Dipyridamole to Prevent Coronavirus Exacerbation of Respiratory Status (DICER) in COVID-19

Statistical Analysis Plan

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Statistical Analysis Plan

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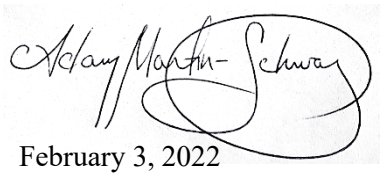
Signature: 

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3 Abbreviations and Definitions

ABG	Arterial blood gases
AE	Adverse event
ALT	Alanine aminotransferase
ARDS	Acute respiratory distress syndrome
AST	Aspartate aminotransferase
BMP	Basic metabolic panel
CBC	Complete blood cell count with differential
COVID-19	Coronavirus disease 2019
CP	Conditional Power
CRF	Case report form
CRP	C-reactive protein
DICER	Name of the Study: Dipyridamole to prevent Coronavirus Exacerbation of Respiratory Status
ECMO	Extracorporeal membrane oxygenation
FiO2	Fraction of inspired oxygen
GFR	Glomerular filtration rate
INR	International normalized ratio
ISTH	International Society on Thrombosis and Hemostasis
LDH	Lactate dehydrogenase
LFT	Hepatic function panel
NEWS2	National Early Warning Score
PTT	partial thromboplastin time
SABER	Statistical Analysis of Biomedical and Educational Research unit in the University of Michigan School of Public Health
SAE	Serious adverse event
SAP	Statistical analysis plan
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SpO2	Peripheral pulse oximetry
ULN	Upper limit of normal

4 Introduction

4.1 Preface

This Statistical Analysis Plan (SAP) describes statistical methods and analyses for the DICER study. This document should be read in tandem with the DICER Study Protocol version 5.0, dated November 23, 2020.

Immune hyperactivation and thrombophilia appear to predict worse outcomes in individuals with COVID-19. The majority of hospitalized patients infected with COVID-19 develop coagulation dysfunction as indicated by elevated levels of D-dimer protein, which predicts acute respiratory distress syndrome (ARDS), venous thromboembolism, and mortality. Dipyridamole is an adenosinergic drug with a favorable safety profile that reduces thrombosis, reduces immune hyperactivation, and directly inhibits SARS-CoV-2 virus replication. A small clinical trial of dipyridamole in 31 patients with moderate- and severe-COVID-19 showed safety of dipyridamole and efficacy in reducing D-dimer levels, and a trend toward improved clinical outcomes (Liu et al, 2020).

The intent of this proof-of-concept study is to evaluate whether the drug dipyridamole will reduce D-dimer elevation in participants with moderate COVID-19 as measured by: increase in D-dimer, respiratory status, ventilation and mortality, and ICU admission. If this novel approach is effective in reducing the progression of D-dimer elevation, it will inform a larger, multi-center trial to determine efficacy in preventing progression of moderate to severe COVID-19 infection. The result may broadly improve care of patients with coronavirus infection.

4.2 Scope of the Analyses

The purpose of this document is to describe primary and secondary statistical analyses to be conducted with regard to the efficacy and safety of dipyridamole versus placebo in the DICER clinical trial.

5 Study Objectives and Endpoints

5.1 Study Objectives

Due to changes in standard of care for COVID-19 patients, this study has two co-primary objectives, with the original secondary endpoint being elevated to a co-primary endpoint. The first and original primary objective of this study is to compare the effect of dipyridamole vs. placebo on plasma D-dimer changes in patients with moderate COVID-19. The second primary objective, formerly a secondary objective, is to compare the effect of dipyridamole vs. placebo on a composite of clinical outcomes including death, survival time, days on mechanical ventilation, decrease in SpO_2/FiO_2 ratio, and cumulative COVID ordinal scores.

5.2 Endpoints

For convenience, we give names to the following times and time windows:

- Baseline or Day 1: Date of the first study drug administration
- Study Hospitalization: Day 1 through either Day 14 or hospital discharge, whichever is first
- Observation Period: The 28-day period following first study drug administration
- Study Duration: The period from baseline until 30 days after last study drug administration

If an endpoint is to be evaluated at all three of the above time points, we use the phrase 'at any time point'.

Endpoints are presented in a numbered list to facilitate references to endpoints when conducting the final analysis.

5.2.1 Primary Endpoint

The first co-primary endpoint is increase in plasma D-dimer levels compared to baseline.

The second co-primary endpoint is a clinical hierarchical composite of:

- Time to death from any cause during study duration
- Number of days on mechanical ventilation during study hospitalization
- A dichotomized (yes/no) decrease in daily average SpO_2/FiO_2 ratio of at least 50 units relative to Day 1 at any time during the observation period
- Cumulative sum of COVID ordinal scores during study hospitalization

5.2.2 Secondary endpoints

1. Days alive and free of organ support, where:
    - Organ support is defined as receipt of invasive mechanical ventilation, vasopressor therapy, ECMO support, or dialysis
2. Individual components of composite endpoint

5.2.3 Safety Endpoints

3. During the observation period, incidence of bleeding associated with: (i) a decrease in the hemoglobin level of at least 2 g per deciliter, (ii) transfusion of at least 2 units of blood, (iii) occurrence in a critical site, or (iv) contributing to death
4. Incidence of serious adverse events (SAEs) during study duration
5. Incidence of hypotension with systolic blood pressure below 90 mmHg and requiring a 1-liter IV fluid bolus during study hospitalization

5.2.4 Exploratory Endpoints

Based on consultation with the DICER researchers, SABER will only analyse a subset of the exploratory endpoints specified in the original protocol. Numbers in the following list (e.g. #17) refer to Section 3.4 Exploratory Outcomes in the protocol, version 5.0.

