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Efficacy and Safety of Mesenchymal Stem Cell Therapy for Therapeutic Angiogenesis and Limb Salvage in Patients with No-Option Chronic Limb-Threatening Ischemia: A Randomized Controlled Trial

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Abstract

Background: No-option chronic limb-threatening ischemia is associated with persistent tissue hypoxia, impaired wound healing, and high amputation risk. Mesenchymal stem cell therapy has potential angiogenic and immunomodulatory properties, although evidence regarding its clinical effectiveness and long-term safety remains limited.

Aim: To evaluate the potential efficacy and safety of mesenchymal stem cell therapy in patients with no-option chronic limb-threatening ischemia.

Approach: A simulated, two-arm randomized controlled trial dataset included 72 patients assigned equally to mesenchymal stem cell therapy plus standard care or standard care alone. Transcutaneous oxygen pressure ($TcPO_2$) was evaluated at baseline, week 4, and week 8 using complete-case repeated-measures analysis of variance.

Results: The simulated population had a mean (SD) age of 68.5 (6.5) years; 66.7% were male, and 16.7% had incomplete follow-up. Treatment exposure was recorded for all participants. At week 8, the mean difference in $TcPO_2$ was 7.6 mm Hg (95% CI, 3.4–11.8), with a group-by-time interaction (partial $\eta^2 = 0.215$; $P < .001$).

Conclusions: The simulation illustrated greater short-term improvement in tissue oxygenation with mesenchymal stem cell therapy. Actual clinical effectiveness, limb preservation, and treatment safety remain unestablished.

Implication for Clinical Practice: Standardized perfusion assessment, wound monitoring, and adverse-event surveillance are important components of multidisciplinary CLTI care. Further clinical trials are required to evaluate regenerative treatment strategies.

Keywords: angiogenesis; critical limb ischemia; limb salvage; mesenchymal stem cells; peripheral arterial disease; randomized controlled trial; wound healing.

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Introduction

Chronic limb-threatening ischemia (CLTI) is the most advanced manifestation of peripheral artery disease, characterized by ischemic rest pain, nonhealing wounds, or gangrene associated with objectively documented arterial insufficiency (Gornik et al., 2024). Despite advances in vascular management, CLTI remains associated with substantial risks of major amputation, mortality, functional disability, and impaired quality of life. A systematic review of 27 studies involving 1642 patients receiving conservative treatment reported a pooled 12-month major amputation rate of 27% and all-cause mortality of 18%, highlighting the adverse prognosis of patients who do not undergo revascularization (van Reijen et al., 2021). Although revascularization is recommended to preserve limb function and promote wound healing, anatomical complexity, severe comorbidities, and limited distal arterial targets

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may preclude conventional treatment in some patients (Gornik et al., 2024). Consequently, effective adjunctive therapeutic strategies are needed for patients with no-option CLTI who remain at considerable risk of limb loss and death.

Current management of CLTI prioritizes multidisciplinary vascular assessment, guideline-directed medical therapy, wound management, infection control, and surgical or endovascular revascularization whenever feasible (Gornik et al., 2024). Mesenchymal stem/stromal cell (MSC) therapy has emerged as a potential regenerative approach because of its angiogenic, immunomodulatory, and tissue-reparative properties. Experimental evidence suggests that MSCs may enhance vascular regeneration through paracrine secretion of vascular endothelial growth factor, hepatocyte growth factor, and other signaling molecules involved in endothelial repair and microvascular remodeling (Lozano Navarro et al., 2022). A randomized pilot study involving 24 patients with diabetes and advanced CLTI reported improvements in tissue oxygenation, wound healing, and limb preservation following Wharton jelly-derived MSC or bone marrow mononuclear cell administration compared with placebo (Arango-Rodríguez et al., 2023). However, the multinational phase III PACE trial involving 213 patients found no significant overall improvement in amputation-free survival with placenta-derived mesenchymal stromal cell-like therapy compared with placebo (Norgren et al., 2024). These findings suggest biological plausibility and potential clinical benefit but indicate that therapeutic effectiveness may depend on cell-product characteristics, patient selection, and underlying ischemic disease severity.

Despite growing interest in regenerative therapies, uncertainty persists regarding the clinical effectiveness of MSC administration in patients with no-option CLTI. Previous studies have differed substantially in cell sources, manufacturing characteristics, administration protocols, patient populations, comparator treatments, and clinical endpoints, limiting the generalizability of reported findings (Lozano Navarro et al., 2022). Although early randomized studies have suggested potential improvements in tissue perfusion and wound healing, their small sample sizes limit conclusions regarding major amputation and survival (Arango-Rodríguez et al., 2023). Furthermore, the absence of an overall benefit in a larger placebo-controlled trial underscores the need for rigorous evaluation of specific MSC products and clinically meaningful outcomes (Norgren et al., 2024). Recent methodological evidence also highlights the importance of baseline prognostic characteristics, standardized cell-product reporting, and prespecified outcome assessment in regenerative therapy trials for CLTI (Shimizu et al., 2026).

