OPENS-2 Statistical Analysis Plan

STATISTICAL ANALYSIS PLAN

OPENS-2: A phase III, multicenter, investigator-initiated, open, randomized, parallel-group, superiority trial to assess the clinical effectiveness of trophic enteral nutrition with supplemental parenteral nutrition versus full enteral feeding in patients with acute severe stroke.

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20/01/2026	2.0	Clarified and optimized study endpoints (Section 3); Added covariates, sensitivity analysis and subgroup Analysis (Section 7.6&10.1); Supplemented and standardized baseline data collection (Section 9); Updated the corresponding methodological descriptions for analysis (Section 10.2).

Approvals by

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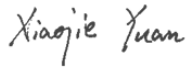
Dr. Xiaojie Yuan (Trial Statistician)		20/01/2026
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List of Abbreviations and Definitions of Terms

AE	Adverse Event
AIS	Acute Ischemia Stroke
APACHE	Acute Physiology and Chronic Health Evaluation
BMI	Body Mass Index
CEC	Clinical Events Committee
CT	Computed Tomography
CRF	Case Report Form
CRRT	Continuous Renal Replacement Therapy
DSMB	Data and Safety Monitoring Board
EAB	Ethics Advisory Board
EAC	Endpoint Adjudication Committee
ECG	Electrocardiogram
EN	Enteral Nutrition
GCP	Good Clinical Practice
GCS	Glasgow coma scale
GEE	Generalized estimating equations
GRV	Gastric residual volume
ITT	Intention-To-Treat
LAR	Legally Authorized Representative
MRI	Magnetic Resonance Imaging
mRS	Modified Rankin Scale
NIHSS	National Institute of Health stroke scale
NRS	Nutritional Risk Screening
PI	Principal Investigator

PN	Parenteral nutrition
PP	Per-Protocol
QC	Quality Control
RCT	Randomized controlled trial
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SPN	Supplemental Parenteral nutrition
TEN	Trophic Enteral Nutrition
TMC	Trial Management Committee
TSC	Trial Steering Committee
PSP	Post stroke pneumonia

1. Background

OPENS-2 is a phase III, multicenter, investigator-initiated, randomized, open-label, blinded-endpoint (PROBE) trial designed to assess the efficacy and safety of combined trophic enteral nutrition (EN) with supplemental parenteral nutrition (SPN) versus full EN in critically ill patients with acute ischemia stroke (AIS). This Statistical Analysis Plan (SAP) describes the statistical methods to be used for the OPENS-2.

Readers of this SAP are encouraged to read the clinical trial protocol to understand the implementation details of this study, the operational aspects of clinical evaluation, and the schedule of patients completing this study.

2. Study objective

2.1 Primary Objectives

To determine whether the combination of trophic EN and SPN reduces the incidence of pneumonia within the first 7 days after enrollment in patients with severe stroke requiring nutritional support.

2.2 Secondary Objectives

- To compare nutritional delivery (caloric/protein intake) and insulin requirements during the first 7 days.
- To assess gastrointestinal tolerance (complications, prokinetic agent use) during the first 7 days.
- To evaluate clinical outcomes, including other infections, ICU length of stay, mortality (ICU & 28-day), and specific complications/interventions.
- To measure neurological function and functional status at ICU discharge and 90 days.

3. Study Endpoints

3.1 Primary Efficacy Endpoint

- Incidence of post-stroke pneumonia from randomization to day 7 (binary).

3.2 Secondary Efficacy Endpoints

- Time from randomization to onset of the post-stroke pneumonia within 7 days post-randomization (time-to-event)
- Daily calorie and protein delivery during the first 7 days post-randomization (continuous)
- Insulin utilization during the first 7 days post-randomization (binary)
- The usage rate of prokinetic agents during the first 7 days post-randomization (binary)
- Occurrence of nosocomial infections from randomization to ICU discharge (binary)
- Length of ICU stay (continuous)
- Incidence of tracheotomy, mechanical ventilation, continuous renal replacement therapy (CRRT), and venous thrombosis from randomization to ICU discharge (binary)
- Usage rate of vasoactive agents from randomization to ICU discharge (binary)
- The score of National Institute of Health stroke scale (NIHSS) at ICU discharge (continuous)
- Glasgow Coma Scale (GCS) at ICU discharge (continuous)
- Modified Rankin scale (mRS) at ICU discharge (ordinal)
- Modified Rankin scale at 90 days (ordinal)
- Excellent functional outcome (mRS 0-1) at 90 days (binary)
- Functional independence outcome (mRS 0-2) at 90 days (binary)

