Statistical Analysis Plan
for the Analysis of the ACE2 RAS Domain
for Patients with COVID-19 Pandemic
Infection Suspected Or Proven (PISOP)

COVID-19 ACE2 RAS Domain SAP Version 1.2 dated 4 August 2022

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1.   COVID-19 ACE2 RAS DOMAIN SAP VERSION

The version is in this document's header and on the cover page.

1.1.   Version history

Version 1:   Finalized on 14 April 2022

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3.   INTRODUCTION

This statistical plan for the analysis of the ACE2 RAS Domain in the pandemic stratum of the REMAP-CAP trial is an appendix to the Pandemic Appendix to Core (PAtC) Statistical Analysis Plan (SAP). This plan details the statistical analyses in the original REMAP-CAP core SAP and the pandemic stratum SAP applied to the analysis of interventions in the ACE2 RAS Domain. This plan is prespecified for the imminent unblinding of data for the ACE inhibitor (ACEi), angiotensin receptor blocker (ARB), ARB + DMX-200, and control interventions in the ACE2 RAS Domain in the severe state, within the pandemic infection suspected or proven (PISOP) (COVID-19) stratum. Presently, the domain has recruited into four intervention arms (no RAS inhibitor [control], ACEi, ARB, and ARB + DMX-200) in two disease states (moderate and severe). While the amended Domain Specific Appendix (Version 2, dated 14 October 2021) includes a fifth intervention arm (TRV-027 + ACEi), at the present time recruitment into this intervention has not yet commenced; therefore, this SAP will not discuss the analysis of data related to this potential intervention.

The ACE2 RAS Domain employed a seamless phase 2-phase 3 design. While the domain was in phase 2, on 24 February 2022, the Data Safety and Monitoring Board (DSMB) communicated safety concerns related to increased risk for acute kidney injury and mortality for severe PISOP patients in both ACEi and ARB treatment arms to the International Trial Steering Committee (ITSC) and made a recommendation to close recruitment in this state. These DSMB recommendations were based on their third, scheduled review of participant safety data, which had included 564 patients in the severe state randomized to either control, ACEi, ARB, or ARB + DMX-200. Enrollment was paused on 24 February, 2022, and subsequently closed in the severe state by the ITSC on 26 February 2022. At the time of initial writing this SAP, enrollment of moderate patients was paused pending assessment of complete data for severe state patients, as well as further review of external data. On 16 June 2022, the trial steering committee voted to close recruitment of patients into the moderate state for operational futility. This SAP prespecifies the analysis plan for both the severe and moderate states. The outcome measures and analysis in SAP is consistent with the trial protocol, including the Core Protocol and Domain Specific Appendix.

4.   DESIGN CONSIDERATIONS

REMAP-CAP explores the effects of treatments in multiple treatment domains on the primary outcome of organ support-free days (OSFDs) by randomizing patients within multiple domains simultaneously. The adaptive platform trial was designed to produce modular results for individual

interventions or full domains. REMAP-CAP is designed with a hierarchical Bayesian analysis as the primary analysis method for the trial. There is one overarching Bayesian model, prespecified in the SAP, driving result summaries. That primary statistical analysis model will be used to report the results for the ACE2 RAS Domain interventions (ACEi, ARB, and ARB + DMX-200) in each of the moderate and severe states of the PISOP stratum.

The decision to use a Bayesian analysis was driven in part by the uncertainty of the extent of the pandemic. The sample size could be small or large, and there may be unexpected external events, such as other trial results, that alter the design of REMAP-CAP. Given the expected evolution of the design and uncertain sample size, the Bayesian approach is more appropriate.

The ACE2 RAS Domain was unique to-date in REMAP-CAP in that the domain employed a seamless phase 2-phase 3 design. All interventions were initially evaluated in phase 2. Blinded graduation to phase 3 could only occur provided: (1) evidence of an acceptable safety profile including on the basis of secondary safety outcomes; and (2) an intermediate probability (>50%) that at least a modest improvement (>20% for ACEi and ARB; >30% for ARB + DMX-200) in the primary outcome of OFSDs was present. The second criterion was required to be met within approximate sample size caps (n=300 for ACE inhibitor and ARB, and n=200 for DMX-200+ARB) – otherwise, intervention(s) would fail to graduate to phase 3. Any intervention could also be dropped in phase 2 for futility based on the usual platform definition of futility (<5% that the median proportional odds ratio [OR] >1.2) at any adaptive analysis. (OR>1 suggests benefit compared with control whereas OR<1 suggests harm.) Once interventions enter phase 3, platform criteria are evaluated at sequential adaptive analyses: efficacy (>99% probability that OR >1; each active intervention in comparison with no RAS inhibitor, and ARB + DMX-200 in comparison with ARB alone), superiority (>99% probability that among all interventions one is the best), equivalence (if two interventions have >90% probability of equivalence [within a 20% odds ratio difference]; among all active interventions), and inferiority (no RAS inhibitor only).

5.   UNBLINDING

REMAP-CAP has multiple domains to which patients can be randomized and multiple interventions within domains. At the time of unblinding of the ACE2 RAS Domain in the severe state, there are other interventions to which patients have been randomized that will not be unblinded at this analysis unless a statistical trigger is hit at the time of the primary analysis. This includes interventions within other domains that are not yet unblinded. In the analysis plan, there will be analyses conducted by the

Statistical Analysis Committee (SAC) using additional randomizations and unblinding of other randomizations. The SAC is unblinded to all interventions and domains as part of their role for REMAP-CAP. There will be other analyses that are conducted with only knowledge of the unblinded RAS inhibitor/control allocation status for patients or the allocation status to other unblinded interventions. These may be conducted by investigators who are blinded to information about other interventions and domains. These analyses are identified below.

