ACT-2

Anti-Coronavirus Therapies (ACT) to prevent progression of COVID-19: Randomized trials

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STATISTICAL ANALYSIS PLAN

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Principal Investigator: John Eikelboom

Study Statistician: Lizhen Xu

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ACT-2	Anti-Coronavirus Therapies (ACT) to prevent progression of COVID-19: Randomized trials
ALI	Acute Limb Ischemia
ASA	Acetylsalicylic Acid (aspirin)
COVID-19	Coronavirus Disease 2019
CI	Confidence interval
ECMO	Extracorporeal membrane oxygenation
ICU	Intensive Care Unit
ITT	Intention-to-treat
HF	Heart failure
HR	Hazard ratio
LTFU	Lost to follow-up
MI	Myocardial infarction
SAP	Statistical Analysis Plan
VTE	Venous thromboembolism

1. INTRODUCTION

The ACT-2 trials are multi-centre, open-label, factorial, randomized control trials that evaluate anti-inflammatory and anti-thrombotic therapies in the prevention of clinical progression of COVID-19 in symptomatic patients outside of hospital with laboratory diagnosis of COVID-19 (Outpatient Trial), and symptomatic patients admitted to hospital with laboratory diagnosis of COVID-19 (Inpatient Trial) over 180 days.

Outpatient trial (at least 3,500 patients)

	Colchicine	**Control**
Aspirin (ASA)	N=875	N=875
Control	N=875	N=875

Inpatient trial (at least 2,500 patients)

	Colchicine	**Control**
Aspirin (ASA) plus Rivaroxaban	N=625	N=625
Control	N=625	N=625

2. STUDY HYPOTHESES/OBJECTIVES

2.1 Outpatient Trial

2.1.1 Primary Objectives

In symptomatic patients outside of hospital with a laboratory diagnosis of COVID-19, the primary objectives are to evaluate if at 45 days following randomization:

1) Colchicine vs. Control prevents hospitalization or death.

2) ASA vs. Control prevents major thrombosis (myocardial infarction, stroke, acute limb ischemia, pulmonary embolism), hospitalization or death.

2.1.2 Secondary Objectives

In symptomatic patients outside of hospital with a laboratory diagnosis of COVID-19, the secondary objectives are to evaluate if at 45 days following randomization:

1) If ASA vs. control prevents the occurrence of:

    a) Any thrombosis

2.1.3 Other Objectives

The other objectives are to evaluate if at 45 days following randomization, both colchicine vs. control and ASA vs. control prevents the occurrence of disease progression by 2 points on a 7-point assessment scale (see Section 7).

Primary, secondary, and other exploratory objectives will be assessed at 6 months following randomization.

2.2 Inpatient Trial

2.2.1 Primary Objectives

In symptomatic patients admitted to hospital with laboratory diagnosis of COVID- the primary objectives are to evaluate if at 45 days following randomization:

1) Colchicine vs. Control prevents high flow oxygen, mechanical ventilation or death

2) ASA plus Rivaroxaban vs. Control prevents major thrombosis (myocardial infarction, stroke, acute limb ischemia, pulmonary embolism), high flow oxygen, mechanical ventilation or death

2.2.2 Secondary Objectives

In symptomatic patients admitted to hospital with a laboratory diagnosis of COVID-19, the secondary objectives are to evaluate if at 45 days following randomization:

1) If Colchicine vs. Control prevents the occurrence of:
    a) high flow oxygen, mechanical ventilation or death due to respiratory causes

2) If ASA plus Rivaroxaban vs. Control prevents the occurrence of:
    a) high flow oxygen, mechanical ventilation or death due to respiratory causes
    b) any thrombosis

2.2.3 Other Objectives

The other objectives are to evaluate if at 45 days following randomization, both colchicine vs. control and ASA plus Rivaroxaban vs. control prevents the occurrence of disease progression by 2 points on a 7-point assessment scale (see Section 7).

Primary, secondary and other exploratory objectives will be assessed on 180 days following randomization.