6. Blood Oxygenation
    - Time to increase in daily average SpO_2/FiO_2 ratio of at least 50 units relative to Day 1 during the observation period (#17)
7. Ventilation
    - Initiation of mechanical ventilation or ICU care during observation period (yes/no) (#6)
    - Initiation of mechanical ventilation during observation period (yes/no) (#11)
    - Initiation of ICU care during observation period (yes/no)
8. Change from baseline at Day 5 for: C-reactive protein, LDH, ferritin, total leukocyte count, neutrophils, lymphocytes, and platelet count in the blood (#4)
    - Daily changes from Day 1 in neutrophils and lymphocytes
9. Hospitalization
    - Time to hospital discharge (#7)

- Incidence of hospital readmission during study duration (#30)

10. Supplemental Oxygen
    - Proportion of surviving patients still requiring supplemental oxygen at the end of the observation period (#8)
    - Number of days on supplemental oxygen during observation period (#9)
11. Imaging: Proportion of study population with each of the following:
    - Arterial or venous thromboembolism diagnosed by imaging study during study duration (#25)
    - Venous thrombosis diagnosed by imaging study during study duration (#26)
    - Arterial thrombosis diagnosed by imaging study during study duration (#27)
12. Change from Day 1 in daily average glomerular filtration rate (GFR) during observation period, assessed daily through Day 5 (#28)
13. An alternative version of the second co-primary endpoint decision-rule:
    - Time to death from any cause during study duration
    - Number of days on mechanical ventilation during study hospitalization
    - A dichotomized (yes/no) decrease in daily average SpO_2/FiO_2 ratio of at least 50 units relative to enrollment date at any time during the observation period
    - A dichotomized (yes/no) increase in Ordinal Score at any time relative to enrollment
        - Analyze dichotomized (yes/no) increase in Ordinal Score at any time relative to enrollment as a standalone endpoint

Additional analyses done separately from SABER (to be done at a future date):

14. A dichotomized (yes/no) decrease for patients with elevated Day 1 neutrophil activation during study hospitalization, where elevated is defined as > 2 SD above established healthy controls (#22)
15. A dichotomized (yes/no) decrease for patients with elevated Day 1 inflammatory, anti-inflammatory, and other cytokines in during study hospitalization, where elevated is defined as blood defined as > 2 SD above established healthy controls (#23)
16. A dichotomized (yes/no) decrease for patients with elevated Day 1 coagulant and anticoagulant cytokines in blood during study hospitalization, where elevated is defined as blood defined as > 2 SD above established healthy controls (#24)
17. Gene expression, function, and activation of peripheral blood leukocytes following treatment on Days 1 or 5 (#29)

6 Study Methods

6.1 General Study Design and Plan

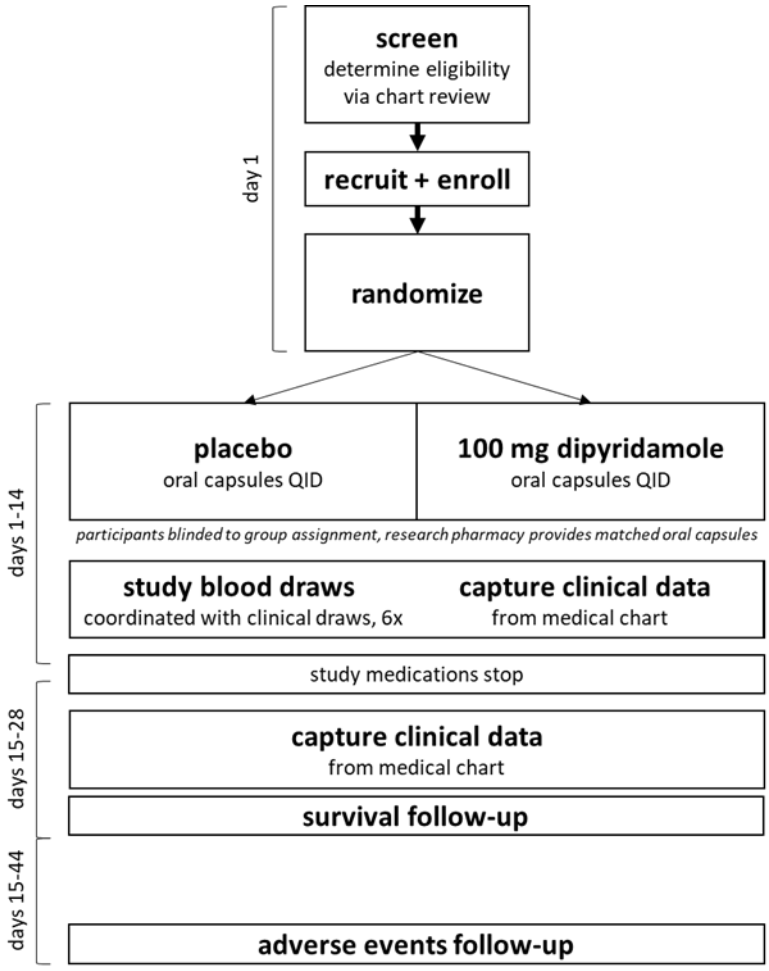
Our primary hypotheses are that patients on dipyridamole will evidence a smaller increase in D-dimer levels and better clinical outcomes on the COVID ordinal score than patients on placebo.

This is a proof-of-concept, placebo-controlled, single-blinded, randomized, 14-day trial of 160 patients.

Participants were assigned treatments to dipyridamole or placebo in 1:1 fashion using stratified, random blocks based on sex and age (above/below 65). Stratified blocks were generated using the 'blockrand' package in R.

After randomization, treatment was administered four times daily up until hospital discharge or 14 days, with clinical evaluations made daily. Clinically significant abnormal lab studies were evaluated for relationship to the study drug. Clinical data capture and survival follow-up occurred on Day 28 ± 1. Patient follow-up occurred again 30 days after last dose of study drug. The study schematic is presented in Figure 1.

