

STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Efficacy of Oral Metformin in Acute Ischemic Stroke (AIS): A Multicentric, Double-Blind, Placebo-Controlled Randomized Controlled Trial

Official Title	Efficacy of Oral Metformin in Acute Ischemic Stroke (AIS): A Multicentric, Double-Blind, Placebo-Controlled Randomized Controlled Trial
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Funding Agency	Indian Council of Medical Research (ICMR) – ICMR ANVESHAN Extramural Grants 2026
Principal Investigator	Dr Deepti Vibha, Department of Neurology, AIIMS, New Delhi
Participating Sites	AIIMS New Delhi; PGIMER Chandigarh; JIPMER Puducherry; IMS-BHU Varanasi

Document History

Version	Date	Description of changes	Author
1.0	12 June 2026	Initial version derived of ICMR ANVESHAN detailed proposal	PI and Co-PIs
2.0	21 June 2026	Edits in the methods section, secondary end points in by the other investigators	PI and Co-PIs
3.0	02 July 2026	Edits after presentation in the institute research section. Addition of incremental doses in intervention group	PI and Co-PIs, institute experts
4.0	31 August 2026	Edits after ethics committee comments. Guidelines to hold/stop intervention defined.	PI and Co-PIs
5.0	12 September 2026	Edits in methodology and SAP by the funding body	PI and Co-PIs

Protocol Approval and Signature Page

By signing below, the investigators confirm that they have read this protocol and agree to conduct the trial in compliance with this protocol, the ICH E6 Good Clinical Practice guideline, the New Drugs and Clinical Trials Rules 2019, the ICMR National Ethical Guidelines for Biomedical and Health Research Involving Human Participants (2017), the Declaration of Helsinki, and all applicable regulatory requirements.

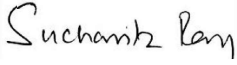
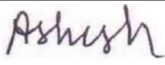
Role	Name and Institution	Signature	Date
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Trial Statistician	Dr Ashish Dutt Upadhyay, Scientist, Clinical Research Unit, AIIMS New Delhi		1st Oct, 2026

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Part A. ClinicalTrials.gov Registration Data Elements

The table below maps trial information to the data elements required in the ClinicalTrials.gov PRS so that the registration record can be completed directly from this document. The same content can be used for the Clinical Trials Registry – India (CTRI) record.

A1. Study Identification

Unique Protocol ID	Pending from the Research Section AIIMS New Delhi
Brief Title	Metformin for Improving Functional Outcome After Acute Ischemic Stroke
Official Title	Efficacy of Oral Metformin in Acute Ischemic Stroke (AIS): A Multicentric, Double-Blind, Placebo-Controlled Randomized Controlled Trial
Acronym	None
Secondary IDs	ICMR grant number IIRPSG(AN)-I-2026-03-00041

A2. Study Status

Record Verification Date	15 September 2026
Overall Recruitment Status	Not yet recruiting
Study Start Date (anticipated)	01 December 2026: planned at approximately month 9 of the 36-month project (after staff recruitment, drug/placebo procurement and training)
Primary Completion Date (anticipated)	30 September 2030
Study Completion Date (anticipated)	31 December 2030

A3. Sponsor / Collaborators and Oversight

Responsible Party	Sponsor-Investigator: Dr Deepti Vibha, Department of Neurology, AIIMS New Delhi
Sponsor	All India Institute of Medical Sciences, New Delhi
Collaborators	Indian Council of Medical Research (funder); Postgraduate Institute of Medical Education and Research, Chandigarh; Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry; Institute of Medical Sciences, Banaras Hindu University, Varanasi
Studies a U.S. FDA-regulated drug product	No
Studies a U.S. FDA-regulated device product	No
Data Monitoring Committee	Yes
Ethics committee Review	

A4. Brief Summary

Acute ischemic stroke (AIS) is a leading cause of death and long-term disability. Fewer than about 10–15% of patients receive reperfusion treatment (clot-dissolving drugs or thrombectomy), so most patients have no treatment that directly limits brain injury. Inflammation after ischemia is a common pathway of brain damage. Metformin, an inexpensive and widely used oral drug for type 2 diabetes, has anti-inflammatory and neuroprotective actions that appear independent of its glucose-lowering effect, and observational studies suggest better outcomes in stroke patients taking metformin. No adequately powered randomized trial has tested it.

This multicentre, double-blind, placebo-controlled trial will randomly assign 440 adults with AIS (within 72 hours of symptom onset, NIHSS score greater than 5, no large vessel occlusion, not already taking metformin) to oral metformin (dose increased from 250 mg twice daily to 1000 mg twice daily by Day 9, as tolerated) or matching placebo for 90 days, in addition to standard stroke care. The main question is whether

metformin increases the proportion of patients with excellent functional recovery (modified Rankin Scale score 0–1) at 90 days. Safety, tolerability, mortality, Barthel Index and blood biomarkers (hsCRP, vitamin B12 and Holotranscobalamin) will also be assessed. Patients with and without diabetes are eligible. Patients already taking metformin before the stroke will be followed in a separate, non-randomized observational cohort.

A5. Detailed Description

The trial is conducted at four tertiary-care academic hospitals in India, coordinated by AIIMS New Delhi. Eligible participants are randomized 1:1 through a central, web-based REDCap system. Study drug is started on Day 0 (day of randomization) at 250 mg twice daily and escalated every three days (500 mg, 750 mg, 1000 mg twice daily) to a maximum of 1000 mg twice daily from Day 9, subject to tolerability, and continued for 90 days. Tablets may be given orally or crushed through a nasogastric (Ryle's) tube in patients with dysphagia. Renal and liver function is monitored every 48–72 hours during hospitalization and every 2 weeks after discharge, with pre-specified rules to hold or stop study drug. Participants are assessed at baseline, Day 7 (± 2), Day 30 (± 7) and Day 90 (± 7). Outcome assessors, participants, treating clinicians and investigators are blinded to allocation. A Data and Safety Monitoring Board (DSMB) will oversee participant safety. Pre-specified subgroup analysis will compare the treatment effect in participants with and without diabetes.

A6. Conditions and Keywords

Conditions	Acute Ischemic Stroke; Ischemic Stroke; Cerebral Infarction
Keywords	Acute ischemic stroke; neuroprotection; metformin; drug repurposing; randomized controlled trial; stroke disability; modified Rankin Scale; neuroinflammation

A7. Study Design

Study Type	Interventional (Clinical Trial)
Primary Purpose	Treatment
Phase	Phase 3
Interventional Study Model	Parallel Assignment
Number of Arms	2 randomized arms (plus a separate non-randomized observational cohort – see A8)
Allocation	Randomized (1:1)
Masking	Quadruple (Participant, Care Provider, Investigator, Outcomes Assessor)
Enrollment (anticipated)	440 randomized participants [plus 40 participants in observational cohort]

A8. Arms and Interventions

Arm Group Label	Arm Type	Description	Intervention(s)
Metformin	Experimental	Oral metformin hydrochloride immediate-release, 250 mg twice daily on Days 0–2, 500 mg twice daily Days 3–5, 750 mg twice daily Days 6–8, and 1000 mg twice daily from Day 9 to Day 90 (maximum dose), titrated to tolerability, plus standard stroke care.	Drug: Metformin hydrochloride
Placebo	Placebo Comparator	Matching placebo tablets identical in appearance, taken on the same escalation schedule and number of tablets for 90 days, plus standard stroke care.	Drug: Placebo
Pre-stroke metformin users (observational cohort)	Other	Patients meeting all other eligibility criteria but already taking metformin before stroke onset. Not randomized; followed prospectively to 90 days. Metformin continued or modified per treating physician.	None assigned by protocol

Intervention: Metformin	Drug; other names: Metformin hydrochloride, dimethylbiguanide. Route: oral or via nasogastric tube. Duration: 90 days.
Intervention: Placebo	Drug; matching inert tablet (manufactured/packed with support of the Department of Pharmacology). Route and schedule identical to active drug.

