Statistical Analysis Plan

Triglyceride-lowering therapy with pemafibrate for prevention of atherosclerotic cardiovascular disease in patients with symptomatic intracranial artery stenosis: a multi-center, open-label, randomized controlled trial (PPAR-ICAS)

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1. Purpose

The purpose of this Statistical Analysis Plan is to prespecify the statistical methods for analyzing the primary and secondary endpoints of the PPAR-ICAS trial and thereby ensure the validity and transparency of the analyses.

2. Primary Endpoint

- Progression in intracranial arterial stenosis on CTA at 12 months from enrollment (progression vs. no progression [no change or improvement]).

Stenosis is assessed by the WASID method[1]; progression is defined as an absolute increase of >=10 percentage points in percent stenosis or the development of occlusion, stability as a change of <10 percentage points in either direction, and improvement as an absolute decrease of >=10 percentage points. For example, a lesion with 50% stenosis at baseline will be considered to have progressed if it worsens to >=60%, and a lesion with >=90% stenosis at baseline will be considered to have progressed if it becomes 100% occluded. Percent stenosis will be measured centrally using automated analysis software (Aquarius iNtuition Viewer; Terarecon, Durham, NC). Head CTA interpretation and stenosis measurement will be performed, with treatment allocation masked, by two independent expert readers (e.g., neurologists or radiologists) who are not involved in clinical care at the participating sites. If the two readers disagree regarding progression, no change, or improvement, the final judgment will be reached by consensus or, when necessary, adjudication by a third evaluator. Representative cases and an evaluation manual will be shared before evaluation begins to standardize assessment criteria.

3. Key Secondary Endpoints (supplementary analyses of the primary endpoint)

- Change in intracranial arterial stenosis on CTA at 12 months from enrollment (three categories: progression, no change, improvement).
- Improvement in intracranial arterial stenosis on CTA at 12 months from enrollment (improvement vs. no improvement [progression or no change]).

In this study, secondary endpoints that are considered most important after the primary endpoint are defined prospectively as key secondary endpoints. These correspond to supplementary analyses intended to aid interpretation of the primary endpoint. Because both key secondary endpoints directly capture change in intracranial arterial stenosis itself, they are positioned separately from other secondary endpoints as outcomes that are particularly relevant to mechanistic understanding and interpretation of the study results.

4. Other Secondary Endpoints

- Change in percent stenosis by the WASID method.
- Proportion of intracranial arterial stenosis progression/improvement per the TOSS[2] and TOSS-2[3] criteria (**Table 1**).

- Proportion of intracranial arterial stenosis progression/improvement per the Wong KS criteria (**Table 2**)[4].
- Major cardiovascular events (MACE): the following individual events and their composite: ischemic stroke (fatal, nonfatal), TIA, intracranial hemorrhage (fatal, nonfatal), any stroke (ischemic stroke, TIA, intracranial hemorrhage), myocardial infarction (fatal, nonfatal), any coronary artery disease event (myocardial infarction or angina treated with PCI or CABG), symptomatic peripheral artery disease (with intermittent claudication, ulceration, or gangrene, or requiring revascularization), vascular death, and all-cause mortality.
- Activities of daily living by mRS score (mRS 0-1, 0-2, and 0-3 proportions, and the overall mRS score distribution).
- Changes in cerebral small vessel disease on brain MRI (Fazekas scale, cerebral microbleeds, total SVD score).
- Changes in ABI, CAVI, and PWV.
- Changes in blood biomarkers: complete blood count; fasting TG, HDL-C, LDL-C, RLP-C; apolipoprotein C-III; lipoprotein(a); fasting plasma glucose; HbA1c; AST, ALT, gamma-GTP; creatinine, eGFR; CK; CRP, high-sensitivity CRP; IL-6; total homocysteine; PT-INR; APTT; fibrinogen; D-dimer.
- Safety: occurrence of adverse events and illnesses.

Table 1. TOSS / TOSS-2 Grading Criteria

Grade	Finding
1	Normal
2	Mild (<50% stenosis)
3	Moderate (>=50% stenosis)
4	Severe (loss of flow signal at the stenotic segment, with preserved distal flow signal)
5	Occlusion (complete loss of flow signal)

An increase of at least one grade is defined as progression, and a decrease in grade is defined as improvement.

Table 2. Wong KS Grading Criteria

Grade	Finding
1	Normal or 0-29% stenosis
2	30-69% stenosis
3	70-99% stenosis
4	Occlusion

An increase of at least one grade is defined as progression, and a decrease in grade is defined as improvement.

