Title: Investigating the relationship between the renin angiotensin system and the coagulopathy associated with COVID-19

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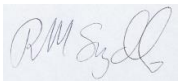
Richard Szydlo, Statistician	13 May 2021	

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Sponsor

Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

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STUDY SUMMARY

TITLE	**Investigating the relationship between the renin angiotensin system and the coagulopathy associated with COVID-19**
DESIGN	Double blind randomised placebo-controlled trial
AIMS	To determine whether RAS inhibition modulates coagulopathy in COVID-19
OUTCOME MEASURES	D-dimer
POPULATION	Inpatients with COVID-19
ELIGIBILITY	Hospitalised, aged 18 or over
DURATION	Each subject will be in the study for 30 days. The study is expected to take 6 months

1. INTRODUCTION

1.1 BACKGROUND

Several clinical trials testing blockade of Angiotensin II (AngII) in COVID-19 using angiotensin converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARBs) are underway (e.g. NCT04330300, NCT04335786 and NCT04355936). The rationale for these trials is based on the two predicates. First, SARS-CoV2 will lead to internalisation and inactivation of ACE2 (the enzyme that metabolises AngII to Ang(1-7)) and cause AngII accumulation, as occurred with SARS-CoV1 (Kuba, Lo, Gralinski, Wang). Second, that COVID-19 has many features which are strikingly similar to the effects of excessive exposure to AngII (Tang (a), Tang (b), Whitaker, Gavras (a), Gavras (b)). However, this approach may fail to yield net benefit, because it overlooks the role of Ang(1-7) and the impact of these drugs on Ang(1-7). Ang(1-7) is the product of ACE2 mediated AngII metabolism, and hence the inactivation of ACE2 induced by COVID-19 will lead not only to AngII accumulation, but also to Ang(1-7) depletion. Because this is due specifically to ACE2 inactivation, it does not occur in more classical conditions featuring renin-angiotensin axis activation, such as heart failure. Ang(1-7) functionally antagonises AngII through its agonist action at the MAS receptor, and may also potentially act as a biased agonist at the AngII receptor (AT1R) (Bader, Galandrin, Montezano, Teixeira, Touyz). Indeed, animal models show that Ang(1-7) depletion causes pathology which aligns with that seen in COVID-19 patients, including lung injury, lung inflammation, myocardial microinfarcts, characteristic glomerular thrombosis and coagulopathy. (Bihl, Bossi, Driggin, Fraga Silva, Hao, Klein, Supe, Xue, Yang (a), Yang (b), Zhang K, Zhang X).

The coagulopathy is particularly noteworthy given an early increase in D-dimer has very high positive predictor value for death in COVID-19, and D-dimer concentrations are unusually high in COVID-19, over and above what would be expected for an acute phase response in an unwell patient (Tang (a), Tang (b)). Given the pleiotropic effects of AngII and Ang(1-7) on the components of the coagulation system, overactivation of the renin angiotensin system may partly drive dysregulation of coagulation. We therefore hypothesise that the coagulopathy associated with COVID-19 is partly explained by both AngII accumulation and Ang(1-7) deficiency. Importantly, whilst ACEI and ARB treatment will antagonise AngII accumulation, they not only fail to address Ang(1-7) deficiency, but would in fact worsen it. This is because ACEI will further deplete Ang(1-7) production, over and above that caused by ACE2 internalization (Luque), and whilst an ARB will not further deplete Ang(1-7), it will prevent it from binding AT1R and hence block its beneficial actions via this receptor. Hence neither ACEi nor ARBs are appropriate tools to address this hypothesis in the context of COVID-19 infection

TRV027 is a similar peptide to Ang(1-7) but is a much more potent biased agonist at AT1R than Ang(1-7), potently and selectively recruiting β-arrestin to the AT1R while antagonizing Ang II-stimulated Gq activation (Violin). This β-arrestin bias of the ligand translates into unique downstream signaling, including extracellular signal-regulated kinases 1 and 2 (ERK1/2) phosphorylation and AT1R internalization. Recruitment of β-arrestin by TRV027 stimulates the activation of endothelial cell nitric oxide synthase (eNOS) and prostacyclin production (Violin). The combination of inhibition of Ang-II-mediated G-protein activation and activation of eNOS and prostacyclin production may contribute to the in vivo vasodilatory properties of TRV027 (Violin). ***In summary, TRV027, a biased agonist at AT1R, would be expected to oppose the effects of AngII accumulation, and functionally correct the Ang(1-7) deficiency.*** Hence it is an appropriate tool to examine the link between RAS activation and coagulopathy in the context of COVID-19 infection.