6. INTERVENTIONS

Presently, there are three active interventions and one control within the ACE2 RAS Domain:

1. No RAS inhibitor (control)
2. Angiotensin converting enzyme inhibitor (ACEi)
3. Angiotensin receptor blocker (ARB)
4. ARB + DMX-200

(As noted, a fifth intervention – TRV-027 + ACE inhibitor – has not yet commenced recruitment at the time of SAP writing and is therefore not included in this SAP.)

7. DISEASE STATES

There are two disease states in the PAtC, which are **moderate** and **severe**. The ACE2 RAS Domain has been open for randomization in patients in the moderate and severe states. The DSMB recommendation could suggest continuation or termination of recruitment for the disease states individually based on the criteria defined in section 4 or due to safety concerns on the basis of secondary outcome measures.

The primary analysis model will estimate distinct effects of each intervention in the moderate and severe states; only the intervention effects for the unblinded intervention(s) and state(s) will be reported. The secondary analysis models run by blinded investigators will be run on only the unblinded intervention(s) and state(s).

8. ANALYSIS POPULATIONS

1. REMAP-CAP COVID-19 severe and moderate state intent-to-treat (ITT). This primary population consists of all PISOP patients in the moderate or severe state randomized within ≥ 1 domain.
2. Unblinded ITT. All PISOP patients randomized in the ACE2 RAS Domain or to any other unblinded interventions/domains within the PISOP stratum in either moderate or severe state.

3. Unblinded non-negative COVID-19. All patients in the Unblinded ITT population after removing those with >1 negative test for COVID-19 **and** no positive tests.

4. ACE2 RAS specific ITT. This population consists of only patients randomized to the ACE2 RAS Domain within the PISOP stratum.

5. DMX-200-eligible ITT. This population consists of only patients randomized to the DMX-200 + ARB intervention, ARB intervention, or the control intervention in the ACE2 RAS Domain within the PISOP stratum who were eligible for the DMX-200 + ARB intervention at the time of randomization

9. ENDPOINTS

The following endpoints will be analyzed, displayed graphically, and summarized through descriptive statistics.

1. **Organ Support-Free Days (OSFD)**
   a. An ordinal endpoint with in-hospital mortality as the worst outcome. The primary endpoint for the REMAP-CAP PISOP stratum. The organ support considered is cardiovascular (vasopressor/inotrope support) and respiratory support. See **Appendix A** for a detailed description.

2. **Survival to Hospital Discharge** (a component of the primary outcome)
   a. A dichotomous endpoint of in-hospital death where the death component corresponds to a –1 on the OSFD endpoint. Follow-up for this outcome is censored at 90 days.
   b. This endpoint will be reported as "survival to hospital discharge" where an OR>1 suggests benefit and OR<1 suggests harm (directionally consistent with OSFD).

3. **90-day Mortality**
   a. This is a time-to-event endpoint through 90-days.
   b. Any patient currently in the hospital or transferred on organ support to an alternative care facility will be censored at their last known status alive.
   c. Any patient successfully discharged from hospital, alive, without organ support, will be assumed to be alive at 90 days, if 90-day mortality data are not yet recorded.

4. **Acute Kidney Injury**

    This is defined as (1) **Stage 2**: serum creatinine increase 2-3x from baseline (time of randomization); and (2) **Stage 3**: serum creatinine increase ≥3x from baseline, *or* increase in serum creatinine by ≥0.5 mg/dL (44 mmol/L) to ≥4 mg/dL (353.6 µmol/L), *or* initiation of renal replacement therapy. Both endpoints are analyzed within two time points: from randomization to study day 7, and from randomization to study day 14.

    a. A dichotomous endpoint of whether a patient developed KDIGO Stage ≥2 AKI within each timeframe (7 and 14 days)

    b. A dichotomous endpoint of whether a patient developed KDIGO Stage 3 AKI within each timeframe (7 and 14 days)

    c. The proportion of patients qualifying for KDIGO Stage 3 AKI on the basis of receiving renal replacement therapy (hemodialysis) will be reported by intervention within each time window.

5. **Change from Baseline to Peak Creatinine within 14 Days**

    a. A continuous endpoint comparing the relative change from baseline to peak creatinine.

6. **Renal Replacement-Free Days**

    a. An ordinal outcome of the number of days free of renal replacement (hemodialysis) through 28 days. This is the exact calculation of OSFD, with renal replacement therapy as the only organ support category. In-hospital death is considered a –1.

7. **Vasopressor/Inotrope-Free Days**

    a. An ordinal outcome of the number of days free of vasopressor/inotropes through 28 days. This is the exact calculation of OSFD, with vasopressor/inotropes as the only organ support category. In-hospital death is considered a –1.

8. **Hypotension While Admitted to a Ward**

    a. A dichotomous endpoint of hypotension while admitted to a ward. This outcome is collected through 14 days after randomization, and is defined as one or more episodes of clinically relevant hypotension while admitted to a ward: clinically relevant hypotension includes hypotension that triggers medical emergency/rapid response team activation, hypotension that requires ICU admission, > 500mL fluid

administration in less than one hour, or administration of vasopressor or inotrope). This outcome is only evaluated in patients randomized in the moderate state.

