Randomized controlled trial of Angiotensin 1-7/ TXA127 for the treatment of severe COVID-19

CUIMC Protocol Number:
AAAT0535

Drug Sponsored by:
Constant Therapeutics LLC

Principal Investigator:
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Statement of Compliance

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- U.S. Code of Federal Regulations applicable to clinical studies (45 CFR 46)
- ICH GCP E6
- Completion of Human Subjects Protection Training

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Principal Investigator: Jeanine D'Armiento, MD, PhD

Signed: _____ Date: _____

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Protocol Summary

Title: Randomized controlled trial of Angiotensin 1-7/ TXA127 for the treatment of severe COVID-19

Population: One hundred Male and Females aged 18-70, with COVID-19 positive status hospitalized in NYP/Columbia Hospital.

Number of Sites: New York Presbyterian Hospital/ Columbia University Irving Medical Center

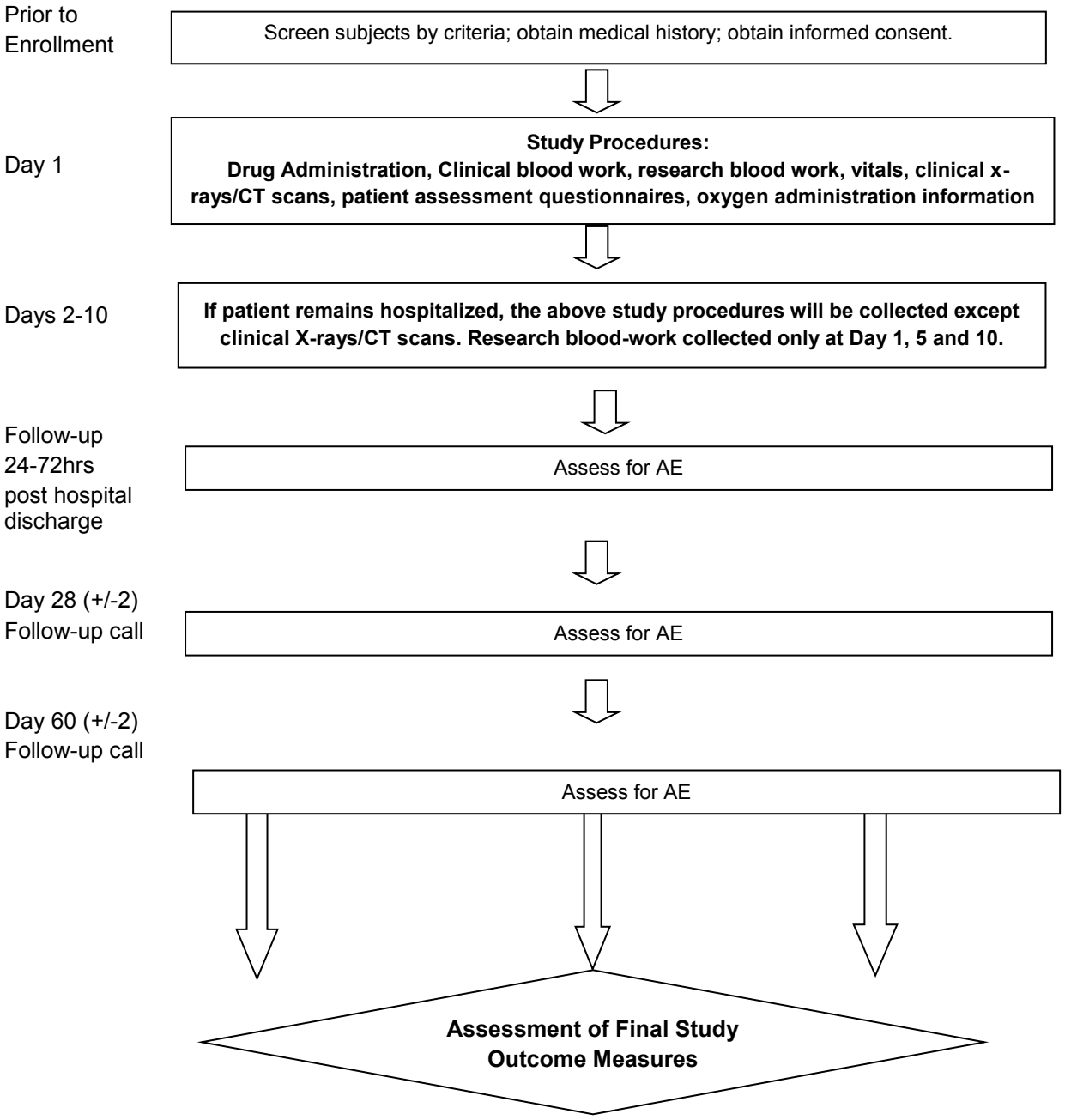
Study Duration: 1 year

Subject Duration: Study duration is 10 days with 3 follow-up calls at 24-72hrs post hospital discharge, at day 28 and day 60 post enrollment date.

Objectives: To determine if administration of angiotensin-(1-7) / TXA127 prevents acute kidney injury and deterioration into multi-organ failure in patients with severe COVID-19.

Schematic of Study Design:

Randomized double-blinded placebo controlled clinical trial proof of concept.



1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

TXA127 is a pharmaceutically-formulated angiotensin (1-7) [A(1-7)] heptapeptide, an endogenously produced, non-hypertensive derivative of angiotensin II (Ang II). TXA127 is currently in development to reduce progression of respiratory disease in COVID-19 patients. TXA127 has been shown to reduce inflammation and fibrosis and restore endothelial and epithelial barrier function in acute lung injury. The mechanism behind the beneficial effect of A(1-7) appears to be mediated via the Mas receptor.