1.2 Study Objectives

Primary

To determine whether the coagulopathy associated with COVID-19 infection is driven by over activation of the renin angiotensin system (RAS)

Secondary objective

To investigate whether dysregulation of other systems associated with COVID-19 infection is driven by over activation of the renin angiotensin system (RAS)

1.3 Study Endpoints

Primary endpoint

Mean change from baseline D-dimer at day 1 to day 3 post randomisation will be compared using paired or unpaired non-parametric methods as appropriate.

Secondary endpoints

Mean change from baseline to day 1 plasma renin and from day 1 to day 3, day 5, day 8 for exploratory variables will be investigated

1.4 Study Design and Population

The study will be run as a double-blind, randomized controlled experimental medicine study in male and female hospitalised patients (n=60) aged 18 or over, with confirmed COVID-19 infection. Patients who are admitted with confirmed COVID-19 infection will be screened with a routine medical assessment, and enrolled if they meet the eligibility criteria. Subjects will be block randomised based on age to continuous intravenous infusion of placebo or TRV027 for 7 days.

1.5 Study sample size

The sample size (30 each group) provides 80% power (alpha 0.05) to detect a 30% reduction in the D-dimer level. Subjects will be block randomised based on age (<60, 60-69, >69 years).

1.6 Study management

The day-to-day management of the study will be co-ordinated by the study project manager.

2. General Considerations

2.1 Analysis Strategy

Histograms and boxplots will be used to check the distribution and possible outliers for continuous variables. Continuous variables will be summarised using medians and ranges. Categorical variables will be summarised using frequencies and percentages.

2.2 Definition of population for analysis

The population for analysis will be the Full Analysis Set (FAS), which consists of all patients who received the full course of TRV027 or placebo. Patients who received at least 1 dose of TRV027 or placebo, but did not complete 3 days of infusion (Incomplete Analysis Set (IAS)) will be included in the baseline data set and summarised with descriptive statistics.

2.3 Data management

Most data is collected and managed using REDCap, an electronic data capture system. The REDCap system includes validation rules for data entry to help ensure data accuracy, and has a full audit trail of data entry and changes. Data will be transcribed into REDCap from the original source (e.g. Cerner electronic medical records) and queries will be raised for inconsistent, impossible or missing data.

2.4 Missing data

For the primary analysis, there will be no data imputation for missing data in the primary endpoint.

2.5 Withdrawal from the study

Patients withdrawn from the study will still be included in the final analysis. Withdrawal can occur at any time according to the following reasons: Patient decision; toxicity, lost to follow-up; Death, or PI decision.

2.6 Interim analysis

There is no plan to conduct any interim analysis.

2.7 Deviations from the SAP

Any deviation from the SAP, e.g. changes of handling and analysis methods of secondary variables following review of data during the study, will be reported in the final analysis report. If problems or fundamental issues become apparent in the on-going checking that forms part of the statistical analysis, the trial statistician will raise these with the appropriate individuals. Protocol deviations identified at any stage during the study will be documented and made available to the statistician and CI. Any such action and subsequent decisions will be documented in the final statistical analysis report.

3 Analysis Plan

3.1 Descriptive Analyses

The baseline characteristics of the patient cohort will be summarised according to section 2.1 (Table 1).

3.2 Efficacy data

DDimer levels will be measured at baseline and at 3 days. Differences in DDimer will be calculated for each patient, enabling an estimate of the mean difference and 95% confidence interval to be calculated for each treatment arm. The differences for the treatment groups will be compared using parametric or non-parametric tests as appropriate (Table 10). In addition, a 30% reduction in DDimer will be considered a response, and so the proportions responding (with 95% CI) will be calculated, and compared for the two groups (Table 11). The results of these two analyses will provide sufficient evidence to differentiate between statistical and clinical significance.

3.3 Exploratory data

Exploratory data variables will be measured at baseline and at days 3,5 and 8 (Tables 9, 11). Differences in exploratory variables will be calculated for each patient, enabling an estimate of the mean difference and 95% confidence interval to be calculated for each treatment arm. The differences for the treatment groups will be compared using parametric or non-parametric tests as appropriate.