A9. Outcome Measures

Primary Outcome Measure

Title	Description	Time Frame
Proportion of participants with good functional outcome (mRS 0–1)	Modified Rankin Scale (range 0 = no symptoms to 6 = death; higher scores indicate worse disability). Good outcome defined as a score of 0 or 1, assessed by a certified assessor blinded to treatment allocation.	90 (± 7) days after randomization

Secondary Outcome Measures

#	Title	Description	Time Frame
1	Proportion of participants with mRS 0–1	As for primary outcome.	30 (± 7) days
2	All-cause mortality	Proportion of participants who died from any cause.	30 days and 90 days
3	Barthel Index (BI) score and change from baseline	Barthel Index of activities of daily living (range 0–100; higher scores indicate greater independence).	Baseline, 30 (± 7) and 90 (± 7) days
4	Early neurological improvement (ENI)	Decrease of ≥ 4 points in NIHSS score from baseline (NIHSS range 0–42; higher is worse).	7 (± 2) days
5	Early neurological deterioration (END)	Increase of ≥ 2 points in NIHSS score from baseline.	7 (± 2) days
6	Serum high-sensitivity C-reactive protein (hsCRP)	Concentration (mg/L) and change from baseline (pre-dose) value.	Baseline, 30 (± 7) and 90 (± 7) days
7	Serum vitamin B12	Concentration (pg/mL) and change from baseline.	Baseline, 30 (± 7) and 90 (± 7) days
8	Serum holotranscobalamin (holoTC)	Concentration (pmol/L) and change from baseline.	Baseline, 30 (± 7) and 90 (± 7) days
9	Incidence of adverse events (AEs)	Number and proportion of participants with any AE, graded by CTCAE v5.0 severity and causality.	Randomization to 90 (± 7) days
10	Incidence of serious adverse events (SAEs)	Number and proportion of participants with any SAE (per ICH E2A/NDCT Rules 2019 definitions).	Randomization to 90 (± 7) days
11	Tolerability of study drug	Proportion of participants reaching and maintaining the target dose (1000 mg twice daily); proportion with dose reduction, temporary hold or permanent discontinuation due to AEs.	Randomization to 90 (± 7) days
12	Glycated haemoglobin (HbA1c)	Change in HbA1c (%) from baseline, overall and by diabetes status.	Baseline and 90 (± 7) days

A10. Eligibility

Minimum Age	18 Years
Maximum Age	N/A (No limit)
Sex	All
Accepts Healthy Volunteers	No
Inclusion / Exclusion Criteria	As listed in Protocol Section 8 (identical text is to be pasted into the PRS eligibility field).

A11. Contacts and Locations

Facility	City, State	Site Principal Investigator	Recruitment Status
All India Institute of Medical Sciences (coordinating site)	New Delhi, Delhi, India 110029	Dr Deepti Vibha	Not yet recruiting
Postgraduate Institute of Medical Education and Research	Chandigarh, India 160012	Dr Sucharita Ray	Not yet recruiting
Jawaharlal Institute of Postgraduate Medical Education and Research	Puducherry, India 605006	Dr Saranya B. Gomathy	Not yet recruiting
Institute of Medical Sciences, Banaras Hindu University	Varanasi, Uttar Pradesh, India 221005	Dr Anand Kumar	Not yet recruiting

Central contact: Dr Deepti Vibha, +91-11-26594485, deeptivibha@aiims.edu

Backup contact: Dr Rajesh Kumar Singh. +91-11-26594049.

A12. IPD Sharing Statement

Plan to Share IPD	Yes
IPD Sharing Plan Description	De-identified individual participant data underlying the published results, together with the data dictionary, will be deposited in a public repository in accordance with ICMR data-sharing guidelines.
Supporting Information	Study Protocol; Statistical Analysis Plan; Informed Consent Form; Clinical Study Report; Analytic Code
Time Frame	Beginning 6 months and ending 5 years after publication of the primary results
Access Criteria	Researchers providing a methodologically sound proposal approved by the trial steering committee, with a signed data access agreement

Part B. Study Protocol

1. Administrative Information

1.1 Trial Committees and Responsibilities

Committee / Role	Composition	Responsibilities
Principal Investigator and Coordinating Centre (AIIMS New Delhi)	PI, Co-PIs, Project Scientist, trial statistician	Overall scientific and operational conduct; protocol development and amendments; central REDCap database and randomization; training; drug/placebo supply; central monitoring; regulatory and EC reporting; publication.
Site Investigators (PGIMER, JIPMER, BHU and AIIMS)	Site PI, Project Technical Support-III, two Project Nurses-II per site	Screening, consent, enrolment, study drug administration, follow-up, data entry, AE/SAE reporting, drug accountability, local quality audit.
Trial Steering Committee	PI, site PIs	Overall supervision of trial; decisions on DSMB recommendations.
Data and Safety Monitoring Board (DSMB)	Independent biostatistician, neurologist, emergency medicine specialist, clinical pharmacologist and laboratory medicine expert	Periodic review of unblinded safety data and trial conduct; recommendation to continue, modify or stop the trial (see Section 20).
Laboratory Coordinator	Prof Ashok Sharma, Neurobiochemistry, AIIMS New Delhi	Standard operating procedures for biomarker sample collection, processing, storage, shipment and assays.
Pharmacy / Pharmacology Support	Department of Pharmacology, AIIMS New Delhi [Dr Harlokesh Narayan Yadav]	Procurement of metformin and matching placebo, blinding, coding, labelling and distribution of drug kits.
Statistician	Dr Ashish Dutt Upadhyay, Scientist, Clinical Research Unit, AIIMS, New Delhi	Framework and monitoring of statistical analysis plan and data management.

1.2 Funding

The trial is proposed for funding by the Indian Council of Medical Research under the ICMR ANVESHAN Extramural Grants 2026 call. The funder has no role in trial design, data collection, analysis, interpretation or the decision to publish.

2. Protocol Synopsis

Title	Efficacy of Oral Metformin in Acute Ischemic Stroke (AIS): A Multicentric, Double-Blind, Placebo-Controlled Randomized Controlled Trial
Design	Multicentric, parallel-group, 1:1, double-blind, placebo-controlled, superiority randomized controlled trial with an additional non-randomized prospective cohort of pre-stroke metformin users
Setting	Four tertiary-care academic hospitals in India (AIIMS New Delhi, PGIMER Chandigarh, JIPMER Puducherry, IMS-BHU Varanasi)
Population	Adults ≥18 years with first-ever, CT-confirmed AIS within 72 hours of onset, NIHSS >5 (item 1a <2), pre-stroke mRS 0–1, without large vessel occlusion, not on metformin
Intervention	Oral metformin 250 mg BD escalated to 1000 mg BD from Day 9 (as tolerated), for 90 days
Comparator	Matching placebo on identical schedule for 90 days
Primary outcome	Proportion with mRS 0–1 at 90 (±7) days
Secondary outcomes	mRS 0–1 at 30 days; mortality at 30 and 90 days; Barthel Index at 30 and 90 days; ENI and END at 7 (±2) days; hsCRP, vitamin B12 and Holotranscobalamin at 30 and 90 days

Safety outcomes	AEs, SAEs, tolerability (dose attainment, holds, discontinuations), renal and hepatic safety, lactic acidosis, hypoglycaemia, HbA1c
Sample size	440 (220 per arm)
Visits	Screening/Day 0, daily during dose escalation (Days 0–9), Day 7 (± 2), discharge, fortnightly safety laboratory tests after discharge, Day 30 (± 7), Day 90 (± 7)
Duration	Individual participation: 90 days. Total project: 36 months (enrolment ~22 months)