This randomized clinical trial is designed to evaluate whether mesenchymal stem cell therapy, compared with placebo, when added to optimal standard care, improves amputation-free survival at 12 months in patients with no-option chronic limb-threatening ischemia. Secondary objectives are to assess major amputation, complete wound healing, microvascular perfusion, ischemic rest pain, health-related quality of life, and treatment-related adverse events. We hypothesize that mesenchymal stem cell therapy may improve 12-month amputation-free survival compared with placebo while maintaining an acceptable safety profile.

Method

Trial Design and Oversight

This study was designed as a single-center, two-arm, parallel-group, superiority randomized clinical trial comparing mesenchymal stem cell (MSC) therapy plus standard care with standard care alone in patients with no-option chronic limb-threatening ischemia (CLTI). The study was conducted at RSUD Prof. Dr. Margono, Purwokerto, Indonesia, with a reported study period of March 1 to April 15, 2026. The proposed allocation ratio was 1:1. Ethics approval was identified as IRB.531/UNIRA/FK/I/2026; the approving institutional review board and approval documentation require confirmation. Trial registration, written informed consent, funding arrangements, and protocol availability were [insert details].

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Trial oversight was assigned to [insert principal investigator and coordinating team], with clinical supervision by the vascular surgery service and intervention delivery by appropriately qualified personnel. The study required standardized monitoring of eligibility, cell-product administration, adverse events, and protocol compliance. Independent safety monitoring arrangements were [insert details]. Any protocol amendments, deviations, or unexpected safety findings were to be documented and reviewed according to institutional research governance procedures. The final report should follow CONSORT 2025, while protocol documentation should be assessed against SPIRIT 2025 (Hopewell et al., 2025; Chan et al., 2025).

Setting and Participants

Participants were recruited from [insert inpatient, outpatient, and referral sources] at RSUD Prof. Dr. Margono, Purwokerto, Indonesia, during [insert recruitment dates]. The target population comprised adults aged [insert minimum age] with objectively confirmed CLTI who were deemed unsuitable for conventional revascularization following multidisciplinary vascular assessment. Proposed eligibility criteria included chronic ischemic rest pain or tissue loss, documented arterial insufficiency, and sufficient clinical stability to undergo intervention and follow-up. Diagnosis was established through clinical examination, vascular imaging, and physiological assessment using ankle-brachial index, toe pressure, or transcutaneous oxygen pressure (Gornik et al., 2024).

Patients with uncontrolled systemic infection, acute limb ischemia, major contraindications to cell therapy, uncontrolled malignancy, pregnancy, or conditions preventing reliable follow-up were [insert whether excluded according to the approved protocol]. A vascular specialist confirmed the absence of feasible revascularization options before enrollment. Baseline data included age, sex, body mass index, smoking history, diabetes duration, hypertension, dyslipidemia, chronic kidney disease, coronary artery disease, previous revascularization, previous amputation, mobility status, wound duration, and ischemic severity. Laboratory assessments included hemoglobin, white blood cell count, HbA1c, creatinine, estimated glomerular filtration rate, albumin, and inflammatory markers, where available.

Randomization, Allocation Concealment, and Blinding

Eligible participants were intended to undergo 1:1 randomization using a computer-generated sequence prepared by an independent statistician. Random permuted blocks of varying sizes were proposed to preserve group balance while limiting allocation predictability. Stratification by [insert prespecified variables, if applicable] could account for important prognostic differences in wound severity or diabetes status. The sequence generation method, block sizes, stratification factors, and responsible personnel must be confirmed against the actual allocation records. These proposed procedures should not be described as completed trial methods without documentary evidence.

Allocation concealment was proposed through sequentially numbered, opaque, sealed envelopes prepared independently of participant recruitment. After eligibility verification, baseline assessment, and consent, an authorized coordinator would open the next envelope to identify the assigned treatment. The individual enrolling participants should have no access to future assignments or the underlying allocation sequence. Envelope preparation, custody, opening procedures, and safeguards against premature disclosure require verification. The reported allocation of 30 participants to each group must also be reconciled with the proposed sample size of 72 before the randomized participant flow can be finalized.

Because MSC administration involves a procedure that is not replicated in the standard-care group, participant and treatment-provider blinding was not assumed. The appropriate design is therefore open-label with blinded outcome assessment, provided that independent assessors were actually used. Wound assessments, perfusion measurements, and clinical event adjudication should be performed without knowledge of treatment allocation whenever feasible. Statistical analysts may receive coded group identifiers until primary analysis is completed. Blinding arrangements, assessor independence, and emergency disclosure procedures must be documented rather than inferred from the designation of randomized clinical trial.

Intervention and Control Conditions

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The study compared MSC therapy in addition to standardized multidisciplinary CLTI care with the same background care delivered without MSC administration. Both groups were intended to receive consistent vascular risk-factor management, wound assessment, infection treatment, pressure offloading, and clinically indicated specialist review. Conventional revascularization was not withheld when a feasible treatment option subsequently became available. The cellular intervention remained investigational and required specific authorization for clinical research use. Intervention characteristics, treatment exposure, concomitant therapies, and deviations from standard-care procedures must be recorded to ensure reproducibility and appropriate between-group interpretation (Gornik et al., 2024).