3.4 Safety data

Adverse events (AE) will be summarised by severity (NCI-CTCAE v4.03), classification, expectedness and relationship to the study drug (Table 3) and also described per patient for each treatment groups (Tables 4 and 5), while serious adverse events (SAE) will be summarised by SAE reason, severity, and causal relationship to study drug (Table 6). The details of each SAE will also be listed with the respective SAE term and outcome (Tables 7 and 8).

3.5 Drug Compliance

Drug compliance will be determined using data entered on to Redcap to calculate patient drug compliance. Number and duration of dose interruptions will be reported for each patient and compliance reported as the percentage of dose completion (Table 2). Although patients may receive up to 7 days infusion, for the primary endpoint, 72hrs infusion will be considered 100%.

3.6 Mortality data

Patient survival status will be monitored up to day 30 and tabulated in Table 9.

3.7 Additional data

Vital signs and cardiovascular monitoring will be documented at days 1, 3 and 5. …... (Table 12).

Table 1. Baseline characteristics

	FAS		IAS	
	Control	TRV027	Control	TRV027
	Median (range) or N (%)	Median (range) or N (%)	Median (range) or N (%)	Median (range) or N (%)
Age (yr)				
Age group **<60** **60-69** **>69**				
Gender **Male** **Female**				
BMI (kg/m^2)				
Ethnicity White Mixed Asian Black Other				
Disease severity? **Ie FDA grade 1-5?**				

Table 2 Drug Compliance

	Control N (%)	TRV027 N (%)
Total Missed doses		
Total % Compliance		
Median missed doses per patient (range)		
Patients with missed dose due to hospital discharge		
Patients with missed dose due to death		

Table 3 Safety data – Pooled Adverse events

	Control		TRV-027	
	No. of Events	**No. of Subjects***	**No. of Events**	**No. of Subjects***
Number of Adverse events				
Severity Grade 1 Grade 2 Grade 3 Grade 4 Grade 5 Missing				
AE classification Not serious Serious				
AE expectedness Expected Unexpected Not Applicable Missing				
Relation to study Definitely Probably Possibly Unlikely Not related Not assessable Missing				

* *A patient may have several AEs*

Table 4 – TRV027 **Safety data –Adverse events details by patient**

Control				TRV-027			
Pt ID	**AE category**	**AE name**	**Grade**	**Pt ID**	**AE category**	**AE name**	**Grade**

Table 6 Safety data – Serious Adverse Events

	Control		TRV027	
	No. of Events	**No. of Subjects**	**No. of Events**	**No. of Subjects**
Number of Serious Adverse Events				
Reasons for SAE Resulted in death Life threatening Congenital anomaly/birth defect Persistent or significant Disability/incapacity Inpatient hospitalisation/prolongation of existing Other				
Severity Mild Moderate Severe Life threatening Death				
Causal relationship to study drug Definitely Probably Possibly Unlikely Not related Not assessable				

Table 7 Safety data – Serious Adverse events details (Controls)

SAE description	SAE Term	Outcome

Table 8 Safety data – Serious Adverse events details (TRV027)

SAE description	SAE Term	Outcome

Table 9 Day 30 Survival

	Control	TRV027
Alive		
Dead		
Lost to follow-up		

Table 10 FAS Efficacy and exploratory parameters

	Control			TRV027		
	Day 1 [N=] Median (range)	**Day 3 [N=] Median (range)**	**Difference D3 – D1**	**Day 1[N=] Median (range)**	**Day 3 [N=] Median (range)**	**Difference D3 – D1**
D-dimer						
Platelets						
aPTT						
INR						
Fibrinogen						
Total Bilirubin						
LDH						
Haptoglobin						
Creatinine						
BNP						
Troponin						
Ferritin						
Pro-calcitonin						
Glucose						

Table 11 Categorical Outcomes

	Control		TRV027		
	Day 1 (Yes/No %)	**Day 3 (Yes/No %)**	**Day 1 (Yes/No %)**	**Day 3 (Yes/No %)**	
Inotrope requirement					
DDimer response (>30% reduction)	NA		NA		

NA=not applicable

Table 12 vital signs and cardiovascular monitoring

	Screening [N=] % or Median (range)	**Day 1 [N=] % or Median (range)**	**Day 3 [N=] % or Median (range)**	**Day 5 [N=] % or Median (range)**
Pulse (Bpm)				
Diastolic blood pressure (mmHg)				
Systolic blood pressure (mmHg)				
Oxygen saturations (%)				
Temperature				