3. Abbreviations

Abbreviation	Definition	Abbreviation	Definition
AE	Adverse event	LAR	Legally acceptable representative
AIS	Acute ischemic stroke	LKW	Last known well
AKI	Acute kidney injury	LVO	Large vessel occlusion
AMPK	5'-AMP-activated protein kinase	mITT	Modified intention-to-treat
ASPECTS	Alberta Stroke Program Early CT Score	mRS	Modified Rankin Scale
BD	Twice daily	NDCT	New Drugs and Clinical Trials (Rules, 2019)
BI	Barthel Index	NIHSS	National Institutes of Health Stroke Scale
CRF	Case record form	Nrf2	Nuclear factor erythroid 2-related factor 2
CTA	CT angiography	PP	Per-protocol
CTCAE	Common Terminology Criteria for Adverse Events	RCT	Randomized controlled trial
DSMB	Data and Safety Monitoring Board	REDCap	Research Electronic Data Capture
EC	Ethics Committee	SAE	Serious adverse event
eGFR	Estimated glomerular filtration rate	SAP	Statistical analysis plan
END / ENI	Early neurological deterioration / improvement	sICH	Symptomatic intracranial haemorrhage
EVT	Endovascular thrombectomy	TNF- α	Tumour necrosis factor alpha
HbA1c	Glycated haemoglobin	TOAST	Trial of Org 10172 in Acute Stroke Treatment
holoTC	Holotranscobalamin	ULN	Upper limit of normal
hsCRP	High-sensitivity C-reactive protein	IVT	Intravenous thrombolysis

4. Background and Rationale

4.1 Burden of disease and unmet need

The management of stroke has advanced substantially in the last decade, driven by reperfusion treatment (RT) with intravenous thrombolysis (IVT) and endovascular thrombectomy (EVT). However, even in high-income countries only about 10–15% of patients with stroke are eligible for and receive RT.^{1–3} The remaining majority receive medical management and rehabilitation alone, leaving considerable scope for additional treatments. Stroke is among the top three causes of death and disability-adjusted life years in India, where access to RT is further constrained.

Ischemia-related inflammation and its downstream effects are a common mechanism of brain injury after ischemic stroke of any aetiology. Numerous neuroprotective or anti-inflammatory approaches – minocycline,^{4,5} Nerinetide,⁶ Edaravone,⁴ Citicoline,⁷ normobaric hyperoxia,⁸ glucagon-like peptide-1 receptor agonists,⁹ Glibenclamide¹⁰ and Colchicine¹¹ – have been studied with and without RT, with mixed results. None has reached level-I evidence sufficient for inclusion in treatment guidelines, and the 2026 AHA/ASA guideline does not recommend any specific anti-inflammatory agent for AIS.¹²

4.2 Metformin as a candidate neuroprotective agent

Metformin is one of the oldest first-line drugs for type 2 diabetes mellitus. Although diabetes and hyperglycaemia are risk factors for poor stroke outcome, the beneficial effects of metformin on stroke

outcomes are probably over and above its glucose-lowering effect.^{13–18} Proposed mechanisms include AMPK-mediated microglia/macrophage polarization and angiogenesis, activation of the Nrf2 antioxidant pathway and inhibition of the NF- κ B cascade to suppress post-ischemic inflammation.^{19–21} Metformin also exerts anti-inflammatory effects through antioxidant activity and insulin sensitization,^{22–24} and reduces inflammatory biomarkers including CRP and TNF- α . Anti-inflammatory effects have been observed irrespective of diabetes status.¹⁵

Retrospective cohort studies suggest improved functional outcomes in AIS patients receiving metformin^{18,25}; in the study by Jian et al., 74.9% of metformin users versus 61.5% of non-users achieved mRS 0–1. Metformin has been used safely at comparable doses in non-diabetic populations for conditions such as heart failure, cancer, dementia, polycystic ovarian syndrome and obesity.^{13,16,26,27} It is inexpensive (less than ₹5 per day), orally available, included in the National List of Essential Medicines, and has a well-established safety profile. Randomized evidence in AIS is, however, lacking.

4.3 Rationale for trial design

A double-blind, placebo-controlled design minimizes performance and assessment bias for a subjective, disability-based primary outcome. A 72-hour window allows inclusion of the majority of patients who present outside RT windows. Patients with large vessel occlusion (who may need EVT) are excluded to reduce heterogeneity from procedural outcomes. Patients without diabetes are included because the hypothesized mechanism is independent of glucose lowering; a pre-specified subgroup analysis will examine effect modification by diabetes status. Patients already on metformin cannot be randomized to placebo without withdrawing an established therapy and are therefore followed in a separate observational cohort. Vitamin B12 and Holotranscobalamin are measured because long-term metformin use is associated with reduced vitamin B12 status.

5. Research Questions, Objectives and Hypotheses

5.1 Research questions

1. Does oral metformin, commenced within 72 hours of AIS onset and continued for 90 days, improve functional outcome (mRS 0–1) compared with placebo?
2. Is oral metformin safe, tolerable and associated with an acceptable adverse-event profile in AIS patients, irrespective of diabetic status?
3. Does metformin change levels of hsCRP, vitamin B12 and Holotranscobalamin at 30 and 90 days?

5.2 Primary objective

- To determine the efficacy of oral metformin in patients with AIS in terms of good functional outcome (mRS 0–1) at 90 ($\pm$ 7) days.

5.3 Secondary objectives

- To determine the efficacy of oral metformin in terms of good functional outcome (mRS 0–1) at 30 ($\pm$ 7) days.
- To determine mortality at 30 and 90 ($\pm$ 7) days.
- To assess Barthel Index scores and their improvement at 30 and 90 days.
- To determine the proportion with early neurological improvement and early neurological deterioration at 7 ($\pm$ 2) days.
- To evaluate levels of biomarkers – hsCRP, vitamin B12 and holotranscobalamin – at 30 and 90 days.
- To determine the safety of the intervention, including tolerability, adverse events, serious adverse events and HbA1c levels.

5.4 Exploratory objectives

- To compare treatment effects in participants with and without diabetes (pre-specified subgroup analysis).
- To describe 90-day outcomes in a prospective cohort of patients taking metformin before stroke onset.

5.5 Hypotheses

Null hypothesis (H0): the proportion of participants with mRS 0–1 at 90 days does not differ between the metformin and placebo arms. Alternative hypothesis (H1): the proportion differs between arms (two-sided superiority test; anticipated 74.9% vs 61.5%).

6. Trial Design

Multicentric, parallel-group, two-arm, double-blind, placebo-controlled, superiority randomized controlled trial with 1:1 allocation, stratified by site and diabetes status and site. A parallel non-randomized prospective observational cohort includes eligible patients already on metformin before stroke.

Figure 1. Trial flow (CONSORT-style)

Patients with acute ischemic stroke presenting within 72 hours of LKW – screened and entered in screening log		
Excluded (not eligible / declined consent) – reasons recorded	Eligible and consented	Eligible but already on metformin → Observational cohort (consented, not randomized) → follow-up to Day 90
Central REDCap randomization 1:1 (n = 440), stratified by site and diabetes status		
Metformin arm (n = 220) 250 mg BD → 1000 mg BD from Day 9, for 90 days + standard care	1 : 1	Placebo arm (n = 220) Matching placebo, identical schedule, 90 days + standard care
Follow-up: Day 7 (±2) – NIHSS (ENI/END) Day 30 (±7) – mRS, BI, biomarkers Day 90 (±7) – mRS (primary), BI, biomarkers, HbA1c, safety		
Analysis: intention-to-treat (primary), per-protocol, safety population; pre-specified diabetes subgroup		

7. Study Setting

Hospital-based trial in the stroke units, neurology wards and emergency departments of four NIRF top-ranked tertiary-care institutes: AIIMS New Delhi (coordinating site), PGIMER Chandigarh, JIPMER Puducherry and IMS-BHU Varanasi. The geographic spread (north, south and east India) supports generalizability to India's varied stroke population. At AIIMS New Delhi, the Department of Geriatric Medicine will assist in identifying eligible patients and the Department of Neurobiochemistry will provide laboratory support. Laboratory equipment for study investigations is available at all sites.

8. Eligibility Criteria

8.1 Inclusion criteria (all must be met)

1. Adults aged ≥18 years.
2. Patient or legally acceptable representative (LAR) willing to give written informed consent before any study procedure.
3. Acute stroke of ischemic origin, radiologically confirmed by computed tomography (CT) before enrolment (exclusion of haemorrhage and mimics).
4. Randomization possible within 72 hours of symptom onset (time last known well).
5. Pre-stroke modified Rankin Scale score of 0 or 1, and first-ever episode of stroke.
6. NIHSS score >5, with NIHSS item 1a (level of consciousness) score <2.
7. No large vessel occlusion (LVO) on vascular imaging (CT angiography or MR angiography) [LVO: ICA, M1, basilar artery].
8. Patients who have received intravenous thrombolysis are eligible.

