STH20412 WILLOW CCS – Statistical Analysis Plan

STATISTICS AND DATA ANALYSIS

10.1 Sample size calculation

The primary hypothesis of this study is that the difference in post-dose bleeding time between aspirin (aspirin lysine) 75 mg once-daily and aspirin (aspirin lysine) 20 mg twice-daily plus rivaroxaban 2.5 mg twice-daily will be less than the difference in bleeding time between aspirin (aspirin lysine) 75 mg once-daily and aspirin (aspirin lysine) 75 mg once-daily plus rivaroxaban 2.5 mg twice-daily.

Statistical power can be estimated based on previous data from our group showing a mean difference in peak effect bleeding time of 154.5 seconds (18%) between aspirin (aspirin lysine) 20 mg BD (plus ticagrelor 90 mg twice-daily, another antithrombotic drug) and 75 mg OD (also plus ticagrelor 90 mg twice-daily), with an interindividual standard deviation of 316 seconds.

Assuming a similar proportionate difference in bleeding time between the aspirin (aspirin lysine) 20 mg BD and aspirin (aspirin lysine) 75 mg OD-based combination regimens seen in our previous study, and a worst case scenario where the intra-individual standard deviation of the difference is 316 secs (likely to be less because of effective pairing of bleeding time data seen in our previous data), we would have 80% power to detect a difference in the change in bleeding time from aspirin (aspirin lysine) 75 mg OD to aspirin (aspirin lysine) 20 mg BD + rivaroxaban 2.5 mg BD vs. aspirin (aspirin lysine) 75 mg OD to aspirin (aspirin lysine) 75 mg OD plus rivaroxaban 2.5 mg BD, of 115 seconds (one-tailed paired-t test), and 90% power to detect a difference of 135 secs.

Based on our previous data for aspirin (aspirin lysine) 75 mg OD + ticagrelor as a baseline, proportionally this would represent a difference of 14% (power 0.8) or 16% (power 0.9), which we would class as meaningful differences with the potential to reduce the incidence or severity of clinical bleeding events. Sample size calculations have not been prepared for the secondary endpoints as these are exploratory.

10.2 Planned recruitment rate

It is aimed to recruit participants at the minimum of one per week. Based on our group's previous experience of similar studies, this is sensibly feasible.

10.3 Statistical analysis plan

10.3.1 Summary of baseline data and flow of patients

The following baseline data will be collected and reported:

From visit 1

- Demographic data (age, sex, ethnicity)
- Vital signs: pulse, blood pressure and temperature
- Weight and BMI
- Full blood count, urea & electrolytes, liver function tests

Categorical data will be reported as proportions and percentages. Continuous data will be reported as mean and standard deviation if normally distributed otherwise median and interquartile range. Logarithmic transformation will be considered if data are skewed.

Differences between the dosing regimens and timepoints groups will be assessed by paired t-tests and by repeated measures ANOVA with timepoint and treatment as factors.

A CONSORT flow diagram will be prepared for inclusion in the report of study findings.

10.3.2 Primary outcome analysis

The primary endpoint will be based on bleeding time at 2 hours following the last dose of IMP of each treatment period and will be determined as the difference in bleeding time between aspirin (aspirin lysine) 75 mg once-daily alone and either aspirin (aspirin lysine) regimen in combination with rivaroxaban 2.5mg bd, allowing comparison of the increase in bleeding time with aspirin (aspirin lysine) 20 mg twice-daily plus rivaroxaban 2.5 mg twice-daily vs. aspirin (aspirin lysine) 75 mg once-daily plus rivaroxaban 2.5 mg twice daily, compared with a one-tailed paired t-test.

10.3.3 Secondary outcome analysis

Secondary analyses will be as follows:

1. Bleeding time immediately before the last dose of IMP of the treatment period assessed within patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

2. Fibrin clot lag time immediately before the last dose of IMP of the treatment period assessed within patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

3. Fibrin clot lag time 2 hours following the last dose of IMP of the treatment period assessed within patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

4. Fibrin clot lysis time immediately before the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

5. Fibrin clot lysis time 2 hours following the last dose of IMP of the treatment period assessed within patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

6. Plasma TNFα immediately before the last dose of IMP of the treatment period assessed within patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

7. Plasma TNFα 2 hours following the last dose of IMP of the treatment period assessed within patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

8. Plasma IL-6 immediately before the last dose of IMP of the treatment period assessed within patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

9. Plasma IL-6 2 hours following the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

10. Serum CRP at 2 hours following the last dose of IMP of the treatment period assessed within patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

11. Circulating leukocyte count immediately before the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

12. Circulating leukocyte count 2 hours following the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

13. Serum TXB2 immediately before the last dose of IMP of the treatment period assessed within patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

14. Serum TXB2 2 hours following the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

15. Urinary PGI-M immediately before the last dose of IMP of the treatment period assessed within patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus

rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

16. Urinary PGI-M 2 hours following the last dose of IMP of the treatment period assessed within patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

17. Urinary TxM immediately before the last dose of IMP of the treatment period assessed within patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

18. Urinary TxM 2 hours following the last dose of IMP of the treatment period assessed within patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

19. Maximum platelet aggregation responses to arachidonic acid (0.3 and 1 mmol/L), collagen (4 and 16 µg/mL) and ADP (20 µmol/L) immediately before the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using a repeated measures ANOVA, with pairwise comparisons if found to be significant.

20. Maximum platelet aggregation responses to arachidonic acid (0.3 and 1 mmol/L), collagen (4 and 16 µg/mL) and ADP (20 µmol/L) 2 hours after the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using a repeated measures ANOVA, with pairwise comparisons if found to be significant.

21. Final clot turbidity immediately before the last dose of IMP of the treatment period assessed within patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

22. Final clot turbidity 2 hours following the last dose of IMP of the treatment period assessed within patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

10.4 Subgroup analyses

No pre-specified subgroup analyses are planned.

10.5 Adjusted analysis

If variables are found to be of skewed distribution, logarithmic transformation will be performed.

10.6 Interim analysis and criteria for the premature termination of the trial

No interim analyses are planned.

10.7Participant population

- The pharmacodynamic analysis set will include all participants who achieve at least 80% compliance with study medication during each of the three periods.
- The safety analysis set (for the purposes of adverse event reporting etc.) will include any participant randomised into the trial that received at least one dose of IMP.

10.8 .Procedure(s) to account for missing or spurious data

- Missing data will be recorded by notating 'NR' (not recorded) in the relevant section of the CRF
- Where analysis is performed using ANOVA, missing data will be estimated by multiple imputation using the IBM SPSS software package. Sensitivity analyses will be carried out to report the robustness of this approach.

10.9 Other statistical considerations.

Not applicable to this study

10.10 Economic evaluation

Not applicable to this study.