TXA127 or A(1-7) has been evaluated in several pre-clinical efficacy models of lung injury, including a bleomycin-induced acute lung injury model, a ventilator-induced acute lung injury model, an oleic acid-induced acute lung injury model and an ovalbumin model of asthma. A favorable safety profile was demonstrated with TXA127 in preclinical toxicology studies. Administration of TXA127 to rats and dogs by intravenous (IV) injection for 1 month at doses up to 1 mg/kg/day resulted in no adverse effects. Similarly, no adverse effects were found when TXA127 was administered by SC injection to rats and dogs at doses up to 10 mg/kg/day for 1 month. In a 100-day toxicity study, the no observed adverse effects level (NOAEL) for systemic toxicity was 50 mg/kg/day in both the rat and dog. No hemodynamic changes were observed when TXA127 was administered to conscious rats by SC injection or IV infusion at doses up to 1 mg/kg. No cardiovascular changes were noted in dogs treated for 4 weeks at doses up to 1 mg/kg/day IV or 10 mg/kg/day SC. Functional assessment of the central nervous system (CNS) in the 100-day toxicity study in the rat showed no effects on functional observational battery (FOB) or locomotor activity after 95 days of treatment with up to 50 mg/kg/day. In the 100-day toxicity study in the dog at Day 94, electrocardiogram (ECG) assessment showed lower heart rate at all doses up to 50 mg/kg/day, a change that was treatment- but not dose-related, longer PR and RR intervals, and no changes in QRS, QT, or QT interval corrected according to the Van de Water formula (QTcV). Respiratory assessments in the same study showed no treatment-related changes in respiratory frequency, tidal volume, or minute volume doses up to 50 mg/kg/day. TXA127 was not mutagenic in a battery of in vivo and in vitro tests.

Ten clinical studies that have completed, terminated early, or are ongoing, have evaluated the safety and effectiveness of TXA127. Seven clinical studies that were either completed or terminated early have analyzed and reported final data on the safety and effectiveness of TXA127. A total of 108 subjects received TXA127 and 38 subjects received placebo or other drug in these studies. Two additional studies are ongoing; final data are being collected for one study that completed enrollment (n = 75, TXA127 or placebo, data are blinded) and for one

study that terminated early (n = 15, all TXA127-treated). One additional study, planned for pediatric patients and submitted under U.S. Investigational New Drug Application (IND) 58,561, was Institutional Review Board (IRB)-approved but was terminated before enrolling patients.

All studies were conducted under U.S. IND 58,561 (oncology) with the exception of two studies that were conducted under different INDs: Study A17-002 (IND 60,159; renal) and Study TXA127-2008-001 (IND 101,736; infectious disease). The dose of TXA127 ranged from 0.0025 to 1.0 mg/kg/day across a range of treatment regimens in the 9 studies that enrolled subjects. In the 7 completed and early terminated studies, TXA127 was found to be well-tolerated with no dose-limiting toxicities (DLTs) identified in any study. Of the 39 serious adverse events (SAEs) reported in these 7 studies, none were classified as definitely related or probably related to the study drug. Only one SAE, a case of anemia in Study A17-002 of patients with end-stage renal disease, was classified as possibly related to the study drug (TXA127). Of the SAEs reported for 16 subjects in the 2 ongoing studies, one SAE of allergic reaction/hypersensitivity in Study TXA127-2010-001 was classified as possibly related to the study drug (TXA127).

2.2 Rationale

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of coronavirus disease 2019 (COVID-19) that can be associated with severe respiratory and multi-organ failure. SARS-CoV-2 is thought to enter cells through angiotensin converting enzyme 2 (ACE-2) on the surface of cells resulting in endocytosis and then translocation. During this process ACE-2 is also internalized and phagocytosed and thereby may become non-functional. ACE-2 mediates the conversion of angiotensin II to angiotensin-(1-7), that opposes the effect of angiotensin II. It is likely that COVID-19 reduces levels of ACE-2 and therefore angiotensin-(1-7). As a result, angiotensin II levels are going to be increased. This hypothesis explains many clinical observations in COVID-19 specifically the high incidence of acute kidney injury that may be due to profound intra-renal vasoconstriction caused by excessive levels of angiotensin II. The synthesized version of angiotensin-(1-7) is TXA127. Administration of TXA127 has been studied in multiple phase I and II studies such as

- A phase I safety and biological activity study in patients with HIV
- A phase II trial for the treatment of skeletal muscle fibrosis and reduced muscle strength in patients with Duchenne muscular dystrophy (DMD) to reduce or to prevent the disease progression
- A phase I study in healthy volunteers
- A Phase IIb Study Evaluating the Safety and Efficacy of TXA127 in the Reduction of Incidence and Severity of Thrombocytopenia in Patients Receiving Combination Gemcitabine and Platinum Therapy for Ovarian, Fallopian Tube, or Peritoneal Carcinoma
- A Phase I/II Single-Blind, Randomized, Placebo-Controlled Trial of TXA127 alone and in combination with erythropoietin on optimization of hemoglobin in end-stage renal disease patients

Except for the last study, TXA127 has been administered subcutaneously with a dose range of 0.01 to 0.5 mg/kg per day with most studies using a dose of approximately 0.3 mg/kg/day. One study ("Effect of TXA127 alone and in combination with erythropoietin on optimization of hemoglobin in end-stage renal disease patients") used a dose range of 0.01-0.05 mg/kg per day intravenously three times per week for 12 weeks.

160 patients received TXA127 in dose ranges from 0.0025 to1.0 mg/kg/day. Only 1 of these patients were found to have a single serious adverse event (anemia) that was considered possibly related to the administration of TXA127.

2.3 Objective

Primary endpoint

- All-cause mortality (from start of experimental drug to day 28) with Acute Kidney Injury as a composite endpoint (two times serum creatinine above normal; this way we avoid potential noise with regard to small changes of serum creatinine).