Figure 1 Schematic Representation of Study Design



6.2 Inclusion-Exclusion Criteria and General Study Population

6.2.1 Inclusion Criteria

1. Age ≥ 18 years
2. Willing and able to provide informed consent prior to performing study procedures unless they have a legally authorized representative (LAR)
3. Confirmed coronavirus (SARS-CoV-2) infection
4. Currently hospitalized or anticipated hospitalization requiring supplemental oxygen

6.2.2 Exclusion Criteria

1. In the opinion of at least two physicians, unlikely to survive for >48 hours from screening
2. Concurrent enrollment in a clinical trial of a cytokine inhibitor (targeting IL-6, IL-6R, IL-1, or Janus kinase). Use of remdesivir is permitted.

3. Currently on invasive mechanical ventilation
4. Hypotension defined as systolic blood pressure < 90 mmHg on two readings at least 4 hours apart
5. Pregnant or breastfeeding
6. Concurrent dual antithrombotic therapy (aspirin or P2Y12 inhibitor (eg. clopidogrel, ticagrelor) plus anticoagulation to treat deep venous thrombosis or pulmonary embolism (single antiplatelet agent, or anticoagulant agent at prophylaxis or therapeutic dose is permitted).
7. Presence of any of the following abnormal laboratory values: aspartate aminotransferase (AST) or alanine aminotransferase (ALT) greater than 5 x upper limit of normal (ULN), platelets <50,000 per mm3, hemoglobin <8 g per deciliter
8. History of recent major bleeding, defined in accordance with the criteria of the International Society on Thrombosis and Hemostasis (ISTH) as overt bleeding that was associated with a decrease in the hemoglobin level of 2 g per deciliter or more, led to a transfusion of 2 or more units of blood, occurred in a critical site within 30 days.
9. Any physical examination findings and/or history of any illness that, in the opinion of the study investigator, might confound the results of the study or pose an additional risk to the patient by their participation in the study.

6.3 Study Assessments

Table 1. Schedule of Events

Study Day	Screening Enrollment	Day 1	Days 3, 7, 11	Days 5, 9, 13	Even Days 2-12	Day 14	Day 28	Early Withdrawal	30 Days After Drug
Screening via chart review	X								
Screening for eligibility	X								
Informed consent	X								
Pregnancy test [a]	X								
Demographics		X							
Medical history		X							
Social history [b]		X							
Physical exam SOC review [c]		X							
Randomization	X								
Study drug administration		X	X	X	X	X			
Vitals/Oximetry SOC review [c]		X	X	X	X	X	X[j]	X	
Respiratory status SOC review[c]		X	X	X	X	X		X	
Clinical progress review		X	X	X	X	X		X	
Clinical care lab result review		X	X	X	X	X		X	
ECG clinical care data review [d]		X	X	X	X	X		X	
Chest imaging review [d]		X	X	X	X	X		X	
BMP [e]		X	X	X				X	
D-dimer [e]		X	X	X		X		X	
Ferritin [e]		X		X				X	
LDH [e]		X		X				X	
CRP [e]		X		X				X	
CBC w/ diff [e]		X	X	X		X		X	
FBN [e]		X	X	X		X		X	
PTT/INR [e]		X	X	X		X		X	
ABG [d]		X							
LFT [e]		X		X				X	
Research blood draw [i]		X[f]	X[f]	X[f]				X	
Concomitant meds/therapies		X	X	X	X	X	X[g]	X	
AE assessment		X	X	X	X	X	X[g]	X	X[h]

a Performed in subjects of child-bearing potential, if not already performed for clinical care reasons
b Social history including pregnancy history, tobacco and alcohol use
c Medical chart review for extraction of standard-of-care data.
d Only if performed while patient is inpatient/hospitalized for clinical care reasons. Data will be recorded.
e Lab results will be extracted from blood drawn for clinical care, whenever possible..
f Blood collection will be coordinated with blood draw for clinical care lab studies, whenever possible.
g Medical chart review for secondary endpoints will continue until Day 28.
h Chart review and phone call for AEs assessment until 30 days after last dose of study drug, maximum of 44 days from initial participation date.
i: Research lab draws on study days 3,5,9,11,13 will have +/- 1 day window.

Prior to conducting any study-related activities, written informed consent containing Health Insurance Portability and Accountability Act (HIPAA) authorization will be signed and dated by the participant.

6.4 Clinical Assessments

6.4.1 Concomitant medications

All concomitant medications will be documented during the study, including early termination when applicable. Dose, route, unit frequency of administration, indication for administration and dates of medication will be captured.

6.4.2 Demographics

Demographic information (race, ethnicity, sex, date of birth, and height and weight) will be recorded at the time of enrollment.

6.4.3 Medical history

Relevant medical history, including history of current disease and information regarding underlying diseases will be obtained from the patient and/or by chart review, and recorded at the time of enrollment.

6.4.4 Social history

Relevant social history, including history of pregnancy, tobacco and alcohol use, will be obtained from the patient and/or by chart review, and recorded at the time of enrollment.

6.4.5 Physical examination

A complete physical examination will be performed by the medical team during the hospitalization. Abnormal physical exam findings will be documented, and the principal investigator will incorporate this information into consideration of continued eligibility from a safety perspective.

6.4.6 Electrocardiogram

Electrocardiogram (ECG) will be performed by the medical team if clinically appropriate and documented in the eCRF if obtained.

6.4.7 Chest imaging

Chest imaging will be performed by the medical team if clinically appropriate and documented in the eCRF if obtained. Imaging findings will be coded in binary variables for infiltrates, pneumonia, pleural effusion, consolidation, pulmonary embolism, RV strain, and cardiomegaly.

6.4.8 Vital signs

Temperature, blood pressure, heart rate, respiration rate, height, weight will be performed per institutional protocol. Blood pressure will be measured at least twice daily.

6.4.9 Oxygen requirements

Pulse oximetry (SpO_2), oxygen requirements (mode of delivery), fraction of inspired oxygen (FiO_2), oxygen flow rate, and mode of mechanical ventilation will be recorded at least daily alongside vital signs measurement. Pulse oximetry will be measured at least twice daily.

