

Angiotensin II for the Treatment of High-Output Shock 3 (ATHOS-3): protocol for a phase III, double-blind, randomised controlled trial

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Shock is common among critically ill patients. Although shock can occur without hypotension, severe shock is almost always associated with hypotension.¹ Severe shock is described as persistent hypotension requiring vasopressors to maintain a mean arterial pressure (MAP) of 65 mmHg and a serum lactate concentration of > 2 mmol/L despite adequate volume resuscitation. In patients with severe shock requiring treatment with high doses of vasopressors (eg, catecholamines and vasopressin), their use is associated with poor outcomes.² Catecholamine-resistant hypotension (CRH) is characterised by inadequate response to usual doses of standard-of-care (SOC) vasopressor therapy, and increased mortality.³ In general, health care professionals use two classes of vasopressors in hypotension: catecholamines and vasopressin-like peptides. However, humans physiologically employ three classes of vasopressors (catecholamines, vasopressin and angiotensins) to maintain blood pressure. Thus, for patients with severe hypotension, the addition of angiotensin II (ANGII) may be beneficial.

ANGII, an octapeptide hormone, is a potent vasopressor that induces vasoconstriction through activation of the ANGII type 1 receptor (AT1R) and functions as an integral component of the renin–angiotensin–aldosterone system.⁴ AT1R activates a G-coupled protein pathway that subsequently activates phospholipase C, inducing vasoconstriction.

Synthetic ANGII shows effective vasopressor activity and has been used to improve MAP in patients with CRH.^{5–9} In a recent pilot study of synthetic human ANGII in patients with CRH, ANGII increased MAP and decreased catecholamine use, both of which could be beneficial.¹⁰ Our study is designed to determine if LJPC-501 (synthetic human ANGII acetate) could increase MAP in patients with CRH. We hypothesise that a greater proportion of patients with CRH treated with ANGII than those treated with placebo will achieve an increase in MAP.

Study rationale

There is an ongoing need for more effective approaches to CRH therapy.¹¹ The Angiotensin II for the Treatment of High-Output Shock 3 (ATHOS-3) trial was designed to compare

ABSTRACT

Objective: Catecholamine-resistant hypotension (CRH) is characterised by inadequate response to standard doses of vasopressors, and increased mortality. Our Angiotensin II for the Treatment of High-Output Shock 3 (ATHOS-3) trial compares the efficacy and safety of angiotensin II (ANGII) versus placebo in CRH.

Design, setting and participants: A phase III, multicentre, randomised, placebo-controlled trial of LJPC-501 (synthetic ANGII) for CRH in up to 120 intensive care units. We have set a target of 300 critically ill patients with CRH receiving standard-of-care (SOC) vasopressor therapy (ie, catecholamine dose >0.2 µg/kg/min for 6–48 hours to maintain a mean arterial pressure [MAP] of 55–70 mmHg). Calculation of a norepinephrine-equivalent vasopressor dose is critical to determining patient eligibility, as ANGII will supplement ongoing vasopressor therapy.

Interventions: Stable patients will be randomised 1:1 to SOC vasopressor plus continuous intravenous infusion of ANGII or placebo for 48 hours, with an aim of achieving MAP of 75 mmHg for the first 3 hours. ANGII (initiated at 20 ng/kg/min) will be titrated according to pre-specified guidelines until 48 hours, with patients followed until Day 7. Frequent vital sign and haemodynamic monitoring will support ANGII titration, safety monitoring and efficacy assessments.

Main outcome measures: The primary efficacy endpoint is MAP ≥75 mmHg or an increase of ≥10 mmHg at treatment Hour 3. Secondary endpoints include change in total and cardiovascular Sequential Organ Failure Assessment scores over 48 hours, and safety data.

Conclusion: Our study will investigate the utility of adding ANGII to current SOC vasopressor options to increase the efficacy and safety of CRH therapy.

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the efficacy and safety of intravenous ANGII with placebo in patients with CRH. The primary endpoint is based on MAP at 3 hours after study drug initiation during maintenance of vasopressor therapy.

Methods

Design

This is a multicentre, randomised, double-blind, placebo-controlled trial of LJPC-501 for improvement of MAP (ClinicalTrials.gov/NCT02338843).

Setting

The trial has been initiated at about 75 investigational sites (intensive care units) in the United States, Canada, Australia, New Zealand, the United Kingdom, Belgium, Finland, France and Germany.