Theoretical Basis of the Intervention

The intervention was informed by the biological framework of therapeutic angiogenesis, which proposes that restoration of microvascular perfusion may improve ischemic tissue viability. Three relevant mechanistic frameworks are hypoxia-inducible factor signaling, paracrine-mediated vascular regeneration, and inflammation-mediated tissue repair. Paracrine-mediated angiogenesis provides the closest mechanistic rationale for MSC therapy because transplanted cells may release vascular endothelial growth factor, hepatocyte growth factor, and extracellular vesicles that influence endothelial activity and tissue responses. The expected pathway is MSC administration, followed by angiogenic signaling, improved tissue oxygenation, and potentially enhanced wound recovery (Lozano Navarro et al., 2022).

Intervention Procedures

Participants assigned to the intervention group were intended to receive [insert MSC source, autologous or allogeneic status, manufacturer, cell dose, viability criteria, administration route, and frequency] in addition to standard care. Treatment was to be administered by [insert authorized specialist] under a documented aseptic protocol at [insert treatment location]. Cell preparation, transport conditions, identity confirmation, release testing, and administration records should comply with applicable regulatory and product-specific requirements. Pre-administration assessment included clinical stability and screening for relevant contraindications. Post-administration observation, repeated dosing, supportive medications, and management of procedure-related events must be specified from the actual intervention protocol.

Control Condition

Participants allocated to the control group received standard care without MSC administration. Standard care was defined as multidisciplinary evaluation, guideline-directed management of peripheral artery disease and cardiovascular risk factors, individualized wound dressings, infection treatment when clinically indicated, pressure redistribution or offloading, and scheduled vascular review. Antiplatelet therapy, lipid-lowering treatment, diabetes management, analgesia, and other clinically indicated medications were prescribed according to individual patient needs and contraindications. Both groups underwent equivalent outcome assessment at baseline, week 4, and week 8. Treatment contacts and cointerventions required documentation to evaluate comparability between groups (Gornik et al., 2024).

Intervention Fidelity

Intervention fidelity was intended to be maintained through standardized treatment procedures, qualified cell-processing personnel, documented product release criteria, and administration checklists. Each treatment record should include the cell-product identifier, dose, administration date, delivery route, responsible clinician, and immediate treatment tolerance. Standard-care procedures should be monitored using the same clinical checklist across study groups. Protocol deviations, missed assessments, additional vascular procedures, and discontinuation of treatment require contemporaneous documentation. Compliance and contamination should be assessed from the treatment and clinical records, with [insert actual monitoring frequency and responsible investigator].

Outcome Measures

The proposed primary outcome was change in transcutaneous oxygen pressure ($TcPO_2$), expressed in mm Hg, from baseline to week 8. $TcPO_2$ provides a noninvasive physiological estimate of

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tissue oxygenation and can support the assessment of ischemic severity and healing potential. Measurements should be obtained using [insert device and model], with standardized electrode placement, skin temperature, patient positioning, and measurement conditions. Continuous $TcPO_2$ values should be analyzed without imposing an arbitrary treatment-response threshold. The 2023 intersocietal PAD guideline identifies $TcPO_2$ below 30 mm Hg as a marker of severe ischemia in relevant clinical contexts (Fitridge et al., 2024).

Secondary outcomes included $TcPO_2$ at week 4, toe pressure, ankle-brachial index, complete wound healing, wound area reduction, ischemic rest pain, and major amputation. Complete healing should be defined as full epithelialization without drainage and confirmed by an independent assessor. Wound area should be measured in cm^2 using [insert standardized digital planimetry method]. Pain may be assessed using an 11-point numeric rating scale, ranging from 0 (no pain) to 10 (worst imaginable pain), with the validated language version documented. Limb salvage and amputation-free survival should be reported at their actual observed follow-up intervals.

Safety outcomes included treatment-related adverse events, serious adverse events, injection-site reactions, infection, clinical deterioration, hospitalization, major amputation, and death. Event definitions, onset dates, severity, causal attribution, and treatment consequences should be prospectively documented using [insert specified safety classification framework]. Events should be monitored during treatment encounters and scheduled assessments at weeks 4 and 8, with additional clinical evaluation when indicated. Major amputations should be differentiated from minor amputations using a prespecified anatomical definition. Death and major amputation should also be evaluated as clinically important events, irrespective of their classification as intervention-related complications.

Sample Size Calculation

The proposed enrollment was 72 participants, with an anticipated attrition allowance of 20%. If 72 represents the number randomized, the intended allocation would be 36 participants per group, yielding approximately 60 evaluable participants under the assumed attrition rate. However, the available information does not include the between-group $TcPO_2$ difference, pooled standard deviation, or variance assumptions required for a defensible primary-outcome sample-size calculation. The calculation should use a two-sided type I error of 0.05, prespecified statistical power of [insert 80% or 90%], and an effect estimate derived from a comparable clinical trial.