5. Definition of Analysis Sets

Full Analysis Set (FAS): The FAS will consist of all registered participants except ineligible cases, untreated cases, and participants with no post-treatment data for efficacy endpoints. In this trial, the primary analysis will follow the Intention-To-Treat (ITT) principle using the FAS. In other words, the analysis population will consist of randomized participants who received at least one dose of study treatment and had at least one post-baseline efficacy assessment.

Per Protocol Set (PPS): The PPS will consist of FAS participants excluding those with major protocol noncompliance or discontinuation. The PPS will be used for supportive analyses to examine the consistency of results with those obtained in the FAS.

Safety Analysis Set: The safety analysis set will consist of all registered participants except untreated cases.

6. Target Sample Size and Rationale

Target sample size: 270 participants.

Rationale: The hypothesis to be tested in this study is that the novel TG-lowering agent pemafibrate prevents progression of intracranial arterial stenosis. Akins et al., using DSA, reported >=10% progression of intracranial arterial stenosis in 40% of patients over 26.7 months.[5] In the TOSS trial using MRA, progression defined by a rough five-grade classification (normal, mild, moderate, severe, occlusion) occurred in 29% of patients in the placebo group over 6 months.[2] In the similarly designed TOSS-2 trial, progression occurred in 16% of patients in the clopidogrel group over 7 months.[3] In our own MRA-based study, progression of intracranial arterial stenosis in pemafibrate-treated patients was 8.3% over 2 years, roughly half the rate reported previously.[6] Because CTA, which will be used in this study, allows more accurate measurement than MRA, more patients may be classified as having progression than in previous reports. Based on previous reports and our clinical experience, the progression rate in the non-pemafibrate group is expected to be at least 30% per year. Accordingly, in this study the stenosis progression rate is assumed to be 30% per year in the non-pemafibrate group, and pemafibrate treatment is assumed to reduce this by 50% to 15% per year. With a two-sided alpha error of 0.05 and a power of 0.8, the required sample size is calculated to be 121 participants per group, for a total of 242. Allowing for dropout, the target sample size required for evaluation of the primary endpoint is set at 270 participants (135 per group).

7. Statistical Analysis Methods

7.1 Descriptive Statistics for Baseline Characteristics

Participant baseline characteristics will be summarized descriptively for the overall population and by treatment group. Continuous variables will be presented as mean and standard deviation, together with median, interquartile range, minimum, and maximum. Categorical variables will be presented as counts and percentages. For between-group comparisons, continuous variables will be analyzed using the t-test and, when normality assumptions are not satisfied, the Wilcoxon rank-sum test as a supplementary method. Categorical variables will be analyzed using the chi-square test or Fisher's exact test.

7.2 Analysis of the Primary and Key Secondary Endpoints

For the primary endpoint, defined as the presence or absence of progression of intracranial arterial stenosis on head CTA at 12 months, and for the key secondary endpoint, defined as the presence or absence of improvement of intracranial arterial stenosis on head CTA at 12 months, the between-group difference in proportions will first be evaluated using the chi-square test (or Fisher's exact test when appropriate). In addition, logistic regression models will be fitted with intervention versus non-intervention as the principal explanatory variable and the presence or absence of progression or improvement as the response variable to estimate odds ratios and 95% confidence intervals. Allocation factors used for randomization may be included as covariates as necessary. Because this is a randomized controlled trial, marked imbalance in baseline factors between groups is not anticipated; however, if clinically non-negligible imbalance is observed, such factors will also be included as covariates and covariate-adjusted odds ratios will be estimated. When appropriate, between-group comparison using the Cochran-Mantel-Haenszel test stratified by allocation factors will also be performed.

For the key secondary endpoint defined as change in intracranial arterial stenosis on head CTA at 12 months (three levels: progression, no change, and improvement), differences in the three-level distribution will first be evaluated using the chi-square test. In addition, an ordinal logistic regression model (proportional odds model; shift analysis) will be fitted with intervention versus non-intervention as the principal explanatory variable and three-level change in intracranial arterial stenosis (progression, no change, or improvement) as the response variable to estimate the common odds ratio and 95% confidence interval. In the ordinal logistic regression model, allocation factors used for randomization may be included as covariates as necessary. If clinically non-negligible imbalance in baseline factors is observed, such factors will also be included as covariates and covariate-adjusted odds ratios will be estimated. When appropriate, between-group comparison using the Cochran-Mantel-Haenszel test stratified by allocation factors will also be performed.