Table 1. Patient enrolment criteria

Inclusion criteria

- ≥ 18 years old with catecholamine-resistant hypotension*
- Central venous access and an arterial line†
- Indwelling urinary catheter‡
- Received ≥ 25 mL/kg of crystalloid or colloid equivalent over previous 24 hours and had adequate volume resuscitation†
- Clinical features of high-output shock§
- Written informed consent
- Exclusion criteria
- Burns covering > 20% of total body surface area
- Cardiovascular SOFA score ≤ 3
- Acute occlusive coronary syndrome
- Receiving VA ECMO; receiving VV ECMO for < 12 hours
- Current bronchospasm or history of asthma, Raynaud phenomenon, systemic sclerosis or vasospastic disease
- Current liver failure¶
- Acute or past mesenteric ischaemia
- Current, past or suspected aortic dissection or abdominal aortic aneurysm
- Requires hydrocortisone > 500 mg/day or equivalent glucocorticoid medication**
- Expected to die within 12 hours
- Active bleeding, need for > 4 units packed red blood cells, or haemoglobin level < 7 g/dL
- Contraindication for serial blood sampling
- Absolute neutrophil count < 1000 cells/mm³
- Known allergy to mannitol
- Current participation in trial of another interventional drug or device
- Pregnant at screening

SOFA = Sequential Organ Failure Assessment. VA = venoarterial. ECMO = extracorporeal membrane oxygenation. VV = venovenous. * Defined as requiring a total sum vasopressor dose of > 0.2 µg/kg/min for 6 to 48 hours to maintain a mean arterial pressure of 55–70 mmHg (see Table 3 for conversion to norepinephrine equivalent). † Must remain present for first 48 hours of the study. ‡ Investigator-assessed. § Central venous oxygen saturation > 70% (measured by oximetry catheter or central venous blood gas) and central venous pressure > 8 mmHg or a cardiac index > 2.3 L/min/m². ¶ Model for End-Stage Liver Disease score ≥ 30. ** See Appendix 2 (online at cicm.org.au/Resources/Publications/Journal) for glucocorticoid-equivalent medication dosing.

Population

We will use trial inclusion criteria (Table 1) to identify a population of about 300 critically ill adults with severe high-output shock, exhibiting CRH, who require high-dose vasopressors. All six inclusion criteria must be fulfilled at the time of screening.

Patient screening

Screening for eligibility will be conducted within the 48 hours immediately before study drug initiation; written informed consent to participate in the study must be provided by each patient or their surrogate before screening. Because study drug is being added to ongoing SOC vasopressor therapy, we have calculated norepinephrine-equivalent vasopressor doses (Table 2) to standardise baseline vasopressor therapy and determine patient eligibility. Eligible patients will be stratified and randomly assigned to treatment (details below). All assessments performed during screening are detailed in Table 3 and Appendix 1 (online at cicm.org.au/Resources/Publications/Journal).

Ethics and consent

The study is being conducted in accordance with good clinical practice, following applicable local regulations and with the ethical principles described in the Declaration of Helsinki. It has been approved by the appropriate ethics committee or institutional review board at each study centre. Patients or their legal surrogate must be willing and able to provide written informed consent and comply with all protocol requirements.

Treatment assignment

Following confirmation of eligibility and consent, central randomisation will be used to assign patients to receive ANGII or placebo (Figure 1). Blocked randomisation within strata will use a 1:1 schedule of placebo:active comparator, with screening MAP (< 65 mmHg and ≥ 65 mmHg) and

Table 2. Conversion of vasopressors to norepinephrine equivalent*

| Drug equivalent | Dose | Norepinephrine |
|-----------------|---------------|----------------|
| Epinephrine† | 0.1 µg/kg/min | 0.1 µg/kg/min |
| Norepinephrine† | 0.1 µg/kg/min | 0.1 µg/kg/min |
| Dopamine† | 15 µg/kg/min | 0.1 µg/kg/min |
| Phenylephrine‡ | 1.0 µg/kg/min | 0.1 µg/kg/min |
| Vasopressin§ | 0.04 U/min | 0.1 µg/kg/min |

* Conversion scale developed by the authors (L C, J R and G T).

† Conversion based on the cardiovascular Sequential Organ Failure Assessment score. ‡ Conversion based on medical literature.

§ Conversion developed (by J R) using Vasopressin and Septic Shock Trial data set.¹¹

Acute Physiology and Chronic Health Evaluation (APACHE) II score (≤ 30 , 31–40 and ≥ 41) as stratification variables.

Dosing regimen

Before study drug initiation, patients will be re-assessed to confirm that they have had adequate volume resuscitation. Patients may receive up to 750 mL of intravenous fluids during the first 3 hours of the study if required for safety reasons.