6.4.10 Modified WHO COVID-19 8-point Ordinal scale assessment

Ordinal scale assessments will be made daily and on Day 28 as below:

Table 2. Modified COVID 8-Point Ordinal Scale

Patient State	Descriptor	COVID Ordinal Score
Dead	Death	8
Hospitalized – severe disease	Ventilation + additional organ support (pressors, renal replacement therapy, ECMO)	7
	Intubation and mechanical ventilation	6
	Non-invasive ventilation or high-flow oxygen	5
Hospitalized – mild disease	Oxygen by mask or nasal prongs	4
	Hospitalized, no oxygen therapy	3
Ambulatory	Limitation of activities	2
	No limitation of activities	1

6.4.11 National Early Warning Scores (NEWS2)

The NEWS2 score will be made daily and on Day 28. We omit details of NEWS2, because it will not be analyzed in this SAP.

6.4.12 Adverse events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates), severity/grade, outcome, treatment and relation to study drug will be recorded on the source document.

6.5 Clinical Laboratory Measurements

6.5.1 D-dimer, Ferritin, lactate dehydrogenase (LDH), C-reactive protein (CRP), complete blood cell count with differential (CBC), fibrinogen, partial thromboplastin time (PTT), international normalized ratio (INR), arterial blood gases (ABG), basic metabolic panel (BMP), hepatic function panel (LFT)

We anticipate blood will be drawn for clinical care at least every other day, and anticipate that most research blood studies will be performed on blood already drawn for clinical care. We will also extract data for any studies done for clinical care purposes. Blood will be obtained and sent to the clinical laboratory to measure LDH, CBC, CRP, D-dimer for standard of care at the discretion of the medical team. D-dimer, fibrinogen, PTT, INR, ferritin, CBC, LFT, LDH, CRP, BMP will be measured on day 1 on blood drawn for clinical care. Although daily measurement of D-dimer would be ideal for the primary endpoint of this research study, we will measure D-dimer every other day given ongoing medical resource constraints. D-dimer, fibrinogen, PTT, INR and CBC will be measured on days 3, 5, 7, 9, 11, 13 and 14 during hospitalization. CRP, ferritin, LDH, BMP will be measured on day 1, 5, 9, and 13, during hospitalization for research study using blood drawn for clinical care wherever possible. LFT will be measured on day 1 for clinical care, and at least every 4 days until end of drug treatment phase in blood drawn for clinical care wherever possible during hospitalization. ABG will only be recorded if performed for clinical care purposes.

6.5.2 Pregnancy test

A urine pregnancy test, if not already performed for clinical care, will be obtained from female patients who are of childbearing age by the medical team prior to obtaining informed consent.

6.5.3 Pharmacokinetic measurements

Not applicable.

6.6 Research Laboratory Measurements (see Schedule of Events)

6.6.1 Coagulation potential

Blood will be obtained to investigate potential mediators of COVID-19 and novel parameters of coagulation and platelet activity measured in the study team's research laboratories.

6.6.2 Biomarkers of blood cell activation

Blood will be collected in sodium citrate, heparin, EDTA or serum separator tubes. The blood sample will be labelled and processed at the local sites before shipping to the study team's research laboratories at the University of Michigan to evaluate novel biomarkers of blood cell activation, coagulation, and cytokines.

6.6.3 Leukocytes

Blood will be collected in EDTA, sodium citrate, or heparin tubes. The blood sample will be labelled and sent for processing to the clinical and research labs.

7 Sample Size

7.1 D-dimer Calculations

Decision thresholds and power calculations are based on a preliminary analysis of 147 D-dimer measurements taken on 41 COVID-19 patients in the University of Michigan hospital in Spring 2020. Quantities were estimated through simulation based on the preliminary data.

The presumed analysis is a variance components regression on log-transformed D-dimer values with fixed effects for ICU status (a binary proxy for case severity) and days since admission. The model assumes random effects for linear subject-level log-D-dimer trends over time. We set an intraclass correlation coefficient (ICC) of 0.7, which reflects large between-subject variation relative to within-subject variation and is conservatively lower than the observed ICC of 0.77.

Power is based on a mean 13% daily increase in D-dimer levels for untreated patients, as observed in preliminary analysis. We posited a 50% reduction in the growth rate (i.e. 6.5% daily increase) for the 100 mg dose of study drug. Based on 2 arms with 40 subjects per arm (80 subjects in all), power is 0.91 for comparing the pooled dipyridamole participants to the controls.

7.2 Win Ratio Calculations

The standard of care treatment for COVID-19 changed with the introduction of anti-inflammatory steroids, which appear to influence D-dimer concentrations. It was therefore determined to elevate the secondary endpoint to become a co-primary endpoint. This endpoint is a composite of clinical outcomes including death, survival time, mechanical ventilation days, decrease in SpO_2/FiO_2 ratio, and cumulative COVID-19 ordinal scores.

This endpoint will be assessed nonparametrically by comparing outcomes across all possible pairs of treatment-placebo patients and evaluating a 'win ratio' using the Mann-Whitney U-statistic (Pocock et al., 2012). See Section 8.6.2.2 for details. The win proportion is interpreted as the probability that a study drug patient experiences a better outcome than a placebo patient, and in this sense, it represents the effect size for the analysis. We powered our analysis on a presumed 0.67 win proportion (i.e. a patient on study drug is twice as likely to have a better outcome as a patient on placebo). We calculated sample size requirements using the formulas provided in Yosef et al. (2019).

Because we set a 0.006 Type I error significance (alpha) for the interim analysis for benefit of D-dimer, we could only use 0.044 of the original 0.05 for the primary endpoints. We divided the 0.044 between the two co-primary endpoints, leaving a 0.022 two-sided Type I error. Accounting for a 10% dropout, we require 127 patients to have 80% power to significantly detect a win ratio of 0.67.