8.2 Exclusion criteria (any one excludes)

1. Receiving endovascular therapy, or candidate for any surgical intervention for treatment of stroke (e.g., decompressive craniectomy).
2. Prior history of stroke of any type (ischemic, intracerebral haemorrhage or subarachnoid haemorrhage).
3. Comatose patients (NIHSS item 1a score ≥2).
4. Evidence of intracranial haemorrhage on baseline CT.

5. Pregnancy or lactation (urine or serum pregnancy test in women of child-bearing potential).
6. Co-morbid illness limiting life expectancy, such as chronic liver disease, chronic kidney disease or malignancy.
7. Concurrent participation in any other therapeutic or rehabilitation clinical trial.
8. Already receiving metformin treatment (eligible for the observational cohort instead).
9. [Proposed additional safety exclusions for investigator confirmation, consistent with metformin prescribing information and the Section 9.4 safety rules: eGFR <45 mL/min/1.73 m² at screening; AST or ALT >2 × ULN; known hypersensitivity to metformin; current sepsis with shock, hypoxaemia or metabolic/lactic acidosis; decompensated heart failure; diabetic ketoacidosis].

8.3 Observational cohort (pre-stroke metformin users)

Patients who fulfil all inclusion criteria and no exclusion criteria other than exclusion criterion 8 (already receiving metformin) will be invited to join a non-interventional, prospective cohort. They will provide informed consent, will not be randomized and will not receive study drug; metformin will be continued, modified or stopped by the treating team according to clinical judgement. Baseline data and 90-day outcomes will be collected using the same CRF modules and assessment methods as the randomized participants.

9. Interventions

9.1 Investigational product and placebo

Active: metformin hydrochloride immediate-release tablets, procured from a licensed manufacturer. Placebo: tablets matching the active tablets in size, shape, colour, taste, packaging and labelling, containing inert excipients only. Procurement, blinding, coding and packaging will be supported by the Department of Pharmacology, AIIMS New Delhi. Certificates of analysis will be retained for both products. Because the placebo is prepared for this non-sponsored study, its manufacture and quality specifications must be documented.

Drug will be supplied in identical, sequentially numbered participant kits labelled “For clinical trial use only”, with the trial title, kit number, dosing instructions, storage conditions, PI name and contact, and expiry date. Kits will be stored at room temperature in a secure, access-controlled location at each site with temperature logs.

9.2 Dose escalation schedule

Day 0 is the day of randomization. Treatment starts after the pre-treatment blood sample has been collected. The target dose is determined by tolerability. Total treatment duration is 90 days.

Study Day	Dose	Frequency	Action
Day 0 (randomization)	250 mg	Twice daily	Collect blood sample before treatment initiation. Monitor for any adverse event (AE).
Days 1 and 2	250 mg	Twice daily	Monitor for any AE. If an AE attributable to study drug occurs, stop the medication. Rechallenge will be done when ascertainment is done as not due to the study drug.
Days 3 to 5	500 mg	Twice daily	If any AE, maintain at 250 mg BD.
Days 6 to 8	750 mg	Twice daily	If any AE, maintain at 500 mg BD.
Day 9 onwards to Day 90	1000 mg (maximum)	Twice daily	If any AE, maintain at 750 mg BD.

Route: oral; crushed and dispersed in water through a Ryle's (nasogastric) tube for patients with dysphagia. Tablets should be taken with or immediately after meals (or feeds) to reduce gastrointestinal intolerance.

9.3 Timing in relation to intravenous thrombolysis

Patients treated with IVT are eligible.

9.4 Safety monitoring and criteria for holding or stopping study drug

Renal function (serum creatinine with eGFR calculated by the CKD-EPI 2021 equation and liver function tests (AST, ALT, bilirubin) will be performed every 48–72 hours during hospital stay and every 2 weeks after discharge. The following rules apply to both arms (the treating team remains blinded):

Criterion	Action on study drug
eGFR 45–59 mL/min/1.73 m ²	Continue; monitor for progressive decline
eGFR 30–44 mL/min/1.73 m ²	HOLD, start when GFR is >59 mL/min/1.73 m ²
eGFR <30 mL/min/1.73 m ²	STOP
Acute kidney injury (KDIGO criteria) or rapidly falling eGFR	Temporarily STOP until renal function and clinical status stabilize
AST or ALT >2 × upper limit of normal	STOP
Sepsis with hypotension/shock	STOP
Sepsis with hypoxaemia/respiratory failure	STOP
Sepsis with acute kidney injury or falling eGFR	STOP
Sepsis with elevated lactate or metabolic acidosis	STOP
Intra-arterial iodinated contrast, or intravenous contrast with eGFR 30–59 / AKI / liver disease]	HOLD at the time of the procedure and for 48 hours afterwards; resume after renal function re-evaluated and stable

Participants whose study drug is held or discontinued will continue all scheduled follow-up assessments and will be analysed in their randomized group.

9.5 Standard care and concomitant medications

All participants will receive standard stroke care according to current national and international guidelines, including antiplatelet or anticoagulant therapy, statins, blood-pressure management, dysphagia screening, venous thromboembolism prophylaxis and rehabilitation. All concomitant medications from 7 days before randomization to Day 90 will be recorded.

- **Prohibited:** open-label metformin or metformin-containing fixed-dose combinations during the 90-day treatment period; participation in another interventional trial.
- **Glycaemic management:** participants with known or newly diagnosed diabetes, and those with stress hyperglycaemia, will be managed by the treating team with glucose-lowering therapies other than metformin (e.g., insulin or other oral agents) in both arms. Capillary blood glucose will be monitored per unit protocol, and hypoglycaemia (<70 mg/dL) recorded as an AE.
- **Use with caution (record and monitor):** drugs that may increase metformin exposure or lactic acidosis risk (e.g., cimetidine, dolutegravir, ranolazine, topiramate and other carbonic anhydrase inhibitors), iodinated contrast, and nephrotoxic agents.
- Endovascular or surgical stroke interventions performed after randomization will be recorded as protocol deviations/intercurrent events; study drug management in such cases will follow Section 9.4.

9.6 Adherence

Adherence will be monitored by direct supervision of administration by project nurses during hospitalization and dose escalation, and thereafter by structured adherence interviews and physical inspection/count of returned empty blister packets and unused tablets at each visit. Adherence (%) = (tablets taken ÷ tablets prescribed for the period) × 100. Adherence ≥80% of prescribed doses will be considered adequate. Participants/caregivers will receive a dosing diary and reminder counselling.

9.7 Drug accountability

Site pharmacists or delegated staff will maintain logs of receipt, dispensing, return, and destruction of kits. Returned packets will be counted and reconciled at Day 30 and Day 90.

10. Outcomes

10.1 Primary outcome

Proportion of participants with good functional outcome, defined as mRS score 0–1, at 90 (± 7) days after randomization. The mRS will be assessed by a trained and certified outcome assessor who is blinded to treatment allocation and not involved in acute care, preferably in person, using a structured interview. Telephone assessment with the patient or primary caregiver using the same structured interview is permitted if an in-person visit is not possible. Participants who die are assigned mRS 6 (poor outcome).

10.2 Secondary outcomes

1. Proportion of participants with mRS 0–1 at 30 (± 7) days.
2. All-cause mortality at 30 days and 90 days.
3. Barthel Index at 30 (± 7) and 90 (± 7) days, and change from baseline.
4. Early neurological improvement (ENI; decrease of ≥ 4 points in NIHSS from baseline) and early neurological deterioration (END; increase of ≥ 2 points in NIHSS from baseline) at 7 (± 2) days.
5. Serum levels of hsCRP, vitamin B12 and Holotranscobalamin at 30 (± 7) and 90 (± 7) days, and change from baseline.