3.3 Safety Endpoints

- All-cause mortality during ICU stay (binary)
- All-cause mortality at 28 days (binary)
- Incidence of cardiac failure from randomization to ICU discharge (binary)
- Incidence of gastrointestinal complications (vomiting, diarrhea, gastric retention, gastrointestinal bleeding) during the first 7 days post-randomization (binary)

3.4 Exploratory endpoints

- Hierarchical composite endpoint of all-cause mortality and post-stroke pneumonia

within 90 days (win ratio).

- Hierarchical composite endpoint of all-cause mortality and post-stroke pneumonia within 7 days (win ratio).
- Time from randomization to the first occurrence of either all-cause death or post-stroke pneumonia within 90 days (time to event).

4. Study Methods

4.1 Overall Study Design and Plan

This is a Phase III, multicenter, investigator-initiated, randomized, open-label, blinded-endpoint (PROBE) trial designed to assess the superiority of combined trophic EN with SPN versus full EN in critically ill patients with AIS. Eligible participants will be randomly assigned (1:1) to one of the two nutritional strategies. The intervention will be administered for 7 days, unless interrupted by the initiation of exclusive oral feeding, discharge from the ICU, or death. All other aspects of clinical management will follow standard care at the discretion of the treating physician. The study patient flow outline following Consort diagram is shown in Figure 1.

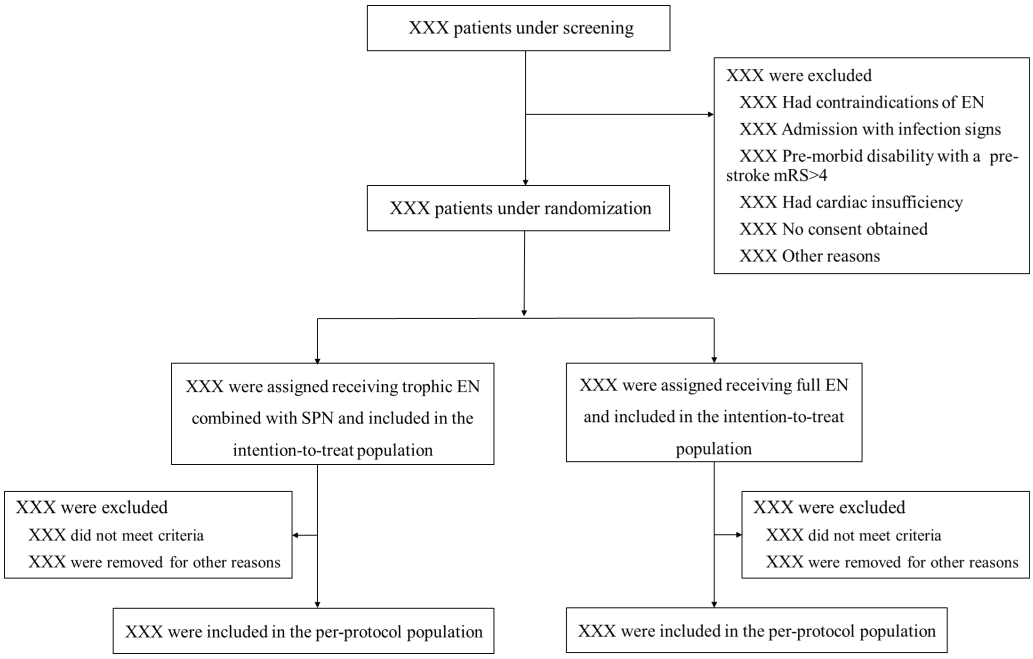

Figure 1. The study patient flow outline following Consort diagram.