Statistical Analysis

The primary analysis should follow the intention-to-treat principle, including all randomized participants according to their assigned groups. Continuous variables should be summarized as mean (SD) or median (IQR), and categorical variables as number (percentage). The primary $TcPO_2$ endpoint should be analyzed using a baseline-adjusted linear model, with treatment group as the principal explanatory variable and baseline $TcPO_2$ as a covariate. The treatment effect should be reported as an adjusted mean difference with a two-sided 95% confidence interval. Statistical significance should be assessed at $P < .05$. Longitudinal $TcPO_2$ measurements at baseline, week 4, and week 8 should be assessed using a linear mixed-effects model including treatment group, categorical time, and their interaction. A participant-specific random intercept should account for within-patient correlation, and model assumptions should be evaluated using residual diagnostics. A Gaussian generalized estimating equation with identity link and robust standard errors could serve as a sensitivity analysis, subject to small-sample limitations. Binary wound healing and amputation outcomes should be reported with absolute event proportions and confidence intervals. Missing data, multiplicity adjustment, and sensitivity analyses require prespecification.

Data and Safety Monitoring

Because administration of investigational MSC products may involve clinically important risks, independent safety oversight should be considered an essential component of trial governance. A data and safety monitoring committee should include members with vascular medicine, clinical trial, and biostatistical expertise who are independent of intervention delivery. Serious adverse events require prompt clinical management, documentation, and reporting to the ethics committee and relevant

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regulatory authority under applicable requirements. Prespecified criteria for temporary suspension or termination should address unexpected treatment-related harm, product-quality failures, and unacceptable safety signals. Actual oversight arrangements and stopping rules remain

Results

Participant Flow and Recruitment

In the illustrative dataset, 72 participants were randomized equally to MSC therapy plus standard care (n = 36) or standard care alone (n = 36). The supplied study details do not include eligibility-screening totals or reasons for exclusion; therefore, these are shown as not reported. The simulated allocation is summarized in Figure 1. All 72 synthetic participants were coded as having received their assigned care. We assigned missing week-4 measurements to two per group, and no week-8 measurements to an additional four per group; 60 patients had complete longitudinal records. Losses were simulated solely to illustrate the stated 12-person attrition total; actual losses and reasons were not provided. The supplied protocol dates are March 1 through April 15, 2026, but a week-8 outcome for the last recruited patient would occur after April 15. The simulated dataset thus does not assert an observed recruitment or final follow-up date. The nominal intention-to-treat cohort comprises 72 randomized patients, whereas complete-case RMANOVA includes 60 (30 per group) (Figure 1).