Doses of SOC vasopressors will be optimised before initiating the study drug, using criteria for conversion of catecholamine and vasopressin dosing to norepinephrine equivalents (Table 2), and kept constant during the first 3 hours of study drug treatment. Inotropes (eg, dobutamine and milrinone) may be titrated during the first 3 hours. Study drug will be titrated based on patient MAP (Table 4). If safety requirements dictate, SOC vasopressor doses may also be titrated to maintain MAP. If a patient’s SOC doses are increased in the first 3 hours, they are classified as having failed treatment but they may continue to receive study drug treatment. Exceptions to failure include epinephrine and dopamine given in the inotropic range.¹²

Drip weight (the patient bodyweight at which SOC vasopressors are being dosed) will be used to calculate study drug dosing, beginning at 20 ng/kg/min. Patients assigned to placebo will receive volume-matched infusion increments of 0.9% NaCl solution. Dose titrations based on MAP can occur as frequently as every 5 minutes, and MAP will be determined as the average of three values obtained ≥ 1 minute apart. Dose titration intervals (Table 4) and permissible dosing adjustments based on MAP at each assessment time point (the key indicator for dosing decisions) are pre-specified for Hours 0–3, 3–48 and, if needed, 48–168.

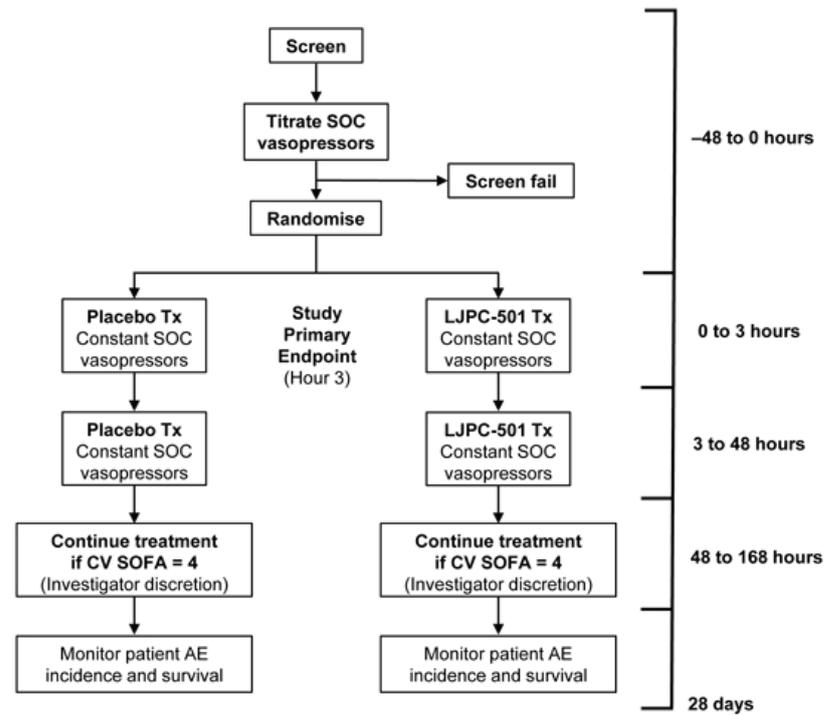
Study drug administration will be initiated at Day 1, Hour 0, and continue until Hour 48, at which time it will be down-titrated and terminated. Subsequently, if a patient’s cardiovascular (CV) Sequential Organ Failure Assessment (SOFA) score is 4 after discontinuing the study drug, dosing may resume within 3 hours and continue

Table 3. Patient assessments at screening

- Titrate SOC vasopressors and document SOFA score
- Obtain a complete medical history*
- Document current medications†
- Perform a limited physical examination‡
- Document MAP hourly over a 6-hour period during SOC vasopressor titration
- Obtain a 12-lead electrocardiogram
- Determine central venous pressure and assess cardiac output when feasible
- Obtain a chest x-ray
- Collect blood samples for analysis§
- Determine arterial blood gases for respiratory component (P_{aO_2}/F_{iO_2}) of SOFA score
- Determine blood oxygenation (alveolar–arterial gradient or P_{aO_2} , depending on F_{iO_2}) and arterial pH for APACHE II score
- Record ventilator settings (tidal volume, PEEP) for patients on mechanical ventilation
- Perform Glasgow Coma Scale assessment to calculate nervous system component of SOFA score, preferably during a sedation holiday
- Collect urine for routine dipstick urinalysis
- Document hourly urine output in the 6 hours before initiation of study drug

SOC = standard-of-care. SOFA = Sequential Organ Failure Assessment. MAP = mean arterial pressure. APACHE II = Acute Physiology and Chronic Health Evaluation II. PEEP = positive end-expiratory pressure. * Including demographic data, catecholamine-resistant hypotension diagnosis, surgical history and concurrent medical conditions. † Including antihypertensive medications taken within 7 days, vasopressors and procedures within 2 days of study drug administration. ‡ Including bodyweight, height and vital signs. § Including clinical chemical and haematological tests with differential blood counts, plasma angiotensin I and II concentrations, and samples to bank for possible later analyses. Total bilirubin levels, serum creatinine levels and platelet counts will be used to calculate the liver, renal and coagulation components of the SOFA score, respectively.