10.3 Safety outcomes

- Adverse events and serious adverse events from randomization to Day 90.
- Adverse events of special interest: lactic acidosis; hypoglycaemia; gastrointestinal intolerance (nausea, vomiting, diarrhoea, abdominal pain, anorexia); acute kidney injury; AST/ALT $> 2 \times$ ULN; sepsis; symptomatic intracranial haemorrhage; recurrent stroke; vitamin B12 deficiency.
- Tolerability: proportion reaching 1000 mg BD; time to maximum tolerated dose; dose reductions, holds and permanent discontinuations and their reasons.
- Change in HbA1c from baseline to 90 days.

10.4 Exploratory outcomes

- Distribution of mRS scores (ordinal shift) at 90 days.
- Primary and secondary outcomes by diabetes status (pre-specified subgroup).
- 90-day outcomes in the observational cohort of pre-stroke metformin users.

11. Participant Timeline – Schedule of Assessments

Assessment	Screen (≤ 72 h)	Day 0 (Rand.)	Days 1–9 inpatient	Day 7 (± 2)	Disch.	q2 wk post-disch.	Day 30 (± 7)	Day 90 (± 7)	Unsch. / Early term.
Screening log	X								
Informed consent	X								
Eligibility checklist	X	X							
Demographics, medical history, risk factors	X								
Pre-stroke mRS	X								
Clinical features, time metrics	X	X							
Vital signs, weight	X	X	X	X	X		X	X	X
NIHSS	X	X		X	X				X
mRS					X		X	X	X
Barthel Index		X			X		X	X	X
Non-contrast CT (and CTA/MRA)	X		(if indicated)						(if indicated)
Pregnancy test (WOCBP)	X								

Assessment	Screen (≤72 h)	Day 0 (Rand.)	Days 1–9 inpatient	Day 7 (±2)	Disch.	q2 wk post-disch.	Day 30 (±7)	Day 90 (±7)	Unsch. / Early term.
Haemogram	X			X			X	X	
Renal function / eGFR, LFT	X		q48–72 h	X	X	X	X	X	X
Glucose; HbA1c	X (HbA1c)		Glucose					X (HbA1c)	
Lipid profile, coagulation	X								
Biomarkers: hsCRP, B12, holoTC		X (pre-dose)					X	X	
TOAST classification					X				
Randomization (REDCap)		X							
Study drug dispensing / escalation		X	X		X		X		
Adherence & packet count			X	X			X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X
AE / SAE assessment		X	X	X	X	X	X	X	X
Blinding assessment (guess of allocation)								X	

WOCBP, women of child-bearing potential. The observational cohort follows the same schedule for clinical assessments, without randomization, study drug, dose-escalation or adherence procedures.

12. Sample Size

The effect size is based on the retrospective cohort study by Jian et al. [14], which reported mRS 0–1 in 74.9% of metformin-treated and 61.5% of non-treated patients (absolute difference 13.4 percentage points). Using a two-sided test of two independent proportions (superiority), $\alpha = 0.05$ and 80% power, approximately 186–189 evaluable participants per arm (372–378 in total) are required, depending on the approximation used (normal approximation without continuity correction). Allowing for 15% loss to follow-up, the target sample size is 220 per arm (440 in total).

13. Recruitment

All patients with suspected AIS presenting to the emergency departments and stroke units of participating sites will be screened daily by the project technical staff. A screening log will record demographics, vascular risk factors, neuroimaging and reasons for exclusion. With a planned enrolment period of approximately 22 months (project months 9–31), about 20 participants per month overall (≈ 5 per site per month) are required. Collaborating sites were added specifically to mitigate the risk of slow recruitment. Recruitment will be monitored monthly against targets by the coordinating centre, and travel support for follow-up visits is budgeted to aid retention.

14. Assignment of Interventions

14.1 Sequence generation

A computer-generated randomization list with 1:1 allocation using permuted blocks of variable size, stratified by site and diabetes status (known diabetes, anti-diabetic therapy, or baseline HbA1c $\geq 6.5\%$), will be prepared by an independent statistician not involved in recruitment or outcome assessment. Block sizes will not be disclosed to investigators.

14.2 Allocation concealment

The allocation sequence will be uploaded to the central REDCap randomization module at AIIMS New Delhi and is accessible only to the independent statistician. After confirming eligibility and consent in REDCap, site staff will obtain a randomization number linked to a pre-coded drug kit number; the allocation (metformin or placebo) is not displayed.

14.3 Implementation

Site investigators enrol participants; REDCap assigns the kit number; project nurses dispense the corresponding kit and administer the first dose.

15. Blinding

Participants, caregivers, treating clinicians, site investigators, nurses, outcome assessors, laboratory personnel and the trial statistician (until database lock) will be blinded. Blinding is maintained by the use of identical placebo tablets and kits. The code list is held by the independent statistician and the pharmacology unit. The DSMB may review unblinded data in closed sessions.

15.1 Emergency unblinding

Unblinding is permitted only when knowledge of allocation is essential for the clinical management of a participant (e.g., suspected lactic acidosis where management would differ). In most situations, study drug can be stopped without unblinding. The site PI will request unblinding through the REDCap emergency unblinding module, the reason, date, time and person will be documented, and the coordinating PI and DSMB notified. Unblinded participants continue follow-up and remain in the ITT analysis. Outcome assessors must remain blinded.

15.2 Assessment of blinding

At the Day 90 visit, participants and outcome assessors will be asked to guess the treatment allocation (metformin / placebo / do not know).

16. Data Collection Methods

16.1 Instruments

Domain	Instrument / Data	Time points
Screening	Screening log: demographics, vascular risk factors, stroke neuroimaging, reasons for exclusion	Screening
Baseline clinical	Demographics; clinical features (time of onset; side of weakness, sensory loss, vision loss, ataxia, loss of consciousness, others); time metrics of interventions including randomized medication administration; vascular risk factors (hypertension, diabetes, rheumatic heart disease, coronary heart disease, atrial fibrillation, smoking, alcohol, genetic risk factors, family history, obesity, obstructive sleep apnoea, etc.)	Screening / Day 0
Stroke severity	National Institutes of Health Stroke Scale (NIHSS, 0–42)	Baseline, Day 7, discharge
Disability	Modified Rankin Scale (0–6), pre-morbid and post-stroke, using a structured interview	Pre-stroke, discharge, Day 30, Day 90
Activities of daily living	Barthel Index (0–100, 10 items)	Baseline, discharge, Day 30, Day 90
Aetiology	TOAST classification	Discharge
Neuroimaging	Non-contrast CT with ASPECTS; CT angiography findings; MRI if performed; follow-up imaging	Baseline; as indicated
Laboratory	Hemogram, biochemistry (renal, liver, glucose, HbA1c, lipids), biomarkers (hsCRP, vitamin B12, Holotranscobalamin)	Per schedule
Hospital course	Concomitant medications; any endovascular or surgical intervention; ENI/END; complications; length of hospital stay	Inpatient
Adherence	Adherence questionnaire and physical inspection of empty packets	Day 7, 30, 90
Safety	AE/SAE forms, protocol deviations	Continuous

16.2 Assessor training and quality

All personnel assessing NIHSS and mRS will complete certification [NIHSS certification and mRS training/certification programmes] before enrolling participants. Standard operating procedures will cover

consent, randomization, drug administration, sample handling, outcome assessment and AE reporting. A site initiation visit and training will be conducted before activation.

16.3 Biological samples

Venous blood will be collected at Day 0 (before first dose), Day 30 and Day 90 for hsCRP, vitamin B12 and Holotranscobalamin.

16.4 Retention and follow-up

Contact details of the participant and at least two alternative contacts will be recorded at enrolment (kept separately from the CRF). Reminder calls will be made before each visit; travel support will be provided. At least 3 documented contact attempts on different days will be made before a participant is considered lost to follow-up. Participants who discontinue study drug are encouraged to remain in follow-up.

16.5 Withdrawal

Participants may withdraw consent at any time without affecting their care. Investigators may discontinue study drug for safety reasons. The CRF will record whether the participant withdrew from treatment only, from further visits, or from all data use. Data collected before withdrawal will be retained unless the participant specifically requests otherwise, as permitted by applicable regulations. Withdrawn participants will not be replaced.

