 30025-1), following the manufacturer's instructions.[85–87] Fluorescence was measured with a Synergy H1 plate reader (BioTek, Winooski, VT, USA), pre-incubated to 37°C. Resazurin buffer solution was prepared by mixing 1 mL resazurin reagent with 9 mL buffer, 100 μl of resazurin buffer solution was added to each well of a 96-well plate, with 4 μg mitochondria per well. ADP (1 mM) and glutamate/malate (5 mM each) were added to appropriate wells, and fluorescence was measured at excitation/emission 550/585 nm every minute, over 20 minutes.

Membrane potential was assessed using tetramethyl rhodamine methyl ester (TMRM, Thermo Fisher Scientific, Waltham, MA, USA; T668). Mitochondria were incubated with 500 nM TMRM and 5 mM glutamate/malate in buffer, and fluorescence was measured at 540-nm excitation and 590-nm emission using a BioTek Synergy H1 microplate reader at room temperature. After carbonyl cyanide m-chlorophenyl hydrazone (CCCP, Sigma-Aldrich, St. Louis, MO, USA; C2759) addition (1 μM final concentration) and 3-minute incubation, a second reading was taken.

Basal ATP concentration was measured using the ATPlite™ luminescence assay kit (Perkin Elmer, Waltham, MA, USA; 6016941), following the manufacturer's instructions. ATP concentrations were assessed using 5 replicates of 20 μg or 10 μg of mitochondrial protein.

### *Statistical analysis*

Descriptive statistics, including mean, median, and interquartile range (IQR), were used to summarize the baseline characteristics of subjects and controls for key variables such as age, NIHSS, ASPECTS, and stroke subtype. Matching was performed using pre-specified covariates, with SMD calculated to assess balance between groups. Paired t-tests and Wilcoxon signed-rank tests were employed to compare laboratory values at baseline, 24 hours, and 48 hours post-thrombectomy between subjects and controls, while t-tests and Wilcoxon rank-sum tests were used to analyze the relationship between procedure time and safety outcomes. Fisher's exact test was applied to assess the association between thrombolytic use and hemorrhage in controls.

Bootstrapping[88,89] (1000 iterations) was performed to estimate variability and generate confidence intervals, with the dataset resampled with replacement to create pseudo-replicates. Mean values from each resample were calculated, and final results were presented as bootstrapped means with 95% confidence intervals. P-values less than 0.05 were considered statistically significant. Analyses were conducted using SAS version 9.4 (SAS Institute Inc.), R version 4.0.5 (R Foundation for Statistical Computing), and Python version 3.8 (Python Software Foundation).

### Ethical considerations

The study was approved by the Institutional Review Board at the University of Washington (STUDY00006638) and adhered to the principles outlined in the Declaration of Helsinki, as well as relevant federal and institutional guidelines for the protection of human subjects. Written informed consent was obtained from all participants or their legally authorized representatives prior to enrollment in the trial. A waiver of consent was granted for the retrospective review of matched subject data.

## Results

### Participant characteristics

Four AIS subjects with MCA occlusion underwent autologous mitochondrial transplantation during thrombectomy. The ages of the subjects ranged from 43 to 80 years, consisting of three females and one male. Initial NIHSS scores varied from 9 to 21, and ASPECTS scores ranged from 6 to 7. Stroke subtypes were categorized as either large artery atherosclerosis (LAA) or cardioembolic (CE) according to prespecified criteria.[90]

The matching process resulted in an effective balance between the subjects and controls across most covariates. No significant differences were observed between subjects and controls for any of the matched variables, with p-values of 0.951 for age, 1.000 for sex, 0.887 for NIHSS, 0.625 for ASPECTS, and 0.505 for stroke subtype (Table 1). The SMD were minimal for age (SMD = −0.035), sex (SMD = 0.000), and NIHSS score (SMD = −0.080), indicating strong comparability between the groups for these variables. The ASPECTS score had a slightly higher SMD (SMD = 0.339), while stroke subtype showed the largest difference (SMD = −0.677).

### Safety outcomes

No periprocedural events, post-procedure events, systemic events, or access site complications were reported. In the matched control group, two cases of reperfusion hemorrhage were observed post-procedure (001 C and 003 A). No other adverse events or complications were reported in the control group. Successful reperfusion (defined by TICI > 2B)[91] was achieved in all subjects and controls. No significant difference in procedure times was observed between subjects and controls (p = 0.83).

### Systemic monitoring

Laboratory values for platelets, WBC, calcium, creatinine, and glucose collected at presentation, 24 hours, and 48 hours post-intervention, along with mean values and corresponding p-values for comparisons between groups at each time point for both mitochondrial transplant subjects and their matched controls (Table 2).