9. **Angioedema**

    a. A dichotomous endpoint of angioedema during hospitalization.

10. **Change in Baseline to Peak Available AST, ALT, and Bilirubin through Study Day 14**

    a. In the DMX-200 + ARB intervention and in DMX-200 + ARB eligible controls, a continuous endpoint evaluating the relative change in baseline to peak biomarker level, included to assess potential toxicity of the DMX-200 + ARB intervention, compared with control.

11. **Respiratory Support-Free Days**

    a. An ordinal outcome of the number of days free of respiratory support through 28 days. This is the exact calculation of OSFD, with mechanical respiratory support as the only organ support category (includes high flow nasal oxygen, non-invasive and invasive mechanical ventilation). In-hospital death is considered a –1.

12. **Ventilator-Free Days**

    a. An ordinal outcome of the number of days free of invasive mechanical ventilation through 28 days. This is the exact calculation of OSFD, with ventilator-free days as the only organ support category. In-hospital death is considered a –1.

13. **Duration of ICU Stay**

    a. A time-to-event endpoint of leaving the ICU alive. If a patient is known to leave the ICU and return to the ICU within 14-days that intervening time will be ignored.

    b. This variable will be truncated at 90-days: all deaths in ICU will be considered 90-days with no liberation of ICU.

    c. Patients still in the ICU at data snapshot will be considered censored.

14. **Duration of Hospital Stay**

    a. A time-to-event endpoint of leaving the hospital alive. If a patient is known to leave and return to the hospital within 14-days that intervening time will be ignored.

    b. This variable will be truncated at 90-days and all deaths in-hospital will be considered 90-days with no discharge event.

      c.   Patients still in the hospital at data snapshot will be considered censored.

15. The World Health Organization (WHO) 8-Point Ordinal Scale, Measured at Day 14.

      a.   A modified WHO ordinal scale will be used:

          i.   0 + 1 + 2 = No longer hospitalized

          ii.   3 = Hospitalized, no oxygen therapy

          iii.   4 = Oxygen by mask or nasal prongs

          iv.   5 = Non-invasive ventilation or high-flow oxygen

          v.   6 = Intubation and mechanical ventilation

          vi.   7 = Ventilation + additional organ support: vasopressors, renal replacement therapy (RRT), ECMO

          vii.   8 = Death

16. At Least One Serious Adverse Event (SAE)

      a.   A dichotomous endpoint of at least one SAE.

      b.   For the DMX-200 + ARB intervention, individual SAEs and SUSARs will be reported.

10. GRAPHICAL DATA SUMMARIES

1. All ordinal endpoints will be plotted using stacked cumulative bar plots and cumulative probability plots.

2. All time-to-event endpoints will be plotted using Kaplan-Meier plots. Positive clinical event outcomes will be plotted as the cumulative rate of event, and negative events will be plotted as the cumulative rate of event-free.

3. All continuous endpoints will be plotted using histograms and boxplots.

11. DESCRIPTIVE STATISTICS

Endpoints will be modeled with the following conventions. For interventions with <10 participants, outcomes may be summarized numerically without the use of modeling due to model estimate instability in view of small sample size. Similarly, endpoints with <5 occurrences will be reported numerically without the use of modeling.

1. Ordinal endpoints will be summarized by the cumulative frequency of each outcome. The 25th, 50th, and 75th percentiles will be summarized.

2. Dichotomous endpoints will be summarized by the proportion in each category.

3. Time-to-event outcomes will be summarized by the 2.5th, 10th, 25th, 50th, 75th, 90th, and 97.5th percentiles from the Kaplan-Meier estimates, as available.

4. Continuous endpoints will be summarized by the mean, standard deviation, median and IQR.

5. Composite endpoints will be summarized overall and for each component individually.

12.   BASELINE CHARACTERISTICS AND CO-INTERVENTIONS

The following demographics and baseline variables will be summarized across arms, stratified by state. More may be added as baseline summaries: Age, sex, BMI, race, ethnicity, illness severity at admission, pre-existing conditions, baseline use of organ support (high-flow nasal oxygenation, non-invasive ventilation, invasive mechanical ventilation, ECMO, vasopressors/inotropes), eGFR, serum creatinine, potassium, C-reactive protein (CRP), APACHE II score, blood pressure and other miscellaneous physiological values. Additionally, exposure to relevant drugs as usual care (e.g., steroids, immunomodulatory therapies, etc.) at baseline will be compared across interventions.

13.   STUDY TREATMENTS

Study drug use will be summarized with the following variables. The specific ACEi and ARB agents used will be summarized as proportions based on the first day's study drug administered. (Study drug agent is generally not anticipated to change over the treatment course, although it could.) Doses will be categorized into intensities reflecting low, moderate, and high doses based on the framework in **Appendix B**. For the ACEi and ARB arms, the following will be summarized in tabular format: Initial dose intensity; maximum dose intensity; total days of treatment; and median time spent receiving each categorized dose intensity (none; low; moderate; high) during days alive in the 10-day treatment window. Total frequencies for the reasons for discontinuing study drug will be tabulated by treatment arm.

14.   ANALYTIC APPROACH

Each inferential analysis will be done using a Bayesian model. Some default frequentist methods are used for exploration and description. Causal forest machine learning methods will evaluate heterogeneity of treatment effect. A summary of the analyses methods is provided below.

14.1. Primary Analysis of Primary Endpoint

The **primary analysis model** is a Bayesian cumulative logistic model for the ordinal primary endpoint. The model is described below.

