Low- versus high-dose glucocorticoids combined with rituximab in the induction therapy for new-onset anti-neutrophilic cytoplasmic autoantibody-associated vasculitis: An open-label, multi-centre, randomised controlled trial

Phase IV clinical trial

Statistical Analysis Plan

Coordinating investigator:
Assistant Professor, Clinical Research Center, Chiba University Hospital, Department of Allergy and Clinical Immunology
Shunsuke Furuta Seal
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Responsible statistician:
Clinical Research Center, Chiba University Hospital
Yohei Kawasaki Seal

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29-Oct-2014 Version 1.0 of the plan prepared

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1. Trial objectives

The objective of this trial is to evaluate the non-inferiority of low- versus high-dose glucocorticoid therapy combined with rituximab in the induction of remission in patients with new-onset anti-neutrophilic cytoplasmic autoantibody (ANCA)-associated vasculitis by the 6-month time point.

1.1. Primary endpoint

[Efficacy]

Percentage of patients achieving remission by the 6-month time point = Number of patients in remission (after 6 months) / Total number of patients

1.2. Secondary endpoints

[Efficacy]

(1) Time to remission

(2) Overall survival (OS), relapse-free survival (RFS), time to end-stage renal disease (ESRD) and time to first serious adverse event (SAE)

- Each endpoint is defined as the time from randomisation to the onset of each event, with all others except the onset of the event censored. The definition of each endpoint and event and the handling of censoring dates are shown in the following table.

Endpoint	**Event (in the event of multiple events, whichever comes first)**	**Censoring date**	**Competing risk**
Time to remission	Remission	Date of the last confirmation that the patient is not in remission	Death
Overall survival	All deaths	Date of the last survival confirmation	None
Relapse-free survival	Any death or relapse	Date of the last confirmation that the patient had not experienced relapse	None
Time to end-stage renal disease (ESRD)	ESRD	Date of the last confirmation that the patient did not have ESRD	Death
Time to first serious	First SAE (including	Date of the last	None

adverse event (SAE)	death and hospitalisation)	confirmation that the patient had not experienced SAEs	

(3) Death, relapse (mild vs. severe), ESRD, percentage of SAEs and corresponding composite outcome rate (after 6 vs. 24 months; *however, only the frequencies of death, ESRD and SAEs were assessed at the 6-month time point)
(4) Percentage of mild and severe relapses (after 24 months)
(5) Percentage of patients who achieved remission and discontinued the use of glucocorticoids
(6) Assessment of disease activity using the Birmingham Vasculitis Activity Score (BVAS) version 3 (after 6 and 24 months)
(7) Assessment of irreversible damage using the Vasculitis Damage Index (VDI) (after 24 months)
(8) Health-related quality of life assessment using the 36-Item Short Form Health Survey (SF-36) (after 6 and 24 months)
(9) Overall assessment of patients using the Vasculitis Damage Index (VAS) (after 6 and 24 months)
(10) Cumulative glucocorticoid use (after 6 and 24 months)

[Safety]
(1) Number and percentage of adverse events (AEs) and SAEs (after 6 and 24 months)
(2) Percentage of new patients with diabetes mellitus, hypertension and dyslipidaemia requiring treatment (after 6 and 24 months)
(3) Percentage of patients with sleep disorders requiring treatment (after 6 and 24 months)
(4) Pathologic fracture rate and lumbar bone mineral density (BMD) (after 6 and 24 months)
(5) Number and percentage of infections for which intravenous or oral antibiotics were administered (after 6 and 24 months)

1.3. Exploratory endpoints
(1) Concentration of immunoglobulins (Ig)
(2) B-cell counts in whole blood
(3) Changes in myeloperoxidase (MPO)-ANCA/proteinase 3 (PR3)-ANCA levels associated with treatment, response to treatment and relapse

Endpoint	**Event (in the event of multiple events, whichever comes first)**	**Censoring date**	**Competing risk**
Time to B-cell recovery	B-cell recovery	Date of the last confirmation that B-cells	Death

		had not recovered	

2. General topics regarding statistical analyses

2.1. Interim analysis

No interim analysis will be performed in this study.

2.2. Handling of missing values

Sensitivity analyses will be performed for dropouts or missing data using the mixed-effects models for repeated measures, last observation carried forward or multiple imputation, as necessary.

2.3. Data transformation

If variable transformations such as square root and logarithmic transformations are performed, the details of the transformation method are to be described under each section of the statistical analysis plan.

2.4. Significance and confidence levels

Unless otherwise specified, the two-sided significance level used in the tests will be 5% (or 2.5% for the one-sided significance level) and the two-sided 95% confidence intervals will be calculated.