Secondary endpoints

- Incidence of respiratory failure requiring intubation and ventilatory support
    - The proportion of patients alive and free of respiratory failure from start of experimental drug/randomization to day 28. With prolonged ventilation considered to be clinically equivalent to respiratory failure.

- Renal failure requiring dialysis (either continuous or intermittent)
    - This will be measured by modality, duration and indications of dialysis from start of experimental drug/randomization to day 28.
- Vasoplegic shock requiring vasopressors
    - Measured by proportion of participants experiencing this event from start of experimental drug/randomization to day 28.
- Inflammatory markers, specifically IL-6 levels
- Return to baseline oxygen requirement from start of experimental drug/randomization to day 28
- ICU Length of stay
- Hospital length of stay
- Time of recovery to return to baseline oxygen requirement

- Clinical status
    - Measured with questionnaires of ordinal scale, NEWS scale and SOFA score. The subcomponents of these scales will be collected daily through Day 10 of patient hospitalization or until discharge, whichever comes first.
- Proportion of patient improvement measured by return to room air and return to normal renal function
- Time of improvement measured by return to room air scaled at days 5, 10, 28 and 60.
- All-cause mortality at day 60

3 STUDY DESIGN

Description of Study Design

This is a Prospective, double-blinded, randomized placebo-controlled trial with two groups: the study drug (treatment group) and the placebo group (control group). We will study severe COVID-19 patients admitted to the hospital with respiratory insufficiency and oxygen requirements.

Approximate time to obtain data:

- ⇒ Data abstraction from EMR for clinical vitals, clinical blood work, clinical chest x-ray/CT chest imaging, COVID-19 history and symptoms: 25-30 minutes
- ⇒ Research Blood draw: 5 minutes
- ⇒ Nurse administration of study drug: 5 minutes
- ⇒ Completion of patient assessments: 10 minutes

Expected duration of subject participation

The participant is expected to complete study procedures during ten days of hospitalization – or until patient is discharged, whichever comes first. Afterwards a study member will call the participant for follow-up AEs at 24-72hrs post discharge, at day 28(+/- 2 days) and at day 60 (+/- 2 days).

4 STUDY POPULATION

4.1 Selection of the Study Population

Sample size

Target sample size is n=50.

Recruitment pool:

Subjects will be drawn from inpatient hospitalized patients at New York Presbyterian Hospital/Columbia University Irving Medical Center (including the Allen Hospital) via person-to-person communication between physicians and review of electronic medical records for COVID-19 admissions.

Screened vs Enrolled:

⇒ **Screened**
A participant is considered to be screened when their medical records have been reviewed by the investigators and eligibility criteria has been met. The CRC will keep record of the total number of eligible and ineligible screened participants.

⇒ **Enrolled**
The participant is considered enrolled when the investigator has determined their eligibility based on the review of their EMR clinical chart and has signed the informed consent form.

4.2 Inclusion/Exclusion Criteria

Inclusion criteria

- Severe COVID-19: Adult patients admitted to the hospital through the ED requiring oxygen therapy (any level) to maintain SaO2 > 90%
- COVID positive by PCR on hospital admission
- Hospitalized patients aged 18 or greater
- have a negative pregnancy test
- willing to use highly effective methods of contraception

Exclusion criteria

- Pre-existing chronic kidney disease
- New use of or change in dose of ACE-inhibitors or ARB within the last 6 months
- Acute kidney injury at the time of enrollment defined as either increase pf serum creatinine by more than 50% or 0.3 mg/dL above baseline or estimated creatinine clearance (by MDRD) of less than 60 ml/min (if no baseline serum creatinine available)
- Pregnant and breastfeeding women
- Contraindicated medications: new use or change of medications from start of trial
    - Start of an ACE inhibitor or ARB within 6 months of trial.

Please Note:

- Guidelines from the FDA and NIH for the definition of severe COPD were followed including: Positive testing by standard RT-PCR assay or an equivalent test; Symptoms suggestive of severe systemic illness with COVID-19, which could include any symptom of moderate illness or shortness of breath at rest, or respiratory distress; and Clinical signs indicative of severe systemic illness with COVID-19, such as respiratory rate ≥ 30 per minute, heart rate ≥ 125 per minute, SpO2 ≤ 93% on room air at sea level or PaO2/FiO2 < 300

- Participants may receive investigational therapies that are used in the context of local standard of care for COVID-19, participation in other clinical studies may complicate the assessment and impact interpretation of efficacy and safety data in this study. Inclusion of other antiviral treatments consistent with standard of care is acceptable.
- On May 1, 2020, FDA authorized use of the unapproved product remdesivir for treatment of suspected or laboratory confirmed COVID-19 in adults and children hospitalized with severe disease. Severe disease, for the purposes of the Emergency Use Authorization (EUA), was defined as patients with an oxygen saturation (SpO2) ≤ 94% on room air or requiring supplemental oxygen or requiring mechanical ventilation or requiring extracorporeal membrane oxygenation (ECMO). Therefore, incorporating remdesivir into clinical trials, as a component of standard of care for patients hospitalized with severe disease, in settings where remdesivir is available via the EUA mechanism is acceptable.

5 STUDY PROCEDURES/EVALUATIONS

5.1 Study Procedures

Methods for collecting Clinical data:

⇒ **Medical History /Medications/Vitals/clinical blood-work/chest x-ray/CT imaging/COVID-19 symptoms and history/AE or SAE**

The clinical data will be abstracted daily from the NYP/Columbia's EMR system called "EPIC." Only individuals that have access to this system will abstract data; this includes the investigators and CRC.