3. POPULATIONS TO BE ANALYZED

3.1 Intention-To-Treat (ITT) Analysis Population: Full Analysis set

The Intention-To-Treat (ITT) analysis population will consist of all patients who are randomized to study treatment, regardless of treatments received or duration of trial participation.

4. PATIENT CHARACTERISTICS

Patient demographics, disease characteristics and severity at time of randomization (e.g. signs and symptoms, on oxygen or in ICU for inpatients), medical history, concomitant medications and laboratory results at baseline will be summarized using descriptive summary measures: mean (± standard deviation) or median (25^{th}-75^{th} percentile) for continuous variables as

appropriate, and counts and (appropriate) percentages for categorical variables. Baseline characteristics will be summarized according to treatment allocation. No statistical tests will be performed to compare these characteristics across treatment groups.

5. COMPLIANCE

5.1 Visit adherence

Participants are followed for a total of 6 months with assessments at day 8, day 45, and 6 months. Adherence to the visit schedule (visit completion and reason for not completing visits) will be summarized using frequency tables by visit (after randomization) and treatment group.

5.2 Study Medication Compliance

Compliance with study medication and reason for non-compliance will be summarized for each active treatment at each follow-up assessment (day 8, day 45) using frequency tables.

6. STUDY FOLLOW-UP TIME

All efforts will be made to collect complete data for all participants in this study.

For the primary and secondary objectives, participants will be followed to the 45 day assessment, day 45 plus 6 days (i.e. 45-51 days post randomization), and will complete all required data collection, regardless of their compliance with study medications, unless they die prior to this expected visit. For long term assessments, the follow-up cut-off dates will be 180 days after randomization.

6.1 Lost to follow-up:

Participants that do not complete the 45 day assessment for vital status, and who have not died prior to the 45 day assessment, will be considered lost to follow-up. All efforts will be made to collect information about the study outcomes for all participants. Participants determined as being lost to follow-up, and who did not experience the study endpoint during the follow-up period, will be censored for the analyzed endpoints on their last day of available endpoint data during the study.

6.2 Follow-up time and censoring

The total follow-up time (in days) is defined as time from randomization to date of death, last study contact, or follow-up cut-off date, whichever is first. Day of randomization is counted as Day 1.

The follow-up time for time-to-event outcomes (in days) is defined as time from randomization to date of outcome event, date of death, last study contact, or follow-up cut-off date, whichever is first. If the date of event is first, then the outcome event is said to occur; otherwise the

patient is censored at the date of death, last study contact, or follow-up cut-off date, whichever is first. Day of randomization is counted as Day 1.

6.3 Missing date information

When an event date is not known, the site investigator will be asked to provide the best estimate as to when the event occurred. Even though the exact date of an event is unknown, the investigator often does know some information that would indicate the approximate date, such as the first week of a month, in the fall of a year, or the middle of a particular year or at least the date when the participant was last seen or contacted. This information can be meaningfully incorporated into the estimated date recorded, as this is likely to be closer to the true date than any produced by an uninformed computer program.

7. 7-Point ASSESSMENT SCALE

The 7-point severity score is defined as the following:

7-point severity score (ordinal scale):
i) Not hospitalized, no limitations on activities
ii) Not hospitalized, limitation on activities;
iii) Hospitalized, not requiring supplemental oxygen;
iv) Hospitalized, requiring supplemental oxygen;
v) Hospitalized, on non-invasive ventilation or high flow oxygen devices;
vi) Hospitalized, on invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO);
vii) Death

A 2-point disease progression is defined as an increase by at least 2 points on the severity scale from baseline score to the maximum score reached during the 45 days of follow-up or 180 days of follow-up for the long term assessment.

8. EFFICACY ANALYSIS

All analyses will be based on the intention to treat principle, i.e. with participants analyzed in the treatment group to which they were randomized.