8 General Analysis Considerations

8.1 Timing of Analyses

Based on interim analyses for futility, it was decided to halt recruitment beginning January 19, 2021. The final data analyses will begin:

- after all 30-day post drug follow-up has been completed for all randomized patients,
- after chart review and data cleaning according to the data management plan are complete, and
- after approval of this SAP document by the study team.

8.2 Analysis Populations

8.2.1 Modified Intent to Treat Population (mITT) and Safety Population

All randomized patients who received at least one dose of dipyridamole or placebo will be included in the mITT population. The safety population will be the same as the mITT population. The mITT population will serve as the basis for comparisons of dipyridamole versus placebo and for assessment of safety.

8.3 Covariates and Subgroups

Models to compare dipyridamole versus placebo will include both stratification factors as predictors: sex and age. The analysis will include interactions between study drug and stratification factors. Age will be modelled both as a continuous covariate and as a grouping factor (in separate models). For longitudinal responses, models will also include days since first drug administration. Where appropriate, models will include baseline values of the response variable.

The win ratio analysis for the clinical hierarchical composite endpoint will be stratified according to age and sex (Dong et al., 2018).

8.4 Missing Data

We anticipate three sources of missing response data in this study: missing data entries, loss to follow-up, and early hospital discharge. We will summarize missingness for primary study endpoints. In preliminary results, a large majority of patients have early hospital discharge.

For the primary response and other longitudinal analyses, we address missing response data through random coefficients regression, which incorporates missingness through the standard errors of subject-level trend estimates. This strategy assumes missing entries are missing at random and that missing entries from early termination or discharge would not differ from the trend in the observed data. For time-to-event responses, we address early termination and discharge by right-censoring.

The DICER team will review data to minimize missingness in sex, age, and baseline values. Following Schafer et al. (1997), if these variables are missing for more than 5% of patients, we will impute them using SAS PROC MI and PROC MIANALYZE if necessary. Otherwise, we will exclude missing records from the analyses.

8.5 Extra Data

The protocol calls for certain procedures only on specified days (e.g. coagulation panels including D-dimer on Days 1, 3, 5, 7, 9, 11, 13, and 14) but allows for collection on other days when ordered by the clinician. We choose to include these ad hoc data in all analyses, because the frequency of sampling the time scale of change in response variables is anticipated to be long compared to sampling, and clinician-ordered sampling should not introduce sizeable bias.

8.6 Interim Analyses and Data Monitoring

8.6.1 Safety Review

The analysis plan includes interim review by the DSMB for the purpose of monitoring study conduct and assessing participant safety after 50% of patients have completed the study or 10 deaths have occurred (whichever happens first). This review includes the number, proportion, and listing of participants with SAEs (including segregation of those involving deaths), treatment-emergent AEs, and discontinuation of study medication due to AEs. These presentations are descriptive, with no formal inferential methods used. No specific rules for halting study enrollment or study interventions for safety are specified; however, the DSMB may request formal inferential testing to assess the risk-benefit profile of these study medications in this study population.

8.6.2 Efficacy Review

8.6.2.1 Primary D-dimer Endpoint

We conduct two interim analyses of efficacy with respect to the D-dimer primary endpoint after 50% of patients have completed the study: a test for benefit and a test for futility. The analyses use the mITT population at the time of reporting.

To analyze for benefit, we separately estimate the change in D-dimer (on the log scale) for each patient and then convert those estimates into a binary outcome indicating whether the patient's change was at least a 5% daily increase. We apply Fisher's exact test to assess whether one treatment arm is clearly superior. We use the O'Brien-Fleming method to correct for interim analyses and the Lan-DeMets spending function to preserve an overall Type I error rate of 0.05. This projects to $\alpha = 0.003$ after 40 patients. We test sensitivity to: (i) the inclusion/exclusion of subjects lacking baseline D-dimer measures, and (ii) the inclusion/exclusion of measures after Day 7, by which time most patients have been discharged.

To analyze for futility, we examine the estimated daily percent change in D-dimer measures within only the dipyridamole treatment arm. This is calculated as in the benefit analysis, except it is dichotomized with respect to a 5% daily increase. The termination for futility criterion is whether the lower 95% confidence bound for mean daily increase is above 9% as determined by a one-sample t-test.

8.6.2.2 Primary Hierarchical Composite Endpoint

We conduct an interim analysis of the efficacy with respect to the hierarchical composite endpoint using data as December 31, 2020. This analysis was added to the protocol at the December 15, 2020 DSMB meeting to assess whether there may be evidence of treatment differences in clinical outcomes.

The analysis uses the win ratio, a modification of the Mann-Whitney U-statistic (Pocock et al., 2012; Finkelstein and Schoenfeld, 2018). A description of the win ratio algorithm is provided in Section 9.2.1 and Table 3. We calculate U-statistics by comparing all possible pairs of treatment-vs-placebo

patients according to the win ratio algorithm. This generates a summary U-statistic for each patient and estimates the win ratio — the proportion of comparisons 'won' by dipyridamole patients. We obtain confidence intervals for the win ratio by bootstrapping the data in-hand and spending only the α for Type I error that remains from the D-dimer analysis according to the Lan-DeMets spending function.

To decide futility or benefit, we apply conditional power (Lan and Trost, 1999; Siu and Lan, 2001; Lachin, 2006). This approach is advantageous, because it does not require an adjustment for the Type I error nor an adjustment on the final critical value to claim significance. Our calculations of conditional power (CP) are based on the estimated effect at the time of analysis for a total sample size of $N = 127$ (based on win ratio sample size calculations in Yosef et al., 2019):

$$\text{CP} = \Pr(\textit{reject } H_0 \textit{ at the end of the study} \mid \text{interim data, estimated effect size is unbiased}).$$

The decision to proceed with the trial depends on the value of CP as follows:

1. CP < 0.3 : Stop for futility
2. If 0.3 ≤ CP < 0.8: Continue the trial but re-estimate sample size up to $N = 160$
3. If 0.8 ≤ CP < 0.9: Continue the trial with $N = 127$
4. CP ≥ 0.9 : Stop for benefit

8.7 Multiple Testing

In the primary analyses, we use the O'Brien-Fleming method to correct for interim analyses and the Lan-DeMets spending function to preserve an overall Type I error rate of 0.05. We will use a Bonferroni adjustment to maintain familywise error for the five secondary analyses. We will not apply a multiple testing adjustment to exploratory endpoints; any findings associated with exploratory endpoints will require confirmation before reporting.