Methods for collecting Research data:

⇒ **Research Blood-work Collection:**

Research blood-work will be collected on Days 1,5 and 10 only and if patient remains hospitalized. A study team member will ask phlebotomist to please assist in drawing blood for research purposes and will provide the proper tubes. If phlebotomist is unavailable, a study team member with certification in phlebotomy or MD degree will gown up wearing appropriate PPE and collect the labs needed.

Procedures for study visits:

⇒ **Registering participant:**
The participant needs to be registered in IBM CTMS system – a secure clinical trial website/tool used to validate research study participants so it may interface with EPIC.

⇒ **Method of Randomization:**
SAS block randomization was used and disclosed to the research pharmacy for study drug randomization and dispensation.

⇒ **Unblinded personnel:**
The Research Pharmacy will remain unblinded for safety purposes. If needed for safety concerns – the DSMB chair will be unblinded. In addition, the CRC is unblinded due to the creation of the SAS block randomization.

⇒ **Ordering study drug / Scheduling study tests:**
Using the new electronic medical record system of EPIC, the PI and co-investigators will need to order and sign off on all study drug ordering.

Calculated dose (mg/kg) x Drug Factor (1.19mg) = Actual dose (mg).

- Calculated Dose - The calculated dosage of the study drug per participant's weight. For example: A participant weight is 75 kg and the dosage of the drug is 0.5mg/kg. Therefore, 75kg x 0.5 mg/kg = 37.5 mg. This is the calculated dose.

- Drug Factor - The drug substance of TXA127 acetate also contains moisture. In order to follow protocol of 0.5mg/kg, we have to weigh out more of the drug substance than corresponds to 0.5 mg/kg so we can end up dosing the patient with 0.5 mg/kg TXA127. In other words, 1 mg of TXA127 would weight out 1.19 mg of drug substance. The Lead pharmacist and drug sponsor have agreed to add the drug factor to the calculated dosage as follows: 0.5 mg/kg x 1.19 = 0.595 mg/kg.

For example: If the calculated dose of a participant is 37.5mg, then we would multiply it by the drug factor as such: 37.5mp x 1.19 mg = 44.625 mg (round to 45 mg). Therefore, the actual dose would be 45 mg.
Angiotensin (1-7) / TXA127: 0.5 mg/kg per day for ten days intravenously over 3 hours or Placebo.

- The PI/Sub-Is/CRC will order the study drug order in EPIC. Within the order there will be an "**order instruction**" instructing the PI/Sub-I/CRC to include the "drug factor" of 1.19 to the calculation of the actual dose. Calculated dose x 1.19 = Actual dose. The patient's weight would be documented in the "**administration instruction**".
- To place the study drug order in EPIC, it must be as an "in-patient" order and linked to the research study.

Type of Information gathered/collected/recorded Day1 through Day 10 (if patient remains hospitalized):

Demographic Information

Demographic information includes: Name, Address, DOB, Telephone number, email, gender, age, race and ethnicity.

Medical History and Medications

Summary of medical history and main diagnosis. All medications currently taken will be collected.

Vital Signs

Clinical vitals will be collected including: heart rate, blood pressure, respiratory rate, temperature, and oxygen saturation. The time of vital collection will also be recorded.

Drug Administration
The drug dosage, maximum flow rate and time of administration will be recorded.

Clinical Bloodwork
Including time of collection, CBC. BMP, Hepatic Panel and other clinical markers (such as ESR, fibrinogen and CRP).

Clinical Imaging
Includes date of imaging and impression summary

Clinical COVID-19 symptoms and history:
Includes clinical review of physicians notes for symptom reporting and of the COVID-19 diagnosis and date of recent positive COVID swab.

Patient Assessments:
The NEWS, Ordinal and Glasgow coma score will be completed by the CRC/Investigators based on the clinical information abstracted. No questions will be directly asked to the patient.

Research Bloodwork
Three tubes of blood collection on Days 1, 5 and 10 will be collected. Plasma, Buffy coat and serum will be abstracted stored in freezer -80 in the laboratory of Dr. D'Armiento.

5.2 Potential Risks and Benefits

5.2.1 Potential Risks

Immediate risks
Extravasation, vasodilation, anemia

Rationale for the necessity of such risks
Improvement in perfusion specifically renal perfusion, replacement of endogenous angiotensin 1-7.

Alternative data gathering procedures that have been considered or will be considered
No alternatives as there are presently no treatment or prevention modalities of COVID-19 associated AKI.

Why alternative procedures may not be feasible
There are presently no treatment or prevention modalities of COVID-19 associated AKI.

Why the value of the information to be gained outweighs the risks involved.
COVID-19 associated AKI has a very high mortality and preventing this AKI by administering angiotensin 1-7 at a dose that can be considered a replacement of physiologic levels and has no substantial adverse effects may improve mortality.

For a list of adverse events previously reported in past human clinical trials, please refer to pages 78-85 of the Investigator Brochure. Below is a summarized list:

Body System	Adverse event	Severity	Relationship to TXA127
General	Injection Site Pain	Mild	Definitely Related
Musculoskeletal	Bone Pain	Mild	Probably Related
	Myalgia	Mild	Probably Related
	Fatigue	Severe	Possibly Related
	Influenza like illness	Mild	Possibly Related
Gastrointestinal	Abdominal distension	Moderate	Possibly Related
	Abdominal pain	Moderate	Possibly Related
	Constipation	Moderate	Possibly Related
	Dry mouth	Mild	Possibly Related
	Gingivitis	Mild	Possibly Related
	Nausea	Moderate	Possibly Related
	Vomiting	Severe	Possibly Related
	Oral pain	Moderate	Possibly Related
	Diarrhea	Mild	Possibly Related
	Bloated Feeling	Mild	Possibly Related
Metabolism	Thirst	Mild	Possibly Related
Nervous system	Tremors	Mild	Possibly Related
	Headache	Moderate	Possibly Related

Skin	Rash	Moderate	Possibly Related
Blood	Neutropenia	Life-threatening	Possibly Related
	Anemia	Severe	Possibly Related

5.2.2 Known Potential Benefits

There are no direct benefits from participating in this study. However, this study will help the researchers determine if this study drug is safe and effectively treats severe COVID-19 patients.