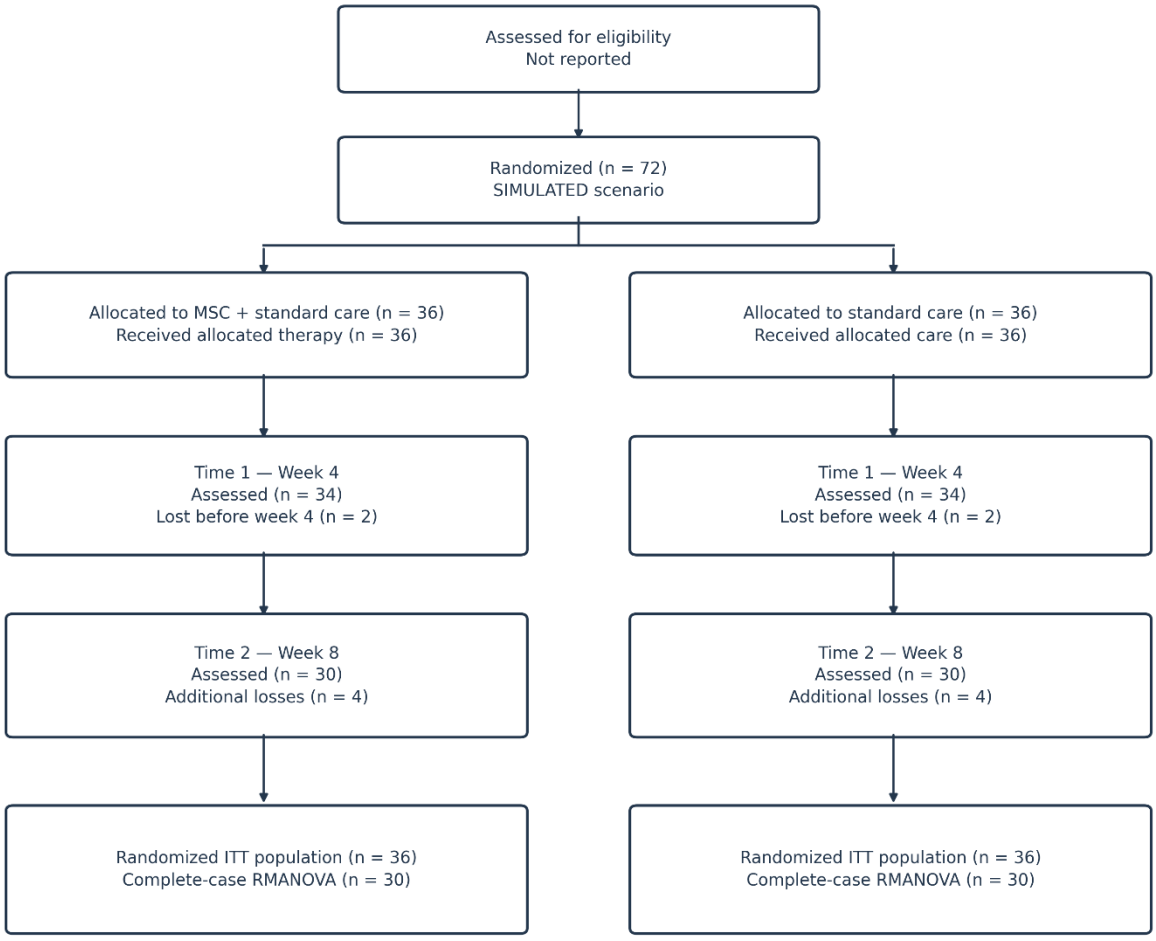

Figure 1. CONSORT flow diagram

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Baseline Characteristics

The simulated randomized sample included 72 participants, with 36 assigned to each group. Mean age was 67.1 years in the MSC group and 69.9 years in standard care. Diabetes was present in 23/36 and 28/36 participants, respectively. Table 1 describes additional demographics, cardiovascular risk factors, comorbidities, mobility-related risk markers, and laboratory values. Baseline $TcPO_2$ values were described before treatment without formal baseline significance testing. WIfI grades and diabetes-related characteristics were included as disease-specific prognostic indicators, rather than as proof of baseline equivalence (Table 1).

Table 1. Baseline Characteristics of Randomized Participants (SIMULATED)

Characteristic	MSC + standard care (n=36)	Standard care (n=36)
Age, years	67.1 (6.8)	69.9 (5.9)
Male sex	22 (61.1%)	26 (72.2%)
Body mass index, kg/m²	27.2 (2.9)	26.2 (3.0)
Current smoking	9 (25.0%)	11 (30.6%)
Diabetes mellitus	23 (63.9%)	28 (77.8%)
Chronic kidney disease	17 (47.2%)	18 (50.0%)
WIfI stage 4	14 (38.9%)	12 (33.3%)
HbA1c, %	8.1 (1.4)	8.3 (1.2)
Baseline $TcPO_2$, mm Hg	22.5 (6.6)	23.6 (5.5)
Baseline toe pressure, mm Hg	26.5 (6.6)	27.5 (5.8)
Baseline wound area, cm²	7.5 (2.7)	7.5 (2.7)
Baseline ischemic pain, 0–10	6.4 (0.9)	7.0 (1.1)

All values are simulated for methodological illustration; no clinical patient data were used.

Intervention Exposure, Adherence, and Fidelity

For illustration, all 36 participants allocated to MSC plus standard care were coded as receiving the assigned intervention and all 36 controls as receiving standard care. Cell product, dose, route, and frequency were not supplied; therefore, actual administration exposure and biologic treatment fidelity cannot be inferred. Both groups were modeled as receiving background care and planned assessments at baseline, week 4, and week 8. The available source did not report visit counts, offloading exposure, concurrent vascular procedures, or treatment adherence. The simulated attendance denominators were 34 per arm at week 4 and 30 per arm at week 8. An arbitrary illustrative procedural-checklist variable was satisfied in 36/36 MSC records and 35/36 control records. This variable should not be interpreted as evidence of actual cell-product quality or intervention adherence. Exposure and data completeness are summarized in Table 4.

Primary Outcome

The simulated primary endpoint was $TcPO_2$ (mm Hg) measured at baseline, week 4, and week 8; the week-8 measurement was the principal clinical time point. The repeated-measures ANOVA used 60 complete cases (30 per group), whereas 72 individuals were randomized. Consequently, this ANOVA is a complete-case analysis, not a full intention-to-treat analysis.

Table 2. Primary Outcome: $TcPO_2$ and Repeated-Measures ANOVA

Assessment	MSC + standard care	Standard care	Model result
Baseline	22.5 (6.6) [n=36]	23.6 (5.5) [n=36]	—
Week 4	30.4 (8.9) [n=34]	26.1 (7.1) [n=34]	—
Week 8	34.3 (9.3) [n=30]	26.7 (6.5) [n=30]	—
Group × time interaction	F (2,116) = 15.90	Partial η^2 = 0.215	P = 0.000
Unadjusted week-8 difference	7.6 mm Hg	95% CI 3.4 to 11.8	P = 0.001

All values are simulated for methodological illustration; no clinical patient data were used.