9 Summary of Study Data

Descriptive summary statistics will be tabulated for baseline patient demographics and clinical characteristics separately by intervention group and overall. All tables will be annotated with the total population size relevant to that table (usually the mITT), including missing observations. For continuous variables, the estimated mean, standard deviation, median, minimum, and maximum will be reported. For categorical variables, number and percentages will be reported (excluding missing values).

9.1 Subject Disposition

Patient consent, screening, randomization, degree of study completion (completion of in-hospital follow-up, 28-day follow-up, and 30-day post-drug follow-up), and reasons for dropout will be summarized in a table and a CONSORT diagram. Numbers will be provided by treatment group and overall where appropriate.

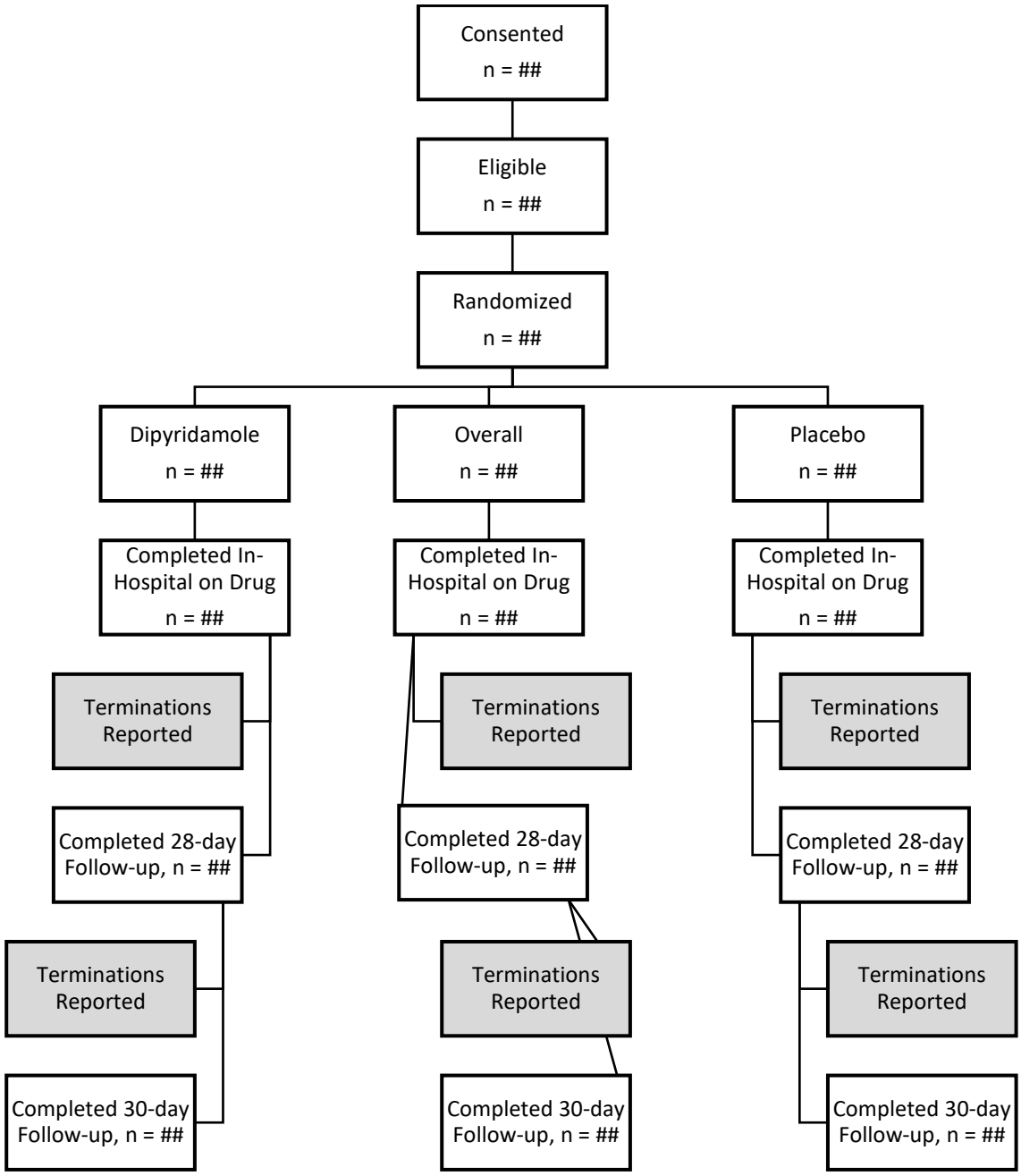


Figure 2. CONSORT Diagram

9.2 Derived variables

9.2.1 Hierarchical Composite Endpoint Used in Win Ratio Analysis

The win ratio analysis of the hierarchical composite outcome requires a direct comparison of outcomes between each dipyridamole patient and placebo patient. The patient with the superior outcome is adjudicated the 'winner' and receives a +1 score, while the 'loser' scores -1. The criteria for comparison are below and are summarized in Table 3:

1. If one participant dies and one lives, the participant who does not die wins (Scenario 1)
2. If both participants die, then the participant who lives longer wins (Scenario 2)

3. If both participants die and live the same number of days, then there is a tie (Scenario 3).
4. If neither participant dies, then the participant with fewer days on ventilator wins (Scenario 4).
5. If the participants are tied after step 4, and only one of the participants has a decrease in daily average SpO2/FiO2 of 50 or greater, then the participant who does not have the decrease in SpO2/FiO2 wins (Scenario 5).
6. If the participants are tied after step 5, then the participant with lower cumulative COVID ordinal score wins (Scenario 6),
7. If the participants are tied after step 6, then their final result is a tie (Scenario 7).