### Mitochondrial characterization

Quality assurance metrics showed that the mitochondrial isolation process produced active mitochondria, indicating their viability at the time of transplantation in AIS subjects. TEM revealed well-preserved mitochondrial morphology with intact double membranes

**Table 1.** Matching criteria of subjects and controls.

| ID | Age (Years) | Sex (M/F) | Intracranial occlusion (site) | NIHSS | ASPECTS | Stroke subtype |
|---|---|---|---|---|---|---|
| Subject 001 | 43 | F | R MCA | 16 | 7 | LAA |
| *Control 001 A* | 48 | F | R MCA | 15 | 7 | CE |
| *Control 001B* | 46 | F | R MCA | 12 | 5 | CE |
| *Control 001 C* | 46 | F | R MCA | 13 | 7 | CE |
| Subject 002 | 72 | F | R MCA | 10 | 7 | LAA |
| *Control 002 A* | 71 | F | R MCA | 14 | 6 | CE |
| *Control 002B* | 72 | F | R MCA | 7 | 8 | CE |
| *Control 002 C* | 72 | F | R MCA | 12 | 8 | CE |
| Subject 003 | 80 | M | L MCA | 21 | 6 | CE |
| *Control 003 A* | 80 | M | L MCA | 20 | 6 | CE |
| *Control 003B* | 82 | M | L MCA | 23 | 7 | LAA |
| *Control 003 C* | 78 | M | L MCA | 22 | 6 | LAA |
| Subject 004 | 48 | F | R MCA | 9 | 6 | CE |
| *Control 004 A* | 48 | F | R MCA | 12 | 5 | CE |
| *Control 004B* | 47 | F | R MCA | 11 | 4 | CE |
| *Control 004 C* | 46 | F | R MCA | 12 | 5 | CE |

ASPECTS: Alberta Stroke Program Early CT Score; CE: cardioembolic; F: female; L: left; LAA: large artery atherosclerosis; M: male; MCA: middle cerebral artery; NIHSS: National Institutes of Health Stroke Scale; R: right.

**Table 2.** Laboratory values of subjects and matched controls.

| Time point | Parameter | Reference range | Subjects (Mean ± SD) | Controls (Mean ± SD) | P-value |
|---|---|---|---|---|---|
| Presentation | Platelet Count | 150–400 × $10^3$/μL | 248.25 ± 42.72 | 259.75 ± 63.31 | 0.76 |
| | WBC | 4.3–10.0 × $10^3$/μL | 11.62 ± 5.02 | 10.09 ± 1.98 | 0.61 |
| | Calcium | 8.9–10.2 mg/dL | 8.35 ± 0.24 | 9.02 ± 0.30 | 0.04 |
| | Creatinine | 0.38–1.02 mg/dL | 0.83 ± 0.17 | 0.79 ± 0.20 | 0.72 |
| | Glucose | 62–125 mg/dL | 126.25 ± 20.69 | 122.00 ± 9.87 | 0.79 |
| 24 Hours | Platelet Count | 150–400 × $10^3$/μL | 270.25 ± 63.23 | 233.75 ± 42.77 | 0.29 |
| | WBC | 4.3–10.0 × $10^3$/μL | 11.17 ± 3.06 | 9.48 ± 1.10 | 0.38 |
| | Calcium | 8.9–10.2 mg/dL | 8.10 ± 0.22 | 8.73 ± 0.21 | 0.04 |
| | Creatinine | 0.38–1.02 mg/dL | 0.82 ± 0.20 | 0.75 ± 0.14 | 0.46 |
| | Glucose | 62–125 mg/dL | 124.00 ± 24.83 | 105.42 ± 5.42 | 0.25 |
| 48 Hours | Platelet count | 150–400 × $10^3$/μL | 239.50 ± 87.85 | 229.58 ± 49.64 | 0.85 |
| | WBC | 4.3–10.0 × $10^3$/μL | 8.45 ± 2.46 | 9.03 ± 1.55 | 0.67 |
| | Calcium | 8.9–10.2 mg/dL | 8.12 ± 0.50 | 8.75 ± 0.23 | 0.16 |
| | Creatinine | 0.38–1.02 mg/dL | 0.79 ± 0.25 | 0.73 ± 0.15 | 0.66 |
| | Glucose | 62–125 mg/dL | 157.25 ± 85.78 | 107.50 ± 14.41 | 0.31 |

ATP: adenosine triphosphate; CI: confidence interval; IQR: interquartile range; pmol/mg: picomoles per milligram; μg: micrograms; μg/μL: micrograms per microliter; SD: standard deviation; WBC: white blood cell.