2.5. Multiplicity adjustments

Unless otherwise specified, no multiplicity adjustments will be performed.

2.6. Subgroup analyses

Not applicable

2.7. Common rules for data handling

<u>Rules on the number of days and duration</u>

- In calculating the number of days, 1 is added after the start date is subtracted from the end date.
  Examples:
  - Days of administration: If administration is completed on the start day of administration, the administration period is 1 day.
  - Time to onset of AE: If an AE occurs the next day after the start of administration, the time to the first onset of AEs is 2 days.
  - OS: If the patient dies the next day after the start of administration, the duration of survival is 2 days.
- The number of days is converted into years, months and weeks using 365.25, 30.4375 and 7 days per year, month and week.
- For the display of the ‘time from the start of administration (day)’ or ‘onset date (day)’, the start

day of administration is Day 1, the day prior to the start day of administration is Day −1 and so on by counting up or down by 1 day.

Decimal places and rules for rounding

- Mean, standard deviation (SD) and median values are displayed up to one digit below the raw data (round off the lower two digits).
- Percentages should be displayed up to the first decimal place (e.g. 12.3%). The second decimal place should be rounded off.
- When calculating statistics, such as the mean, SD and median, rounding off should not be performed during the calculation but should be performed on the final calculation result.

3. Statistical analysis populations

3.1. Definition of analysis populations

3.1.1. Full analysis set

The full analysis set (FAS) will include all patients enrolled in the study with no major protocol violations, such as consent not obtained and enrolment outside the contract period, and who have received at least one study drug dose after randomisation and have undergone at least one efficacy endpoint measurement.

Baseline efficacy data	Study drug administration after randomisation	Efficacy data after randomisation	FAS
None	None	None	x
None	None	Yes	x
None	Yes	None	x
None	Yes	Yes	○
Yes	None	None	x
Yes	None	Yes	x
Yes	Yes	None	○
Yes	Yes	Yes	○

3.1.2. Per protocol set

The per protocol set (PPS) will include all patients from the FAS excluding those with protocol violations, such as study procedure or combination therapy violations, as described below.

- Inclusion criteria violations
- Exclusion criteria violations
- Compliance <75%

  Compliance = Cumulative study drug (rituximab) dose/planned cumulative study drug dose

3.1.3. Safety analysis set

The safety analysis set will include all patients enrolled in the study and who have received at least one study drug dose. In addition, patients will be included in the treatment group of the actual study treatment administered.

3.2. Handling of the elements of statistical analyses

The FAS will be used for all primary analyses of efficacy, and as necessary, sensitivity analyses will be performed in the PPS. Furthermore, safety analyses will be performed in the safety analysis set.

4. Analysis plan for patient disposition

The breakdown of the following will be tabulated, and the frequencies and percentages will be shown.

- Enrolled patients
- Safety analysis set
- Untreated patients
- FAS
- Patients for whom consent was not obtained
- Patients enrolled outside the contract period
- PPS
- Patients with inclusion criteria violations
- Patients with exclusion criteria violations
- Patients with <75% compliance
- Patients discontinued from the study (summarised by reason for discontinuation)

5. Analysis plan for patient background characteristics

The distribution of patient background characteristic data and summary statistics for each analysis set will be calculated by group. For nominal variables, the frequency and percentage of categories are presented by group. For continuous variables, summary statistics, such as number of patients, mean, SD, minimum, median, maximum, and interquartile ranges (IQR), will be calculated by group. Moreover, for group comparisons, Pearson's chi-square test will be used for nominal variables. However, if the expected frequency of cells that are <5 is ≥20%, Fisher's exact test (Cochran, *Ann Math Stat* 1952; **23**:315-345) and *t*-tests will be used for nominal and continuous variables, respectively. In addition, the two-sided significance level will be set at 5%. The following is a list of nominal and continuous variables:

- Nominal variables

  Age (<65 vs. ≥65 years), renal function (estimated glomerular filtration rate [eGFR] <50 mL vs. ≥50 mL per min), ANCA (MPO-ANCA vs. PR3-ANCA), sex, diagnosis, medical history, complications and use or non-use of prednisolone during the period from screening to enrolment
- Continuous variables

  Prednisolone dose from screening to patient enrolment

6. Primary endpoint analysis plan

The primary objective of this study is to evaluate the non-inferiority of low- versus high-dose glucocorticoid therapy combined with rituximab in the induction of remission in patients with new-onset ANCA-associated vasculitis at the 6-month time point.