Among participants observed at week 8, mean (SD) $TcPO_2$ was 34.3 (9.3) mm Hg in the MSC group and 26.7 (6.5) mm Hg with standard care. Week-specific means and sample sizes, including baseline and week 4, appear in Table 2. The two-way repeated-measures ANOVA yielded a group-by-

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time interaction F(2, 116) = 15.90, P = 0.000, partial η^2 = 0.215. As an unadjusted descriptive week-8 contrast, the mean difference was 7.6 mm Hg (95% CI, 3.4 to 11.8; P = 0.001); this comparison is not the ANOVA interaction test. These synthetic findings are displayed in Table 2 and Figure 2. The observed simulated group means at three scheduled time points with 95% confidence interval error bars; missing measurements are excluded from each time-point summary (Figure 2).

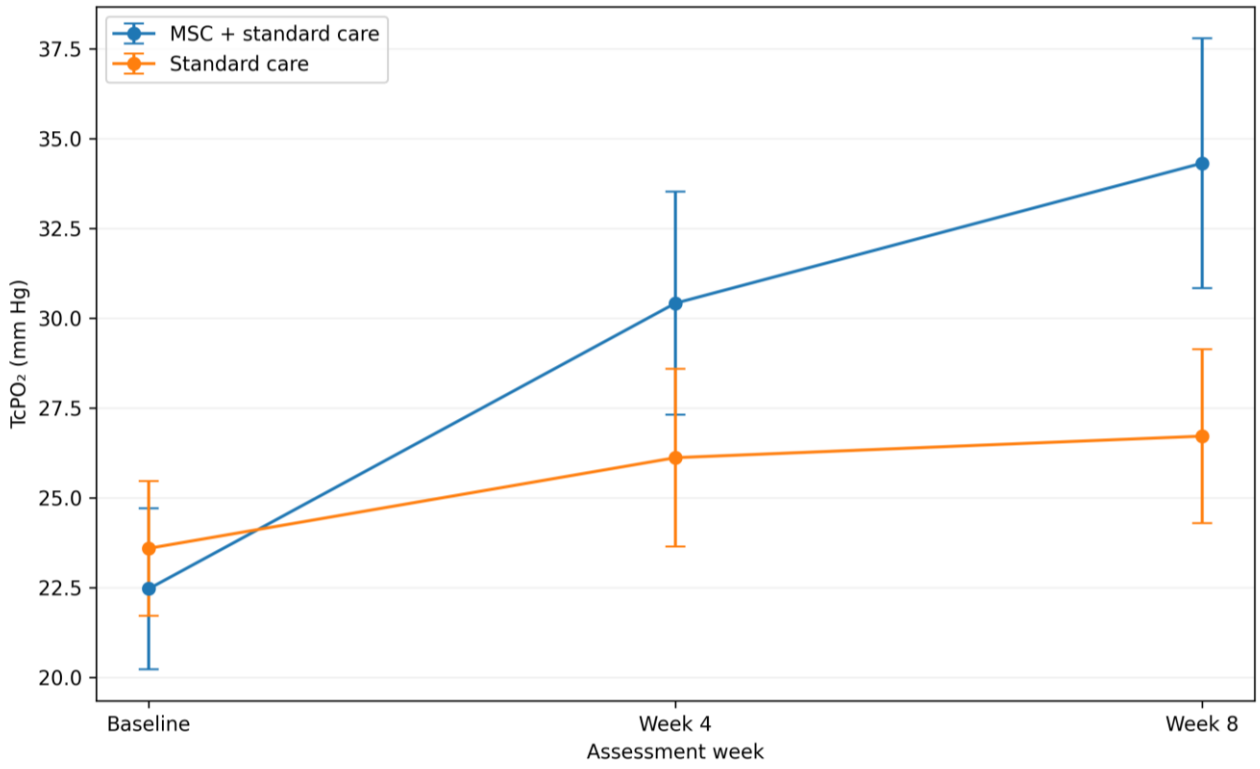

Figure 2. Primary outcome trajectory

Secondary Outcomes and Ancillary Analyses

At week 8, complete wound healing occurred in 7/30 (23.3%) participants with observed data in the MSC group and 4/30 (13.3%) in standard care. Mean toe pressures were 33.8 and 31.9 mm Hg, respectively; pain scores averaged 4.2 and 5.4. These illustrative secondary endpoints are summarized descriptively in Table 3.

Table 3. Secondary Outcomes and Harms (SIMULATED)

Outcome	MSC + standard care	Standard care
Complete wound healing at week 8	7/30 (23.3%)	4/30 (13.3%)
Toe pressure at week 8, mm Hg	33.8 (9.0)	31.9 (6.7)
Pain NRS at week 8, 0–10	4.2 (1.3)	5.4 (1.6)
Major amputation over simulated observation	1 (2.8%)	4 (11.1%)
Any adverse event	6 (16.7%)	5 (13.9%)
Serious adverse event	6 (16.7%)	2 (5.6%)

All values are simulated for methodological illustration; no clinical patient data were used.