4.2 Selection of Participants

4.2.1 Inclusion Criteria

- Age≥18 years
- Definite diagnosis of acute stroke (GCS ≤12 or NIHSS≥11)
- The randomized nutritional treatment could be initiated up to 72 hours after symptom onset
- Any cases of profiles #3 through 5 in Water Swallowing Test or with disorder of consciousness
- Plan to receive enteral feeding for at least 7 days
- Informed consent

4.2.2 Exclusion Criteria

- Receiving parenteral nutrition support
- Contraindications of enteral nutrition
- Complicated with the disease which only have life expectancy < 7 days
- Admission with infection signs
- Dementia or severe disability (mRS>4) before stroke
- Antibiotics were used within the previous 7 days
- Subarachnoid hemorrhage, cerebral arteriovenous malformation
- Presence of coexisting medical conditions that could interfere with outcome assessment and/or follow-up (a. advanced cancer; b. severe pulmonary dysfunction [forced expiratory volume in 1 second < 50% or/and moderate to severe acute lung injury (PaO2/FiO2)<200mmHg]; c. cardiac insufficiency (NYHA class > I; cardiac structural and/or functional abnormalities such as EF< 50%, abnormal cardiac chamber enlargement, moderate/severe ventricular hypertrophy, or moderate/severe valvular stenosis); d. severe liver failure [Child-Pugh score⩾7]; e. severe renal failure [glomerular filtration rate ⩽30mL/min or serum creatinine ⩾4mg/dL]
- Currently participating in other clinical trial
- Pregnant woman

- Patient who is considered highly likely not to adhere to the study treatment or follow-up protocol.

4.3 Method of Treatment Assignment and Randomization

Randomization will be performed using a web-based system accessible through mobile devices or computers (http://www.epiedc.com/kf.php). The system will assign participants in a 1:1 ratio to either trophic EN with SPN group or full EN group with stratification by study site. The block size will not be disclosed, to ensure concealment. Once eligibility is confirmed and informed consent procedures are completed, randomization will occur immediately, with the aim of initiating nutritional support within 24 hours after the patient's admission to the intensive care unit. Each randomized patient will receive a unique trial identification number.

4.4 Blinding/Unblinding

Given the practical nature of the nutritional interventions, this trial adopts an open-label design. The treating clinical team is aware of treatment assignments to ensure proper protocol implementation, while most severe stroke patients are expected to experience impaired consciousness during the intervention period. To ensure objective endpoint assessment and data integrity, blinding is strictly maintained for the Endpoint Adjudication Committee, Core Imaging Laboratory personnel, and study statisticians throughout the trial period. These parties remain completely unaware of treatment group allocations until database lock and completion of the final analysis.

4.5 Interim analysis

No interim analysis is planned for this study.

5. Sample Size Estimates

The sample size is based on the primary efficacy endpoint. We expect an 12.0% absolute difference between patients receiving trophic EN combined with SPN and patients receiving full EN (expected rates of post stroke pneumonia: 28% in patients receiving trophic EN combined with SPN and 40% in patients receiving full EN), which corresponds to a relative risk (RR) of 0.70[1,2]. In order to demonstrate the expected

treatment effect with a type-1 error alpha of 0.05 (two tailed) and a power of 80%, a sample size of n= 490 patients (n=245 per treatment group) is required. To safeguard against dilution of the treatment effect associated with an approximate 10% attrition rate (due to loss to follow-up, consent withdrawal and other reasons), we plan to enroll n=546 patients (n=273 per treatment group) for this study.

6. Analysis Population

6.1 Intention-to-Treat (ITT) Population

The ITT[3] population includes all patients randomized into the trial, even if the subject does not receive the correct nutritional support, or does not follow the protocol until completion. This population will serve as the primary analysis set for efficacy endpoints, with all subjects analyzed according to the treatment group assigned during randomization.

6.2 Per-Protocol (PP) Population

The PP population consists of patients from the ITT population who received the assigned nutritional support as randomized and complied with key trial procedures without major protocol violations that could impact efficacy outcomes. As this is an open-label trial, major protocol deviations will be identified through unblinded review prior to database lock. Patients will be excluded from the PP population based on the following criteria at a minimum:

- Deviations in Nutrition Support Administration.