The primary endpoint for the severe state has 23 and the moderate state has 24 possible ordered outcomes respectively. Let the outcome for a patient be labeled as Y_i, with possible values, –1 (death), 0, 1, …, 21, 22. The outcome of 22 for the severe state (never received organ support) is not possible. A cumulative logistic model is specified. The model is structured so that an odds-ratio >1 implies clinical benefit. The full details of the model are specified in the Current State of The Statistical Model, Version 4.3 dated 28 July 2022. The model has factors for:

- Each level of the ordinal endpoint
- State at randomization
- Each global site, nested within country
- Age; ≤39, 40-49, 50-59, 60-69 (reference), 70-79, 80+
- Sex; Male (reference) or female
- Time; 2-week buckets of time working backwards from the last enrolled patient, with the most recent bucket being 4 weeks.
- For each domain, an effect for being randomized to the domain
- For each domain, an effect for being ineligible for the domain
- An effect for each intervention within each domain
- Specified interactions in the model between interventions across domains

The primary analysis uses the following rules:

• All sites within a country that have <5 patients randomized will be combined into a single site within that country.

• If there is an outcome in the ordinal scale that did not occur in the data in a given state, then that outcome will be combined with a neighboring outcome (the worse outcome). This is done for model stability. For example, if the outcome 11 never occurred in the severe state, then a combined outcome of 10 & 11 will be modeled for the severe state in that analysis.

• Time buckets with <5 randomized subjects in a state may be combined with the more recent neighboring bucket for that state.

The primary analysis model will be referenced with certain model assumptions for sensitivity analyses. For example, the "time effects" in the model could be assumed to be 0.

14.1.1.   Proportional Odds Assumption

The primary analysis model is based on an assumption of a proportional effect of treatment across the scale of the ordinal outcome. In order to assess the robustness of the results to this assumption, a dichotomous model is fit to every level of the ordinal outcome across the scale and the odds-ratio

for each dichotomous break is presented. No statistical test of proportional odds is conducted. This sensitivity analysis will be run in models including both the severe and moderate states.

14.2.   Analytic Approach for Secondary Dichotomous Endpoints

A Bayesian logistic regression model will be used for each dichotomous outcome. The model is the standard logistic link function model:

$$log\left(\frac{\pi}{1-\pi}\right) = \alpha - [factors]$$

References will be made to the factors in the model and their prior distribution. Many of these factors will be the same as the primary analysis model, with the same priors, as the parameters have similar interpretation. For example, all in-hospital mortality models should use the Beta prior distribution implied by the Dirichlet prior in the OSFD model. If not otherwise specified, the prior distribution for the main effect is $\alpha \sim N(0, 1.82^2)$ (similar to a uniform prior on the probability scale).

14.3.   Analytic Approach for Secondary Time-To-Event Endpoints

All inferential time-to-event analyses will be done using a Bayesian piecewise exponential model. The Bayesian time-to-event model is intended to mirror a Cox proportional hazards model, with the underlying hazard rate modeled with a piecewise exponential model. The underlying hazard will be modeled with a hazard rate for each 10-day period in the model. The prior distribution for the hazard rate for each day is a gamma distribution with 1 day of exposure and a mean equal to the total exposure divided by the total number of events. This prior will have very little weight but will provide numerical stability to the model. Each factor is incorporated as a proportional hazard rate through an additive linear model of the log-hazard. The default prior for each factor is the same as for the log-odds in the ordinal model. If other non-specified variables are added to the model, then a normal distribution with mean 0 and standard deviation 10 will be utilized.

14.4.   Analytic Approach for Secondary Continuous Endpoints

A Bayesian linear regression model will be used for each continuous outcome. The model is a standard linear model:

$$Y = \alpha + Y_{baseline} + [factors] + \epsilon$$
$$\epsilon \sim Normal(0, \sigma^2)$$

Priors:

$$\alpha \sim N(0,1)$$
$$\left(\frac{1}{\sigma^2}\right) \sim Gamma(0.5, 0.5)$$

Each continuous outcome Y will be centered and scaled, and other transformations of the outcome may be considered if the normality assumption is violated. The term $Y_{baseline}$ refers to the baseline measurement of the (standardized) outcome. The prior on the coefficient for $Y_{baseline}$ will be a normal distribution with mean 0 and standard deviation 1. The formulation of this continuous model will be the same as the primary analysis model. Unless a hierarchical distribution is specified, all coefficients in the model will have a prior that is normally distributed with mean 0 and standard deviation 1. All hierarchical distribution hyperpriors for precision parameters will have a Gamma prior with shape 0.5 and rate 0.5. If other non-specified variables are added to the model, then a normal distribution with mean 0 and standard deviation 1 will be utilized. Model parameters may be summarized on the scale of the standardized outcome and/or on the scale of the original outcome measure.

14.5. Markov Chain Monte Carlo (MCMC) Model Stability

The Bayesian models have many parameters and there may be risk of poor model stability, including convergence and mixing behavior of the MCMC sampler. These instabilities may be based on sparse data on the outcome or covariates. The statisticians running the model may make changes that do not affect the overall interpretation but provide reliable model diagnostics and scientific rigor. Any alterations will be noted.

14.6. Model Outputs

The standard model outputs for each treatment effect will be the mean, standard deviation, median, and 95% credible intervals (all credible intervals will be equal-tailed intervals, so 95% credible intervals will range from the 2.5th percentile to the 97.5th percentile of the posterior distribution). For the ordinal endpoints, the odds-ratios will be summarized. For the dichotomous endpoints, the odds-ratio will be summarized. For the time-to-event endpoints, the hazard ratios will be summarized. For continuous endpoints, the estimated change in mean will be summarized.