Therefore, the risk differences in the percentages of patients in remission at 6 months (percentage of patients in remission in the low-dose group − percentage of patients in remission in the high-dose group) between the high- and low-dose glucocorticoid therapy combined with rituximab groups (high- and low-dose groups, respectively) will be assessed in the primary analysis. If the lower limit of the two-sided 95% confidence interval for the risk difference is >−0.2, non-inferiority will be considered statistically significant. In addition, the *P*-value for the non-inferiority hypothesis test (H_0: percentage of patients in remission in the low-dose group − percentage of patients in remission in the high-dose group, ≤−0.2 vs. H_1: percentage of patients in remission in the low-dose group − percentage of patients in remission in the high-dose group, >−0.2) will be calculated. The interval estimates of risk differences and calculation of *P*-values will be based on Wald statistics.

As a sensitivity analysis, 95% confidence intervals for adjusted risk differences will also be calculated. Adjustment factors will be used as stratification factors (age at the time of informed consent, eGFR, ANCA). Moreover, the interval for the adjustment of risk differences will be estimated using the two-sided 95% confidence intervals for the Mantel–Haenszel risk difference by Sato (1989).

7. Secondary endpoint analysis plan

7.1. Secondary efficacy endpoints

The secondary efficacy endpoints will be analysed to supplement the main analysis results of this study. Multiplicity adjustments will not be performed for secondary endpoint analyses. Furthermore, the two-sided significance level of the hypothesis test will be set at 5%, and the two-sided 95% confidence intervals will be calculated.

(1) Duration of response (DOR), OS, RFS, time to ESRD and time to first SAE

The duration of survival and competing risk analyses will be performed for the time to each event, starting at the time of randomisation.

The log-rank test will be used to compare OS, RFS and time to first SAE between treatment groups, the Kaplan–Meier method will be used to estimate survival function and the median time to event will be calculated. The Kaplan–Meier plots for survival functions will be shown. Furthermore, the Cox proportional hazards model will be used to estimate the hazard ratio for the high- versus low-dose groups and the corresponding two-sided 95% confidence intervals. In addition, a hypothesis test will be conducted on the assumption that the hazard ratio for the null hypothesis is 1. The two-sided significance level will be set at 5%.

For the DOR and time to ESRD, cumulative incidence function (CIF) will be estimated using survival functions estimated on the basis of Kaplan–Meier method, and CIF plots will be shown. Moreover, the Cox proportional hazards and Fine–Gray models will be used to estimate the cause-specific and sub-distribution hazard ratios and the corresponding two-sided 95% confidence intervals for the low- versus high-dose groups. A hypothesis test will also be performed on the assumption that each hazard ratio is 1 and that CIF by event will be equal for the null hypothesis. The two-sided significance level will be set at 5%.

(2) Percentage of deaths, relapses (mild vs. severe), ESRD and SAEs and corresponding composite outcome rates (after 6 vs. 24 months, with only the percentages of deaths, ESRD and SAEs assessed at the 6-month time point)

For deaths, SAEs and corresponding composite outcome rates, the percentage of events occurring at each time point will be estimated using the Kaplan–Meier method and the two-sided 95% confidence intervals will be estimated using the Greenwood method.

For relapses (mild vs. severe) and ESRD, survival functions estimated on the basis of Kaplan–Meier method will be used to estimate the CIF and two-sided 95% confidence intervals at individual time points.

(3) Percentage of mild and severe relapses (after 24 months)

The frequencies and percentages will be presented by group, and Pearson's chi-square test will be used for comparisons between treatment groups (Fisher's exact test will be used if the expected frequency of cells that are <5 is ≥20%). Furthermore, the risk differences and two-sided 95% confidence intervals for each endpoint in the low- versus high-dose groups will be estimated.

(4) Percentage of patients who achieved remission and discontinued glucocorticoids

For the low-dose group, the two-sided 95% confidence intervals for frequency, percentage and percentage using the F distribution will be estimated.

(5) BVAS ver. 3 (after 6 and 24 months), VDI (after 24 months), SF-36 (after 6 and 24 months) and VAS (after 6 and 24 months)

Changes will be summarised using summary statistics (number of patients, mean, SD, minimum, median, maximum, and IQR) and compared between groups using an analysis of variance (ANOVA) model. In addition, for each endpoint, the differences in the population means of the low- versus high-dose groups at each time point and the corresponding two-sided 95% confidence intervals will be estimated. However, for data with repeated measures, the treatment group, time point and correlation between the treatment group and time point are assumed to be fixed effects, and the differences in population means and corresponding two-sided 95% confidence intervals will be estimated using the linear mixed-effects models assuming unstructured correlations for individual patient measurements. In addition, the boxplots of changes will be shown.