6 STUDY SCHEDULE

Please refer to the Schedule of Assessments (Appendix A).

6.1 Screening

⇒ Screening will be done by the PI/Sub-Is on a weekday daily basis. Emails will be sent to the study group with list of new COVID-19 admissions and brief description of the potential participant's medical history. Based on this the physicians would narrow which patients would be a good candidate for the study. Then a further in-depth review of their medical chart is done to ascertain the eligibility criteria.

⇒ The physicians or CRC will appropriately gown up in PPE and approach the patient for informed consent.

6.2 Day 1 through Day10

⇒ Day 1 through 10 procedures is listed in the Schedule of Assessments. These procedures will be done for 10 consecutive in-patient hospitalization day from date of consent or until patient is discharged (in the case patient is discharge prior to Day 10).

6.3 Follow-up phone calls

⇒ Assess for AE: Ask questions related to any possible adverse reaction to the drug and overall health status of the patient.

7 ASSESSMENT OF OUTCOME MEASURES

7.1 Specification of the Appropriate Outcome Measures

7.1.1 Primary Outcome Measures

Table2:

Outcome Measures	Efficacy Endpoints
All-cause mortality (from start of experimental drug to day 28) including subcomponent of Acute Kidney Injury	Primary
Incidence of respiratory failure requiring intubation and ventilatory support; defined as the proportion of patients alive and free of respiratory failure from start of experimental drug to day 28.	Secondary
Renal failure requiring dialysis (either continuous or intermittent) Measured by modality, duration and indications of dialysis from start of experimental drug to day 28.	Secondary
Return to baseline oxygen requirement from start of experimental drug to day 28	Secondary
Vasoplegic shock requiring vasopressors Measured by proportion of participants experiencing this event from start of experimental drug to day 28.	Secondary
Inflammatory markers, specifically IL-6 levels	Secondary
ICU Length of stay	Secondary
Hospital length of stay	Secondary
Time of recovery to return to baseline oxygen requirement	Secondary
Clinical status measured on a ordinal scale of mechanical ventilation, requiring dialysis and death.	Secondary

Proportion of patient improvement measured by return to room air and return to normal renal function	Secondary
Time of improvement scaled at days 5, 10, 28 and 60	Secondary

8 SAFETY ASSESSMENT AND REPORTING

All AE and SAE will be captured and assessed at the end of each study visit and via AE phone calls done at 24-72hr post discharge and at Day 28 (+/- 2 days) and at Day 60 (+/- 2 days) post enrollment.

8.1 Definition of Adverse Event (AE) and Serious Adverse Event (SAE)

Adverse Events
An adverse event (AE) is any untoward medical occurrence in a study subject. This may include symptom(s), illness, clinically significant abnormal laboratory value or change in value, or worsening in a subject during a clinical study.

Procedures for Adverse Events

During Day 1 through Day 10:
AEs will be extracted from Standard Medical Treatment and clinical notes within EPIC. These will be reported in AE log within the regulatory binder. The PI/co-I will complete the Adverse Event Form within seven days of learning of the event (if life-threatening or death) and within 15 days for all other SUSARs. The CRC will comply with local regulations and policies when notifying the institutional IRB.

During follow-up phone calls:
The CRC will record the AE within the CRF, as well as complete the AE log in the regulatory binder. Information recorded includes: name of event, date of onset, date of awareness, severity, duration, and relationship to study drug (if it can be determined). Severe adverse events will be followed until they are adequately resolved or stabilized during the study. The CRC will then notify the PI (or co-investigators if the PI is unavailable) and complete the Severe Adverse Event Form within seven days of learning of the event.

The following general definitions for rating severity should be used for this study:

Mild: Awareness of signs or symptoms, but easily tolerated and transient; causing no loss of time from normal activities; symptoms would not require medication or a medical treatment; signs and symptoms are transient.

Moderate: Marked symptoms and discomfort severe enough to cause moderate interference with the subject's usual activities. Symptomatic treatment is possible.

Severe: Incapacitating with inability to do work or usual activities; signs and symptoms may be of systemic nature or require medical intervention and/or treatment. Hospitalization may be required or prolonged.

The relationship of an AE or SAE to the underlying disease or to the procedure will be attributed using the following definitions:

Unrelated: There is no evidence that the event has a relationship to the drug(s).

Unlikely: There is no evidence that the event has a relationship to the drug(s). However, the potential association to the drug cannot be totally eliminated.

Possibly Related: The event has a timely relationship to the drug(s) used. However, a potential alternative etiology may be responsible for the adverse event.

Probably Related: The event has a timely relationship to the drug(s) used and the causative relationship can clearly be established. No potential alternative etiology is apparent.

Related: There is evidence that the event has a relationship to the drug(s).

There is no standard scale for COVID patients, thus we will use the Common Terminology Criteria for Adverse Events (CTCAE) v5.0

- **Grade 1**

Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

- **Grade 2**

Moderate; minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental ADL*.

- **Grade 3**

Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL**.

- **Grade 4**

Life-threatening consequences; urgent intervention indicated.

- **Grade 5**

Death related to AE.