  Failure to achieve 7 days of nutrition support duration, unless the discontinuation is due to patient death, discharge from the ICU, the achievement of adequate oral intake, or is supported by a valid clinical justification;

- Deviations in Nutrition Route.

a) Failure to implement the assigned nutritional strategy according to randomization;

b) (Trophic EN with SPN group) Exceeding the enteral nutrition rate limit (as defined per protocol) for more than 24 consecutive hours;

c) (Full EN group) Initiation of parenteral nutrition (PN) without appropriate clinical

justification;

d) Cessation of all nutritional support for more than 48 consecutive hours without valid medical reasons;

- Other Major Deviations.

a) Violation of inclusion/exclusion criteria with subsequent randomization

b) Failure to perform protocol-required nutrition monitoring and data collection

c) Serious violations of enteral/parenteral nutrition preparation and administration protocols.

All protocol deviations must be thoroughly documented in the Case Report Forms (CRFs) with explanations. The Steering Committee will provide final confirmation of all deviations prior to database lock and any analysis by treatment group.

6.3 Safety Population

The Safety Population comprises all randomized patients who initiated nutritional support regimen. Patients who violated the randomization protocol will be additionally analyzed according to the actual nutritional strategy they received in a supplementary analysis. The classification of patients for the 'as-treated' analysis must be completed prior to database unblinding. Patients who immediately withdrew consent after randomization without receiving any nutritional support will be excluded from the Safety Population.

7. General Issues for Statistical Analysis

7.1 General Principles

Data were summarized by means and standard deviations, or medians (interquartile ranges) for continuous variables, and frequency along with percentage for categorical variables. All statistical analyses will be performed using R version 4.4.0.

7.2 Methods for Withdrawals and Missing Data

7.2.1 Withdrawals

All subjects who withdraw from the study prematurely are required to complete a

withdrawal visit, and the reasons for withdrawal will be documented in a summary table. Subjects who do not complete the 90-day follow-up but have available post-baseline data will therefore be included. For subjects who are randomized but do not receive any study intervention, endpoint data will be collected and analyzed whenever available. However, they may not contribute data to endpoints that require the administration of intervention for assessment (e.g., total calorie and protein intake). These subjects will be listed separately, and their characteristics and reasons for non-intervention will be described to allow for an assessment of potential bias.

7.2.2 Missing Data

All efforts will be undertaken to ensure complete follow-up, with the highest priority given to the assessment of the primary outcome.

7.2.2.1 Baseline Covariates

Missing baseline covariates will be imputed using simple imputation methods in the covariate adjusted analysis based on the covariate distributions, should the missing values for a particular covariate be less than 5%. For a continuous variable, missing values will be imputed from random values from a normal distribution with mean and SD calculated from the available sample. For a categorical variable, missing values will be imputed from random values from a multinomial distribution with probabilities P1, P2, …, and Pk from the sample. If the proportion of subjects with missing values for a covariate is ≥ 5% then the missing values will be imputed using Multiple Imputation by Chained Equations (MICE). A total of 10 multiply imputed dataset will be generated to generate the single combined dataset to be used for covariate adjusted analyses. The seed for the imputations will be 128.

7.2.2.2 Efficacy Outcomes

Missing primary outcome values will be replaced using multiple imputation method. Logistic regression model with the following covariates: allocated treatment, age, NIHSS at ICU admission, stroke type, history of diabetes, history of chronic respiratory disease, and smoking will be used and implemented using SAS PROC MI with a seed

being 224. A total of 10 multiply imputed datasets will be generated to get the final treatment effect estimate and associated confidence interval (CI) from the modified Poisson regression model.

Sensitivity analyses based on different hypotheses about the missingness pattern of the primary outcome will be performed to test for the robustness of the primary analysis results (see section 'Primary Efficacy Analysis').

7.3 Data Transformations

The analysis of continuous variables may require transformation to normalize the distributions, but these variables will be mainly analyzed using nonparametric statistical tests. Decisions to transform the distribution of some variables for analysis will be taken after the blind review of the data, and before unblinding the treatment code.

7.4 Multicenter Data[4]

In this analysis, the center effect will be considered as a cluster effect in the models in the sensitivity analysis of the primary outcome to evaluate the robustness of the primary analysis to the specification of the center effect using a generalized estimating equation (GEE) approach with an exchangeable correlation structure, treating center as a cluster.

7.5 Multiplicity

Analyses of secondary outcomes, safety outcomes and additional analyses for the primary outcome are regarded as exploratory in nature, therefore, multiplicity adjustment will not apply to the primary and secondary outcome analyses[5].

7.6 Covariates

In the covariates-adjusted analysis, the following variables will be adjusted.

