Protocol Amendment to ACE2 Renin-Angiotensin System (RAS) Domain-Specific Appendix

Summary of changes

REMAP-CAP: Randomized, Embedded, Multifactorial Adaptive Platform trial for Community-Acquired Pneumonia

REMAP-CAP COVID-19 ACE2RAS Modulation Domain-Specific Appendix Amendment Summary

Version 1 dated 16 November 2021

TABLE OF CONTENTS

1. CURRENT VERSIONS OF PROTOCOL DOCUMENTS

1.1. The current versions of ACE2 RAS Modulation Domain specific protocol documents

- REMAP-CAP Core Protocol Version 3, dated 10 July 2019
- REMAP-CAP Pandemic Appendix to Core Version 2, dated 18 May 2020
- REMAP-COVID Core Protocol Version 1, dated 27 March 2020
- REMAP-CAP COVID-19 ACE2 RAS Modulation Domain-Specific Appendix Version 1, dated 08 November 2020

2. AMENDMENT 1

The COVID-19 ACE2 RAS Modulation Domain-Specific Appendix Protocol document underwent an amendment in October 2021. The primary objective of this amendment is to add a new intervention – TRV-027, a novel AT1 receptor-selective agonist, as a potential treatment for acute respiratory distress syndrome (ARDS) and abnormal clotting in patients with COVID-19. Additionally, details of the statistical analysis are clarified, as well as the specification of an additional secondary outcome and clarification of an exclusion criterion.

The REMAP-CAP COVID Core Protocol has been created as an alternative core protocol document for submission in some regions. This document removes any information from the REMAP-CAP Core Protocol that is not relevant to the COVID-19 pandemic, and integrates this information with the Pandemic Appendix to the Core Protocol into a single document. It is intended that the REMAP-COVID Core Protocol may be used by some regions as an alternative to the REMAP-CAP Core Protocol and the Pandemic Appendix to the Core Protocol. The language in this DSA has been modified to refer to either set of core protocol documents.

2.1. Summary of changes

Section	Original text	New Text	Reason
Front page and whole document header	REMAP-CAP COVID-19 ACE2 RAS Modulation Domain-Specific Appendix Version 1 dated 08 October 2020	REMAP-CAP COVID-19 ACE2 RAS Modulation Domain-Specific Appendix Version 2 dated 14 October 2021	Administrative change
Whole document	Version 1.0 (page 13) 8th November 2020 (page 15) 7.3.4 (page 44)	Version 1 14th October 2021 8.4.4	Minor administrative corrections to align new sections referenced in the text and with nomenclature across protocol documents. Updated appendix approval date by Chair
Summary Page 2	In this domain of the REMAP-CAP trial, participants meeting platform entry criteria with suspected or microbiological testing confirmed COVID-19 will be randomized to receive one of up to three renin-angiotensin system (RAS) blockade strategies, depending on availability and acceptability, or control: • No RAS inhibitor (no placebo) • Angiotensin converting enzyme inhibitor (ACEi) • Angiotensin II receptor blocker (ARB)	In this domain of the REMAP-CAP trial, participants meeting platform entry criteria with suspected or microbiological testing confirmed COVID-19 will be randomized to receive one of up to four renin-angiotensin system (RAS) modulation strategies, depending on availability and acceptability, or control: • No RAS inhibitor (no placebo) • Angiotensin converting enzyme inhibitor (ACEi) • Angiotensin II receptor blocker (ARB)	Inclusion of a new potential intervention.

<table>
<tr>
<td></td>
<td>• ARB in combination with DMX-200, a chemokine receptor-2 [CCR2] inhibitor (ARB + DMX-200)
At this participating site the following interventions have been selected within this domain:
☐ No RAS inhibitor
☐ ACEi
☐ ARB
☐ ARB + DMX-200</td>
<td>• ARB in combination with DMX-200, a chemokine receptor-2 [CCR2] inhibitor (ARB + DMX-200)
• ACEi in combination with TRV-027, an angiotensin-(1,7) analogue (ACEi + TRV-027)
At this participating site the following interventions have been selected within this domain:
☐ No RAS inhibitor
☐ ACEi
☐ ARB
☐ ARB + DMX-200
☐ ACEi + TRV-027</td>
<td></td>
</tr>
<tr>
<td>Summary
Page 3</td>
<td colspan="2">Original table
<table>
<tr><td>Stratum</td><td colspan="2">Pandemic infection suspected or proven (PISOP)</td><td>Pandemic infection neither suspected nor proven (PINSNP)</td></tr>
<tr><td>Core protocol documents</td><td colspan="2">REMAP-CAP Core Protocol + Pandemic Appendix, or
REMAP-COVID Core Protocol</td><td>REMAP-CAP Core Protocol</td></tr>
<tr><td>Illness Severity State</td><td>Moderate State</td><td>Severe State</td><td>Severe State</td></tr>
<tr><td>Interventions specified in this DSA</td><td>No RAS inhibitor
ACEi
ARB</td><td>No RAS inhibitor
ACEi</td><td>Not available</td></tr>
</table></td>
<td>Update to the standard table with the addition of the new intervention</td>
</tr>
</table>

	ARB + DMX-200		ARB ARB + DMX-200	
Interventions submitted for approval in this jurisdiction	☐ No RAS inhibitor ☐ ACEi ☐ ARB ☐ ARB + DMX-200		☐ No RAS inhibitor ☐ ACEi ☐ ARB ☐ ARB + DMX-200	Not available
Interventions offered at this site	Ward	ICU	ICU	ICU
	☐ No RAS inhibitor ☐ ACEi ☐ ARB ☐ ARB + DMX-200	☐ No RAS inhibitor ☐ ACEi ☐ ARB ☐ ARB + DMX-200	☐ No RAS inhibitor ☐ ACEi ☐ ARB ☐ ARB + DMX-200	Not available

New text

Stratum	Pandemic infection suspected or proven (PISOP)		Pandemic infection neither suspected nor proven (PINSNP)
Core protocol documents	REMAP-CAP Core Protocol + Pandemic Appendix, or REMAP-COVID Core Protocol		REMAP-CAP Core Protocol
Illness Severity State	Moderate State	Severe State	Severe State
Interventions specified in this DSA	No RAS inhibitor ACEi ARB	No RAS inhibitor ACEi	Not available