8.1 Outpatient Trial

8.1.1 Primary Outcome

In symptomatic patients, outside of hospital with a laboratory diagnosis of COVID-19, the primary efficacy outcomes at 45 days of follow-up are:

Colchicine compared with Control:

Primary outcome : Time from randomization to the first occurrence of composite of hospitalization or death

ASA compared with Control:

Primary outcome : Time from randomization to the first occurrence of the composite of major thrombosis (myocardial infarction, stroke, acute limb ischemia, pulmonary embolism), hospitalization, or death

8.1.2 Secondary Outcomes

The secondary outcomes at 45 days of follow-up are the time from randomization to the first occurrence of the following events:

Colchicine compared with Control: none

ASA compared with Control: Any thrombosis

8.1.3 Other outcomes

For both comparisons of Colchicine being compared with Control and ASA being compared with Control, the other outcome is time to the first occurrence of the disease progression by 2 points on a 7-point assessment scale.

8.2 Inpatient Trial

8.2.1 Primary Outcome

In symptomatic patients admitted to hospital with laboratory diagnosis of COVID-19, the multiple primary outcomes at 45 days of follow-up are:

Colchicine compared with Control:

Primary outcome :

For patients who have not yet received high flow oxygen or mechanical ventilation before randomization, the primary outcome is time from randomization to the first occurrence of the composite of high flow oxygen, mechanical ventilation, or death. For patients who have already received high flow oxygen or mechanical ventilation before the randomization, the primary outcome is time to death.

ASA plus Rivaroxaban compared with Control:

Primary outcome : Time from randomization to the first occurrence of the composite of major thrombosis (myocardial infarction, stroke, acute limb ischemia, pulmonary embolism), high flow oxygen, mechanical ventilation, or death

8.2.1 Secondary Outcomes

The secondary outcomes at 45 days of follow-up are time from randomization to the first occurrence of the following events:

Colchicine compared with Control:

a) composite of high flow oxygen, mechanical ventilation or death due to respiratory causes

ASA plus Rivaroxaban compared with Control:

a) composite of high flow oxygen, mechanical ventilation or death due to respiratory causes
b) any thrombosis

8.2.2 Other Outcomes

For both comparisons of Colchicine being compared with Control and ASA plus Rivaroxaban being compared with Control, the other outcome is time to the first occurrence of the disease progression by 2 points on a 7-point assessment scale.

8.3 Analysis Methods

8.3.1 Comparisons of the Treatment Arms

All comparisons will be based on the margins of the factorial design, as described by the objectives, and analyses performed stratified by the opposite arm of the factorial design.

i.e. Outpatient trial: Colchicine vs. Standard Care (stratified by Aspirin/Standard Care)

Outpatient trial: Aspirin vs. Standard Care (stratified by Colchicine/Standard Care)

Inpatient trial: Colchicine vs. Standard Care (stratified by the ASA plus Rivaroxaban/Standard Care)

Inpatient trial: ASA plus Rivaroxaban vs. Standard Care (stratified by the Colchicine/Standard Care arm)

Colchicine and antithrombotic therapies have different targets and there is no biological or pharmacological rationale for expecting an interaction between these treatments when being co-administered.

In each trial separately, a possible interaction between the two treatment arms will be assessed by inclusion of an interaction term in the model. In the unlikely event of a significant interaction between the two treatment arms, the effects of colchicine vs. standard care will be reported separately in those randomized to the antithrombotic intervention and those not randomized to antithrombotic intervention (i.e. standard care), and conversely, the effect of antithrombotic intervention vs. standard care will be reported separately in the strata defined by colchicine/standard care randomization.

8.3.2 Analysis of Primary outcomes

The time to event endpoints will be assessed by the Kaplan-Meier method. Kaplan-Meier curves between the two treatment arms will be compared with a log-rank test stratified by the other treatment arm of the factorial design. Hazard ratios and their corresponding two-sided 95% confidence intervals will be estimated using a stratified Cox proportional hazards regression model, with treatment group as a predictor variable and stratified by the other treatment arm of the factorial design. The proportional hazards assumption will be assessed by visually examining the plot of the log of the negative log of Kaplan-Meier survival function vs. the log of

time for evidence of non-parallelism and by including a time by treatment interaction term (time log transformed) in the Cox model. Significance of the interaction term will be tested at the 5% type I error level using the likelihood ratio test. If the interaction is significant and there is strong evidence of non-proportionality from the log-log plot, time-dependent hazard ratios will be estimated with the Cox model that includes the interaction term.