For each inferential model, a posterior probability that one arm is superior will be provided for each comparison between arms. This posterior probability has been identified as the primary analysis metric between arms. A posterior probability greater than 99% has been identified as statistically significant in REMAP-CAP.

14.7. Subgroup Analyses

The following subgroups will be evaluated: age (3 subgroups: <50, 50-70, 70+); sex (2 subgroups); baseline invasive mechanical ventilation (2 subgroups); baseline vasopressors/inotropes (2

subgroups); baseline eGFR (3 subgroups: <60 ml/min/1.73 m2, ≥60 mL/min ml/min/1.73 m2, unknown); region (feasible subgroups will be determined pending review of final enrollment by region); race (4 subgroups: white, black, Asian, other/unknown).

A prospective analysis using causal forest machine learning methods will evaluate heterogeneity of treatment effects. The analysis will proceed in two phases. In the first phase, the consideration of subgroup effects will be limited to the above list of pre-specified subgroups, and the results compared to those from the Bayesian model. The second phase will assess a wider range of possible subgroup effects, by considering all baseline covariates and combinations thereof as possible subgroup effects. We will avoid overfitting by using 'honest estimation', whereby an observation is either used to determine the splits, or to estimate the effects but not both.

The causal forest method will be applied to survival to hospital discharge and 90-day mortality in accordance with the currently available implementations of the method that accommodates binary or continuous but not ordinal endpoints. Full details are provided in Appendix C.

14.8. Exploratory Analyses

Exploratory analyses after unblinding will not be considered inferential and no p-values will be presented. Any post-hoc exploratory analyses will be clearly labeled as exploratory and will use the conventions below. Post-hoc mediation analysis may be considered to evaluate whether the occurrence of intermediate outcomes (e.g., acute kidney injury or vasopressor use) mediates a potential association between treatment allocation and other outcomes (e.g., OSFDs or hospital survival). Additionally, exploratory analyses may consider evaluating predicted versus observed control event rate (CER) to contextualize the findings.

1. Ordinal endpoints will be compared using a cumulative proportional odds model with summaries of the OR, 95% credible intervals, and Wilcoxon tests for robustness against a lack of proportional odds.

2. Time-to-Event analyses will utilize a Cox proportional hazards model, summarizing the hazard ratios and 95% confidence intervals.

3. Continuous endpoints will compare means with 95% confidence intervals based on two-sample t-test procedures.

4. Dichotomous proportions will be compared using logistic regression summarizing the OR and 95% confidence intervals. Differences between proportions will be summarized using observed differences and normal approximations for the 95% credible intervals.

15.   SPECIFIC PROSPECTIVE ANALYSES

Table 1. Prospective analysis. Modeling results will not be reported for any intervention with <10 patients

#	Status	Population	Endpoint	State(s)	Intervention	Other
15.1	Primary	REMAP-CAP COVID-19 moderate and severe state ITT	OSFD	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	Includes all interventions and pre-specified interactions.
15.2	Primary	REMAP-CAP COVID-19 moderate and severe state ITT	Survival to hospital discharge	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	Includes all interventions and pre-specified interactions.
15.3	Sensitivity	REMAP-CAP COVID-19 moderate and severe state ITT	Dichotomized OSFD	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	A logistic regression will be run for each dichotomization of OSFDs as a robustness check.
15.4	Sensitivity	Unblinded ITT population	OSFD	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	Includes all unblinded interventions and pre-specified interactions.
15.5	Sensitivity	Unblinded ITT population	Survival to hospital discharge	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	Includes all unblinded interventions and pre-specified interactions.
15.6	Sensitivity	ACE2 RAS specific ITT	OSFD	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	Only ACE2 RAS interventions are modeled
15.7	Sensitivity	ACE2 RAS specific ITT	Survival to hospital discharge	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	Only ACE2 RAS interventions are modeled
15.8	Secondary	Unblinded ITT population	90-day mortality	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	A time-to-event analysis.
15.9	Secondary	ACE2 RAS specific ITT	Acute kidney injury (KDIGO Stage ≥2 AKI) within 7 days	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	A dichotomous endpoint of KDIGO Stage ≥2 AKI within 7 days of randomization.

15.10	Secondary	ACE2 RAS specific ITT	Acute kidney injury (KDIGO Stage ≥2 AKI) within 14 days	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	A dichotomous endpoint of KDIGO Stage ≥2 AKI within 14 days of randomization.
15.11	Secondary	ACE2 RAS specific ITT	Acute kidney injury (KDIGO Stage 3 AKI) within 7 days	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	A dichotomous endpoint of KDIGO Stage 3 AKI within 7 days of randomization.
15.12	Secondary	ACE2 RAS specific ITT	Acute kidney injury (KDIGO Stage 3 AKI) within 14 days	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	A dichotomous endpoint of KDIGO Stage 3 AKI within 14 days of randomization.
15.13	Secondary	ACE2 RAS specific ITT	Change from baseline to peak creatinine	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	A continuous endpoint.
15.14	Secondary	Unblinded ITT population	Renal replacement-free days	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	An ordinal outcome.
15.15	Secondary	Unblinded ITT population	Vasopressor/ inotrope-free days	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	An ordinal outcome.
15.16	Secondary	Unblinded ITT population	Respiratory support-free days	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	An ordinal outcome.
15.17	Secondary	Unblinded ITT population	Ventilator-free days	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	An ordinal outcome.
15.18	Secondary	Unblinded ITT population	Duration of ICU Stay	Severe	ACEi, ARB, DMX-200 + ARB, control	A time-to-event outcome.