<table>
<tr><td></td><td>
<table>
<tr><td></td><td colspan="2">ARB + DMX-200
ACEi + TRV-027</td><td>ARB
ARB + DMX-200
ACEi + TRV-027</td><td></td></tr>
<tr><td>Interventions submitted for approval in this jurisdiction</td><td colspan="2">☐ No RAS inhibitor
☐ ACEi
☐ ARB
☐ ARB + DMX-200
☐ ACEi + TRV-027</td><td>☐ No RAS inhibitor
☐ ACEi
☐ ARB
☐ ARB + DMX-200
☐ ACEi + TRV-027</td><td>Not available</td></tr>
<tr><td rowspan="2">Interventions offered at this site</td><td>Ward</td><td>ICU</td><td>ICU</td><td>ICU</td></tr>
<tr><td>☐ No RAS inhibitor
☐ ACEi
☐ ARB
☐ ARB + DMX-200
☐ ACEi + TRV-027</td><td>☐ No RAS inhibitor
☐ ACEi
☐ ARB
☐ ARB + DMX-200
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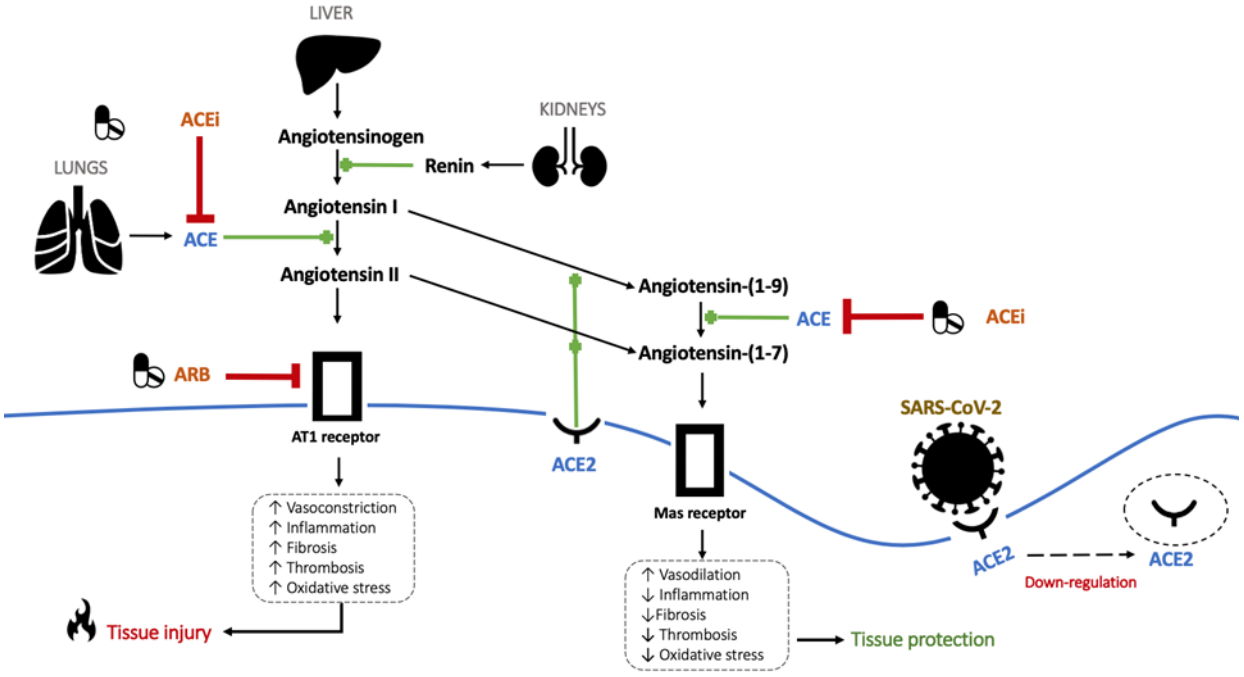
☐ ACEi
☐ ARB
☐ ARB + DMX-200
☐ ACEi + TRV-027</td><td>Not available</td></tr>
</table>
</td><td></td></tr>
<tr><td>Summary table
Page 4</td><td></td><td>Changes to Interventions, Domain-Specific exclusions, Intervention-Specific exclusions, and Outcome measures sections:
(1) Clarification of wording around exclusion criteria for history of angioedema
(2) Addition of secondary outcomes, “hypotension while admitted to a ward”</td><td>Updated summary table which provides an overview of the document.</td></tr>
</table>

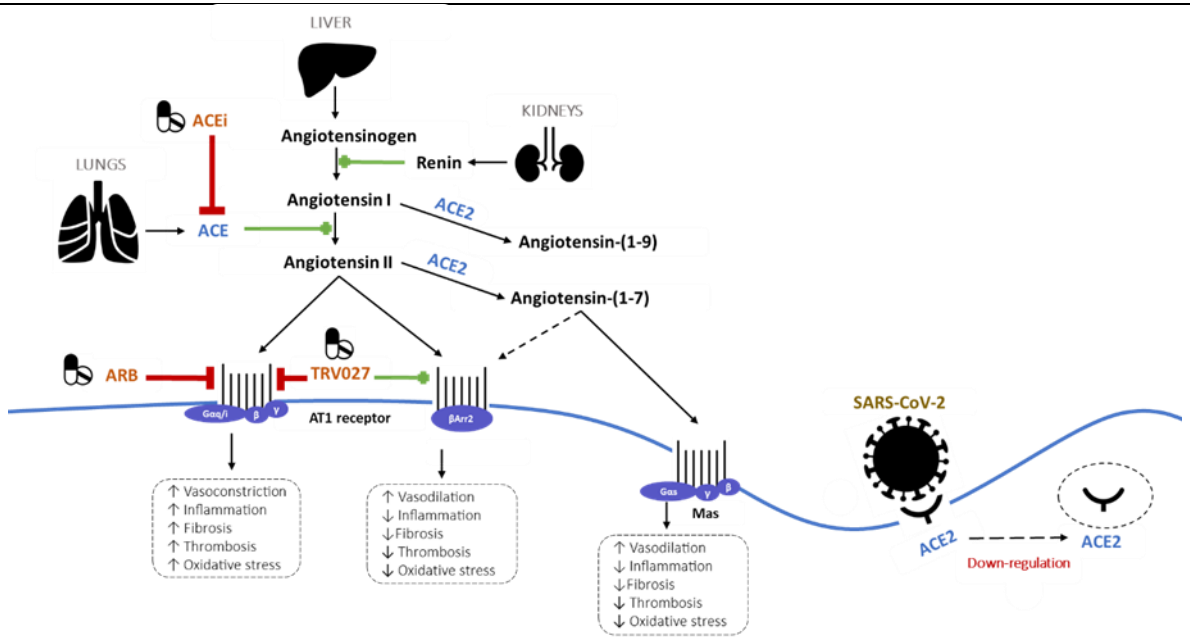
		(3) Explicit mention of vasopressor-free days as a secondary safety outcome on which phase 2 graduation decisions are based. Details are in this summary of changes of document.	
SECTION 3 COVID-19 ACE2 RAS MODULATION DOMAIN-SPECIFIC APPENDIX VERSION	**Original text**	**New Text**	**Reason**
3.1. Version History Page 13	Version 1.0: Approved by the COVID-19 ACE2 RAS Modulation Domain-Specific Working Group (DSWG) on 8th November 2020	Version 1: Approved by the COVID-19 ACE2 RAS Modulation Domain-Specific Working Group (DSWG) on 8th November 2020 Version 2: Approved by the COVID-19 ACE2 RAS Modulation Domain-Specific Working Group (DSWG) on 14 October 2021	Administrative update
SECTION 4 VITAMIN C DOMAIN GOVERNANCE	**Original text**	**New Text**	**Reason**
4.1. Domain members Page 13	Dr. Rebecca M. Baron Dr. Slava Epelman Dr. Claudia Frankfurter Dr. David Gattas Dr. Frank Gommans Prof. Anthony Gordon Dr. Rashan Hanifa Prof. David Huang Dr. Edy Kim	Dr. Rebecca M. Baron Dr. Slava Epelman Prof. Justin Ezekowitz Dr. Claudia Frankfurter Dr. David Gattas Dr. Frank Gommans Prof. Anthony Gordon Dr. Rashan Hanifa Prof. David Huang	Updated with new members