Table 3. Description of Hierarchical Composite Endpoint Win Scenarios

Scenario	Death [1]	Survival [1]	Number of days on mechanical ventilation [2]	Decrease from baseline in SpO2/FiO2 ≥ 50 [3]	Sum of COVID ordinal scores [4]	Score
1	Dead					-1
	Alive					+1
2	Dead	Shorter				-1
	Dead	Longer				+1
3	Dead	Tied				0
	Dead	Tied				0
4	Alive	N/A	Longer			-1
	Alive	N/A	Shorter or None			+1
5	Alive	N/A	Tied	Yes		-1
	Alive	N/A	Tied	No		+1
6	Alive	N/A	Tied	Tied	Higher	-1
	Alive	N/A	Tied	Tied	Lower	+1
7	Alive	N/A	Tied	Tied	Tied	0
	Alive	N/A	Tied	Tied	Tied	0

[1] Death and survival are assessed from the time of study medication to 30 days after the last dose of study drug (a maximum of 44 days from consent). Participants are censored at the time of drop-out or the data cutoff date (if no event has occurred).
[2] Number of days on mechanical ventilation are counted from the time of study medication until hospital discharge. If a participant is re-hospitalized and receives ventilation, these data are not counted.
[3] Oxygenation measurements are collected from EHR records. Baseline is defined as the average of measurements taken between 8 hours prior to start of study medication and the start of study medication administration. A 50-point drop is defined as a participant who experiences two consecutive measures of at least 50 points below baseline. Post-baseline measurements are assessed up to and including day 44 (from consent).
[4] COVID ordinal scores are defined in Table 2.

The cumulative sum of COVID ordinal scores used in Step 6 is calculated as the time-weighted sum of daily ordinal scores, with units of ordinal score days. For example, if a subject started medication at 9:00 pm on Day 1 with a score of 4, and that score decreased to 3 at 6:00 am the next day, and that patient was discharged at noon on the third day, then the cumulative sum of ordinal scores would be:

$$\frac{\{3\ hrs \times 4 + (6\ hrs \times 4 + 18\ hrs \times 3) + 12\ hrs \times 3\}}{24\ hrs/day} = \frac{12 + (24 + 54) + 36}{24} = 5.25$$

The use of the cumulative ordinal score in Step 6 of the above algorithm is meant as a combined measure of the severity and duration of a patient's illness, since longer hospitalizations and larger daily ordinal scores will both increase the cumulative total.

9.3 Protocol Violations

A protocol violation occurs when the participant, study coordinator or the investigator fails to adhere

to significant protocol requirements affecting the inclusion, exclusion, participant safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

1. Use of a prohibited concomitant medication
2. Non-compliance with study drug regimen

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The principal investigator in consultation with IND Sponsor will determine if a protocol violation will result in withdrawal of a participant. A listing of protocol deviations by intervention group and overall will be provided.

9.4 Demographic and Baseline Variables

Demographic variables for participants will be:

1. Age in years at consent
2. Dichotomized age (≤ 65 or > 65)
3. Sex
4. Race (American Indian / Alaska Native, Asian, Black / African American, Native Hawaiian / Other Pacific Islander, White, Unknown)
5. Ethnicity (Hispanic, non-Hispanic)
6. Height
7. Weight

Baseline variables for participants will be:

8. D-dimer
9. SpO_2 and SpO_2/FiO_2
10. COVID ordinal scale score
11. Hematology labs: C-reactive protein, LDH, ferritin, leukocyte count, and platelet count in the blood
12. Temperature
13. Glomerular filtration rate (GFR)

9.5 Concurrent Illnesses and Medical Conditions

All participants' non-study medications will be administered at the discretion of the treating medical provider. Any medications and supplements are allowed except:

- Dual antithrombotic therapy (aspirin or P2Y12 inhibitor plus anticoagulation to treat deep venous thrombosis or pulmonary embolism). Single antiplatelet agent, or anticoagulant agent at prophylaxis or therapeutic dose is permitted.
- Any medication deemed by the PI to be unsafe or likely to confound analysis.

9.6 Treatment Compliance

We will summarize participant medication exposure, including missed doses, using descriptive statistics including n, mean, median, standard deviation, minimum, and maximum. The summary will include the total number of patients on drug from Day 1 through Day 14 separately by treatment.

10 Efficacy Analyses

Continuous variables will be summarized using descriptive statistics including n, mean, median,

standard deviation, minimum, and maximum. Qualitative variables will be summarized using counts and percentages. Summaries will be provided by treatment groups and overall. Two-sided hypotheses will be tested for efficacy endpoints at the 5% level.

Unless otherwise stated, analyses will be performed on the mITT population and will be programmed in SAS software (SAS Institute, Inc., Cary, NC), while graphics will be generated using R (R Core Team, 2020).

10.1 Primary Efficacy Analysis: D-dimer

The first co-primary endpoint is mean daily change in D-dimer during up to 14 days of hospitalization. We will assess longitudinal trends in the mITT analysis set via linear mixed effects regression of log-transformed D-dimer values:

$$\log(\text{D-dimer}_{it}) = \text{Intercept}_i + \text{Slope}_i \times t$$

where i indexes the patient, and t is time since first drug administration. The intercept will consist of an overall intercept term plus terms for treatment, both stratification factors (sex and an indicator for age ≥ 65 years), baseline log(D-dimer), and a random patient effect. We will model treatment interactions by stratification factor.

The hypothesis is that patients in the treatment arm will have a smaller mean slope than those in the placebo arm. Because we are conducting an interim analysis for efficacy (Section 8.6.2.1), we adopt the O'Brien-Fleming method to partition the overall Type I error rate: $\alpha = 0.006$ for the interim analysis, and 0.022 for each primary efficacy analysis based on an interim population of $n = 45$.