Serious Adverse Events

In accordance with 21 CFR Parts 803 and 812, an SAE is defined as any untoward medical occurrence that results in:

- death,

- is life-threatening; any adverse experience that places the subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred (i.e., it does not include a reaction that, had it occurred in a more serious form, might have caused death),
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect,
- requires intervention to prevent permanent impairment or damage
- Important medical events that do not result in death, are not life-threatening, or do not require hospitalization may be considered serious adverse events when, based upon appropriate medical judgment, they might jeopardize the subject and might require medical or surgical intervention to prevent one of the outcomes listed above.

All SAE will be reported to the PI and co-investigators within 24 hours after knowledge of the occurrence. If a serious adverse event (SAE) occurs during an in-person study visit or by phone, the CRC will follow the same steps as with the AE reporting.
The IND sponsor has the obligation to report all serious adverse events that are 'Probably' or 'Related' and 'Unexpected' in relation to the study drug to the FDA. SAEs are reported to the IRB only if they meet the definition of a UP and these will be submitted by the PI. All events reported to the FDA by the IND sponsor are to be submitted utilizing the Form FDA 3500A (MedWatch Form), if applicable.

Unanticipated Problem (UP)

"Unanticipated problem": any incident, experience or outcome involving risk to subjects or others in any human subjects' research that meets **all** of the following criteria:

⇒ Unexpected (in terms of nature, severity or frequency) given (a) the research procedures that are described in the IRB approved protocol and informed consent document, and (b) the characteristics of the subject population being studied;

⇒ Related or possibly related to participation in such research (i.e., there is a reasonable possibility that the incident, experience or outcome may have been caused by the procedures involved in such research); and

⇒ Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic or social harm) than was previously known or recognized.

8.2 Criteria for Discontinuation or Withdrawal of a Subject (or a Cohort), if applicable

Withdrawal:
The only reason for a patient being considered withdrawn should be withdrawal of consent. If this occurs. data collected up to the date of withdrawal may be used in data analysis. No further data collection will be done, and no follow-up calls will be made.
Withdrawal will in no way affect the care management of the participant.

Discontinuation:
Treatment discontinuation from the study might occur if the participant experiences a serious adverse event per definition and the investigator decides to discontinue drug administration for the safety of the subject. However, patients who discontinue treatment will remain in the study and continue to be assessed for efficacy and safety.

For example, for acute kidney injury: The study drug will be continued after development of acute kidney injury and we will continue to monitor the patient. However, if a patient starts dialysis, we will discontinue study drug but will still follow the patient clinically per protocol.

Patients who become pregnant during the study will immediately discontinue the study drug but will continued to be followed per schedule of assessments. The outcome of pregnancy (spontaneous abortion, therapeutic abortion, live birth, fetal death in utero) will be recorded.

Loss to Follow-up:
To minimize loss to follow-up, we will continue to follow patients after discharge via phone calls post discharge date, at day 28 and day 60 and ascertain vital status in all patients.

8.3 Halting Rules

The decision to halt the study will be left at the discretion of the DSMB, after the DSMB has had the opportunity at each DSMB meeting to review cumulative unblinded comparative safety results as well as cumulative unblinded comparative efficacy results.

9 CLINICAL MONITORING STRUCTURE

Study team (PI, co-investigators and CRC) research meetings will be held quarterly (less or more frequent depending on participant enrollment) to review the study status, any AEs or SAEs, primary and secondary outcome measures, among other topics. Any clinical safety modifications will be discussed and made during these meetings.

The Anesthesiology department implemented a Compliance Research Coordinator whose responsibilities include quarterly reviews of the regulatory binder, consent and HIPAA forms and subject case histories. After the compliance coordinator reviews the study materials, a copy of the monitoring report is filed in the Regulatory Binder. Any revisions listed in the reporting are promptly addressed by the clinical research coordinator.

9.1 Data Safety and Monitoring Board – DSMB

An independent Data Safety Monitoring Board (DSMB) composed of 4 individuals: 2 pulmonary specialists, one regulatory/statistical clinical trial specialist and one kidney specialist will be established to provide independent benefit/risk oversight during the conduct of the study. The DSMB will: 1) Review the protocol and consent form prior to study initiation, 2) Evaluate Serious Adverse Events on an “as needed” basis and all adverse events on a quarterly basis, 3) Recommend discontinuation of the study in the event of the occurrence of Serious or Unexpected Adverse Events that are determined by the DSMB to pose a significant safety concern. The Chairperson of the DSMB will notify the HRPO and Dr. D’Armiento who will in turn notify the FDA or other regulatory bodies of adverse safety outcome information sufficient to stop the study. This information will also be reported as part of required regulatory progress update reports.

10 STATISTICAL CONSIDERATIONS

10.1 Study Outcome Measures

Severe COVID-19

Primary endpoints

- All-cause mortality (from start of experimental drug to day 28) including subcomponent of Acute Kidney Injury

Secondary endpoints

- *Incidence of respiratory failure requiring intubation and ventilatory support*
- *Renal failure requiring dialysis (either continuous or intermittent)*
- *Vasoplegic shock requiring vasopressors*
- *Inflammatory markers, specifically IL-6 levels*
- *Reduction in supplemental oxygen requirements from start of experimental drug*
- *ICU Length of stay*
- *Hospital length of stay*
- *Time of recovery to return to baseline oxygen requirement*
- *Clinical status (mechanical ventilation, requiring dialysis and death)*
- *Proportion of patient improvement measured by return to room air and return to normal renal function*
- *Time of improvement scaled at days 5, 10, 28 and 60.*

10.2 Sample Size Considerations

If we assume a proportion of 65% mortality in patients with severe COVID-19 and further assume that administration of angiotensin (1-7) / TXA127 will reduce the proportion of mortality to 15% we will require a total sample size of 50 subjects (25 in each group) to reach a power of 92% with a 95% confidence level.