	Dr. Francois Lamontagne Dr. David Leaf Prof. John Marshall Dr. Colin McArthur Dr. Bryan McVerry Prof. Danny McAuley Dr. Muthiah Vaduganathan Dr. Roland van Kimmenade Prof. Frank van de Veerdonk Prof. Steve Webb	Dr. Edy Kim Ms. Yvonne Kwan Dr. Francois Lamontagne Dr. David Leaf Prof. John Marshall Dr. Colin McArthur Dr. Bryan McVerry Prof. Danny McAuley Dr. David Owen Dr. Katrina Pollock Dr. Michael Puskarich Dr. Muthiah Vaduganathan Dr. Roland van Kimmenade Prof. Frank van de Veerdonk Prof. Steve Webb	
SECTION 6 BACKGROUND AND RATIONALE	**Original text**	**New Text**	**Reason**

6.2.1. SARS-CoV-2, ACE2, the renin-angiotensin-system, and its modulators Page 15	The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), originating from Wuhan, Hubei province, China, has resulted in a global pandemic of an infectious respiratory disease known as COVID-19 (Huang et al., 2020, Zhu et al., 2020). A rapid understanding of the disease has evolved over the months since its identification, supported by observations from severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1), the virus responsible for the SARS epidemic in 2002-2004.	Deleted	Shortened for brevity
6.2.1. SARS-CoV-2, ACE2, the renin-angiotensin-system, and its modulators Page 16	Angiotensin-(1-7) exerts counter-regulatory effects to angiotensin II by binding to the Mas receptor, eliciting vasodilation as well as anti-inflammatory and anti-thrombotic effects (Mehta and Griendling, 2007, Santos et al., 2003, Santos et al., 2018.	Angiotensin-(1-7) exerts counter-regulatory effects to angiotensin II both by binding to the Mas receptor and by acting as a biased agonist of AT1, eliciting vasodilation as well as anti-inflammatory and anti-thrombotic effects (Mehta and Griendling, 2007, Santos et al., 2003, Santos et al., 2018, Galandrin et al., 2016, Gaidarov et al., 2018, Teixeira et al., 2017).	Updated to include reference to new intervention's putative mechanism of action. References added in this section also added to Section 13 References (page 57)
6.2.1. SARS-CoV-2, ACE2, the renin-angiotensin-system, and its modulators Page 17	Original Figure 1		Pictorial description of the renin-angiotensin system amended to include where the new intervention acts


LIVER
KIDNEYS
ACEi
LUNGS
Angiotensinogen
Renin
Angiotensin I
ACE
Angiotensin II
Angiotensin-(1-9)
ACE
ACEi
Angiotensin-(1-7)
ARB
AT1 receptor
ACE2
SARS-CoV-2
Mas receptor
↑ Vasoconstriction
↑ Inflammation
↑ Fibrosis
↑ Thrombosis
↑ Oxidative stress
↑ Vasodilation
↓ Inflammation
↓Fibrosis
↓ Thrombosis
↓ Oxidative stress
ACE2
Down-regulation
ACE2
Tissue injury
Tissue protection

New Figure 1

			
6.2.1. SARS-CoV-2, ACE2, the renin-angiotensin-system, and its modulators Page 18	Activation of the AT1 receptor results in vasoconstriction, aldosterone release, as well as an increased inflammation, fibrosis, thrombosis, and oxidative stress, which precipitates tissue injury, notably in the lungs. ACE2, a homologue of ACE, cleaves angiotensin I into angiotensin-(1-9) and angiotensin II into angiotensin-(1-7) that acts on the Mas receptor. Activation of the Mas receptor results in vasodilation and decreases inflammation, fibrosis, thrombosis, and oxidative stress, in counterbalance to the negative	Activation of the AT1 receptor by angiotensin II results in vasoconstriction, aldosterone release, as well as an increased inflammation, fibrosis, thrombosis, and oxidative stress, which precipitates tissue injury, notably in the lungs. ACE2, a homologue of ACE, cleaves angiotensin I into angiotensin-(1-9) and angiotensin II into angiotensin-(1-7) that acts on the Mas receptor. Activation of the Mas receptor results in vasodilation and decreases inflammation, fibrosis, thrombosis, and oxidative stress, in counterbalance to the negative	Explanation of the renin-angiotensin system amended to include where the new intervention acts.

<table>
<tr>
<td></td>
<td>effects of angiotensin II. ACE inhibitors and angiotensin receptor blockers (ARBs) inhibit ACE and AT1 receptors, respectively. The surface spike protein of SARS-CoV-2 attaches to the ACE2 receptor, and subsequently downregulates expression of ACE2, which may induce acute lung injury. Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; AT1, angiotensin II type 1. Overall, this domain seeks to study several modulators of the RAS system, as well as related targets outlined below, overviewed in the Box Insert.</td>
<td>effects of angiotensin II. The AT1 receptor can also be activated by angiotensin-(1-7), which acts as a biased agonist inducing a different (β-arrestin) signaling pathway from the angiotensin II G protein activation pathway (Teixeira et al., 2017); ACE inhibitors and angiotensin receptor blockers (ARBs) inhibit ACE and AT1 receptors, respectively. The surface spike protein of SARS-CoV-2 attaches to the ACE2 receptor, and subsequently downregulates expression of ACE2, which may induce acute lung injury. Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; AT1, angiotensin II type 1.</td>
<td></td>
</tr>
<tr>
<td>6.2.1. SARS-CoV-2, ACE2, the renin-angiotensin-system, and its modulators
Page 18</td>
<td>Original text - blank
New text
<table>
<tr><th colspan="3">Box Insert: Summary of possible interventions in the domain. Not all sites offer all interventions.</th></tr>
<tr><th>Intervention</th><th>Targeted mechanism of action</th><th>Possible beneficial effects</th></tr>
<tr><td>ACEi</td><td>Reduce levels of angiotensin II</td><td>Reduced inflammation, reduced fibrosis, reduced thrombosis</td></tr>
<tr><td>ARB</td><td>Block the receptor for angiotensin II</td><td>Reduced inflammation, reduced fibrosis, reduced thrombosis</td></tr>
<tr><td>TRV-027 + ACEi</td><td>TRV-027 stimulates the β-arrestin component of the angiotensin II receptor pathway as well as Mas receptor pathway; ACEi is given in combination to reduce levels of angiotensin II</td><td>Reduced inflammation, reduced fibrosis, reduced thrombosis, reduced oxidative stress</td></tr>
</table></td>
<td>Addition of a Box Insert summary table of the domain interventions, including where the new intervention acts</td>
</tr>
</table>