Figure 1. Study design



SOC = standard-of-care. Tx = treatment. CV SOFA = cardiovascular Sequential Organ Failure Assessment score. AE = adverse event.

Table 4. Study drug titration schema

| Mean arterial pressure (mmHg) | Initial dose (ng/kg/min) | Titration interval (min) | Dose titration (ng/kg/min) | Max. dose (ng/kg/min) | Min. dose (ng/kg/min) |
|---|--------------------------|--------------------------|--|-----------------------|-----------------------|
| Hours 0–3 (target MAP, ≥ 75 mmHg) | | | | | |
| ≤ 59 | 20 | 5 | Increase to 80 then by increments of 20* | 200 | 2.5 |
| 60–74 | 20 | 15 | Increase by 10 | 200 | 2.5 |
| 75–84 | na | 15 | Maintain dose | 200 | 2.5 |
| ≥ 85 | na | 5 | Decrease by 10 [†] | 200 | 2.5 [‡] |
| Hours 3–48 (target MAP, 65–70 mmHg) | | | | | |
| ≤ 59 | – | 5 | Increase to 40 | 40 | 2.5 |
| 60–64 | – | 15 | Increase by 10 | 40 | 2.5 |
| 65–70 | – | 15 | Maintain dose [§] | 40 | 2.5 |
| ≥ 70 | – | 15 | Decrease by 10 [¶] | 40 | 2.5** |
| Hour 48 to Day 7 (target MAP, 65–70 mmHg) | | | | | |
| ≤ 59 | – | 5 | Increase to 40 | 40 | 2.5 |
| 60–64 | – | 15 | Increase by 10 | 40 | 2.5 |
| 65–70 | – | 15 | Maintain dose [§] | 40 | 2.5 |
| ≥ 70 | – | 15 | Decrease by 10 [¶] | 40 | 2.5** |

max. = maximum. min. = minimum. MAP = mean arterial pressure. na = not applicable (patients not eligible for study participation). SOC = standard-of-care. * Dosing schema may be modified by consensus of data and safety monitoring board to as low as 60 ng/kg/min and as high as 120 ng/kg/min, if deemed necessary for safety purposes. † Once a dose of 10 ng/kg/min is reached, study drug dose may be further reduced by halving each titration until the minimum dose is achieved. ‡ Dosing may be modified to as low as 1.25 ng/kg/min for patients considered hyper-responders (ie, MAP remains ≥ 85 mmHg despite discontinuation of vasopressin and all catecholamines). § If the sum of the norepinephrine and epinephrine doses is ≥ 0.03 but < 0.1 $\mu\text{g/kg/min}$, the study drug dose should be maintained. ¶ If vasopressin is being used, the patient should be weaned off vasopressin first, then titrate SOC vasopressors until the sum of the norepinephrine and epinephrine dose is as low as 0.03 $\mu\text{g/kg/min}$. ** Dosing may be modified to as low as 1.25 ng/kg/min for patients considered hyper-responders (ie, MAP remains ≥ 70 mmHg despite discontinuation of vasopressin and reduction of sum norepinephrine and epinephrine dose to as low as 0.03 $\mu\text{g/kg/min}$).

through to Day 7 (168 hours), at which time it must be titrated off.

Assessments

Pre-defined on-treatment and post-treatment assessments (Appendix 1) will include very frequent monitoring of vital signs and haemodynamic parameters to support study drug titrations and assessments of therapeutic responses.

Study endpoints

Primary efficacy endpoint

The primary efficacy endpoint is MAP response, defined as a mean MAP of ≥ 75 mmHg or a ≥ 10 mmHg increase in MAP from baseline at treatment Hour 3. For the primary efficacy endpoint, MAP will be measured at 15 minutes before, at, and 15 minutes after the Hour 3 time point on Day 1. No MAP response will be recorded if neither of these endpoints are reached, the patient dies before all three MAP measurements can be taken, or the patient requires an increase in SOC vasopressor dose. In the absence of all three MAP measures, the average of available measures will be used.