In both the outpatient and inpatient trials, the interaction effect of the two treatment arms in the factorial design on the primary endpoints will also be accessed. For details, please refer to Section 8.3.1.

8.3.3 Analysis of Secondary and Other Outcomes

The analysis of the secondary and other outcomes will be based on the ITT population. Hypothesis testing and parameter estimations will use a similar approach to that described in Section 8.3.2.

8.3.4 Sensitivity Analysis

Sensitivity analyses will be performed that adjust for demographics, exposure characteristics, , and severity of disease upon admission, in the stratified Cox proportional hazard models.

Sensitivity analysis will also be performed by treating the outcomes as binary variables. In these models, participants determined as being lost to follow-up, and who did not experience the study endpoint during the follow-up period, will be excluded from the analysis. Logistic regression models including treatment group (as randomized) and other possible confounding factors as covariates, will be used in these analysis.

Additionally, the primary and secondary efficacy endpoints as well as the safety, expected, known adverse events will be further analysed when the treatment groups are based on “as treated”.

8.3.5 Additional Analyses

8.3.5.1 Analysis on Primary, Secondary and Other Outcomes During the 180-day Follow-up Period

The analysis of primary, secondary, and other outcomes during the 180-day follow-up period will be performed in the same way as the primary analysis (Section 8.3.2). therapy is considered as recurrent only if the patient is re-hospitalized.

The outcomes that will be analyzed are defined as the following:

(For outpatient trials)

Colchicine compared with Control:

Composite of death and total (first and recurrent) hospitalization ,

ASA compared with Control:

Composite of death, total (first and recurrent) hospitalization, total (first and recurrent) MI, total (first and recurrent) stroke, total (first and recurrent) ALI, and total (first and recurrent) pulmonary embolism.

(For inpatient trials)

Colchicine vs control:

Composite outcome of death, total (first and recurrent) high flow oxygen, total (first and recurrent) mechanical ventilation

ASA plus Rivaroxaban compared with Control:

Composite outcome of death, total (first and recurrent) high flow oxygen, total (first and recurrent) mechanical ventilation, total (first and recurrent) MI, total (first and recurrent) stroke, total (first and recurrent) ALI, total (first and recurrent) pulmonary embolism

Recurrent events will be analyzed using the Andersen-Gill (AG) approach (Andersen and Gill, 1982), a generalization of Cox proportional hazard model which considers each repeated event as a separate term in the partial likelihood. The AG model assumes that the increments between events are conditionally uncorrelated, given the covariates. The robust information sandwich estimate of the covariance matrix of the coefficient estimates will be employed to relax this somewhat unrealistic assumption (Lin et al., 2000).

8.3.5.3 Analysis on the Disease Progression

In this additional analysis, an ordinal logistic regression model (also called as proportional odds logistic regression) will be applied to the 7-point scale disease progression endpoint. The model will have the scale disease progression score as the dependent variable, and the treatment group as the covariate. The proportional odds assumption will be checked in the crude model with treatment group as the only covariate by using the Brant test (Brant, 1990). Other variables such as demographics, exposure characteristics and severity of disease upon admission will also be explored for adjustment. We will also consider clinical meaningful scoring of disease progression in an exploratory way.

9. SAFETY ANALYSIS

9.1 Expected, Known Adverse Events

Colchicine, ASA and rivaroxaban have been extensively studied and used for many years in many thousands of patients (and in some cases millions of patients), and their adverse effects profiles are well described.