	DMX-200 + ARB	DMX-200 inhibits chemokine receptor-2; ARB is given in combination to reduce angiotensin II signalling, as there is evidence that these two receptors communicate and that combined blockade of both receptors is more effective than blockade of only one receptor	Reduced macrophage-related inflammation, reduced fibrosis, reduced thrombosis	
6.2.2. Adverse sequelae of unregulated angiotensin II activity and impaired angiotensin 1-7 activity Page 18	6.2.2. Adverse sequelae of unregulated angiotensin II activity The entry of SARS-CoV2 via membrane fusion down regulates ACE2 abundance on cell surface membranes, resulting in subsequent loss of the enzyme's beneficial effect to regulate angiotensin II activity (Kuba et al., 2005). Reduced ACE2 expression may be associated with both an attenuation of the conversion of angiotensin II into angiotensin 1-7 and concomitant amplification of angiotensin II activity.		6.2.2. Adverse sequelae of unregulated angiotensin II activity and impaired angiotensin 1-7 activity The entry of SARS virus via membrane fusion is believed to downregulate ACE2 abundance on cell surface membranes, resulting in subsequent loss of the enzyme's beneficial effect to regulate angiotensin II activity (Kuba et al., 2005). Reduced ACE2 expression may be associated with both an attenuation of the conversion of angiotensin II into angiotensin 1-7 and concomitant amplification of angiotensin II activity. and reduction in angiotensin 1-7 activity.	Updated to expand the discussion to include the relevance of the new intervention
6.2.2. Adverse sequelae of unregulated angiotensin II activity and impaired angiotensin 1-7 activity Page 18	Blank		Much of the pathology associated with unregulated angiotensin II activity is also reproduced by angiotensin 1-7 depletion, as would be expected given angiotensin 1-7 functionally and pharmacologically antagonizes angiotensin II. Indeed, the animal models of angiotensin 1-7 depletion produce pathology which aligns with that seen in COVID-19 patients, including lung injury, lung	Updated to expand the discussion to include the relevance of the new intervention

		inflammation, myocardial microinfarcts, characteristic glomerular thrombosis and coagulopathy.	
6.2.3. RAS inhibition: possible mechanisms of benefit Page 19	As infection with SARS-CoV-2 results in down-regulation of ACE2 expression with concomitant amplification of angiotensin II, use of ACEi or ARB may mitigate the potentially deleterious effects of unchecked RAS activation (Kuba et al., 2005).	As infection with SARS-CoV-2 results in down-regulation of ACE2 expression with concomitant amplification of local angiotensin II and reduced angiotensin 1-7, use of ACEi or ARB may mitigate the potentially deleterious effects of unchecked RAS activation (Kuba et al., 2005).	Updated to expand the discussion to include the relevance of the new intervention
6.2.4.2. Concerns about Potential Harm Give Way to Hypotheses on Potential Efficacy Page 24	These observational studies suggest that ACEi/ARB use may be safe, and furthermore possibly beneficial, in patients with COVID-19, although these observational findings may be limited by uncontrolled confounding and bias. RCTs studying the efficacy and safety of ACEi and ARB in patients with COVID-19 are being undertaken, with initial results (discussed below) from BRACE-CORONA providing reassurance and results from REPLACE-COVID pending.	These observational studies suggest that ACEi/ARB use may be safe, and furthermore possibly beneficial, in patients with COVID-19, although these observational findings may be limited by uncontrolled confounding and bias. RCTs studying the efficacy and safety of ACEi and ARB in patients with COVID-19 are being undertaken, with initial results (discussed below) from BRACE-CORONA and REPLACE-COVID providing reassurance, (Cohen et al., 2021, Lopes et al., 2021).	Updated background to include new data References added in this section also added to Section 13 References (page 57)
6.2.7. Concomitant angiotensin II inhibition with an angiotensin 1-7 analogue (TRV027) Page 32	Original text: Blank New text: 6.2.7. Concomitant angiotensin II inhibition with an angiotensin 1-7 analogue (TRV027) Whilst ACEi and ARB treatment antagonize angiotensin II accumulation, either by inhibiting its production or blocking its access to AT1, they may contribute to angiotensin 1-7 deficiency, as ACEi may further deplete angiotensin 1-7 production as it is a product of angiotensin II, and ARBs may prevent angiotensin 1-7 from binding ATR1, and hence block its beneficial biased agonist actions at this receptor. However, a combination of ACEi with an		Addition of background and rationale for new intervention (TRV027)

	angiotensin 1-7 analogue may plausibly mitigate both angiotensin II activity and angiotensin 1-7 depletion. An ACEi would reduce angiotensin II formation, and although it would further deplete endogenous angiotensin 1-7, co-administration with an angiotensin 1-7 analogue would restore the beneficial bias agonist signaling at AT1. TRV027 is a similar peptide to angiotensin 1-7, but is a more potent biased agonist at AT1R than angiotensin 1-7, potently and selectively recruiting β-arrestin to the AT1 while antagonizing Ang II-stimulated Gq activation (Violin et al., 2010). This β-arrestin bias of the ligand translates into unique downstream signaling, including extracellular signal-regulated kinases 1 and 2 (ERK1/2) phosphorylation and AT1 internalization. Recruitment of β-arrestin by TRV027 stimulates the activation of endothelial cell nitric oxide synthase (eNOS) and prostacyclin production. 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. 6.2.7.1. Safety of TRV027 Nonclinical safety pharmacology and toxicology studies have shown that TRV027 is compatible with blood, non-genotoxic, and that continuous IV infusions up to 14 days duration are well tolerated. The no observed adverse effect level (NOAEL) in 2-week continuous IV infusion studies in rats and dogs was 500 µg/kg/min, the highest dose tested. Measured TRV027 plasma concentrations at the NOAEL dose in rats and dogs were approximately 20-times higher than extrapolated steady-state plasma concentrations in humans administered the highest Phase 2 clinical dose of 25 mg/hr. TRV027 has been studied in healthy volunteers (n=20) and in three studies in patients with heart failure. In the FTIH study (NCT01514578), a dose range of 0.01 to 20 µg/kg/min for 4 hours (2.4 to 4800 µg/kg) was studied in 20 healthy subjects; the highest dose was expected to be approximately 20-fold higher than the efficacious dose in acute decompensated heart failure (ADHF) patients. All AEs reported were mild and transient. There were three AEs, in two subjects, that were considered possibly related to study drug: mild fatigue, and mild dizziness and paresthesia. All resolved within 2 hours without medical intervention. There were no deaths, no SAEs, and no AEs that led to discontinuation from the study. There were no clinically significant changes in laboratory findings, vital	