17. Data Management

Data will be recorded on paper CRFs at each site; source data will be captured as images and data entered into the central REDCap database hosted at AIIMS New Delhi, which is also used for randomization. REDCap provides role-based access, audit trails, range and logic checks, and automated backups. Participants are identified in the database only by study ID; the key linking study IDs to identities is held securely at each site. Anonymised and blinded data will be audited periodically for quality and completeness by the PI and Co-PIs, and data queries resolved with sites. Source documents and essential documents will be retained for [at least 5 years after trial completion, per NDCT Rules 2019]. The database will be locked after all queries are resolved and before unblinding for final analysis.

18. Statistical Methods (Summary)

The full Statistical Analysis Plan is provided in Part C. In brief: the primary analysis follows the intention-to-treat principle, comparing the proportion with mRS 0–1 at 90 days between arms, reported as a risk ratio and risk difference with 95% confidence intervals, with adjustment for stratification factors. Missing primary outcome data will be handled by multiple imputation under a missing-at-random assumption, with sensitivity analyses for data missing not at random. Secondary outcomes will be analysed with appropriate regression models. A pre-specified subgroup analysis will examine effect modification by diabetes status. Safety will be summarised descriptively.

19. Data Monitoring

19.1 Data and Safety Monitoring Board

An independent DSMB comprising a biostatistician, neurologist, emergency medicine specialist, clinical pharmacologist and laboratory medicine expert, none of whom are trial investigators, will operate under a DSMB charter. The DSMB will meet before enrolment and review cumulative safety data (AEs, SAEs, deaths, lactic acidosis, renal/hepatic events, discontinuations) at least every 6 months and after approximately 25%, 50% and 75% of participants have completed 30-day follow-up. It may recommend continuation, protocol modification or termination to the Trial Steering Committee.

19.2 Interim analyses

No formal interim analysis for efficacy is planned; therefore no alpha is spent before the final analysis. The DSMB may recommend stopping for safety if there is a clear excess of SAEs or deaths in either arm, using clinical judgement supported by descriptive statistics.

19.3 Quality assurance and auditing

Quality audits will be performed periodically by site PIs and Co-PIs, and central monitoring by the coordinating centre will include review of consent documentation, eligibility, primary outcome data, SAE reporting and drug accountability. The trial may be audited by the Ethics Committees, ICMR or regulatory authorities.

20. Harms: Adverse Event Definitions and Reporting

20.1 Definitions

- **Adverse event (AE):** any untoward medical occurrence in a participant after randomization, whether or not considered related to study drug.
- **Serious adverse event (SAE):** any AE that results in death; is life-threatening; requires in-patient hospitalization or prolongs existing hospitalization; results in persistent or significant disability/incapacity; is a congenital anomaly/birth defect; or is an important medical event requiring intervention to prevent one of these outcomes.
- **Adverse event of special interest (AESI):** lactic acidosis (blood lactate >5 mmol/L with pH <7.35); hypoglycaemia (blood glucose <70 mg/dL; severe <54 mg/dL or requiring assistance); gastrointestinal intolerance leading to dose change; AKI (KDIGO); AST/ALT >2 × ULN; symptomatic intracranial haemorrhage (per ECASS III / SITS-MOST definitions; recurrent stroke; vitamin B12 deficiency.
- **Expected events:** events related to the natural history of stroke (e.g., stroke progression, pneumonia, urinary tract infection) and known metformin effects listed in the prescribing information (gastrointestinal symptoms, metallic taste, vitamin B12 reduction). Expected events are still recorded.

20.2 Assessment

Severity will be graded using CTCAE version 5.0. Causality will be assessed by the site investigator using the WHO-UMC system (certain, probable, possible, unlikely, conditional/unclassified, unassessable). Actions taken with study drug and outcome will be documented.

20.3 Recording and reporting

AEs will be recorded from randomization until the Day 90 visit; SAEs will be followed until resolution or stabilization. All SAEs will be reported by the site investigator to the coordinating PI, the site Ethics Committee. Deaths and SAEs will be reported to the DSMB. Compensation and free medical management for trial-related injury will be provided in accordance with applicable rules and the ICMR National Ethical Guidelines.

21. Ethics and Dissemination

21.1 Regulatory and ethics approval

The trial will be conducted in accordance with the Declaration of Helsinki, ICH E6 Good Clinical Practice, the ICMR National Ethical Guidelines (2017) and the New Drugs and Clinical Trials Rules 2019. Approval will be obtained from the Ethics Committee of each participating centre before site activation. Metformin is an approved, marketed drug being repurposed for a new indication; the trial is planned as an academic clinical trial whose results are not intended for regulatory submission. The trial will be registered prospectively with CTRI and ClinicalTrials.gov before enrolment of the first participant.

21.2 Protocol amendments

Substantive amendments will be submitted to all Ethics Committees (and regulators where applicable) and implemented only after approval, except where necessary to eliminate immediate hazards. Registry records will be updated.

21.3 Informed consent

Written informed consent will be obtained by trained investigators from the patient or, where the patient lacks capacity (e.g., aphasia or reduced consciousness), from the legally acceptable representative, using EC-approved participant information sheets and consent forms in English, Hindi and regional languages (Punjabi, Tamil) as required. An impartial witness will be present if the patient/LAR is illiterate. Participants

enrolled via LAR consent will be re-consented when they regain capacity. Separate optional consent will be sought for storage and future use of biological samples.

21.4 Confidentiality

Personal information will be kept confidential and accessed only by authorized trial personnel, auditors and regulators. CRFs and the database will use study IDs only. Published data will not identify participants.

21.5 Ancillary and post-trial care

Participants will continue standard stroke care. At the end of the 90-day treatment period, participants will return to routine care; post-trial provision of metformin is not planned unless clinically indicated for another reason.

21.6 Dissemination

Results will be published in peer-reviewed indexed journals irrespective of outcome, including a protocol paper (years 1–2) and the primary results paper (year 3), with further publications on secondary and biomarker analyses. Authorship will follow ICMJE criteria. Results will be posted on ClinicalTrials.gov and CTRI, shared with participants and communicated to policymakers (including national stroke guideline and NPCDCS programme stakeholders).

22. Project Timeline

Activity	Project months	Year 1	Year 2	Year 3
Staff recruitment	0 – 6	■		
Procurement and packaging of trial drug and placebo	2 – 9	■		
Staff training, site initiation	4 – 11	■		
Patient enrolment	9 – 31	■	■	■
Collection and processing of blood samples	9 – 34	■	■	■
Patient follow-up	12 – 34	■	■	■
Data cleaning and analysis	33 – 36			■
Progress reports	12, 24, 36	■	■	■
Publications: protocol paper; primary results paper	12–24; 36		■	■

23. Potential Risks and Mitigation

Risk / challenge	Mitigation
Slow recruitment and randomization	Four collaborating high-volume centres; daily screening by dedicated staff; monthly recruitment monitoring
Procurement of matching placebo in a non-sponsored study	Support from Department of Pharmacology; early procurement phase (months 2–9)
Laboratory quality for biomarkers	Neurobiochemistry Co-PI; common SOPs
Loss to follow-up	Multiple contacts; travel support; permitted telephone mRS assessment
Gastrointestinal intolerance / lactic acidosis	Slow dose escalation; renal/hepatic monitoring; explicit hold/stop rules; DSMB oversight

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Part C. Statistical Analysis Plan

C1. Administrative Information

Trial title	Efficacy of Oral Metformin in Acute Ischemic Stroke (AIS): A Multicentric, Double-Blind, Placebo-Controlled Randomized Controlled Trial		
Trial registration	Pending		
SAP version and date	Version 2.0, [DD/MM/YYYY]		
Protocol version	Version 6.0, [DD/MM/YYYY]		
SAP authors	Dr Deepti Vibha (PI)		
Timing of SAP finalization	The SAP will be finalized and signed before database lock and before any unblinded analysis of outcome data.		
Role	Name	Signature	Date
Author – Trial Statistician	Dr Ashish Dutt Upadhyay		
Principal Investigator	Dr Deepti Vibha		
Senior Statistician / DSMB statistician (review)			

C2. Introduction

C2.1 Background and rationale

See Protocol Section 4. This SAP describes the planned analyses for the primary, secondary, safety and exploratory outcomes of the randomized trial and the observational cohort.