7.3 Analysis of Other Secondary Endpoints

Other secondary endpoints will be analyzed using appropriate statistical methods according to their characteristics. Continuous variables (e.g., TG levels, degree of stenosis, blood pressure, inflammatory markers, glycemic indices, renal function indices, and ABI/CAVI/PWV values) will be compared between groups using t-tests, Wilcoxon rank-sum tests, ANCOVA, or other appropriate methods, with adjustment for baseline values as covariates when necessary. Three-level change in intracranial arterial stenosis (progression, no change, and improvement) will be analyzed using the chi-square test, Mantel-Haenszel test, and ordinal logistic regression model (shift analysis). For mRS, distributional comparison (e.g., Wilcoxon rank-sum test) and shift analysis (ordinal logistic regression) will be performed, and supplementary binary analyses using cutoffs at mRS 2 or 3 will also be conducted. For event occurrence, the chi-square test or Fisher's exact test will be used, and endpoints involving time-to-event will additionally be analyzed using Kaplan-Meier methods and Cox proportional hazards models.

As exploratory analyses, waterfall plots will be used to visualize the distributions of change in TG and change in percent stenosis for individual participants. By arranging participants in order of the magnitude of change before and after intervention, waterfall plots will be used as a means of intuitively assessing heterogeneity of treatment effect.

In addition, exploratory analyses will evaluate the correlation between the rate of change in TG and the rate of change in percent stenosis. The relationship between the two variables will be analyzed using Pearson or Spearman rank correlation coefficients and, when appropriate, visualized using scatter plots and regression lines. Stratified comparisons according to baseline TG level and the magnitude of TG change will also be performed. Similarly, correlation analyses between change in percent stenosis and rates of change in inflammatory markers (e.g., hs-CRP and IL-6), other lipid parameters (e.g., HDL-C and LDL-C), blood pressure (systolic and diastolic), glycemic indices (HbA1c and fasting glucose), and renal function indices (e.g., eGFR) will be conducted.

Within-subject changes before and after treatment will be analyzed according to the type of outcome. Continuous variables will be analyzed using the paired t-test and, when the normality assumption is not satisfied, the Wilcoxon signed-rank test. For categorical variables, McNemar's test will be used for binary variables and Bowker's test for multinomial variables.

7.4 Sensitivity Analyses

Sensitivity analyses are intended to examine the consistency of results for the primary endpoint under different assumptions, with and without imputation of missing data, and across analysis populations (FAS and PPS). In particular, stratified analyses will be performed according to the following baseline factors: age, sex, BMI, hypertension, diabetes mellitus, CKD, TG level, HDL-C level, LDL-C level, degree of stenosis, stenotic lesion site, time from TIA/ischemic stroke onset, antiplatelet therapy, and statin use. Analyses excluding outliers and re-analyses after imputation of missing data will also be performed as sensitivity analyses.

7.5 Subgroup Analyses

In addition to sensitivity analyses, differences in treatment effect on the primary and secondary endpoints will be explored in prespecified clinically important subgroups. Major stratification factors will include TG level (<150 mg/dL vs. >=150 mg/dL), LDL-C level (<100 vs. >=100 mg/dL), HDL-C level, concomitant statin use, antithrombotic therapy, degree of stenosis (moderate, 50-69%, vs. severe, 70-99%), time from TIA/ischemic stroke onset (<1 month vs. >=1 month), sex, and age group (<65 years vs. >=65 years), among others. Between-group differences within each subgroup will be evaluated and presented visually using forest plots.

7.6 Analysis of Safety Endpoints

Analyses will be performed in the safety analysis set. Death and the occurrence of adverse drug reactions will be evaluated using chi-square tests and, where appropriate, Kaplan-Meier curves with log-rank tests.

7.7 Interim Analysis
No interim analysis is planned for this trial.

7.8 Final Analysis
Analyses will be performed after the available data have been fixed. The lead statistician will prepare an analysis report and submit it to the principal investigator.

7.9 Presentation of P values and Confidence Intervals
For all statistical analyses, the significance level will be two-sided 5%. P values will be displayed to three decimal places; values less than 0.001 will be reported as 'P < 0.001'. Analysis results will present effect estimates and 95% confidence intervals as the primary results, with P values shown as supplementary information. The types of effect measures are as follows:
- Binary outcomes: odds ratio (OR) or risk ratio (RR).
- Time-dependent events: hazard ratio (HR).
- Continuous variables: between-group difference in means.

P values and effect measures from exploratory analyses will be presented as reference information and interpreted with appropriate caution.