- Age (continuous)
- Stroke severity (continuous)
- Stroke type (ischemic vs. hemorrhagic)

- Diabetes (no vs. yes)
- Chronic respiratory disease (no vs. yes)
- Smoking (Binary: ever-smoker and never-smoker)

If a covariates-adjusted model does not converge, inverse probability of treatment weighting (IPTW) method will be used[6].

7.7 Planned Subgroups

Subgroup analyses will be performed to supplement the primary findings by examining the consistency of the treatment effect across key patient populations. These analyses are considered exploratory and will be conducted regardless of the significance of the overall treatment effect. The predefined subgroups include:

- Age (≤70 years vs. >70 years)
- Stroke severity (NIHSS 11-19 vs. NIHSS ≥20)
- Stroke type (ischemic vs. hemorrhagic)
- Diabetes (no vs. yes)
- History of chronic respiratory disease (No vs. Yes)
- Smoking status (ever-smoker and never-smoker)
- Consciousness level (GCS 9-12 vs. GCS ≤8)
- Time from onset to nutrition initiation (≤24 hours vs. >24 hours)

The presence of effect modification will be formally tested by including a multiplicative interaction term between the treatment group and each subgroup variable in the primary analysis model.

8. Disposition of Subjects and Withdrawals

The number of patients for each of the following categories will be summarized by treatment group.

- Total number of assessed patients
- Number of randomized patients
- Number of patients completing the study and not completing the study (grouped by treatment and main reason)
- Number of patients included in the ITT population
- Number of patients included in the PP population
- Number of patients included in the Safety population

9. Demographics and Baseline Characteristics

Clinically important demographic and disease characteristics at screening will be summarized with descriptive statistics for each treatment group. Summaries will be produced for the ITT population and PP population.

If the missing value exceeds 5%, the denominator will be added in the footnote of the corresponding summary table.

The variables to be summarized include but are not restricted to the following:

- Age
- Sex
- BMI
- Weight
- Medical history (Hypertension, Diabetes mellitus, Atrial fibrillation, Chronic respiratory disease and Previous stroke)
- Smoking status (Never-smoker / Ever-smoker)
- Premorbid mRS
- APACHE-II score at ICU admission
- NIHSS at ICU admission

- GCS at ICU admission
- Type of stroke (ischemic vs. hemorrhagic)
- Acute stroke treatment (Intravenous thrombolysis and Endovascular therapy)
- Albumin at admission
- Triglycerides at admission
- Total cholesterol at admission
- Blood glucose at admission
- Time from stroke onset to randomization (For patients with wake-up stroke, Onset to Randomization will be recorded as the time from last known without progression to randomization)
- Time from stroke onset to initiation of nutritional support

10. Efficacy Analyses

Efficacy analyses will be performed using the ITT population, with patients grouped according to the treatment assigned at randomization. All available data, regardless of protocol adherence, will be included in the efficacy analyses. Secondarily, the same analyses will be repeated on the PP population. All efficacy analyses will provide the point estimate of the treatment effect with associated two-sided 95% CIs.

All clinical efficacy assessments will adhere to the predefined target visit windows specified in the protocol. If multiple assessments occur within the same window, the value closest to the target time point will be selected. Assessments falling outside the permissible windows will be excluded from the analysis.

10.1 Primary Efficacy Analysis

The primary outcome is the incidence of post-stroke pneumonia within 7 days after

randomization. If a patient dies within 7 days, pneumonia is counted only if it occurred before death. If a patient is discharged from the ICU or transitions to oral feeding within 7 days, pneumonia occurring after that point but still within the 7-day window is included. The primary analysis will assess the effect of the nutritional strategy using a modified Poisson regression model to calculate the risk ratio (RR). For any missing pneumonia assessment in patients otherwise under follow-up, multiple imputation will be employed using the strategy described in Section 7.2.2.2.

10.1.1 Crude Analysis

Analysis of the primary efficacy outcome will test the hypothesis as follows:

H_0: $P_{\text{Intervention}} = P_{\text{Control}}$

H_1: $P_{\text{Intervention}} \neq P_{\text{Control}}$

where $P_{\text{Intervention}}$ and P_{Control} represent the proportion of patients with post-stroke pneumonia within 7 days after randomization in the Trophic EN with SPN group and the Full EN group, respectively. Superiority of the combined nutritional strategy will be declared if the two-sided P-value of the estimated treatment effect is < 0.05.