	signs (heart rate, blood pressure, oxygen saturation, respiratory rate and temperature), ECGs or physical examinations. Study CP120027.1002 enrolled 17 patients with heart failure and renal dysfunction. Each patient received both placebo and a dose (1.25-31.25 mg/hr) of TRV027 as a 6.5 hr continuous infusion (NCT01444872). All AEs reported in this study were mild or moderate in nature. There were no serious adverse events or clinically significant adverse events reported. In CP120027.2001, 33 patients with NYHA class 3-4 heart failure and a clinical indication for right-heart catheterization received either a dose regimen of TRV027 or volume-matched placebo (NCT01187836). The doses studied were as follows: • Cohort 1: 0.1, 0.2, 0.4, 0.7 µg/kg/min each for 1 hour over hours 1-4, followed by 1 µg /kg/min for 10 hours • Cohorts 2 and 4: 0.3, 0.6, 1.2, 2.4 µg/kg/min each for 1 hour over hours 1-4, followed by 3 µg/kg/min for 10 hours • Cohort 3: 1, 2, 4, 8 µg/kg/min each for 1 hr over hours 1-4, followed by 10 µg/kg/min for 10 hours. There were no serious adverse events attributed to TRV027 treatment. In the first cohort, one subject experienced hypotension necessitating dose reduction and then discontinuation of the study drug infusion. No other TRV027-related clinically significant adverse events were reported. In the CP120027.2002 study (n= 621 patients with ADHF) which used doses ranging from 1-25 mg/hr for 48-96 hours, no safety issues were identified that affected the safety of trial subjects (NCT01966601). There was no difference from placebo in non-serious and serious treatment-emergent adverse events, events leading to discontinuation, or deaths. In a small (n=29) experimental medicine study in hospitalized patients admitted with COVID-19 comparing 7 days treatment with TRV027 (12mg/hr continuous IV infusion) or placebo (NCT04419610), there was no difference from placebo in non-serious and serious treatment-emergent adverse events, events leading to discontinuation, or deaths.	

	6.2.7.2. Pharmacokinetics and Dose Rationale of TRV027 The pharmacokinetics of TRV027 has been studied in healthy volunteers, in patients with stable mild/moderate heart failure, patients with stable NYHA class III/IV CHF, and in 621 adults with ADHF. The clearance of TRV027 is rapid (ranging between 51.3-127 L/hr) and exhibits a short half-life (4.2-15.8 minutes). Heart failure, sex, or renal dysfunction had no significant effect on the clearance of TRV027. TRV027 is not metabolized by, nor does it inhibit any of the CYP450 enzymes. The plasma protein binding of TRV027 is low at 53.4%. TRV027 has been studied in patients with acute heart failure in doses ranging from 1.25 – 31.25 mg/hr. Using in vitro-derived AT1 receptor binding data as well as population PK data in healthy volunteers, simulations were performed to explore a range of doses of TRV027 which might be expected to result in at least 80% receptor occupancy, correcting for protein binding. The results (Figure 3) suggest that 12 mg/hr will result in median receptor occupancy well in excess of 80%. This dose is in the mid-range of what has been studied clinically and is expected to be well-tolerated.	

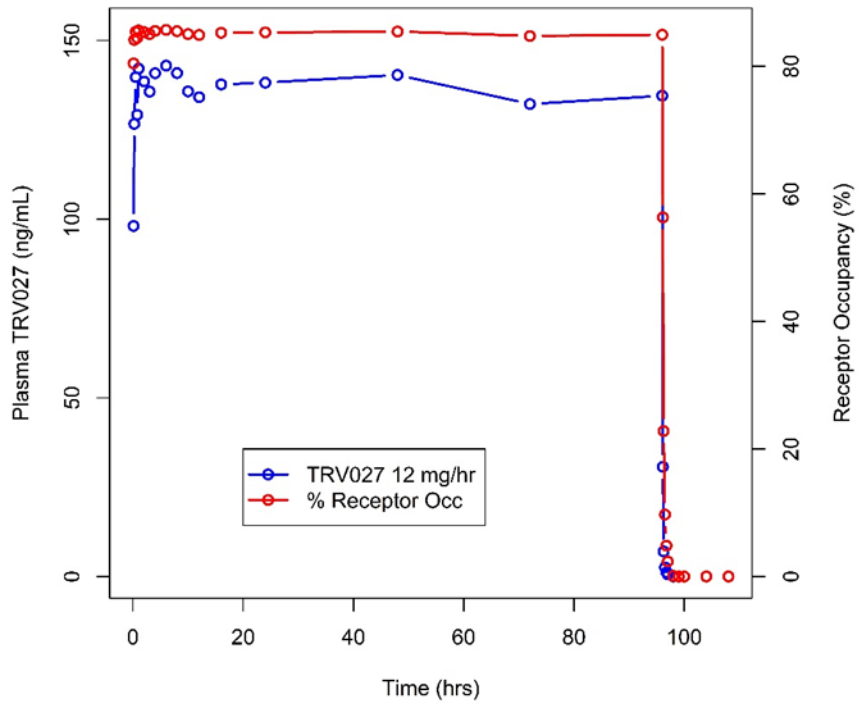


Figure 3. Estimation of the median AT1R receptor occupancy resulting from TRV027 given at a dose of 12 mg/hr for 96 hours.

SECTION 7 DOMAIN OBJECTIVES	**Original text**	**New Text**	**Reason**
Page 35	The following interventions will be available: • No RAS inhibitor • ACEi • ARB • ARB + DMX-200	The following interventions will be available: • No RAS inhibitor • ACEi • ARB • ARB + DMX-200	Administrative update adding the new intervention