Secondary efficacy endpoints

The secondary efficacy endpoints are change in CV SOFA score and total SOFA score between baseline measurement and Hour 48. The total SOFA score is the sum of the six individual SOFA item scores, including CV score. Missing secondary endpoint values at Hour 48 due to death will be assigned the worst possible SOFA score. The last observation will be carried forward for values missing for reasons other than death. We will compare the change in CV SOFA scores between treatment arms using the van Elteren-stratified Wilcoxon rank-sum test, with strata defined by the randomisation strata. The change in total SOFA score will be analysed using the general linear model including all randomisation strata. We will use a norepinephrine-equivalent calculation (Table 3) for all vasopressor dose and CV SOFA score analyses.

Safety monitoring

Safety will be monitored by assessing adverse events (AEs), blood pressure and heart rate, and using routine clinical safety laboratory testing including urinalysis and urine output measurement and testing of blood chemical and haematological parameters (see Appendix 1). All serious and non-serious AEs will be recorded from the first dose

of study drug to Day 7 or the end-of-study visit. Deaths will be captured from the first dose of study drug to Day 28. Serious AEs reportable to regulatory authorities will be captured after Day 7 or at the end of the study through to Day 28. Patients with AEs will be monitored until resolution or stabilisation of the AE. All serious AEs will be reported to the sponsor or its designee and monitored until they are resolved or are clearly determined to be due to a chronic condition or concurrent illness. Reportable safety AEs occurring between study Day 7 and Day 28 will be assessed by a follow-up phone call or chart review to occur about 28 days after study drug initiation.

Vasopressor toxicity

Vasopressor toxicity monitoring will occur throughout the study, and event details will be systematically collected. Safety AEs indicative of vasopressor toxicity include but are not limited to: myocardial infarction and/or ischaemia (beyond simple troponin elevation); relevant dysrhythmias, including atrial fibrillation; re-entrant atrioventricular nodal tachycardia; ventricular tachycardia; cerebral ischaemia; hypoperfusion (eg, digital ischaemia, mesenteric ischaemia, shock liver); renal hypoperfusion and local vasoconstriction or necrosis at or near the infusion site.¹¹

Termination of treatment and/or study participation

Patients may be withdrawn from the study for clinically significant concurrent illness, occurrence of an AE, patient request, major protocol violation, non-compliance, administrative reason, failure to return for follow-up on Day 7, or changes in the patient's condition making further study treatment unacceptable (investigator determination). All study procedures intended for study Day 7 or the end of the study are to be completed at the time of withdrawal. Patients who withdraw after receiving any amount of study drug will be included in the modified intention-to-treat analysis (mITT) population.

Concomitant treatment and supportive care

All patients will be given SOC therapy during the course of the study. Supportive therapy and use of concomitant medications for other medical conditions that are ongoing at baseline (subject to specific protocol requirements) will be permitted during the treatment phase of the study. No other experimental therapy is permitted. All medications and supportive therapies that are administered during the study will be recorded.

Statistical considerations and analysis plan

Sample size estimation

The sample size for our study is based on a hypothesised success rate in the primary efficacy endpoint of 40% in the placebo arm and 60% in the ANGI arm. A two-by-two χ^2

test with a two-sided α of 0.05 will have > 90% power to show superiority of ANGI over placebo with a sample size of 150 evaluable patients per treatment arm.

Stratification and randomisation

We will use central randomisation. Screening MAP (< 65 mmHg and \geq 65 mmHg) and APACHE II score (\leq 30, 31–40 and \geq 41) will be used as stratification variables. Blocked randomisation within strata with a 1:1 schedule of placebo:active will be used.

Analysis populations

The primary efficacy analysis population will be the mITT population that includes all randomised patients who receive study drug. The per protocol (PP) analysis population will include all mITT patients who were not considered to be major protocol violators. Analyses based on the PP population will be used to support the mITT analyses. The safety population will be all patients who received study drug.

Handling of missing, unused and spurious data

For mITT and PP analyses, primary or secondary endpoint values missing due to death will be imputed as failures. Values will also be imputed when missing due to reasons other than death in the mITT but not the PP analysis population. In the mITT population, the last observed value will be carried forward as a primary imputation method. Multiple imputation, using PROC MI and PROC MIANALYZE (SAS Institute), will be used as a sensitivity analysis for the primary efficacy analysis. No imputation of values for missing data will be performed for the safety population. We will use standard clinical monitoring and data management practices to ensure data integrity.

Statistical methods

We will summarise data by treatment arm and by measurement time using descriptive statistics for continuous and ordinal variables, with 95% confidence intervals. For statistical comparisons of differences between treatment groups, we will use the Wilcoxon rank-sum test or analysis of variance for continuous or ordinal variables and the χ^2 or Fisher exact test for discrete variables.