The primary analysis of the primary efficacy outcome will be based on the ITT population. The hypothesis will be tested using a modified Poisson regression model with treatment as the study variable to derive the RR and its 95% CI[7,8]. A crude analysis without covariate adjustment will serve as the primary approach to draw the main conclusion regarding the efficacy of the intervention, which will be supported by a secondary analysis in the PP population.

10.1.2 Covariates Adjusted Analysis

We will also perform an adjusted analysis of the primary outcome. The analysis will be adjusted for the following pre-specified baseline covariates: age, NIHSS at ICU admission, stroke type, history of diabetes, history of chronic respiratory disease, and

smoking. This model will be used to calculate an adjusted RR with its 95% CI, providing an estimate of the treatment effect after accounting for potential imbalances in prognostic factors. For this adjusted analysis, any missing data in the baseline covariates will be handled using multiple imputation, as detailed in Section 7.2.2.1.

10.1.3 Sensitivity Analysis

To assess the influence of the missing primary endpoints on the treatment effect estimate[9], sensitivity analyses will be performed using the same statistical methods as described in Section 10.1.1, under the different assumptions of the missing outcome below:

A. Influence of pneumonia occurring prior to nutritional support

Excluding patients having pneumonia before receiving nutritional support.

B. Influence of excluding pneumonia occurring after change in clinical status

Excluding patients having pneumonia after ICU discharge or transition to oral feeding.

C. Influence of missing data

The impact of missing primary outcome data will be evaluated under different assumptions:

- Complete case analysis: Excluding patients with missing pneumonia assessment.
- Worst-case scenario: Imputing all missing outcomes as events (pneumonia).

D. Influence of center effect

A generalized estimating equations (GEE) approach with an exchangeable correlation structure and robust standard errors, treating center as a cluster. If the covariates adjusted GEE Poisson model does not converge, IPTW method will be used.

10.1.4 Subgroup Analysis

To explore the potential heterogeneity of the treatment effect on the primary endpoint, subgroup analyses will be performed on the following baseline characteristics:

- Age (≤70 years vs. >70 years)
- Stroke severity (NIHSS at ICU admission 11-19 vs. ≥20)
- Stroke type (ischemic vs. hemorrhagic)
- Diabetes (no vs. yes)
- History of chronic respiratory disease (No vs. Yes)
- Smoking status (ever-smoker and never-smoker)
- Consciousness level (GCS 9-12 vs. GCS ≤8)
- Time from onset to nutrition initiation (≤24 hours vs. >24 hours)

The treatment effect (RR) and its 95% confidence interval will be estimated for each subgroup using a modified Poisson regression model. The formal test for interaction, which assesses whether the treatment effect differs significantly across subgroups, will be conducted by including a treatment-by-subgroup interaction term in the model. The P-value for this interaction term will be reported. The primary subgroup analyses will be performed on the ITT population, with supportive analyses in the PP population.

10.2 Secondary Efficacy Analysis

Secondary efficacy outcomes will be analyzed according to their variable types as detailed below. All models will include treatment group as the primary predictor. Adjusted analyses will incorporate pre-specified baseline covariates where appropriate.

10.2.1 Binary Outcomes

The following dichotomous endpoints will be analyzed using modified Poisson regression (with robust error variances) to calculate crude and adjusted RR with corresponding 95% CI:

- Usage rate of insulin during the first 7 days post-randomization
- Usage rate of prokinetic agents during the first 7 days post-randomization
- Occurrence of nosocomial infections from randomization to ICU discharge
- Incidence of tracheotomy from randomization to ICU discharge
- Incidence of mechanical ventilation from randomization to ICU discharge
- Incidence of CRRT from randomization to ICU discharge
- Incidence of venous thrombosis from randomization to ICU discharge

- Usage rate of vasoactive agents from randomization to ICU discharge
- Excellent functional outcome (mRS 0-1) at 90 days
- Functional independence outcome (mRS 0-2) at 90 days

10.2.2 Continuous Outcomes

The following continuous endpoints will be analyzed using linear regression models with treatment group as the primary predictor and baseline measurement as covariate if available[10]:

- Daily calorie delivery during the first 7 days post-randomization
- Daily protein delivery during the first 7 days post-randomization
- Length of ICU stay
- National Institutes of Health Stroke Scale (NIHSS) score at ICU discharge
- Glasgow Coma Scale (GCS) score at ICU discharge

For each outcome, the crude and adjusted mean difference with corresponding 95% CI will be reported. The normality of model residuals will be assessed using Q-Q plots. If the normality assumption is seriously violated, the win ratio method will be applied[11,12]. Crude and adjusted win ratio will be calculated using WINS package. Adjusted win ratio will be calculated using IPTW approach.