		• ACEi + TRV-027	
SECTION 8 TRIAL DESIGN	**Original text**	**New Text**	**Reason**
Page 35	This domain will be conducted as part of the REMAP-CAP trial. Treatment allocation will not be adaptive, although this may be revised if the ARB + DMX-200 intervention is withdrawn from the domain.	This domain will be conducted as part of the REMAP-CAP trial. Treatment allocation proportions will not be adaptive, although this may be revised if the ARB + DMX-200 and ACEi + TRV-027 interventions are withdrawn from the domain.	Administrative update to include the new investigational product intervention
8.1. Phases of evaluation Page 36	A decision to proceed from phase 2 to phase 3 will be dependent on meeting a pre-specified statistical trigger, comprising a greater than 0.5 probability of a more than 20% (for the ACEi and ARB interventions compared to no RAS inhibitor) or 30% (for the ARB + DMX-200 intervention compared to ARB alone and compared to no RAS inhibitor) improvement in odds ratio for the primary endpoint in the absence of an unsatisfactory safety profile, as judged by the DSMB.	A decision to proceed from phase 2 to phase 3 will be dependent on meeting a pre-specified statistical trigger, comprising a greater than 0.5 probability of a more than 20% (for the ACEi and ARB interventions compared to no RAS inhibitor) or 30% (for the ARB + DMX-200 intervention compared to ARB alone and compared to no RAS inhibitor; and also for the ACEi + TRV-027 intervention compared to ACEi alone and compared to no RAS inhibitor) improvement in odds ratio for the primary endpoint in the absence of an unsatisfactory safety profile, as judged by the DSMB.	Administrative update to include the new intervention
8.3.2. Domain exclusion criteria Page 37	• Known hypersensitivity to ACEi or ARB, including angioedema	• Hypersensitivity to ACEi or ARB, including any history of angioedema	Minor refinements to language for improved clarity and consistency with the Case Report Form (CRF)

8.3.3. Intervention exclusion criteria Page 38	Blank	• Hypersensitivity to TRV-027 will result in exclusion from receiving ACEi + TRV-027.	Added to include the exclusion relevant to the new intervention
8.4.1. Domain interventions Page 38	Patients will be randomly assigned to receive one of the following open-label strategies. All interventions will be commenced immediately after allocation status is revealed. □ No RASi □ ACEi □ ARB □ ARB + DMX-200	Patients will be randomly assigned to receive one of the following open-label strategies. All interventions will be commenced immediately after allocation status is revealed. □ No RASi □ ACEi □ ARB □ ARB + DMX-200 □ ACEi + TRV-027	Administrative update adding the new intervention
8.4.5 Combination CCR2 inhibition and ARB intervention Page 44	Patients allocated to the ARB+CCR2 inhibitor intervention arm will receive the DMX-200 (repagermanium) investigational medicinal product administered concurrently with an ARB as per the ARB inhibition treatment arm (7.3.4) with concurrent DMX-200.	Patients allocated to the ARB+CCR2 inhibitor intervention arm will receive the DMX-200 (repagermanium) investigational medicinal product administered concurrently with an ARB as per the ARB inhibition treatment arm (8.4.4) with concurrent DMX-200.	Minor administrative correction to align new sections referenced in the text
8.4.5.1. Combination CCR2 inhibitor (DMX-200) and ARB dosing Page 44	8.4.5.1. Combination CCR2 and ARB Dosing	8.4.5.1. Combination CCR2 inhibitor (DMX-200) and ARB dosing	Minor refinements to language of heading for improved clarity
8.4.6. Combination angiotensin 1-7 and ACEi intervention	Blank	8.4.6. Combination angiotensin 1-7 and ACEi intervention	Updated with the new intervention

Page 44		Patients allocated to the ACEi+TRV-027 intervention arm will receive the TRV-027 investigational medicinal product administered concurrently with an ACEi as per the ACEi inhibition treatment arm (8.4.3). 8.4.6.1. Combination ACEi and TRV027 dosing TRV-027 will be prepared by dilution in normal saline. To prepare the infusion, withdrawal of 28.8 mL of normal saline from a 250 mL normal saline bag is undertaken, followed by replacement with 28.8 mL of TRV-027. This is then administered by continuous intravenous infusion at 11 mL/hour (corresponding to 12 mg/hour). No titration of the TRV-027 infusion is required. Dosing of the ACEi is as above (section 8.4.3.1). Consideration of temporary discontinuation of ACEi therapy should occur as in the ACEi intervention above (section 8.4.3.1); in these circumstances, the ACEi should be discontinued, but TRV-027 may continue to be administered (without ACEi). If hypotension continues or worsens then consideration of temporary discontinuation of TRV-027 should also be considered. By contrast, the development of a SUSAR felt attributable to the study drugs should prompt the	

		permanent discontinuation of both the ACEi and TRV-027.	
8.6.2. Secondary endpoints Page 46	• acute kidney injury defined as KDIGO Stage ≥2 acute kidney injury: • stage 2: serum creatinine increase 2-3x from baseline within 7 days • stage 3: serum creatinine increase ≥3x from baseline within 7 days, 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 • change from baseline to peak creatinine • angioedema • change in baseline to peak available AST, ALT, and bilirubin during the treatment period (14-day peak; these should be performed at a minimum once within blocks of post-randomization days 1-5, 6-10, and 11-14; preferably these should be from standard of care labs, and if not available should be checked at least once within each of these time blocks) • in the ARB + DMX-200 intervention only: • occurrence of suspected unexpected serious adverse reactions (SUSAR).	• acute kidney injury defined as KDIGO Stage ≥2 acute kidney injury: • stage 2: serum creatinine increase 2-3x from baseline within 7 days and also within 14 days • stage 3: serum creatinine increase ≥3x from baseline within 7 days and also within 14 days, 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 • change from baseline to peak creatinine • angioedema • change in baseline to peak available AST, ALT, and bilirubin during the treatment period (14-day peak; these should be performed at a minimum once within blocks of post-randomization days 1-5, 6-10, and 11-14; preferably these should be from standard of care labs, and if not available should be checked at least once within each of these time blocks) • clinically relevant hypotension while admitted to a ward (defined as one or more episodes of clinically relevant hypotension while admitted to a ward; clinically relevant hypotension includes hypotension	14-day timepoint added to more completely assess the risk of renal injury and for consistency with the Case Report Form (CRF)

	• serious adverse events as defined in relevant core protocol documents and this DSA	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) through 14 days after randomization • in the ARB + DMX-200 intervention and ACEi + TRV-027 interventions only: • occurrence of suspected unexpected serious adverse reactions (SUSAR). • serious adverse events as defined in relevant core protocol documents and this DSA	One new endpoint added to more completely assess risk of hypotension while the patient is not in the ICU Administrative update to include the new intervention
SECTION 9 TRIAL CONDUCT	**Original text**	**New Text**	**Reason**
9.2. Domain-specific data collection Page 47	• Administration of ACEi, ARB, and ARB + DMX-200 as per study protocol (including daily doses) • Baseline and peak creatinine • Occurrence of angioedema • Baseline and peak AST, ALT, and bilirubin • SUSARs (for patients allocated to the ARB + DMX-200 intervention)	• Administration of ACEi, ARB, ARB + DMX-200, and ACEi + TRV-027 as per study protocol (including daily doses) • Baseline and peak creatinine • Occurrence of angioedema • Baseline and peak AST, ALT, and bilirubin • Clinically relevant hypotension while admitted to a ward • SUSARs (for patients allocated to the ARB + DMX-200 and ACEi + TRV-027 interventions)	Administrative updates to include the new intervention. The addition of a new data variable relevant to the new intervention
SECTION 10 STATISTICAL CONSIDERATIONS	**Original text**	**New Text**	**Reason**