We will analyse the primary efficacy endpoint using logistic regression. Covariates will include dichotomised baseline MAP, APACHE II score, vasopressor use in the 6 hours before randomisation (with "yes/no" data) and quantity of vasopressor use over the 6 hours before randomisation. A two-tailed α of 0.05 will be used in testing the hypothesis of treatment difference. We will also evaluate the presence of a site effect and site-by-treatment interaction with this model.

We will summarise all AEs, serious AEs and AEs leading to discontinuation, along with their relationship to the study

drug. Summaries will be based on Medical Dictionary for Regulatory Activities terminology (www.meddra.org).

Exploratory analyses

We will conduct exploratory analyses to compare treatment effects in patients stratified according to certain characteristics, including sex, age, baseline MAP, baseline vasopressor dose, baseline APACHE II score, geographical region, recent exposure to angiotensin-converting enzyme (ACE) inhibitors, recent exposure to angiotensin receptor blockers, history of acute respiratory distress syndrome, history of sepsis, baseline plasma angiotensin I and II concentrations and their ratio, and drug sensitivity as measured by study drug dose 30 minutes after initiating the study drug.

Additional exploratory endpoints include mortality at Day 7 and Day 28, analysed by Kaplan–Meier methods and log-rank test; MAP response at Hour 1 and Hour 2, analysed by the same method as MAP response at Hour 3; change in SOFA component scores, analysed by the same method as CV SOFA score analysis; changes in heart rate, vasopressor use, lactate level, serum creatinine level, urine output; and length of time on vasopressor, time on ventilator, and time in the ICU.

Trial oversight

Monitoring and auditing procedures were developed by La Jolla Pharmaceutical Company or its designee in line with good clinical practice and the principles and guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. A site monitor representing the sponsor will conduct periodic site visits to review study source documents and ensure that the investigation is conducted according to protocol design and regulatory requirements. Case report forms will be monitored for completeness, clarity and consistency with source documents. This study will be overseen by a data safety monitoring board, which will be conducted according to the guidelines outlined in a separate, pre-approved charter.

Discussion

Patients with CRH have limited treatment options and, despite some longitudinal improvements in patient survival, mortality remains high for patients with septic shock.¹³ Available rescue therapies, including methylene blue, high-volume haemofiltration and extracorporeal therapies, have not been tested in large, randomised controlled trials, are largely ineffective and are associated with increased AEs.^{14–16} ANGII has shown clinical effectiveness in case reports of patients with CRH.^{5–9} A randomised, placebo-controlled pilot study has also shown that ANGII increased MAP and

decreased catecholamine use.¹⁰ However, because of the limited sample size (10 patients per treatment arm) and short treatment duration (6 hours), statistical significance was not achieved for the primary endpoint and safety was not adequately established. The ATHOS-3 trial is the first phase III trial to compare the efficacy and safety of ANGII with placebo in patients with CRH.

Humans have evolved three classes of vasopressor hormones: catecholamines, vasopressin and angiotensins. Catecholamines and vasopressin, but not angiotensin, are currently approved in most regions for vasoactive support in shock. Reduced renin–angiotensin system activity, whether due to endogenous variability or exogenous pharmaceutical manipulation (inhibition of ACE or AT1R), is associated with increased risk of shock and increased patient mortality in severe sepsis.^{17,18} Further, polymorphism in the AT1R-associated protein gene was associated with increased AT1R protein, decreased blood pressure and increased sepsis mortality,¹⁹ providing additional evidence that inadequate ANGII signalling contributes to poor outcomes.

Our ATHOS-3 trial examines the hypothesis that intensivist access to ANGII in addition to SOC vasopressors could improve blood pressure support and patient outcomes when homeostatic mechanisms fail during an occurrence of shock. Modern advances in the treatment of many complex disease states, such as acquired immune deficiency syndrome, rheumatoid arthritis, psoriasis and refractory hypertension, have relied on combination therapies that target multiple physiological pathways, thereby increasing synergy and avoiding toxicity.^{20–22} Similarly, because both catecholamines and vasopressin have significant toxicities, ANGII may enable synergy by targeting a distinct signalling pathway and facilitate improved outcomes.

Trial status

Recruitment to the trial is complete. This study protocol manuscript was accepted for publication before study completion.

Acknowledgements

This trial is sponsored and supported by La Jolla Pharmaceutical Company. We thank Christopher Barnes, ArticulateScience, for medical editorial assistance with the manuscript; Gerard Smits for sample size calculations; and Stewart Kroll, lead statistician for the study. We thank the ATHOS-3 investigators and study site personnel for their dedication to and support of the study.