10.2.3 Ordinal Outcomes

The mRS is an ordered scale of global disability ranging from 0 (no symptoms) to 5 (severe disability), with death assigned a score of 6. The distribution of the 90-day mRS will be summarized for each treatment group and presented graphically using Grotta bars. The primary analysis for this outcome will employ an ordinal logistic regression model, fitted to estimate the common odds ratio (cOR) and its 95%CI, representing the odds of a shift towards better functional outcomes with the intervention. The proportional odds assumption will be formally assessed. If this assumption holds (score test p-value > 0.05), the cOR from the ordinal model will be reported. If the assumption is violated, the generalized odds ratio (GenOR) and its 95% CI will be derived as a robust alternative measure of treatment effect[13].

10.2.4 Time-to-Event Outcomes

Time from randomization to onset of post-stroke pneumonia within 7 days will be analyzed using survival methods. The Fine-Gray subdistribution hazards model will be employed to account for the competing risk of death and successful transition to oral feeding prior to pneumonia onset[14]. Subdistribution hazard ratios (sHR) and 95% confidence intervals will be reported.

10.2.5 Composite Outcomes

The hierarchy of composite endpoint of all cause death or incidence of pneumonia at 7 days will be primarily analyzed using a win ratio method. The hierarchy of the composite endpoint is death due to all cause (time-to-event) and then incidence of pneumonia at 7 days (time-to-event). Similarly, the corresponding composite endpoint within 90 days will be analyzed using the same method. Crude and adjusted win ratio via IPTW will be estimated using WINS package. Additionally, the time from randomization to the first occurrence of either all-cause death or pneumonia within 90 days will be analyzed as a time-to-event outcome using the Fine-Gray subdistribution hazards model.

11. Safety Analysis

Safety analyses will be conducted on the Safety population only.

11.1 Binary Outcomes

The following endpoints will be analyzed using modified Poisson regression (with robust error variances) to calculate crude and adjusted RR with corresponding 95% CI:

- All-cause mortality during ICU stay
- All-cause mortality at 28 days
- Incidence of cardiac failure from randomization to ICU discharge
- Incidence of individual gastrointestinal complications (vomiting, diarrhea, gastric retention, and gastrointestinal bleeding) within the first 7 days post-randomization.

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Summary of Changes - Statistical Analysis Plan OPENS-2 Version 1.0- Version 2.0

Below is the table of changes. Deleted items are identified with Strikethrough font. Additional wording is in bold font. Revised text is formatted as bold and underlined.

Section(s)	**Protocol Version 1.0 Change From:**	**Protocol Version 2.0** **Change To:**	**Rationale**
Section 3.2 Secondary Efficacy Endpoints	Insulin dose during the first 7 days post-randomization (continuous)	• Insulin **utilization** during the first 7 days post-randomization (Binary) • **Excellent functional outcome (mRS 0-1) at 90 days (binary)** • **Functional independence outcome (mRS 0-2) at 90 days (binary)**	To enhance clinical interpretability and reduce variability from dose titration
Section 3.4 Exploratory endpoints		Add: **Exploratory endpoints** • **Hierarchical composite endpoint of all-cause mortality and post-stroke pneumonia within 90 days (win ratio).** • **Hierarchical composite endpoint of all-cause mortality and post-stroke**	To provide a holistic assessment of the intervention's clinical benefit

		pneumonia within 7 days (win ratio). • **Time from randomization to the first occurrence of either all-cause death or post-stroke pneumonia within 90 days (time to event).**	
Section 4.2.2 Exclusion Criteria		Add: • **Admission with infection signs** • **Antibiotics were used within the previous 7 days** • **Subarachnoid hemorrhage, cerebral arteriovenous malformation**	To be consistent with the Change of Inclusion Criteria of Protocol
Section 7.2.2 Missing data	Logistic regression model with the following covariates: allocated treatment, age, NIHSS at ICU admission, stroke type, and history of diabetes will be used and implemented using SAS PROC MI with a seed being 224.	Logistic regression model with the following covariates: allocated treatment, age, NIHSS at ICU admission, stroke type, history of diabetes, **history of chronic respiratory disease, and smoking** will be used and implemented using SAS PROC MI with a seed being 224.	To improve the accuracy of missing data imputation and reduce potential confounding in outcome analysis