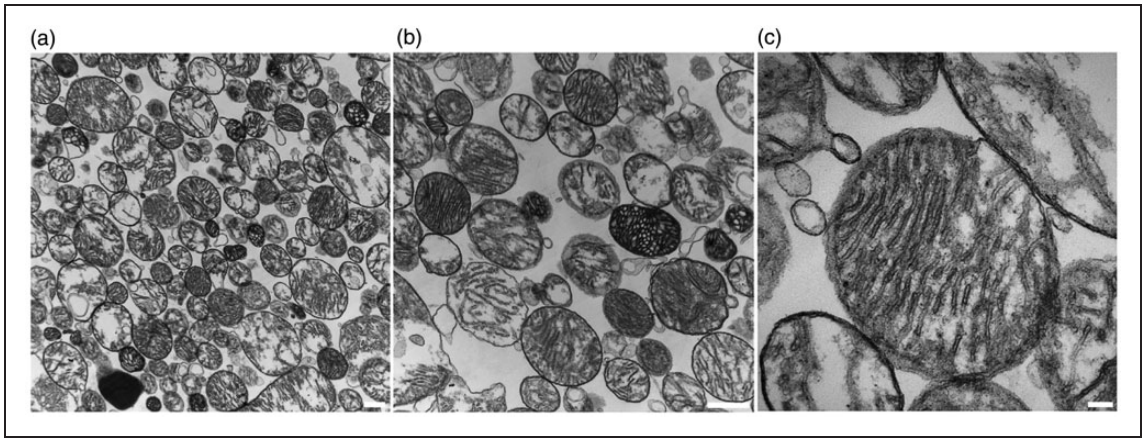


**Figure 1.** Mitochondrial isolate from patient skeletal muscle tissue shows well-preserved mitochondrial morphology with minimal contamination. Representative mitochondrial sample isolated from skeletal muscle tissue in patient undergoing mitochondrial transplantation during thrombectomy. Electron microscope images of mitochondria in patient sample at (a) 11,000× magnification (scale bar, 500 nm), (b) 18,500× magnification (scale bar, 500 nm), and (c) 68,000× magnification (scale bar, 100 nm).

and cristae structure, indicating minimal contamination from non-mitochondrial components (Figure 1). Mitochondrial function testing demonstrated high metabolic activity, as measured by resazurin reduction assays, viability and function under experimental conditions (Figure 2(a) and (b)). Mitochondrial membrane potential, assessed using TMRM, was robust and stable, indicating functional integrity and bioenergetic capacity (Figure 2(c)).

Descriptive statistics across key metrics, including protein concentration, ATP concentration, and mitochondrial counts, demonstrated consistency in the results (Table 3). The bootstrapped confidence intervals confirm minimal variability, with no significant deviations observed in mean values between samples.

## Discussion

No safety endpoints were met in this Phase 1 trial of autologous mitochondrial transplantation for AIS, and no adverse effects on cerebral artery patency were observed. Serial imaging and continuous monitoring in study participants revealed no vasospasm, thromboembolism, reperfusion injury, or hemorrhagic transformation. Post-procedure imaging and laboratory

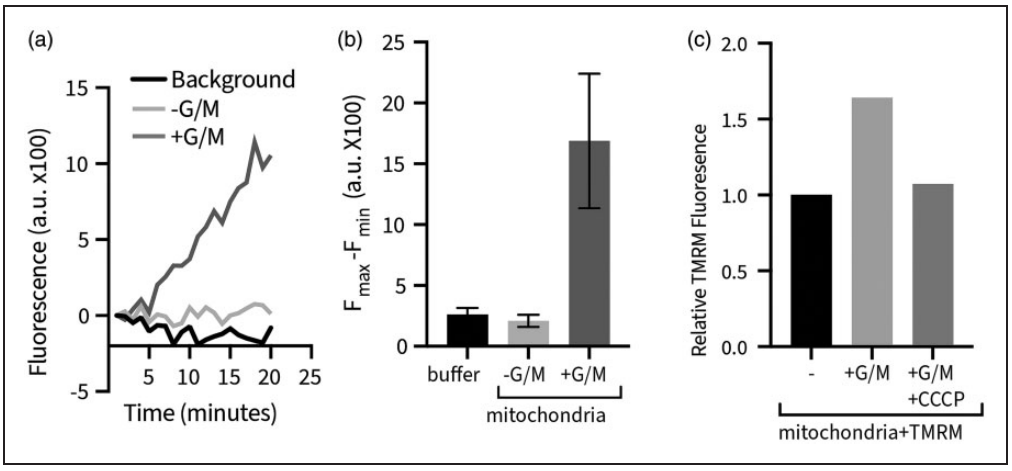


**Figure 2.** Functional testing of mitochondrial isolates demonstrates high metabolic activity. (a) Resazurin assays were performed on mitochondrial isolate from patient muscle tissue, incubating 4 μg mitochondria with or without 5 mM glutamate/malate each. Data from representative wells are shown. Background indicates wells with resazurin reagent only. (b) The difference between maximum and minimum resazurin fluorescence in each well is shown, data from five technical replicates were averaged. Error bars indicate standard deviation. (c) TMRM was incubated with mitochondrial isolate in respiration buffer alone or with 5 mM glutamate/malate each. CCCP was added after the initial read and incubated for 3 additional minutes.