10.1.1. Stopping and graduation rules during phase 2 Page 48	During phase 2, a 'stop-go' decision for efficacy will be applied at series of interim analyses; for each intervention, the first of these interim analyses will occur once 50 patients have been assigned to that intervention, and then will occur monthly on the first day of each month thereafter. These 'stop-go' rules are applied separately within patients in the Moderate and Severe States, although borrowing across states will permit statistical sharing of observations between states if treatment effects are similar. There are three mutually exclusive outcomes at each interim analysis: • Graduate to phase 3. For the ACEi and ARB interventions, graduation can only occur after assignment of at least 100 patients and will occur if the posterior probability of a greater than 20% improvement in odds ratio is higher than 0.5 compared with the 'no RAS inhibitor' control (i.e., efficacy not domain superiority). For the ARB + DMX-200 intervention, graduation can only occur after assignment of at least 100 patients and will occur if the following are true: 1) posterior probability of a greater than 30% improvement in odds ratio is higher than 0.5 in the ARB + DMX-200 compared with the ARB intervention as a control (i.e., efficacy compared to ARB	During phase 2, a 'stop-go' decision for efficacy will be applied at series of interim analyses; for each intervention, the first of these interim analyses will occur as soon as possible after a minimum of 50 patients have been assigned to that intervention, and then will occur at the time of regular adaptive analyses, with the aim of occurring approximately monthly thereafter. These 'stop-go' rules are applied separately within patients in the Moderate and Severe States, although borrowing across states will permit statistical sharing of observations between states if treatment effects are similar. There are three mutually exclusive outcomes at each interim analysis: • Graduate to phase 3. For the ACEi and ARB interventions, graduation can only occur after assignment of at least 100 patients to that intervention and will occur if the posterior probability of a greater than 20% improvement in odds ratio is higher than 0.5 compared with the 'no RAS inhibitor' control (i.e., efficacy not domain superiority). For the ARB + DMX-200 intervention, graduation can only occur after assignment of at least 100 patients and will occur if the following are true: 1) posterior probability of a greater than 30% improvement in odds ratio is higher than 0.5	Clarifications to the existing statistical analysis section, and addition of new text to address the inclusion of an additional intervention

	alone, not domain superiority) AND 2) posterior probability of a greater than 30% improvement in odds ratio is higher than 0.5 in the ARB + DMX-200 compared with the 'no RAS inhibitor' intervention. • Withdraw intervention and not proceed to phase 3. Withdrawal of an intervention will occur if there is evidence of futility, defined as the posterior probability of a more than 20% improvement in odds ratio being less than 0.05 or, if at the maximum available sample size for that intervention, the posterior probability of a more than 20 or 30% improvement in odds ratio is less than 0.5 compared with the pre-specified paired control group (lack of efficacy). • Retain intervention. At any interim that occurs prior to the maximum sample size for an intervention being reached, if neither the criteria for graduation nor the criteria for withdrawal are met, then the intervention is retained. The 'stop-go' rules are applied at the level of an intervention (i.e., one or more interventions may have graduated from phase 2 to phase 3, while other interventions active in the domain remain at phase 2). The number of interim analyses available to each intervention (i.e. 100 patient blocks) is specified to be:	in the ARB + DMX-200 compared with the ARB intervention as a control (i.e., efficacy compared to ARB alone, not domain superiority) AND 2) posterior probability of a greater than 30% improvement in odds ratio is higher than 0.5 in the ARB + DMX-200 compared with the 'no RAS inhibitor' intervention. For the ACEi + TRV-027 intervention, graduation can only occur after assignment of at least 100 patients and will occur if the following are true: 1) posterior probability of a greater than 30% improvement in odds ratio is higher than 0.5 in the ACEi + TRV-027 compared with the ACEi intervention as a control (i.e., efficacy compared to ACEi alone, not domain superiority) AND 2) posterior probability of a greater than 30% improvement in odds ratio is higher than 0.5 in the ACEi + TRV-027 compared with the 'no RAS inhibitor' intervention. • Withdraw intervention and not proceed to phase 3. Withdrawal of an intervention will occur if there is evidence of futility, defined as the posterior probability of a more than 20% improvement in odds ratio being less than 0.05 or, if at the maximum available sample size for that intervention, the posterior probability of a more than 20% (ACEi/ARB) or 30% (ARB + DMX-200/ACEi + TRV-027) improvement in odds ratio is less	

	• ACEi = 300 patients • ARB = 300 patients • ARB + DMX-200 = 200 patients The selection of these maximum sample sizes is based on simulations which are available as an appendix. The rationale for a larger maximum sample size for the ACEi and ARB interventions is that these drugs are not expensive and are widely available, providing a rationale for tolerating a smaller risk of type II error. An intervention can be withdrawn for safety end-points, at the discretion of the DSMB, at any time including after any interim analysis. The safety end-points that will be evaluated are: • commencement of renal replacement therapy (dialysis) • administration of vasopressors (evaluated using vasopressor-free days at 21 days) • reported SAEs • In the ACEi intervention only: o angioedema • in the ARB + DMX-200 intervention only: o change in baseline to peak available AST, ALT, and bilirubin during the treatment period o occurrence of SUSARs	than 0.5 compared with the pre-specified paired control group (lack of efficacy). • Retain intervention. At any interim that occurs prior to the ~~maximum~~ sample size cap for an intervention being reached, if neither the criteria for graduation nor the criteria for withdrawal are met, then the intervention is retained. The 'stop-go' rules are applied at the level of an intervention (i.e., one or more interventions may have graduated from phase 2 to phase 3, while other interventions active in the domain remain at phase 2). In the event that the ARB or 'no RAS inhibitor' interventions are withdrawn when ARB + DMX-200 is in phase 2, the graduation criteria for the ARB + DMX-200 intervention will be based only on comparison with the retained comparator (ARB or 'no RAS inhibitor' – but no longer both). In the event that the ACEi or 'no RAS inhibitor' interventions are withdrawn when ACEi + TRV-027 is in phase 2, the graduation criteria for the ACEi + TRV-027 intervention will be based only on comparison with the retained comparator (ACEi or 'no RAS inhibitor' – but no longer both). Decisions to graduate from phase 2 to phase 3 are hence based only on the treatment effect relative to the relevant concurrently active	

		comparator. The use of a single comparator only arises in the event that one of the two comparators is dropped. In the event of graduation from phase 2 to phase 3, the treatment effect in phase 3 is evaluated as per below. In the unlikely event that both either the ARB or ACEi and no RAS inhibitor control groups are dropped (the latter of which could only happen in phase 3 for efficacy or superiority of ARB or ACEi), then the remaining intervention would be implied as the new standard of care based on the phase 3 evaluation and the results of the remaining ARB + DMX-200 or ACEi + TRVB-027 interventions compared with this active new standard of care comparator. It is also acknowledged that, although the above represents the framework for graduation decisions, analyses with all available comparators, possibly accounting for the effects of time, will be presented in the final report. The following sample size caps will be applied with the cap being operationalized at the first adaptive analysis that occurs after the maximum sample size has been attained: • ACEi = 300 patients • ARB = 300 patients • ARB + DMX-200 = 200 patients	