(6) Glucocorticoid use (after 6 and 24 months)

The time courses of doses will be summarised using summary statistics (number of patients, mean, SD, minimum, median, maximum, and IQR), and the Wilcoxon rank sum test will be used to compare the cumulative values between the groups. In addition, the boxplots of changes will be shown.

7.2. Secondary safety endpoints

This is a non-inferiority trial, and the expectation is that the frequency of the side effects of glucocorticoids will be reduced in the low-dose group. Multiplicity adjustments will not be performed for the analysis of secondary safety endpoints. Moreover, the two-sided significance level of the hypothesis test will be set at 5%, and the two-sided 95% confidence intervals will be calculated.

(1a) Number and frequency of AEs and SAEs (after 6 and 24 months)

The frequencies and percentages of AEs and SAEs will be presented by treatment group for each category using CTCAE ver. 4. Pearson's chi-square test will be used for comparisons between treatment groups (Fisher's exact test if the expected frequency of cells that are <5 is ≥20%). Also, the risk differences and two-sided 95% confidence intervals for each endpoint in the low- versus high-dose groups will be estimated.

(1b) Number and frequency of ≥grade 3 AEs and SAEs (after 6 and 24 months)

The frequencies and percentages of ≥grade 3 AEs and SAEs will be presented by group using CTCAE ver. 4. Pearson's chi-square test will be used for comparisons between treatment groups (Fisher's exact test if the expected frequency of cells that are <5 is ≥20%). Also, the risk differences and two-sided 95% confidence intervals for each endpoint in the low- versus high-dose groups will be estimated.

(2) Percentage of new patients with diabetes mellitus, hypertension and dyslipidaemia requiring treatment (after 6 and 24 months)

The frequencies and percentages will be presented by group, and Pearson's chi-square test will be used for comparisons between treatment groups (Fisher's exact test if the expected frequency of cells that are <5 is ≥20%). Also, the risk differences and two-sided 95% confidence intervals for each endpoint in the low- versus high-dose groups will be estimated.

(3) Percentage of patients with sleep disorders requiring treatment (after 6 and 24 months)

The frequencies and percentages will be presented by group, and Pearson's chi-square test will be used for comparisons between treatment groups (Fisher's exact test if the expected frequency of cells that are <5 is ≥20%). Also, the risk differences and two-sided 95% confidence intervals for each endpoint in the low- versus high-dose group will be estimated.

(4a) Rate of pathologic fractures (after 24 months)

The frequencies and percentages will be presented by group, and Pearson's chi-square test will be used for comparisons between treatment groups (Fisher's exact test if the expected frequency of cells that are <5 is ≥20%). Also, the risk differences and two-sided 95% confidence intervals for each endpoint in the low- versus high-dose groups will be estimated.

(4b) Lumbar BMD (after 24 months)

Summary statistics (number of patients, mean, SD, minimum, median, maximum, and IQR) will be used to tabulate and compare treatment groups with an ANOVA model. In addition, the

differences in the population means of the low- versus high-dose groups and corresponding two-sided 95% confidence intervals will be estimated.

(5) Number and frequency of infections (for which intravenous or oral antibiotics were administered) (after 6 and 24 months)

The frequencies and percentages will be presented by group, and Pearson's chi-square test will be used for comparisons between treatment groups (Fisher's exact test if the expected frequency of cells that are <5 is ≥20%). Also, the risk differences and two-sided 95% confidence intervals for each endpoint in the low- versus high-dose groups will be estimated.

8. Exploratory analysis plan

The changes in eGFR, immunoglobulin G (IgG) and B-cell counts will be summarised using summary statistics (e.g. number of patients, mean, SD, minimum, median, and maximum) and compared between groups with an ANOVA model. In addition, the differences in the population means of the low- versus high-dose groups and corresponding two-sided 95% confidence intervals will be estimated. The Modification of Diet in Renal Disease Study equation (corrected using the Japanese coefficient) will be used to calculate eGFR, and patients undergoing haemodialysis will be treated as having eGFR = 0 mL/min/1.73 m^2.

The numbers and percentages of B-cell loss/recovery and persistent positive MPO-ANCA/PR3-ANCA conversion to MPO-ANCA/PR3-ANCA negative and prednisolone discontinuation/continuation will be summarised, and Pearson's chi-square test will be used for comparisons between treatment groups (Fisher's exact test if the expected frequency of cells that are <5 is ≥20%). The risk differences and two-sided 95% confidence intervals for each endpoint in the low- versus high-dose groups will also be estimated. In addition, a subgroup analysis for the presence or absence of relapse and a comparison between groups for the presence or absence of relapse will be performed.