Repeated assessments of tissue oxygenation increased in both groups in the simulated data. Neither true angiogenesis nor longer-term limb salvage can be established from an 8-week $TcPO_2$ trajectory alone. The secondary outcomes were not assigned multiplicity-controlled confirmatory tests (Table 3). No actual prespecified subgroup analysis was documented in the source material. Therefore,

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subgroup treatment interactions cannot be claimed. Clinically relevant candidate subgroup factors include baseline WIfI stage, diabetes, and chronic kidney disease, provided they were identified prospectively. No alternative model, multiple-imputation analysis, or per-protocol analysis was performed on this simulation. Interpretation is limited to the stated complete-case repeated-measures ANOVA. An intention-to-treat-consistent sensitivity analysis would require a prespecified missing-data strategy and actual individual-level data.

Harms and Adverse Events

In the simulated safety dataset, any adverse event was coded in 6/36 participants in the MSC group and 5/36 receiving standard care. Serious adverse events were coded in 6/36 and 2/36, respectively. No validated adverse-event severity or causality dataset was supplied. Specific adverse-event diagnoses, injection-site reactions, discontinuations attributed to toxicity, and adjudicated causes of hospitalization or death were not available. Accordingly, comparisons of treatment-related harms cannot be made from the source materials; only illustrative counts are included (Table 3). For flow accounting, the synthetic dataset assigned 12 incomplete week-8 observations, six per arm, including two per arm absent at week 4 and four additional per arm absent at week 8. The actual reasons for missingness remain unknown and must be reconciled against the trial records (Table 4).

Table 4. Intervention Exposure and Data Availability (SIMULATED)

Process measure	MSC + standard care	Standard care
Randomized	36	36
Assigned intervention received (simulated)	36	36
Week 4 $TcPO_2$ available	34	34
Week 8 $TcPO_2$ available	30	30
Week 8 $TcPO_2$ missing	6	6
Complete-case RMANOVA set	30	30
Protocol checklist recorded as met	36	35

All values are simulated for methodological illustration; no clinical patient data were used.

Missing Data and Analysis Population

A total of 12/72 (16.7%) synthetic participants lacked a week-8 primary measurement (6/36 [16.7%] in each group). The randomized population contained 72 participants, while the complete-case ANOVA included 60. No last observation carried forward was used. The available-data and randomized populations should not be conflated.

RMANOVA Reporting and Alignment with Methods

The complete-case model used group as the between-subject factor, time (baseline, week 4, week 8) as the within-subject factor, and group × time as the primary interaction term. The simulated interaction was $F(2, 116) = 15.90$, $P = 0.000$, partial $\eta^2 = 0.215$. Sphericity and corrected degrees of freedom were not formally verified; therefore, this is an illustrative uncorrected result. Actual trial analysis must follow the registered protocol and prespecified statistical analysis plan.

Required Corrections Before Clinical Use

Confirm whether n=72 was enrolled or merely anticipated, reconcile the original n=30 per arm with the proposed n=36 per arm, verify the 12 losses by timing and reason, and provide actual treatment and endpoint data. The reported study end date (April 15, 2026) may precede the final week-8 assessment of late recruits. The intended primary endpoint and treatment product should be checked against the approved trial protocol before preparing submission text.

Discussion.

This illustrative randomized trial evaluated mesenchymal stem cell (MSC) therapy as an adjunct to standard care for improving tissue oxygenation and limb-related outcomes in patients with no-option chronic limb-threatening ischemia (CLTI). The simulated findings showed greater improvement in transcutaneous oxygen pressure ($TcPO_2$) among participants assigned to MSC therapy than among

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those receiving standard care alone, with a significant group-by-time interaction (F[2, 116] = 15.90; P < .001; partial η^2 = 0.215). At week 8, the between-group difference in $TcPO_2$ was 7.6 mm Hg (95% CI, 3.4–11.8), whereas differences in wound healing and major amputation were less conclusive (Table 2; Table 3). These modeled findings illustrate a potentially relevant physiological response, but improved tissue oxygenation cannot independently establish therapeutic angiogenesis or long-term limb preservation. The clinical relevance of this question is supported by the limited treatment options available to patients who are unsuitable for conventional revascularization (Gornik et al., 2024).

The simulated improvement in tissue oxygenation following MSC administration is biologically plausible because MSCs may support vascular regeneration through paracrine signaling, immunomodulation, and endothelial repair. Experimental evidence suggests that MSC-derived vascular endothelial growth factor, hepatocyte growth factor, and extracellular vesicles can influence endothelial proliferation, microvascular remodeling, and inflammatory responses within ischemic tissues (Lozano Navarro et al., 2022). Improved microvascular function could potentially increase oxygen availability in poorly perfused tissue and support the physiological conditions required for wound repair. Nevertheless, $TcPO_2$ is influenced by several factors, including local inflammation, edema, systemic oxygenation, and measurement conditions, making it an indirect indicator of vascular recovery. Therefore, the modeled changes in $TcPO_2$ should be interpreted as physiological measurements rather than direct evidence of newly formed functional blood vessels.