Competing interests

Lakhmir Chawla and George Tidmarsh are employees of and own stock in La Jolla Pharmaceutical Company, are medical monitors for the study, and have assigned patents relating to the use of angiotensin II for hypotension.

James Russell is an inventor on patents owned by the University of British Columbia that are related to PCSK9 inhibitor(s) and sepsis and related to the use of vasopressin in septic shock. He is a founder, director and shareholder of Cyon Therapeutics, has share options in Leading Biosciences, and is a shareholder in Molecular You Corp. He has received consulting fees from Cubist Pharmaceuticals, Leading Biosciences, Ferring Pharmaceuticals, Grifols, La Jolla Pharmaceutical Company, CytoVale and Asahi Kasei Pharma America. He received an investigator-initiated grant from Grifols that is provided to and administered by the University of British Columbia.

Stuart Goldstein and Andrew Shaw are consultants to La Jolla Pharmaceutical Company to adjudicate the appropriateness of extending treatment with study drug beyond 7 days. Sean Bagshaw is supported by a Canada Research Chair in Critical Care Nephrology. Sean Bagshaw and James Russell were consultants to La Jolla Pharmaceutical Company on the design and development of the ATHOS-3 protocol. They now serve as Chair (J R) and a member (S B) of the ATHOS-3 trial Data Safety Monitoring Board and receive consulting fees for this service.

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* Posthumously published

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Appendix 1. This appendix was part of the submitted manuscript and has been peer reviewed. It is posted as supplied by the authors.

| Table 1. Schedule of study procedures | | | | | | | |
|---|------------------------|----------------------|-----------------------|------------------------|-------------|------------------------------|--------------------|
| Activity | Screening ¹ | Day 1 (0–3 hours) | Day 1 (3–24 hours) | Day 2 (24–48 hours) | Days 3–6 | Day 7 (EOS ²) | Day 28 (Safety) |
| Informed consent ³ | X | | | | | | |
| Titrate SOC vasopressors ⁴ | X | | | | | | |
| Inclusion/exclusion criteria evaluation | X | | | | | | |
| Demographics | X | | | | | | |
| Medical/surgical history | X | | | | | | |
| Concomitant medications and concomitant procedures ⁵ | X | X | X | X | X | X | |
| Limited physical exam ⁶ | X | | | | | X | |
| Chest x-ray | X | | | | | | |
| Hemodynamic vital signs ⁷ | X | X | X | X | X | X | |
| Other vital signs ⁸ | X | X | X | X | X | X | |
| 12-Lead ECG ⁹ | X | | | X | | | |
| Central venous pressure ¹⁰ | X | X | X | X | | | |
| Cardiac output ¹⁰ | X | X | X | X | | | |
| Urine output ¹¹ | X | X | X | X | | | |
| Arterial blood gas ¹² | X | X | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SOFA scoring ¹³ | | | | | | | |
| a. Respiratory system (PaO ₂ /FiO ₂) | | | | | | | |
| b. Nervous system (Glasgow Coma Scale) | | | | | | | |
| c. Cardiovascular system (MAP or vasopressors required) | X | X | | X | | | |
| d. Liver (bilirubin) | | | | | | | |
| e. Coagulation (platelets) | | | | | | | |
| f. Renal (creatinine or urine output) | | | | | | | |
| Hematology ¹⁴ | X | X | | X | | | |
| Serum chemistry ¹⁴ | X | X | | X | | | |
| Blood for serum angiotensin I and II concentrations | X | X | | | | | |
| Blood for banked storage sample ¹⁵ | X | | | | | | |
| Routine urinalysis ¹⁶ | X | | | | | | |
| Randomise to study | X | | | | | | |
| Pharmacy ANGII preparation | X | | | | | | |
| ANGII administration/titration ¹⁷ | | X | X | X | X | X | |
| Safety assessments (AEs/SAEs) ¹⁸ | | X | X | X | X | X | X |

AE = adverse event. ECG = electrocardiogram. ANGII = angiotensin II. EOS = end of study. FiO₂ = fraction of inspired oxygen. HR = heart rate. MAP = mean arterial pressure. PaO₂ = partial pressure of dissolved oxygen. SAE = serious adverse event. SOC = standard of care. SOFA = Sequential Organ Failure Assessment.

1. Screening procedures may be conducted up to 48 hours prior to the anticipated initiation of ANGII dosing.
2. EOS to be performed no earlier than 168 hours (7 days) ± 12 hours after initiation of ANGII. All patients will be followed for a minimum of 72 hours (3 days) ± 6 hours following discontinuation of ANGII.
3. Informed consent must be obtained before any study-related activities can occur.
4. All patients will have their SOC vasopressors titrated for a minimum of six hours but not for longer than 48 hours prior to initiation of ANGII dosing. The screening MAP is defined as the average of six hourly MAP measurements collected during the period of time SOC vasopressors are being titrated.