C2.2 Objectives

See Protocol Section 5. The primary objective is to determine whether oral metformin increases the proportion of participants with mRS 0–1 at 90 (± 7) days compared with placebo.

C3. Study Methods

C3.1 Trial design

Multicentre, parallel-group, 1:1, double-blind, placebo-controlled superiority RCT (Protocol Section 6).

C3.2 Randomization

Central web-based randomization via REDCap using permuted blocks of variable size stratified by site and diabetes status (Protocol Section 14). Stratification factors will be included as covariates in the primary adjusted analysis.

C3.3 Sample size

440 participants (220 per arm), providing 80% power at two-sided $\alpha = 0.05$ to detect an absolute increase in mRS 0–1 from 61.5% to 74.9% (risk ratio 1.22), allowing for 15% attrition (Protocol Section 12).

C3.4 Framework

Superiority. Two-sided tests with $\alpha = 0.05$.

C3.5 Interim analyses and stopping guidance

No formal interim efficacy analyses. DSMB safety reviews will use descriptive, arm-level summaries of SAEs, deaths, AESIs and discontinuations prepared by an unblinded DSMB statistician independent of the trial team. The final analysis will use $\alpha = 0.05$ without adjustment.

C3.6 Timing of final analysis

The final analysis will be performed after the last participant completes the Day 90 visit (or the window closes), all data queries are resolved, a blinded data review meeting is held (to confirm analysis populations and protocol deviation classification), and the database is locked.

C3.7 Timing of outcome assessments

Visit	Target day (Day 0 = randomization)	Window
Day 7	7	Days 5–9
Day 30	30	Days 23–37
Day 90 (primary)	90	Days 83–97

Assessments obtained outside the window will be included in the primary analysis if obtained between [Day 60 and Day 120] for the 90-day visit; a sensitivity analysis will restrict to within-window assessments. Deaths occurring at any time before the assessment are counted as mRS 6.

C4. Statistical Principles

C4.1 Confidence intervals and P values

All estimates will be presented with two-sided 95% confidence intervals. The primary outcome test will use $\alpha = 0.05$. No adjustment for multiplicity will be made for secondary outcomes; these will be reported with point estimates and 95% CIs, and any P values will be regarded as nominal and interpreted as supportive/exploratory.

C4.2 Adherence and protocol deviations

Adherence will be computed from supervised inpatient administration and returned packet counts: percentage of prescribed tablets taken over the 90-day period (or until death). Adequate adherence is $\geq 80\%$. Adherence will be summarised by arm (mean, SD, median, IQR; proportion $\geq 80\%$).

Major protocol deviations (defined before unblinding) include: randomization of an ineligible participant (violation of key inclusion/exclusion criteria); receipt of the wrong allocated treatment; receipt of open-label metformin during the treatment period; adherence $< 80\%$; EVT or surgical stroke intervention after randomization; primary outcome assessed by an unblinded assessor; primary outcome outside the extended window. Deviations will be tabulated by arm.

C4.3 Analysis populations

Population	Definition	Use
Intention-to-treat (ITT)	All randomized participants, analysed according to allocated arm regardless of treatment received, adherence or deviations	Primary analysis of all efficacy outcomes
Per-protocol (PP)	ITT participants without major protocol deviations who took $\geq 80\%$ of prescribed doses (or until a protocol-mandated stop for safety) and have a primary outcome assessment	Sensitivity analysis for primary and key secondary outcomes
Safety population	All randomized participants who received at least one dose of study drug, analysed according to treatment actually received	All safety analyses
Observational cohort	All consented participants in the pre-stroke metformin cohort	Exploratory analyses

C5. Trial Population

C5.1 Screening and eligibility

Numbers screened, eligible, consented and randomized, with reasons for exclusion (from the screening log), will be presented in a CONSORT flow diagram, along with numbers enrolled in the observational cohort.

C5.2 Recruitment, withdrawal and follow-up

The CONSORT diagram will show allocation, receipt of treatment, discontinuations (with reasons), withdrawals, losses to follow-up and deaths at each visit, and numbers included in each analysis population. Recruitment by site and month, and timing of follow-up visits relative to target days, will be summarised.

C5.3 Baseline characteristics (Table 1)

Summarised by arm, without formal statistical testing. Continuous variables: mean (SD) or median (IQR) as appropriate; categorical: n (%).

- Demographics: age, sex, site.
- Vascular risk factors: hypertension, diabetes (known/new), dyslipidaemia, coronary heart disease, atrial fibrillation, rheumatic heart disease, smoking, alcohol, obesity (BMI), obstructive sleep apnoea, family history.
- Pre-stroke mRS (0 vs 1); pre-stroke medications.
- Stroke characteristics: baseline NIHSS (median, IQR; categories 6–10, 11–15, >15), side and clinical syndrome, onset-to-randomization time (≤ 24 h, >24 –48 h, >48 –72 h), IVT (yes/no; agent), ASPECTS, TOAST subtype.
- Laboratory: glucose, HbA1c, eGFR, AST/ALT, hsCRP, vitamin B12, Holotranscobalamin.
- Baseline Barthel Index.

C6. Analysis

C6.1 Primary estimand

Attribute	Specification
Population	Adults with first-ever AIS within 72 hours, NIHSS >5 , pre-stroke mRS 0–1, without LVO, not on metformin (as defined by eligibility criteria)
Treatment conditions	Metformin with dose escalation for 90 days plus standard care versus matching placebo plus standard care
Variable (endpoint)	mRS 0–1 (yes/no) at 90 (± 7) days
Intercurrent events and strategies	Discontinuation, dose reduction or hold of study drug: treatment-policy strategy. Use of rescue EVT/surgery: treatment-policy strategy. Open-label metformin: treatment-policy strategy (PP sensitivity). Death: composite strategy (death = mRS 6, i.e., not a good outcome).
Population-level summary	Risk ratio (metformin vs placebo) adjusted for stratification factors; risk difference as supportive absolute measure

C6.2 Primary analysis

Data will be entered and managed electronically using REDCap. Continuous variables will be summarized as mean $\pm$ SD or median (IQR), as appropriate, and categorical variables as frequencies and percentages. Normality will be assessed using the Shapiro–Wilk test. Baseline characteristics between treatment groups will be compared using the independent t-test or Mann–Whitney U test for continuous variables and the Chi-square or Fisher's exact test for categorical variables. The primary analysis will follow the intention-to-treat (ITT) principle. The primary outcome will be favorable functional outcome (mRS 0–1) at 90 days, reported as adjusted RR and RD with 95% CIs. Unadjusted RR and RD will also be presented. Missing 90-day mRS data will be assessed for extent and c and, assuming MAR, addressed using MICE with 50 imputed datasets, with estimates combined using Rubin's rules or alternative method based on missing-data pattern. A per-protocol (PP) analysis will be performed as a sensitivity analysis among participants with adequate treatment adherence and no major protocol deviations. Secondary outcomes, including mRS at 30 days, mortality, Barthel Index, ENI, END, hsCRP, vitamin B12, holoTC, HbA1c, and treatment tolerability, will be analysed using appropriate statistical methods according to the outcome type and distribution. AEs, SAEs, deaths, and other safety outcomes will be summarized descriptively. A pre-specified subgroup analysis by baseline diabetes status will assess treatment effect modification using the treatment-by-diabetes interaction. Other appropriate statistical analyses may be performed based on the study objectives and data characteristics at the time of analysis. All tests will be two-sided, with 95% CIs; $\alpha=0.05$ will be used for the primary analysis, while secondary-outcome P values will be considered nominal and exploratory.