The expected, known adverse events of special interest are the following:

Colchicine:

a) Severe cytopenias
b) Severe allergy to active study medication
c) Study intervention overdose causing severe adverse drug reaction

ASA plus rivaroxaban:

a) Intracranial bleeding
b) Fatal bleeding
c) Serious bleeding in a major organ
d) Severe allergy to active study medication
e) Study intervention overdose causing severe adverse drug reaction

The expected, known adverse events of special interest will be summarized with frequency and percentages within each of the respective study intervention group (i.e. the Colchicine expected, known adverse events will be summarized for all participants randomized to Colchicine and the ASA plus rivaroxaban expected, known adverse events will be summarized for those participants randomized to the ASA plus rivaroxaban).

9.2 Adverse Events and Serious Adverse Events

Participant safety will be summarized in a series of tables including reported Adverse Events as well as Serious Adverse Events (SAEs). All safety summaries will be tabulated by MedDRA

System Organ Class and Preferred Term, by randomized treatment group, with frequencies and percentages, and will consider all adverse events that occur on or after date of randomization up to the 45 day assessment and 180 day assessment.

Effect of the related study medication on AEs and SAEs will be assessed by comparing total number of participants with reported outcomes in the intervention group and the standard care group, using Chi-square test or Fisher exact test, as appropriate.

10. SUBGROUP ANALYSIS

10.1 Outpatient Trial Subgroups

The consistency of treatment effects on the primary outcome of the outpatient trial will be explored in predefined subgroups, including

a) Sex (Male,Female)
b) Age group (<50, 50-69, >70)
c) Comorbidities at baseline
    i. Current Smoking (Yes, No)
    ii. History of Diabetes (Yes, No)
    iii. History of Diabetes (Yes, No)
    iv. History of Hypertension (Yes, No)
    v. BMI (≤30 kg/m^2, > 30 kg/m^2)
    vi. Cancer (Active Cancer, No Active Cancer)
    vii. CV disease (Yes, No)
    viii. Respiratory Disease (Yes, No)
    ix. Renal Disease (Yes, No)
    x. Liver Disease (Yes, No)
    xi. Immunosuppressed Status (Yes, No)
d) Co-interventions at baseline
    i. ACE-I use (Yes, No)
    ii. NSAID use (Yes, No)

e) Covid-19 vaccine status (none, partially vaccinated, and fully vaccinated)

10.2 Inpatient Trial Subgroups

The consistency of treatment effects on the co-primary outcomes of the inpatient trial will be explored in predefined subgroups, including

a) Sex (Male, Female)
b) Age group (<50, 50-69, >70)
c) Co-morbidities at baseline
    i. Current Smoking (Yes, No)
    ii. History of Diabetes (Yes, No)
    iii. History of Diabetes (Yes, No)
    iv. History of Hypertension (Yes, No)
    v. BMI (≤30 kg/m^2, > 30 kg/m^2)
    vi. Cancer (Active Cancer, No Active Cancer)
    vii. CV disease (Yes, No)
    viii. Respiratory Disease (Yes, No)
    ix. Renal Disease (Yes, No)
    x. Liver Disease (Yes, No)
    xi. Immunosuppressed Status (Yes, No)
d) On oxygen at baseline (Yes, No)
e) Admission to ICU at randomization (Yes, No)
f) Ventilation at randomization (Yes, No)
g) Co-interventions at baseline
    i. ACE-I use (Yes, No)
    ii. NSAID use (Yes, No)

h) Covid-19 vaccine status (none, partially vaccinated, and fully vaccinated)

10.3 Subgroup Analyses

The effect of the intervention on the multiple endpoints will be assessed in the predefined subgroups using time to event analysis methods as described in section 8.3.2. The consistency of treatment effects across predefined subgroups will be explored with respect to the multiple endpoints using tests for interactions by including the subgroup, and subgroup-treatment allocation interaction term in the Cox proportional hazard model.

11. POOLED ANALYSES

Two types of prospective pooled analyses are planned.

1. ACT2 Outpatient and Inpatient combined trial data
2. Patient level meta-analysis combining ACT2 trial data with the ECLA COVID-19 trial data

Separate SAPs will be defined for both of these pooled analyses.