5. Record all concomitant medications taken (including all vasopressors), any procedures performed within two days of administration of ANGII, and continue to do so through Day 7/EOS. Record any concomitant anti-hypertensive medications, including angiotensin-converting enzyme inhibitors and angiotensin-receptor blockers, taken within seven days of administration of ANGII through Day 7/EOS.
6. A limited physical examination should be performed at screening that includes documentation of body weight and height.
7. It is anticipated that patients will be continuously monitored during the first 48 hours following initiation of ANGII. Haemodynamic vital signs (ie, HR and MAP) must be documented hourly during screening for a minimum of six hourly readings. The average of these six readings will determine the screening MAP for purposes of randomisation and stratification. MAP must be measured –30 minutes, –15 minutes, and 0 minutes (or just prior) to initiation of ANGII. The average of these three MAP values will determine the baseline MAP (ie, how a patient should begin ANGII dose titration). MAP and HR will be recorded on Day 1 prior to every dose titration and minimally every 15 minutes during hours 0–3:15, and prior to any dosing adjustments, and a minimum of hourly thereafter beginning with Day 1 at 4 hours through Day 2 at 48 hours. During hours 0–3, MAP will be measured in triplicate and averaged to determine MAP value for dosing modifications. Evaluation of MAP and HR should continue once daily on Days 3–7. If a patient remains on ANGII beyond Day 2 at 48 hours, documentation of MAP and HR should continue as during hours 3–48 (ie, just prior to any dose adjustment and a minimum of hourly through discontinuation of ANGII).
8. Other vital signs (blood pressure, respiratory rate, body temperature) should be assessed at screening, just prior to initiation of dosing on Day 1, Day 1 at 3 hours, Day 1 at 24 hours, Day 2 at 48 hours and once daily on Days 3–7.
9. ECG assessments will be performed at screening and on Day 2 at 48 hours.
10. An assessment of cardiac output will be conducted by an acceptable method at screening to determine eligibility, if available. A central venous pressure measurement will be conducted at screening to determine patient eligibility. In addition, cardiac output (if available) and central venous pressure will be collected a minimum of every 15 minutes during hours 0–3, and a minimum of hourly during hours 3–48.
11. Urine output will be measured and recorded hourly in the six hours prior to the initiation of ANGII. Upon start of ANGII titration, urine output will be measured and recorded hourly from hours 0–3. Urine output between hours 0–3 should be summed and documented in the chart. The total amount of intravenous fluid administered during hours 0–3 will also be documented in the chart. Hourly urine output measurement and recording should continue during hours 3–48.
12. An arterial blood gas will be conducted during screening and just prior to initiation of ANGII dosing on Day 1 at hour 0.
13. The screening cardiovascular SOFA score must be determined with data obtained within six hours prior to initiation of ANGII dosing. All parameters of the SOFA index should be performed at screening, on Day 1 at 3 hours and on Day 2 at 48 hours.
14. Blood for clinical chemistry and haematology will be collected at screening, on Day 1 at 3 hours and on Day 2 at 48 hours.
15. Blood will be collected to bank as a storage sample that may be evaluated at a later time point for measures to be determined.
16. It is not necessary to wait for results of the screening routine urinalysis to randomise the patient to the study, if otherwise eligible, at the investigators' discretion.
17. See Table 5 for study periods and dose titration schemes.

18. Safety will be evaluated at every time point. On Day 28, a follow-up phone call to the patient, the patient's primary care physician and/or a chart review will be conducted to determine if reportable safety events occurred between Day 7/EOS and Day 28. Investigators should pay particular attention to signs of vasopressor toxicity. Information about these events will be systematically collected throughout the duration of the study on a once-daily basis.

Appendix 2. This appendix was part of the submitted manuscript and has been peer reviewed. It is posted as supplied by the authors.

| Table 1. Corticosteroid conversion table* | | |
|--|----------------------------------|----------------|
| Glucocorticoid | Approximate equivalent dose (mg) | Half-life (hr) |
| Short-acting | | |
| Cortisone | 25 | 8–12 |
| Hydrocortisone | 20 | 8–12 |
| Intermediate-acting | | |
| Methylprednisolone | 4 | 18–36 |
| Prednisolone | 5 | 18–36 |
| Prednisone | 5 | 18–36 |
| Triamcinolone | 4 | 18–36 |
| Long-acting | | |
| Betamethasone | 0.60–0.75 | 36–54 |
| Dexamethasone | 0.75 | 36–54 |

* Conversions based on published values.^{1–3}

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