Section 7.6 Covariates		Add: • **Chronic respiratory disease (no vs. yes)** • **Smoking (Binary: ever-smoker and never-smoker)**	To strengthen the statistical model by more comprehensively adjusting for known prognostic factors and potential confounders
Section 7.7 Planned Group		Add: • **History of chronic respiratory disease (No vs. Yes)** • **Smoking status (ever-smoker and never-smoker)**	To allow for a more comprehensive exploration of treatment effect heterogeneity.
Section 9 Demographics and Baseline Characteristics		Add: • **Previous stroke** • **For patients with wake-up stroke, Onset to Randomization will be recorded as the time from last known without progression to randomization.**	• To better describe the enrolled population. • Specify how patients who wake from sleep having progress will be handled.

Section 10.1.2 Covariates Adjusted Analysis	The analysis will be adjusted for the following pre-specified baseline covariates: age, baseline NIHSS score, stroke type, and diabetes.	The analysis will be adjusted for the following pre-specified baseline covariates: age, baseline NIHSS score, stroke type, history of stroke, diabetes, **chronic respiratory disease, and smoking status.**	To strengthen the statistical model by more comprehensively adjusting for known prognostic factors and potential confounders, leading to a more reliable and unbiased estimate of the treatment effect.
Section 10.1.3 Sensitivity Analysis		Add: **A. Influence of pneumonia occurring prior to nutritional support** **Excluding patients having pneumonia before receiving nutritional support.** **B. Influence of excluding pneumonia occurring after change in clinical status** **Excluding patients having pneumonia**	To provide a more accurate estimate of the pneumonia risk.

		after ICU discharge or transition to oral feeding.	
Section 10.1.4 Subgroup Analysis		Add: **History of chronic respiratory disease (No vs. Yes)** **Smoking status (ever-smoker and never-smoker)**	To allow for a more comprehensive exploration of treatment effect heterogeneity.
Section 10.2.1 Binary Outcomes		Add: • **Usage rate of insulin during the first 7 days post-randomization** • **Excellent functional outcome (mRS 0-1) at 90 days (binary)** • **Functional independence outcome (mRS 0-2) at 90 days (binary)**	To be consistent with the Change of Secondary Efficacy Endpoints

Section 10.2.2 Continuous Outcomes	• Daily calorie delivery during the first 7 days post-randomization • Daily protein delivery during the first 7 days post-randomization • Insulin dose during the first 7 days post-randomization • Length of ICU stay • National Institutes of Health Stroke Scale (NIHSS) score at ICU discharge • Glasgow Coma Scale (GCS) score at ICU discharge	• Daily calorie delivery during the first 7 days post-randomization • Daily protein delivery during the first 7 days post-randomization • Length of ICU stay • National Institutes of Health Stroke Scale (NIHSS) score at ICU discharge • Glasgow Coma Scale (GCS) score at ICU discharge	To be consistent with the Change of Secondary Efficacy Endpoints
`10.2.5 Composite Outcomes		Add: Composite Outcomes **The hierarchy of composite endpoint of all cause death or incidence of pneumonia at 7 days will be primarily analyzed using a win ratio method. The hierarchy of the composite endpoint is death due to all**	To provide a holistic assessment of the intervention's clinical benefit

		cause (time-to-event) and then incidence of pneumonia at 7 days (time-to-event). Similarly, the corresponding composite endpoint within 90 days will be analyzed using the same method. Crude and adjusted win ratio via IPTW will be estimated using WINS package. Additionally, the time from randomization to the first occurrence of either all-cause death or pneumonia within 90 days will be analyzed as a time-to-event outcome using the Fine-Gray subdistribution hazards model.	