The time to B-cell recovery will be analysed for competing risks, starting at the time of randomisation. CIF will be estimated using survival functions assessed on the basis of Kaplan–Meier method. Furthermore, the Cox proportional hazards and Fine–Gray models will be used to estimate the cause-specific and sub-distribution hazard ratios and corresponding two-sided 95% confidence intervals for the low- versus high-dose groups, and CIF plots will be shown. In addition, a hypothesis test will be performed on the assumption that each hazard ratio is 1 and that CIF by event will be equal for the null hypothesis. The two-sided significance level will be set at 5%.

9. Analysis plans for laboratory values and vital signs

The changes in laboratory values, vital signs, body weight and urinalysis (urine creatinine) will be analysed. For each test item, the summary statistics of measurements (number of patients, mean, SD, minimum, median, maximum, and IQR) at each time point will be calculated, and *t*-tests will be used for comparisons between treatment groups. The two-sided significance level will be set at 5%. In addition, the boxplots of changes will be shown.

However, for urinalysis (general urinalysis tests and urine sediment), the frequency and percentage of each test item at each time point will be shown by group, and the Wilcoxon rank sum test will be used for comparisons between treatment groups.

Individual assessments are listed below.

Test	Test item	Time point
Haematology	Red blood cells, haemoglobin, platelets, white blood cells, differential white blood cell and B-cell count	0, 1, 2, 4, 6, 9, 12, 18 and 24 months; treatment completion and study discontinuation
Biochemistry	Total protein, albumin, electrolytes (sodium, potassium, chloride), blood urea nitrogen, serum creatinine, creatine phosphokinase, total bilirubin, aspartate transaminase, alanine transaminase, alkaline phosphatase, lactate dehydrogenase, gamma-glutamyl transferase, C-reactive protein, IgG, IgA, IgM, C3, C4, complement titre, total cholesterol, low- and high-density lipoprotein cholesterol, triglycerides, blood glucose, haemoglobin A1c and enzyme-linked immunosorbent assay method/IF MPO-ANCA and PR3-ANCA	0, 1, 2, 4, 6, 9, 12, 18 and 24 months; treatment completion and study discontinuation
Vital signs	Heart rate, blood pressure (systolic, diastolic) and body temperature	Screening; 0, 1, 2, 4, 6, 9, 12, 18 and 24 months; treatment completion and study discontinuation
Weight	Weight	Screening; 0, 1, 2, 4, 6, 9, 12, 18 and 24 months; treatment completion and study discontinuation

Test	Test item	Time point
Urinalysis	General urinalysis tests (glucose, protein and occult blood) and urinary sediment and creatinine	0, 1, 2, 4, 6, 9, 12, 18 and 24 months; treatment completion and study discontinuation

10. Amendment history

10.1. Changes from the protocol

Date	Name	Distributed to	List of changes
Not applicable			

10.2. Amendment history of the statistical analysis plan

Date	Name	Distributed to	List of changes
2014/04/09	Kengo Nagashima	None	Draft version 0.1 (first draft)
2014/05/13	Kengo Nagashima Yasunori Sato	Study coordinating investigator	Draft version 0.2 (revision based on reviews)
2014/08/28	Kengo Nagashima	None	Draft version 0.3 (revision based on amendment of the protocol draft)
2014/10/29	Kengo Nagashima	None	Draft version 0.4 (revision based on reviews)

11. Statistical analysis administrative structure and environment

11.1. Responsible statistician

Lecturer, Clinical Research Center, Chiba University Hospital

Yasunori Sato

11.2. Statistician responsible for statistical analyses

Assistant Professor, Clinical Research Center, Chiba University Hospital

Kengo Nagashima

11.3. Hardware environment

Analyses will be performed in the following hardware environment:

- OS version higher than Microsoft Windows XP

11.4. Software environment

The following software will be used:

- SAS 9.3 and above
- Microsoft Office 2013 and above
- Adobe Acrobat XI and above

11.5. General information on the format of tables or listings

The paper size will be A4. Figures and summary tables will be in portrait, and listings and data listings will be in landscape orientation in general. However, the format will depend on the characteristics of individual tables and listings. Also, tables and listings will be printed out as Excel or PDF files.

12. Signature/seal

The signature/seal in the following signifies that the content of this statistical analysis plan is valid as a statistical analysis plan for an open-label, multi-centre, randomised controlled trial of low- versus high-dose glucocorticoids combined with rituximab in the induction therapy of new-onset ANCA-associated vasculitis. In addition, it indicates that this statistical analysis plan was finalised prior to the final analysis.

2014 (Month) (Day)

(Seal)