8. Data Lock and Quality Control
8.1 Procedures for Data Lock
Statistical analyses will be performed after treatment with medical products has been completed in all participants, all data including the primary endpoint have been entered and verified, and the database has been locked. Analyses will be conducted according to prespecified programs. Data quality control will be performed through monitoring and data cleaning.

8.2 Handling of Missing Data
If missing data exist for primary or secondary endpoints, the occurrence and reasons for missingness will be classified and recorded, and appropriate methods for imputation or exclusion will be applied as appropriate for the analysis. When missing-data imputation methods such as multiple imputation are used, results before and after imputation will be compared to assess the impact.

8.3 Handling of Dropouts
The timing and reasons for dropout will be recorded, and participants will be included in the primary analysis to the extent that observational data are available. Analyses will use data obtained up to the time of dropout, and the consistency of results will be examined in sensitivity

analyses. Even when imaging assessment at 1 year is incomplete, events occurring during the preceding follow-up period (e.g., vascular events or adverse events) and test data (e.g., blood test results) will be included in analyses to the extent available.

8.4 Handling of Outliers and Abnormal Values

Outliers and abnormal values will be identified on the basis of prespecified thresholds and statistical criteria such as Z scores. In sensitivity analyses, such outliers will be excluded and the analyses repeated to assess their impact on the results.

8.5 Definition of Censoring

In survival analyses, loss to follow-up or the end of observation at study completion will be treated as censoring. For participants without an event who become lost to follow-up, the date of last confirmation will be used as the censoring time in the analysis. Even after study completion, follow-up will be continued to the extent possible for ascertainment of events (e.g., death and stroke), and additional submission of case report forms may be requested.

9. Statistical Software

Statistical analyses will be performed using JMP (version Pro 17 or later) and Stata (version 18 or later). The versions used and major packages will be recorded and specified in the analysis report.

10. Criteria for Discontinuation of the Trial

Continuation of the trial will be reviewed if any of the following applies:

1. Recruitment is difficult and achievement of the target sample size is judged to be virtually impossible.
2. The study objective has been achieved before the planned sample size or study period is reached.
3. Continuation of the study is judged to provide no benefit.
4. An unpredictable serious adverse event has occurred for which a causal relationship cannot be ruled out.
5. The Certified Clinical Research Review Board or the Minister of Health, Labour and Welfare requests discontinuation.
6. A major study violation has been identified.

11. Handling of Protocol Deviations

If a protocol deviation occurs, its details, cause, and impact will be documented, and a decision will be made as to whether the affected participant should be excluded from the analysis set, taking into account the impact on the primary endpoint. Major deviations (e.g., violation of the randomization procedure or deviation from the dosing schedule) will result in exclusion from the PPS.

12. Amendment Procedures

If any change is made to the original statistical analysis plan, the study protocol or this Statistical Analysis Plan will be revised, and the change will also be described in the final clinical study report.

13. Trial Registration Information and Data Sharing Policy

This study is planned to be prospectively registered in the following clinical trial registries:
jRCT: jRCTs031260001
ClinicalTrials.gov: registration number to be provided later.

In addition, after completion of the analyses, the principal dataset will be shared and made publicly available in accordance with the policy of the relevant journal, with due consideration for the protection of personal information.

14. References

1. Chimowitz MI, et al. Comparison of warfarin and aspirin for symptomatic intracranial arterial stenosis. N Engl J Med. 2005 Mar 31;352(13):1305-16.
2. Kwon SU, et al. Cilostazol prevents the progression of the symptomatic intracranial arterial stenosis: the multicenter double-blind placebo-controlled trial of cilostazol in symptomatic intracranial arterial stenosis. Stroke. 2005 Apr;36(4):782-6.
3. Kwon SU, et al. Efficacy and safety of combination antiplatelet therapies in patients with symptomatic intracranial atherosclerotic stenosis. Stroke. 2011 Oct;42(10):2883-90.
4. Wong KS, et al. Variability of magnetic resonance angiography and computed tomography angiography in grading middle cerebral artery stenosis. Stroke. 1996 Jun;27(6):1084-7.
5. Akins PT, et al. Natural history of stenosis from intracranial atherosclerosis by serial angiography. Stroke. 1998 Feb;29(2):433-8.
6. Hoshino T, et al. Effect of Pemafibrate on Cerebrovascular Atherosclerosis in Patients with Stroke and Hypertriglyceridemia. J Atheroscler Thromb. 2025 Jun 1;32(6):676-687.