**Table 3.** Summary of mitochondrial characterization.

| Metric (units) | QA sample (Mean ± SD) | Calculated transplant (Mean ± SD) |
|---|---|---|
| Total protein (μg) | 333.75 ± 41.36 | 1,335 ± 165.44 |
| ATP concentration (pmol/μg[a]) | 2.94 ± 1.13 | 11.77 ± 4.52 |
| Estimated mitochondria (number) | 333,750 ± 41,360 | 1,335,000 ± 165,440 |

[a]ATP concentration is reported as *pmol/μg of protein*, representing measurements from QA aliquots taken prior to any further processing or administration. This baseline level aligns with expected viability benchmarks for isolated mitochondria and is comparable to the reported values of ATP amount per microgram of protein.[92]

monitoring also showed no adverse events. The findings further demonstrate the reliability and consistency of the standardized[21,22,24] sampling methods, with reproducibility across subjects supporting the robustness of the mitochondrial isolation process.

While these preliminary findings provide promising safety insights, several key translational challenges must be addressed for broader clinical application. These challenges include localizing and verifying the functionality of transplanted mitochondria within the brain; determining the appropriate dose and frequency for transplantation; optimizing the timing of the intervention to prevent IRI; and addressing potential systemic interactions and delivery methods.

In addition to localization, ensuring engraftment and functionality of transplanted mitochondria is a key challenge in determining the neuroprotective benefits of mitochondrial transplantation and differentiating its effects from those of reperfusion alone. Unlike cardiac applications, where real-time functional metrics can be measured by echocardiography[9], brain imaging techniques such as perfusion MRI or spectroscopy are delayed, complicating the differentiation between the effects of the transplant and the benefits of recanalization after thrombectomy.

Determining the appropriate dose, timing, and frequency for transplantation is complex due to the lack of in vivo data on human cortical mitochondrial function and energetic states in both healthy and diseased conditions.[33,34,93,94] The risks of intravascular volume overload, thromboembolic events, and cerebral edema complicate dosing strategies in the ischemic brain during the procedure, while administering multiple doses over time may further increase procedural risk or impact the timing and effectiveness of revascularization.[46,47] The optimal window for mitochondrial transplantation is likely during the acute phase of ischemia, coinciding with reperfusion. However, there may be benefits to delayed transplantation, particularly in the subacute phase, as the blood-brain barrier remains permeable for up to several weeks.[95–97] This could be particularly relevant for patients with large volumes of ischemic brain tissue or core infarct, where the risks associated with dosing strategies are heightened, but the potential benefits related to mitigating reperfusion injury may also be significant. Balancing the risks and

benefits of acute versus delayed interventions, along with potential repeated mitochondrial dosing, is an area of ongoing research.

The potential for allogeneic mitochondrial transplantation[98–101] is compelling, as it could broaden availability and shorten the time required for isolation and preparation. Products like mitochondria organelle complex-Q (MRC-Q$^{TM}$, LUCA Science, Tokyo, Japan), which can be frozen for distribution and storage, offer a practical solution. While preclinical studies[101–103] have shown success, their application in human trials remains unexplored.

Another significant challenge lies in the route of mitochondrial delivery. Intra-arterial delivery offers targeted therapy to specific arterial territories but carries invasive risks, while systemic dispersion via intravenous delivery may require larger doses, increasing the potential for off-target effects. Optimizing or combining these delivery methods will be important in the design of future trials.

## Conclusion

This Phase 1 study demonstrated that autologous mitochondrial transplantation during mechanical thrombectomy for AIS is safe. The standardized methods for mitochondrial isolation and quality testing confirmed the viability and functionality of the isolated mitochondria, consistent with previous findings. This study represents the first use of mitochondrial transplantation in the human brain, highlighting specific logistical challenges related to the acute clinical setting, such as limited tissue samples and constrained time for isolation and transplantation. Ongoing research and rigorous scientific exploration will refine and support the clinical translation of mitochondrial transplantation in the context of acute cerebral ischemia and beyond.

### Data availability

The data supporting this study are available from the corresponding author upon reasonable request.

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### Declaration of conflicting interests

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### Authors' contributions

MW conceived the study, and the trial design was developed by MW and MRL. Data acquisition, analysis, and interpretation were performed by MW, MRL, YS, FJM, EF, KP, AW, SHSL and JWS. MW drafted the manuscript, with critical revisions by MRL and YS. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for the accuracy and integrity of the work.

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