10.3 Analysis Plan

The definition of the primary efficacy endpoints:

Primary endpoint of this study will be all-cause mortality (from start of experimental drug to day 28) including subcomponent of Acute Kidney Injury.
Any cause mortality including palliative withdrawal or withholding of support from start of experimental drug to day 28. For intercurrent events besides death, the main estimand for each key efficacy endpoint will follow the treatment policy strategy. Therefore, the analyses will include all observed on-study data regardless of patients' use rescue medications, discontinuation of the investigational product, or other violations of the protocol.

Secondary endpoints are defined as follows:

Respiratory failure requiring intubation and ventilatory support
Any endotracheal intubation for respiratory failure within 28 days after initiation of study drug/placebo; we will exclude intubations that are required for providing anesthesia for any surgeries or procedures. Patients who require intubation for the provision of anesthesia will remain in the study as prolonged ventilation is considered to be clinically equivalent to respiratory failure.

Renal failure requiring dialysis (either continuous or intermittent)
Any use of dialysis (continuous or intermittent hemodialysis or peritoneal dialysis) within 28 days after initiation of study drug/placebo

Vasoplegic shock requiring vasopressors
Proportion of patient requiring any vasopressor drug such as phenylephrine, norepinephrine, vasopressin, epinephrine or dopamine for more than two hour within 28 days after initiation of study drug/placebo. The time of possible procedures or operations are not included in this definition

Inflammatory markers, specifically IL-6 levels
Absolute levels of the following inflammatory markers (continuous variables):
IL-6, IL-7, IL-8, C-reactive protein, erythrocyte sedimentation rate. We will determine the peak value within 28 days after start of experimental drug/placebo as a secondary endpoint

Return to baseline oxygen requirement from start of experimental drug/placebo to day 28
Time to ability to maintain oxygen saturation (SaO2) >95% or above baseline in patients with baseline low sAo2 (for example due to COPD) with supplemental oxygen administration

The multiplicity control procedure to be used to control the family-wise type I error:

We acknowledge that family-wise type errors are a concern considering that many of our primary and secondary endpoints are interdependent. For example, vasopressor use (a secondary endpoint) is frequently associated with AKI (our primary endpoint). We will attempt control for the multiplicity by

1. Using all-cause mortality with a subcomponent of acute kidney injury to aligned with the type of study of a proof of concept study
2. Use of a combined secondary endpoint; this endpoint is achieved if the patient either dies, requires intubation or renal replacement therapy within 28 of initial administration of study drug / placebo

Main analysis for each of the key primary and secondary efficacy endpoints:

Primary endpoint of all-cause mortality will be used as a binary variable and compared between patients receiving study drug and placebo using Chi-Square test. We will analyze all randomized patients in the main efficacy analysis for primary and secondary endpoints, regardless of whether they have missing data.

Respiratory failure requiring intubation and ventilatory support, Renal failure requiring dialysis and vasoplegic shock requiring vasopressors will also be determined as binary variables. Peak Inflammatory markers within 28 days, specifically IL-6 level will be analyzed as a continuous variable.
Intercurrent events are a concern and some of these patients may for example receive rescue medications or treatments. For intercurrent events besides death, the main estimand for each key efficacy endpoint will follow the treatment policy strategy. Therefore, the analyses will include all observed on-study data regardless of patients' use rescue medications, discontinuation of the investigational product, or other violations of the protocol.

We will use the appropriate statistical methods to analyze the primary and secondary endpoints such as Ch-Square for binary variables, t-test or Mann-Whitney test for continuous variables. Significance will be determined at two-sided $p < 0.05$. Variables that are significant in a univariate analysis will be included in a multi-variate analysis in addition to demographic variables such as sex, age or race, presence of co-morbidities and severity of COVID-19 as described below.
More detailed statistical methods will be determined with the consultation of a statistician prior to unblinding and data analysis.

Subgroup analyses

We will analyze the following subgroups separately

- Age below and above 60 years
- History of diabetes
- History of hypertension
- Race, sex
- Severity of COVID-19 disease at enrollment
  - Mild COVID-19
    - No clinical signs indicative of Moderate, Severe, or Critical Severity
  - Moderate COVID-19
    - Respiratory rate ≥ 20 breaths per minute, saturation of oxygen (SpO2) > 93% on room air at sea level, heart rate ≥ 90 beats per minute
  - Severe COVID-19
    - Shortness of breath at rest, or respiratory distress and respiratory rate ≥ 30 per minute, heart rate ≥ 125 per minute, SpO2 ≤ 93% on room air or PaO2/FiO2 < 300

Safety analyses

Safety outcomes and adverse events that will be included in a safety analysis will include among others:

- Anemia
- Arrhythmia
- Hypotension
- Concurrent infections
- Liver dysfunction

Grading will occur as described by the FDA in the “Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0”
Patients will be monitored closely for drug-drug interactions. Daily drug administration will be recorded and any potential drug-drug interaction reported to the DSMB that will then review these cases within 48 hours. Possible drug-drug interactions will include pharmacodynamic interactions with antihypertensives, specifically ACE inhibitors or Angiotensin receptor blocker and pharmacokinetic interactions.

11 ACCESS TO SOURCE DATA/DOCUMENTS

Any information collected during this study (coded or containing identifiers) will be kept confidential. Specimens will be assigned a code number, and separated from the participants' name or any other information that could identify the participants. The research file that links the participants name to the code number will be kept in a locked file cabinet and only the investigator and study staff will have access to the file.

12 ETHICS/PROTECTION OF HUMAN SUBJECTS

12.1 Declaration of Helsinki

"The investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki, or with the International Conference for Harmonisation Good Clinical Practice (ICH-GCP) regulations and guidelines, whichever affords the greater protection to the subject."