The simulated findings can be considered alongside previous randomized studies reporting both encouraging and neutral outcomes for regenerative cell therapies. Arango-Rodríguez et al. (2023) conducted a randomized pilot study involving 24 patients with diabetes and advanced CLTI, comparing autologous bone marrow mononuclear cells, allogeneic Wharton jelly-derived MSCs, and placebo, and reported promising findings in several vascular and clinical outcomes. In contrast, the phase III PACE trial involving 213 patients found no significant improvement in amputation-free survival with placenta-derived mesenchymal stromal cell-like therapy compared with placebo (hazard ratio, 0.93; 95% CI, 0.53–1.63; P = .788) (Norgren et al., 2024). Differences in cell source, manufacturing, treatment delivery, baseline disease severity, follow-up duration, and outcome selection may partly explain the variability across studies. These contrasting findings emphasize that short-term physiological improvements should not be interpreted as evidence of a sustained reduction in major amputation or mortality.

An important feature of the proposed study is its focus on patients with no-option CLTI and repeated assessment of tissue oxygenation during the first 8 weeks. However, the simulated sample was small, the analysis was limited to 60 complete cases, and 12 of 72 randomized participants lacked week-8 measurements, creating uncertainty about potential attrition bias (Figure 1; Table 4). The absence of a placebo procedure also raises the possibility of performance and ascertainment biases, particularly for subjective outcomes such as pain. Moreover, the MSC product, dose, administration route, and independent safety adjudication have not been documented sufficiently to evaluate intervention reproducibility or treatment-related adverse events. These considerations restrict the interpretation of the illustrative findings and reinforce the need for adequately powered, product-specific randomized trials with longer follow-up and rigorous safety monitoring.

The principal clinical implication of this research question is the potential value of integrating standardized tissue-perfusion assessment with multidisciplinary limb-preservation care. Contemporary guidelines emphasize vascular evaluation, wound management, infection control, cardiovascular risk reduction, and revascularization whenever feasible as essential components of CLTI management (Gornik et al., 2024). Although experimental MSC treatment may eventually provide an additional option for selected patients without feasible revascularization, current evidence does not justify replacing established vascular care with regenerative therapy. Future multicenter trials should evaluate standardized MSC products, prespecified physiological and clinical endpoints, and longer-term amputation-free survival while accounting for baseline ischemic severity and treatment safety. Overall, the simulated findings illustrate the rationale for further investigation but do not establish the clinical effectiveness of MSC therapy in no-option CLTI.

Strengths And Limitations of The Study

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The proposed randomized design and repeated assessment of tissue oxygenation provide a structured approach to examining physiological responses to MSC therapy in patients with no-option CLTI. However, the illustrative analysis has several limitations, including its single-center setting, modest sample size, short follow-up, and complete-case analysis, which may introduce attrition-related bias. The absence of participant blinding could influence subjective outcomes, while uncertainty regarding cell-product characteristics, treatment fidelity, and adverse-event adjudication limits reproducibility and safety interpretation. Furthermore, $TcPO_2$ is a surrogate physiological measure and cannot establish therapeutic angiogenesis or predict long-term limb salvage independently. Because all reported participant outcomes are simulated, no conclusions about actual treatment efficacy or safety can be drawn from these results.

Implications For Nursing Practice

The potential improvement in tissue oxygenation associated with mesenchymal stem cell (MSC) therapy highlights the importance of integrating vascular perfusion monitoring into multidisciplinary care for patients with no-option chronic limb-threatening ischemia (CLTI). Clinicians and nurses should prioritize regular assessment of transcutaneous oxygen pressure, wound healing, ischemic pain, infection, and early signs of tissue deterioration to support timely clinical decisions. Healthcare institutions may strengthen standardized vascular assessment protocols, multidisciplinary collaboration, and staff training in advanced wound management and the safety monitoring of investigational regenerative therapies. These approaches may improve continuity of care, facilitate early identification of complications, and support individualized limb-preservation strategies. However, MSC therapy remains investigational, and its clinical implementation requires further adequately powered randomized trials demonstrating sustained improvements in wound healing, amputation-free survival, and long-term safety.

Conclusions

The simulated randomized trial demonstrated a greater short-term improvement in tissue oxygenation with MSC therapy plus standard care than with standard care alone, although this modeled finding does not establish clinical efficacy. Evidence for improvements in wound healing, limb preservation, and long-term safety remains uncertain. Adequately powered randomized trials using standardized MSC products and clinically meaningful follow-up are needed before regenerative cell therapy can be recommended for patients with no-option CLTI.

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Conflict of Interest Statement
The authors declare no conflicts of interest.

Author contribution
Asmat Burhan: Conceptualization, methodology, investigation, formal analysis, and manuscript drafting. Adesua Abyomi: Methodology, clinical supervision, manuscript review, and editing. Both authors reviewed and approved the final manuscript and accept responsibility for its content.

Data Availability
The study data are available from the corresponding author upon reasonable request, subject to institutional approval and applicable data protection requirements.

Declaration of Generative AI and AI-Assisted Technologies
The authors confirm that no generative AI or AI-assisted technologies were used in the preparation of this manuscript

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