15.19	Secondary	Unblinded ITT population	Duration of Hospital Stay	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	A time-to-event outcome.
15.20	Secondary	Unblinded ITT population	WHO Ordinal Scale	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	An ordinal outcome.
15.21	Secondary	ACE2 RAS specific ITT	Hypotension while admitted to a ward	Moderate	ACEi, ARB, DMX-200 + ARB, control	A dichotomous outcome.
15.22	Secondary safety analysis	DMX-200-elidgible ITT	Change in baseline to peak available AST	Moderate and Severe	DMX-200 + ARB, control	A continuous outcome.
15.23	Secondary safety analysis	DMX-200-elidgible ITT	Change in baseline to peak available ALT	Moderate and Severe	DMX-200 + ARB, control	A continuous outcome.
15.24	Secondary safety analysis	DMX-200-elidgible ITT	Change in baseline to peak bilirubin	Moderate and Severe	DMX-200 + ARB, control	A continuous outcome.
15.25	Secondary safety analysis	ACE2 RAS specific ITT	Occurrence of angioedema	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	A dichotomous outcome.
15.26	Primary safety analysis	ACE2 RAS specific ITT	Serious adverse events	Moderate and Severe	ACEi, ARB, DMX-200 + ARB, control	A dichotomous outcome.
15.27	Subgroup	Unblinded ITT population	OSFD	Severe and Moderate	ACEi, ARB, DMX-200 + ARB, control	Including differential treatment effect by age (<50, 50-70, 70+)
15.28	Subgroup	Unblinded ITT population	Survival to hospital discharge	Severe and Moderate	ACEi, ARB, DMX-200 + ARB, control	Including differential treatment effect by age (<50, 50-70, 70+)

15.29	Subgroup	Unblinded ITT population	OSFD	Severe and Moderate	ACEi, ARB, DMX-200 + ARB, control	Including differential treatment effect by sex.
15.30	Subgroup	Unblinded ITT population	Survival to hospital discharge	Severe and Moderate	ACEi, ARB, DMX-200 + ARB, control	Including differential treatment effect by sex.
15.31	Subgroup	Unblinded ITT population	OSFD	Severe and Moderate	ACEi, ARB, DMX-200 + ARB, control	Including differential treatment effects by invasive mechanical ventilation at baseline (yes/no)
15.32	Subgroup	Unblinded ITT population	Survival to hospital discharge	Severe and Moderate	ACEi, ARB, DMX-200 + ARB, control	Including differential treatment effects by invasive mechanical ventilation at baseline (yes/no)
15.33	Subgroup	Unblinded ITT population	OSFD	Severe and Moderate	ACEi, ARB, DMX-200 + ARB, control	Including differential treatment effects by vasopressors/ inotropes at baseline (yes/no)
15.34	Subgroup	Unblinded ITT population	Survival to hospital discharge	Severe and Moderate	ACEi, ARB, DMX-200 + ARB, control	Including differential treatment effects by vasopressors/ inotropes at baseline (yes/no)
15.35	Subgroup	Unblinded ITT population	Vasopressor/ inotrope-free days	Severe and Moderate	ACEi, ARB, DMX-200 + ARB, control	Including differential treatment effects by vasopressors/ inotropes at baseline (yes/no)
15.36	Subgroup	Unblinded ITT population	OSFD	Severe and Moderate	ACEi, ARB, DMX-200 + ARB, control	Including differential treatment effects by eGFR at baseline (<60 ml/min/1.73 m^2; ≥60 mL/min ml/min/1.73 m^2; unknown).
15.37	Subgroup	Unblinded ITT population	Survival to hospital discharge	Severe and Moderate	ACEi, ARB, DMX-200 + ARB, control	Including differential treatment effects by eGFR at baseline (<60 ml/min/1.73 m^2; ≥60 mL/min ml/min/1.73 m^2; unknown).
15.38	Subgroup	ACE2 RAS specific ITT	Acute kidney injury (KDIGO Stage ≥2 AKI)	Severe and Moderate	ACEi, ARB, DMX-200 + ARB, control	Including differential treatment effects by eGFR at baseline (<60 ml/min/1.73 m^2; ≥60 mL/min ml/min/1.73 m^2; unknown).

			within 14 days			
15.39	Subgroup	ACE2 RAS specific ITT	Acute kidney injury (KDIGO Stage 3 AKI) within 14 days	Severe and Moderate	ACEi, ARB, DMX-200 + ARB, control	Including differential treatment effects by eGFR at baseline (<60 ml/min/1.73 m^2; ≥60 mL/min ml/min/1.73 m^2; unknown).
15.40	Subgroup	Unblinded ITT population	Renal replacement therapy-free days	Severe and Moderate	ACEi, ARB, DMX-200 + ARB, control	Including differential treatment effects by eGFR at baseline (<60 ml/min/1.73 m^2; ≥60 mL/min ml/min/1.73 m^2; unknown).
15.41	Subgroup	Unblinded ITT population	OSFD	Severe and Moderate	ACEi, ARB, DMX-200 + ARB, control	Including differential treatment effect by region.
15.42	Subgroup	Unblinded ITT population	Survival to hospital discharge	Severe and Moderate	ACEi, ARB, DMX-200 + ARB, control	Including differential treatment effect by region.
15.43	Subgroup	Unblinded ITT population	OSFD	Severe and Moderate	ACEi, ARB, DMX-200 + ARB, control	Including differential treatment effect by race (4 subgroups: white, black, Asian, other/unknown).
15.44	Subgroup	Unblinded ITT population	Survival to hospital discharge	Severe and Moderate	ACEi, ARB, DMX-200 + ARB, control	Including differential treatment effect by race (4 subgroups: white, black, Asian, other/unknown).
15.45	Subgroup	ACE2 RAS specific ITT	Survival to hospital discharge	Severe and Moderate	ACEi, ARB, control	Heterogeneity of treatment effect using causal forest machine learning methods