C6.3 Handling of missing primary outcome data

The extent and pattern of missing data will be described by arm, and baseline characteristics compared between those with and without the primary outcome. Under an assumption of missing at random (MAR), missing 90-day mRS will be imputed by multiple imputation using chained equations (ordinal logistic model

for mRS 0–6, subsequently dichotomized), generating 50 imputed datasets, separately by arm. The imputation model will include: treatment arm, site, diabetes status, age, sex, baseline NIHSS, pre-stroke mRS, IVT, onset-to-randomization time, TOAST, Day 7 NIHSS, discharge mRS and Day 30 mRS. Estimates will be combined using Rubin's rules. Participants who died are not missing (mRS 6).

C6.4 Sensitivity analyses for the primary outcome

1. Complete-case analysis (participants with observed 90-day mRS).
2. Missing not at random: tipping-point (delta-adjustment) analysis, shifting imputed log-odds of good outcome in the metformin arm downwards (and placebo arm upwards) until the conclusion changes; best-case/worst-case single imputation.
3. Per-protocol population.
4. Restricting to 90-day assessments within the ± 7 -day window and to in-person assessments.
5. Covariate-adjusted analysis: primary model additionally adjusted for age, baseline NIHSS, IVT and onset-to-randomization time.
6. Unadjusted analysis (chi-square; unadjusted RR and RD).

C6.5 Secondary outcome analyses

Outcome	Summary measure	Method
mRS 0–1 at 30 days	RR and RD (95% CI)	As for primary analysis; MI for missing data
Mortality at 30 and 90 days	RR (95% CI); HR (95% CI)	Modified Poisson for binary mortality; Kaplan–Meier curves to Day 90 and Cox proportional hazards model (stratified by site, adjusted for diabetes), censoring at last known alive date; proportional hazards checked with Schoenfeld residuals. Firth correction if events are few.
Barthel Index at 30 and 90 days	Median difference (95% CI); adjusted mean difference	Main: quantile (median) regression of BI at each visit adjusted for baseline BI, site and diabetes, with deaths assigned BI = 0. Sensitivity: among survivors, linear mixed model for repeated measures (visit $\times$ arm, baseline BI) and proportion with BI ≥ 95 .
ENI at Day 7 (NIHSS decrease ≥ 4)	RR and RD (95% CI)	Modified Poisson adjusted for stratification factors. Participants who died before Day 7 classified as not having ENI. If discharged before Day 7, NIHSS measured at the Day 7 visit.
END at Day 7 (NIHSS increase ≥ 2)	RR and RD (95% CI)	As for ENI; deaths before Day 7 classified as END.
hsCRP at 30 and 90 days	Geometric mean ratio (95% CI)	Log-transformed values; linear mixed model with arm, visit, arm $\times$ visit, log baseline value, site and diabetes; random participant intercept. Values below the limit of detection set to LOD/ $\sqrt{2}$.
Vitamin B12 and holotranscobalamin at 30 and 90 days	Adjusted mean difference or GMR (95% CI)	As for hsCRP (log-transform if skewed). Proportion with low vitamin B12 / holoTC compared by modified Poisson.

C6.6 Safety analyses

Conducted in the safety population, by treatment received, descriptively. No formal hypothesis testing will be performed except that differences in proportions with 95% CIs and Fisher's exact test P values (nominal) may be presented for SAEs and AESIs.

- Number of AEs and participants with ≥ 1 AE, by system organ class, severity (CTCAE v5.0), seriousness and relatedness.
- SAEs and deaths (with causes) by arm; AEs leading to dose reduction, hold or discontinuation.
- AESIs: lactic acidosis, hypoglycaemia, GI intolerance, AKI, hepatic enzyme elevation, sepsis-related stops, symptomatic ICH, recurrent stroke, vitamin B12 deficiency.
- Dose attainment: proportion reaching 1000 mg BD; maximum tolerated dose distribution; time to maximum dose; cumulative dose received.
- Laboratory shifts: eGFR categories (≥ 60 , 45–59, 30–44, < 30) and AST/ALT (≤ 2 , $> 2 \times$ ULN) from baseline to worst post-baseline value.
- HbA1c: ANCOVA of 90-day HbA1c adjusted for baseline HbA1c, site and diabetes status, overall and by diabetes status.

C6.7 Subgroup analyses

The pre-specified subgroup analysis will assess heterogeneity of the treatment effect on the primary outcome by diabetes status (diabetes vs no diabetes) by adding a treatment × subgroup interaction term to the primary model. Additional exploratory subgroups: age (<65 vs ≥65 years), sex, baseline NIHSS (6–10 vs >10), onset-to-randomization (≤24 h vs >24 h), IVT (yes vs no), TOAST subtype (large artery, small vessel, cardioembolic, other/undetermined), baseline hsCRP (above vs below median), and site. Results will be displayed as a forest plot with subgroup-specific RRs and interaction P values. Because the trial is not powered for subgroup comparisons, these analyses are hypothesis-generating.

C6.8 Exploratory analyses

- **Ordinal mRS (shift) analysis at 90 days:** common odds ratio from proportional odds logistic regression adjusted for stratification factors; proportional odds assumption assessed with the Brant test; if violated, the generalized odds ratio (Wilcoxon–Mann–Whitney) will be reported. Distribution displayed as Grotta bars.
- **Observational cohort (pre-stroke metformin users):** baseline characteristics and 90-day outcomes will be summarised descriptively. Exploratory comparison of mRS 0–1 at 90 days with the randomized placebo arm will use inverse probability of treatment weighting based on a propensity score including age, sex, baseline NIHSS, diabetes duration, HbA1c, IVT, TOAST subtype, pre-stroke mRS and site; balance assessed with standardized mean differences (<0.1). Results will be interpreted as observational and not used to support the randomized comparison.
- Correlation between changes in hsCRP and 90-day functional outcome, adjusted for arm.

C6.9 Additional considerations

- **Clustering by site:** accounted for by including site as a fixed effect; a sensitivity analysis will use a mixed-effects model with random site intercept.
- **Outliers and data checks:** implausible values will be queried before database lock; no values will be excluded without documented justification.
- **Assumption checks:** model diagnostics (residual plots, normality for linear models, influence statistics) will be reported in the statistical report.
- **Blinding assessment:** Bang's blinding index by arm for participants and outcome assessors.

C6.10 Statistical software

Analyses will be performed using R (current version available at the time of analysis (packages: sandwich, mice, survival, ordinal, quantreg) and/or Stata version xx]. Analysis code will be version-controlled and retained.

C6.11 Planned tables and figures

No.	Title
Figure 1	CONSORT flow diagram (screening, allocation, follow-up, analysis) including observational cohort
Table 1	Baseline characteristics by arm
Table 2	Primary and secondary efficacy outcomes (ITT)
Table 3	Safety outcomes: AEs, SAEs, AESIs, dose attainment and discontinuations (safety population)
Table 4	Sensitivity analyses of the primary outcome
Figure 2	Distribution of mRS scores at 90 days by arm (Grotta bars)
Figure 3	Forest plot of subgroup analyses (including diabetes vs non-diabetes)
Figure 4	Kaplan–Meier curves for survival to Day 90
Figure 5	Biomarker trajectories (hsCRP, vitamin B12, holoTC) at baseline, Day 30 and Day 90 by arm
Supplementary	Adherence; protocol deviations; site-wise recruitment; observational cohort results; blinding assessment

Table 2 – Primary and secondary efficacy outcomes (ITT)

Outcome	Metformin (n = 220)	Placebo (n = 220)	Adjusted RR / effect (95% CI)	Adjusted RD (95% CI)	P value
mRS 0–1 at 90 days, n (%)					
mRS 0–1 at 30 days, n (%)					—
Death by 30 days, n (%)					—
Death by 90 days, n (%)					—
Barthel Index at 90 days, median (IQR)					—
ENI at Day 7, n (%)					—
END at Day 7, n (%)					—
hsCRP at 90 days, geometric mean					—

C7. Deviations from the SAP

Any changes to this SAP after finalization will be documented with justification and dated in a revised version before database lock. Any analysis not pre-specified here, or deviations from planned analyses, will be clearly identified as post hoc in the final report and publications.