		• ACEi + TRV-027 = 200 patients It is noted that if one or more of these sample size caps are applied, that the total sample size will be slightly larger than the specified cap and may also include patients who continue to be randomized until the results of the first adaptive analysis that occurs after the cap is attained are available to the DSMB. The selection of these ~~maximum~~ sample sizes is based on simulations which are available as an appendix. The rationale for a larger maximum sample size for the ACEi and ARB interventions is that these drugs are not expensive and are widely available, providing a rationale for tolerating a smaller risk of type II error. An intervention can be withdrawn for safety end-points, at the discretion of the DSMB, at any time including after any interim analysis. The safety end-points that will be evaluated are: • commencement of renal replacement therapy (dialysis) • administration of vasopressors (evaluated using vasopressor-free days at 21 days) • clinically relevant hypotension while admitted to a ward • reported SAEs	

		• In the ACEi intervention only: o angioedema • in the ARB + DMX-200 intervention only: o change in baseline to peak available AST, ALT, and bilirubin during the treatment period • in the ARB + DMX-200 and ACEi + TRV-027 intervention: o occurrence of SUSARs	
10.1.2. Stopping rules during phase 3 Page 52	• Efficacy (all active interventions in comparison with no RAS inhibitor and ARB- DMX-200 in comparison with ARB alone) • Superiority (among all interventions) • Futility (all active interventions in comparison with no RAS inhibitor) • Equivalence (among all active interventions)	• Efficacy (all active interventions in comparison with no RAS inhibitor, ARB + DMX-200 in comparison with ARB alone, and ACEi + TRV-027 in comparison to ACEi alone); however, if the 'no RAS inhibitor' or ARB is dropped, then efficacy of the ARB + DMX-200 intervention is primarily evaluated relative only to the retained comparator intervention ('no RAS inhibitor' or ARB), while if the 'no RAS inhibitor' or ACEi is dropped, then efficacy of the ACEi + TRV-027 intervention is evaluated relative only to the retained comparator intervention ('no RAS inhibitor' or ACEi) • Superiority (among all interventions) • Futility (all active interventions in comparison with no RAS inhibitor and, if the no RAS inhibitor intervention was dropped, ARB + DMX-200 in comparison with ARB	Clarifications to the existing statistical analysis section,

		alone and ACEi + TRV-027 in comparison with ACEi alone) • Equivalence (among all active interventions)	
10.2. Unit-of-analysis and strata Page 52	Response adaptive randomization will not be conducted in this domain, as long as the ARB + DMX-200 is retained. All interventions will be assigned equally but response adaptive randomization may be instituted if the ARB + DMX-200 intervention is dropped.	Response adaptive randomization will not be conducted in this domain, as long as the ARB + DMX-200 and ACEi + TVR-027 interventions are retained. All interventions will be assigned equally but response adaptive randomization may be instituted if the ARB + DMX-200 and ACEi + TRV-027 interventions are dropped. The blinded International Trial Steering Committee will determine whether response adaptive randomization is implemented if the ARB + DMX-200 and ACEi + TRV-027 interventions are dropped from the domain. Such a decision may take into account the possible inclusion of additional interventions in the domain for which current interventions may serve secondarily as active comparators.	Clarifications to the existing statistical analysis section,
10.4. Interactions with interventions in other domains Page 46	An a priori interaction with the Antibiotic Domain of REMAP-CAP is not able to be evaluated as analysis occurs in different statistical models. An a priori interaction with the Macrolide Duration Domain of REMAP-CAP is not considered possible and will not be incorporated into the statistical models used to analyze this domain.	Interactions with other domains are either not evaluable or not considered possible and will not be incorporated into the statistical model or models in which this domain is evaluated. If an interaction is specified with a future domain, it is sufficient for the interaction to be specified only in the DSA of such a future domain.	Clarifications to the existing statistical analysis section.

	An a priori interaction with the Corticosteroid is not able to be evaluated as analysis occurs in different statistical models. An a priori interaction with the COVID-19 Antiviral Therapy Domain is not considered possible and will not be incorporated into the statistical models used to analyze this domain. An a priori interaction with the Therapeutic Anticoagulation Domain is not considered possible and will not be incorporated into the statistical models used to analyze this domain. An a priori interaction with the Vitamin C Domain is not considered possible and will not be incorporated into the statistical models used to analyze this domain or is not able to be evaluated for PINSNP patients as analysis occurs in different statistical models. An a priori interaction with the COVID-19 Immune Modulation Domain is not considered possible and will not be incorporated into the statistical models used to analyze this domain. An a priori interaction with the (Influenza) Antiviral Domain is not able to be evaluated as analysis occurs in different statistical models.		

	No interaction is evaluable between the Ventilation Domain and this domain.		
SECTION 11 ETHICAL CONSIDERATIONS	**Original text**	**New Text**	**Reason**
11.3. Domain-specific consent issues Page 55	The use of DMX-200 (repagermanium) is as Investigational Medicinal Product and should not be used outside a clinical research study. The process of obtaining informed consent must be in accordance with applicable regulatory requirement(s) for Investigational Medicinal Products and must adhere to ICH GCP guidelines.	The use of DMX-200 (repagermanium) and TRV-027 are as Investigational Medicinal Products and should not be used outside a clinical research study. The process of obtaining informed consent must be in accordance with applicable regulatory requirement(s) for Investigational Medicinal Products and must adhere to ICH GCP guidelines.	Administrative updates to include the new intervention
SECTION 12 GOVERNANCE ISSUES	**Original text**	**New Text**	**Reason**
12.2. Funding of domain interventions and outcomes measures Page 55	Dimerix will provide DMX-200 through a centrally-regulated and overseen process. All other study drugs will come from local supply. Dimerix may also contribute to platform-level and site-level costs. Dimerix have had input into the design of the domain but analysis and reporting will be conducted independently of Dimerix.	Dimerix will provide DMX-200 through a centrally-regulated and overseen process. All other study drugs will come from local supply. Dimerix may also contribute to platform-level and site-level costs. Dimerix have had input into the design of the domain but analysis and reporting will be conducted independently of Dimerix. Trevena will provide TRV-027 through a centrally-regulated and overseen process. Trevena may also contribute to platform-level and site-level costs. Trevena will review the study design prior to initiation of this intervention in the domain and may provide feedback, subject to approval of the investigators and	Addition of information regarding the funding for the new intervention.

		ITSC. Analysis and reporting will be conducted independently of Trevena.	