10.2 Primary Efficacy Analysis: Hierarchical Composite Endpoint

The second co-primary endpoint is a hierarchical composite endpoint of death, survival, mechanical ventilation, SpO_2/FiO_2, and cumulative COVID ordinal scores. With one exception, the analysis of the mITT population will be identical to the interim analysis described in Section 8.6.2.2 based off the win ratio algorithm described in Section 9.2.1. The interim analysis was pooled across stratification factors (age and sex). The primary efficacy analysis will stratify win-ratio calculations by age and sex and estimate an overall win ratio across strata (Dong et al., 2018).

The hypothesis is that patients in the treatment arm will experience better outcomes than patients in the placebo arm, leading to a win proportion significantly higher than 0.5. Because we are conducting an interim analysis for efficacy (Section 8.6.2.1), we adopt the O'Brien-Fleming method to partition the overall Type I error rate: $\alpha = 0.006$ for the interim analysis, and 0.022 for each primary efficacy analysis based on an interim population of $n = 40$.

10.3 Secondary Efficacy Analyses

We will conduct secondary analyses on each component of the hierarchical composite endpoint:

- Time to death
  - *Analysis:* Cox proportional hazards regression
- Number of days on mechanical ventilation during study hospitalization
  - *Analysis:* Negative binomial regression
- Dichotomized (yes/no) decrease in SpO_2/FiO_2 ratio of at least 50 units relative to baseline at any time during the observation period
  - *Analysis:* Logistic regression

- Cumulative sum of COVID ordinal scores during study hospitalization
    - *Analysis:* Mann-Whitney U-test (nonparametric)

We will analyze an additional secondary endpoint:

- Days alive and free of organ support, where organ support is defined as receipt of invasive mechanical ventilation, vasopressor therapy, ECMO support, or incident dialysis
    - *Analysis:* Mann-Whitney U-Test (nonparametric)

Each analysis will include interactions of the study drug by stratification factor.

10.4 Exploratory Efficacy Analyses

The DICER study contains four major kinds of exploratory endpoints (listed in Section 5.2.4): continuous, change over time (continuous), binary, and time to event. All analyses will be based on the mITT population except where specified in the definition of the endpoint.

We will analyze continuous endpoints by ANOVA with treatment and stratification factors (sex and age) as predictors.

We will analyze continuous change over time endpoints by mixed linear effect models using the same methodology described in Section 10.1 for the D-dimer primary efficacy endpoint.

We will analyze binary endpoints by logistic regression with treatment and stratification factors (sex and age) as predictors.

We will analyze time-to-event by Cox proportional hazards regression with treatment and stratification factors (sex and age) as predictors.

In all analyses, the only estimate of interest will be the difference between treatment arms. We will include treatment interactions by stratification factor in all models, and we will fit separate models where age is continuous versus where age is represented by an indicator variable for age ≥ 65 years. Hypothesis tests will be two-sided.

11 Safety Analyses

Safety analyses will be performed on the mITT analysis set. Summaries of safety data and will include frequency, proportion, and listing of participants experiencing events. All summaries will be by treatment group and overall. Safety endpoints are:

- Incidence of serious adverse events (SAEs) during study duration,
- Proportion of participants with hypotension (systolic blood pressure below 90 mmHg and requiring a 1-liter IV fluid bolus) during study hospitalization,
- Proportion of participants during the observation period, with major bleeding as defined by the ISTH: (i) a decrease in the hemoglobin level of at least 2 g per deciliter, (ii) transfusion of at least 2 units of blood, (iii) occurrence in a critical site, or (iv) contributing to death.

11.1 Extent of Exposure

We will summarize participant medication exposure, including missed doses, using descriptive statistics including n, mean, median, standard deviation, minimum, and maximum. The summary will include the total number of patients on drug from Day 1 through Day 14 separately by treatment.

11.2 Adverse Events

11.2.1 AE Severity

The guidelines shown in Table 4. AE Severity Grading will be used to grade severity. We point out that a grading of "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 4. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The participant may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The participant is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

11.2.2 AE Relationship to Study Drug

The relationship of an AE to the study drug will be assessed using the guidelines in .

Table 5. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the participant's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

11.3 Deaths, Serious Adverse Events and other Significant Adverse Events

An SAE is defined as any AE that results in any of the following outcomes:

- Major bleeding as defined by the ISTH
- AST or ALT > 10 times the upper limit of normal

Other important medical events may also be considered a SAE when, based on appropriate medical

judgement, they jeopardize the participant or require intervention to prevent one of the outcomes listed.

Study personnel will document all SAEs that occur (whether or not related to study drug). The collection period for all SAEs will begin after informed consent is obtained and end 30 days after administration of the last dose of study drug. SAEs will be graded for severity and relationship to study drug. In accordance with the standard operating procedures and policies of the local IRB, the study team will report SAEs to the IRB.

11.4 Clinical Laboratory Evaluations

As detailed in Section 6.5.1, blood will be drawn for clinical care at least every other day, and we anticipate that most research blood studies will be performed on blood already drawn for clinical care.

Clinical laboratory evaluations will be summarized using number, mean, standard deviation, median, minimum and maximum. Relative changes from baseline to end of study hospitalization will be similarly summarized. These summaries will be done by treatment group and overall. In addition, we will produce box plots overlaid with dot plots of lab values at each time point (overall and by treatment group).

Spaghetti plots will be provided to evaluate lab value patterns for patients who have out of range values (either high or low).

11.5 Prior and Concurrent Medications

The total number and proportion of patients taking concurrent medications will be summarized descriptively by treatment group and overall.

12 Other Analyses

None at this time.

13 Reporting Conventions

P-values ≥0.001 will be reported to 3 decimal places; p-values less than 0.001 will be reported as "<0.001". The mean, standard deviation, and any other statistics other than quantiles, will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g., regression coefficients) will be reported to 3 significant figures.

14 Summary of Changes to the Protocol and/or SAP

No changes to the protocol and/or SAP have affected the analysis plan as of January 19, 2022.

15 References

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