15.1. Reporting of Analysis Results

For each analysis model, the following summaries will be reported when applicable:

Parameter	Mean	SD	Median	95% Credible Interval
Age < 39				
Age 40-49				

Age 50-59				
Age 70-79				
Age 80+				
Female				
Time Bucket 1				
…				
Time Bucket k-1				
Moderate to severe transition				
ACEi or ARB or ARB + DMX?				
Main effect of subgroup				
ACEi or ARB therapy by subgroup				

For each analysis model, the following comparisons will be made by state, when applicable:

- ACEi, ARB, and ARB + DMX-200 interventions will be compared to the control (no RAS inhibitor) arm. The posterior probability that OR>1 will be used to define efficacy, whereas this probability may also be reported as the probability of harm if OR<1. In subgroup models, this probability will be provided by subgroup. If sample size permits (minimum of 10 patients in each intervention), ARB + DMX-200 will be compared with ARB.

- ACEi, ARB, and DMX-200 + ARB interventions will be compared for futility. A 95% probability of a smaller than 1.2 odds ratio for ACEi, ARB, and DMX-200 + ARB therapy relative to no RAS inhibitor will be used as a statistical trigger for futility. In subgroup models, this probability will be provided by subgroup.

- For the sensitivity analysis assessing the proportional odds assumption, the ACEi and ARB intervention ORs will be reported for each dichotomization of OSFD and each unblinded state.

15.2. Graphical summaries

The following graphical summaries will be provided for all endpoints:

- Population: ACE2 RAS specific ITT

- Endpoint: all endpoints

- Factors: ACE2 RAS and no ACE2 RAS interventions

The following additional graphical summaries will be provided for OSFD and in-hospital mortality:

- Population: ACE2 RAS specific ITT

- Endpoint: OSFD, in-hospital mortality

- Factors:

    o ACEi and ARB use and no RAS inhibitor use interventions

- Analysis: Conducted by the ITSC Analysis Center

Appendix A. Definition of organ support-free days

This outcome is an ordinal scale of integers from –1 to 22 for each state (Moderate or Severe) derived from a composite of the patient's vital status at the end of acute hospital admission and days spent receiving organ failure support while admitted to an ICU (including a repurposed ICU) during the 21 days (504 hours) after randomization.

A patient enrolled in the Severe State while still in an Emergency Department is regarded as 'admitted to an ICU' and the time of commencement of organ failure support is the time of randomization, as it is for all other patients in the Severe State.

Patents who survive to hospital discharge and are enrolled in one or more domains in the Moderate State and are enrolled in one or more domains in the Severe State have a primary end point value for each state, which may be different.

If deceased between first enrolment and ultimate hospital discharge, code OutcomeDay21 as -1

If not deceased, ModerateOutcomeDay21 = 21 – (the sum of the length of time in days and part-days between time of first commencement of organ failure support while admitted to an ICU and the time of last cessation of organ failure support during that ICU admission plus time between first commencement and last cessation of organ failure support during any and all subsequent readmissions to ICU, censored at the 504 hours after enrolment in the Moderate State)

- A patient who is enrolled in the Moderate State who never receives organ failure support while admitted to an ICU has a ModerateOutcomeDay21 = 22.
- A patient who is enrolled in the Moderate State in a ward location who commences organ failure support on the ward and is transferred to an ICU while receiving organ failure support has a commencement time of organ failure support corresponding to the time of ICU admission.

If not deceased, SevereOutcomeDay21 = 21 – (the sum of the length of time in days and part- days between time of enrolment and the time of last cessation of organ failure support during that ICU admission plus the lengths of time between first commencement and last cessation of organ failure support during any and all subsequent readmissions to ICU, censored at 504 hours after the time of enrolment

Decimals are rounded up or down to nearest whole day.

If transferred between hospitals before the last study day 21 and known to be alive at ultimate hospital discharge use all available information to calculate Outcome Day21 with an assumption that no subsequent organ failure support in an ICU was provided.

If transferred between hospitals before the last study day 21 and vital status at ultimate hospital discharge is not known, code as follows:

- If last known to be on a ward use all available information to calculate OutcomeDay21 with an assumption that the patient has not died prior to ultimate hospital discharge and that there were no subsequent ICU admissions.
- If last known to be in an ICU, code OutcomeDay21 as missing (999)

If a patient is discharged alive from the ultimate hospital before 504 hours from each enrolment, assume all subsequent time is alive and without provision of organ failure support in an ICU.

If the patient is alive at the end of one or both censoring time points, the hours will be calculated as above. If the patient dies after the end of one or both of the censoring time points and before hospital discharge, the value will be updated to -1

A patient who remains admitted to an acute hospital and is still alive at the end of study day 90 no further changes to coding will be made.