12. ADHERENCE TO THE PROTOCOL

Summary statistics (counts and % of participants) for reported protocol deviations as outlined in the protocol deviation-reporting plan will be presented as the total number of deviations and deviations within each category (e.g. inclusion/exclusion criteria, randomization).

13. INTERIM ANALYSES

The DSMC may make recommendations regarding any study-conduct issues (e.g., recruitment, adherence, completeness of follow-up, outcome assessments, data quality, patterns of protocol violations) that are identified.

13.1 Monitoring for Efficacy

The DSMC will follow a flexible pragmatic monitoring approach for efficacy, considering each trial separately. They will follow a modified Haybittle-Peto boundary of 3 s.d. for benefit and harm as a general guideline. The modification is that if the boundary of 3 s.d. is crossed in a given analysis, it will have to be crossed at the subsequent analysis (i.e. two consecutive crossings). They will also be looking for consistency of efficacy results across both trials, as well as the totality of the evidence of efficacy and harm, prior to any potential recommendation of stopping any trial early.

No modification of the level of significance of the final results is being considered. The standard Haybittle-Peto procedure (Haybittle 1971, Peto et al 1976) states that if at an interim analysis, the P-value is ≤0.001 (Z-statistic ~3), then the trial should be stopped early. The final analysis can still be evaluated at the planned level of significance (usually 0.05). The main advantage of the Haybittle–Peto boundary is that the same threshold is used at every interim analysis, and does not vary by the number of interim looks.

13.2 Safety

The DSMC will also be monitoring the safety of the participants, focusing on adverse events and other safety indicators. No formal statistical criteria are being used, but the 2.5 or 3.0 s.d. cutpoint will serve as a guideline for harm, in addition to the DSMC members' clinical judgement. Usually a lower threshold for harm is used.

13.3 Futility

The ACT trials are not expected to be stopped for futility and will only be stopped for this reason under exceptional circumstances. If a lack of sufficient recruitment impedes the ability of the trial to achieve its goals, or the intervention arms are contaminated, the DSMC may bring this to the attention of the PI and possibly recommend early termination.

Futility is also possible in the presence of lack of effectiveness of the active intervention. The DSMC will consider both the clinical outcomes data and (if available) the translation substudy data (i.e. viral titres, inflammatory markers, markers of tissue damage). The DSMC will be presented with conditional power analyses (Lachin, 2005) of interim results to help identify when a significant difference between treatment groups is unlikely to be identified (e.g., conditional power <20%) if the trial continues to the preplanned sample size. However, such findings would not necessarily indicate that a trial should be stopped (Tyson et al, 2016), but are merely provided as information to help the DSMC in making recommendations. If there is also lack of effect on viral titres for antiviral therapy, this will lend support to termination. If an effect on viral titres is observed, it may prompt the DMSC to suggest the trial should continue.

13.4 Considering External Data

The DSMC may review data external to the study in order to provide perspective, for instance, on recent developments or changing requirements of health regulatory agencies, especially when such data might suggest early termination of the trial for safety issues seen with the same or a similar compound.

13.5 External Subject Matter Expert (SME)

The DSMC may request consultation with an independent Subject Matter Expert with relevant expertise in the study area. The PI and Steering Committee Chair will be responsible for identifying an appropriate SME. The DSMC may consult the SME regarding perceptions of safety issues relevant to the study area and clinical considerations.

14. References

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APPROVAL

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By signing the below, I designate my approval of the above-named version of the STUDYNAME Statistical Analysis Plan on behalf of all named authors.

Name	**John Eikelboom**
Role	**Principal Investigator**
Signature	
Date (yyyy/mm/dd)	

By signing the below, I designate my approval of the above-named version of the STUDYNAME Statistical Analysis Plan on behalf of PHRI Statistics.

Name	**Lizhen Xu**
Role	**Lead Blinded Statistician** **SAP Author**
Signature	
Date (yyyy/mm/dd)	