Appendix B. Study Drug Intensity Dose Equivalents

Agent	Approximate Dose Equivalent* (based on total daily dose)		
	Low	Moderate	High
ACE inhibitors			
Ramipril	≤2.5 mg	>2.5 to 10 mg**	>10 to 20 mg
Lisinopril	≤10 mg	>10 to 20 mg	>20 to 80 mg
Perindopril	≤4 mg	>4 to 8 mg	>8 to 16 mg
Enalapril	≤5 mg	5-20 mg	>20 to 40 mg
Trandolapril	≤1 mg	>1 to 4 mg	>4 to 8 mg
Captopril	≤37.5 mg	>37.5 to 100 mg	>100 to 450 mg
Angiotensin receptor blockers			
Losartan	≤50 mg	>50 to 100 mg	>100 to 150 mg
Valsartan	≤80 mg	>80 to 160 mg	>160 to 320 mg
Candesartan	≤8 mg	>8 to 16 mg	>16 to 32 mg
Irbesartan	≤150 mg	>150 to 300 mg	>300 mg***
Telmisartan	≤40 mg	>40 to 80 mg	>80 mg***
Olmesartan	≤20 mg	>20 to 40 mg	>40 mg***

*Categorization is based on the available dose range and (particularly in the low dose ranges) approximate equivalency in blood pressure effect when used for the treatment for hypertension, as well as on guideline-directed starting doses and available dose ranges for use in heart failure with reduced ejection fraction.
**Ramipril 5 mg total daily dose is considered a starting dose when used in heart failure.
***Doses in this range are generally not used in routine clinical practice.

Appendix C. Causal forest evaluation of treatment effect heterogeneity

Analysis population
ACE2 RAS specific intention-to-treat population excluding patients assigned to the investigation agent DMX-200 plus an ARB given the extremely small numbers (<10) assigned to this intervention. Moderate and Severe disease states will be combined for this analysis as the co-variates (factors) describing baseline organ support fully distinguish these two severity states.

Endpoints
1. <u>Survival to hospital discharge</u>. The causal forest method will be applied to survival to hospital discharge (a component of the primary outcome) in accordance with the currently available implementations of the method that accommodates binary or continuous but not ordinal endpoints.
2. <u>90-day mortality</u>. A secondary causal forest analysis will be performed using 90-day mortality

Co-variates / factors
In the first phase, co-variates will be limited to the pre-specified subgroups as per section (age group, sex at birth, baseline invasive, baseline vasopressors/inotropes, baseline eGFR, region, race) and the results compared to those from the Bayesian model.
In the second phase, all baseline covariates as per section 12 will be considered. Where appropriate, composite scores in addition to component variables will be used (e.g. APACHE II score).

Method
As this domain includes 3 interventions, the "*multi_arm_causal_forest*" function in the *grf* package in R will be used to evaluate all treatment contrasts (Tibshirani et al, 2022). An initial causal forest consisting of 50,000 trees will be 'grown' and the included variables then ranked in terms of their variable importance in determining splits within these trees. Those variables with variable importance above a threshold (e.g 0.2*mean importance) will be retained and training repeated to obtain the final causal forest.

The following hyperparameters will be tuned: number of trees (num.trees), the fraction of the data used to build each tree (sample.fraction), the number of variables tried for each split (mtry), the targetted minimum number of observations in each tree leaf (min.node.size), the fraction of data that will be used for determining splits (honesty.fraction) and a penalty to reduce the likelihood of splits that result in a large size imbalance between the split groups, or leaf nodes that are too small (imbalance.penalty). To tune these hyperparameters, we will draw a number (100) of random points in the space of possible parameter values, train the multi-arm CF using these parameter values, and compute the out-of-bag errors and use these to determine the optimal hyperparameter values.

The following hyperparameters will not be tuned and will have the following settings:
 sample.weights = NULL
 equalize.cluster.weights = FALSE,
 honesty = TRUE,
 honesty.prune.leaves = TRUE,
 alpha = 0.05,
 stabilize.splits = TRUE,
 ci.group.size = 2,
 compute.oob.predictions = TRUE,
 num.threads = NULL,
 seed = a fixed value for reproducability (e.g. 1344876)

Cluster-robust estimations will be obtained by training the forest using clusters of site within country, weighted by the number of individuals within each cluster and optimised using simulations. A time co-variate in days since first Domain randomisation will be used. Missing values will be handled implicity by the *grf* package using the 'missing incorporated in attributes' criterion (Twala, 2008) which assumes that data are missing at random.

If omnibus testing demonstrates no significant evidence of heterogeneity, forest training will be repeated with both active intervention arms (ACEi and ARB) pooled using the standard "*causal_forest*" function.

A data adaptive approach will be used to identify additional subgroups using an honest estimation approach whereby a random forest will be estimated on half of the data and the most representative tree used to identify potential subgroups of interest. Effects and standard errors for these groups will then be estimated using the remainder of the data (not used to identify the subgroups, thereby avoiding overfitting).

Outputs

The final forests will be used to estimate the Conditional Average Treatment Effect (CATE) for each individual along with their standard errors, by predicting from the forest using their covariate values. These individual CATEs will then be aggregated to obtain conditional ATEs for each subgroup using augmented inverse propensity weighting as implemented in the '*average_treatment_effect*' function in the *grf* package. Subgroup conditional ATEs will be reported as both odds ratios and risk differences with 95% confidence intervals.

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Twala, B. E. T. H., M. C. Jones, and David J. Hand. Good methods for coping with missing data in decision trees. *Pattern Recognition Letters* 29, 2008