12.2 Institutional Review Board

This protocol along with the associated informed consent documents and recruitment material will be reviewed by an appropriate independent ethics review committee (IEC) or Institutional Review Board (IRB) registered with OHRP. Any amendments to the protocol or consent materials must be reviewed before they are placed into use. No study procedures will be conducted until the aforementioned is approved by the CUIMC IRB.

12.3 Informed Consent Process

***"Informed consent** is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation in this study will be provided to the subjects and their families.*

***Consent forms** describing in detail the study procedures and risks are given to the subject and written documentation of informed consent is required prior to enrolling in the study. Consent forms will be IRB approved and the subject will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the subject and answer any questions that may arise. The subjects will sign the informed consent document prior to being enrolled in the study. The subjects should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of the study. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study."*

Waiver of pre-screening consent

⇒ **Initial Screening of Medical Records:**
Referrals of potential participant candidates will be provided by colleagues of the PI/Sub-Is. Prior to approaching the potential patient, the CRC, PI and CO-I will review the

medical records in EMR system to determine further eligibility based on the Inclusion/Exclusion Criteria.

⇒ **Signed Informed Consent Form**
The clinical research coordinator or PI or co-investigator will thoroughly go over the consent form and provides the participant ample time for them to read it and ask any questions or concerns they may have. After participant reviews the consent form, written consent is obtained by the participant. At that moment, the participant is considered to be enrolled in the study and an ID# is given. A copy of the consent and HIPAA forms are provided to the participant.

12.4 Subject Confidentiality

All subject identifiers will be removed after collection of the data and the data will receive a code that will allow us to link this data to the medical record number and will be stored in a locked and secured, encrypted computer. Access to any of the data will be restricted to the principal investigator, coinvestigators and CRC by assignment of a password.

12.5 Data Capture Methods

Study data is captured by paper and electronic forms. Any self-reported data by the patients will be captured and incorporated into CRF including timing of drug administration. Electronic data such as Medical Record abstraction will be extracted from EPIC. Excel tracking sheets are maintained by the CRC as internal electronic documents which will eventually be transferred to RedCap. Trained study staff will enter data from the paper CRFs into the excel file. This method, once implemented will serve as the source documents and may be printed for audit/review purposes if need be.

12.6 Study Records Retention

All source documents including but not limited to data collection, study materials, reports and internal documents will be retained within the CUIMC/NYP premise in a locked file cabinet, inside a locked office for the longest duration dictated by applicable regulations (6 years for HIPAA, 3 years for HHS, 2 years for FDA, contract with drug sponsor may require longer duration).

13 REFERENCES

Investigator Brochure Manual provided by the drug sponsor as well as the CUMC IRB Standard of Care policies.

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Siew ED, Peterson JF, Eden SK, Moons KG, Ikizler TA, Matheny ME. Use of multiple imputation method to improve estimation of missing baseline serum creatinine in acute kidney injury research. Clin J Am Soc Nephrol. 2013;8(1):10-18.

Aroda VR, Saugstrup T, Buse JB, Donsmark M, Zacho J, Davies MJ. Incorporating and interpreting regulatory guidance on estimands in diabetes clinical trials: The PIONEER 1 randomized clinical trial as an example. Diabetes Obes Metab. 2019;21(10):2203-2210.

Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. (https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf). Accessed 05/30/

14 APPENDICES

14.1 Appendix A: Schedule of Assessments

Event	Screen	Day 1 +/- 2 days of screen	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	24-72 hrs*	Day 28 +/- 2 days	Day 60 +/- 2 days
			If patient remains hospitalized, the below assessments will be done											
Medical History	X													
Informed Consent	X													
Demographics		X												
Clinical Vitals[1]		X	X	X	X	X	X	X	X	X	X			
Drug/Placebo Administration[2]		X	X	X	X	X	X	X	X	X	X			
Clinical Blood Work[3]		X	X	X	X	X	X	X	X	X	X			
Research Blood Work		X				X					X			
Clinical Chest x-ray + Chest CT		X												
Clinical COVID-19 symptoms and history		X	X	X	X	X	X	X	X	X	X			
Clinical Patient Assessments[4]		X	X	X	X	X	X	X	X	X	X			
Follow-up AE phone call												X	X	X

*Post hospital discharge date
1-Vitals include: HR, RR, BP, O2Sat, Weight, Height
2-Drug/Placebo Administration includes: Time drug was given, dosage, vitals within 3 hours of drug given
3-Clinical Blood work includes: Chemistry, Hematology, Hepatic and BMP.

4-Patient Assessments include: collection of COVID-19 symptoms, SOFA, NEWS, Ordinal scale and Glasgow coma scale.

14.2 Appendix B: Questionnaires

Ordinal Scale:

• 1.Death	• 5.Hospitalized, not requiring supplemental oxygen
• 2.Hospitalized, on invasive mechanical ventilation or ECMO	• 6.Not Hospitalized, limitation on activities
• 3.Hospitalized, on non-invasive ventilation or high flow oxygen devices	• 7.Not Hospitalized, no limitation on activities
• 4.Hospitalized, requiring supplemental oxygen	

NEWS Scale:

Respiratory Rate	Point Value
• <=8	+3
• 9-11	+1
• 12-20	0
• 21-24	+2
• >25	+3
Oxygen Sat %	
• <91	+3
• 92-93	+2
• 94-95	+1
• >=96	0
Any supplemental oxygen	
• Yes	+2
• No	0
Heart rate	Point Value
• <=40	+3
• 41-50	+1
• 51-90	0
• 91-110	+1
• 111-130	+2
• >=131	+3

Temperature in C	Point Value
• <=35 (95F)	+3
• 35.1-36 (95.1-96.8)	+1
• 36.1-38.0 (96.9-100.4	0
• 38.1-39.0 (100.5-102.2)	+1
• >=39.1 (>=102.3)	+2
Systolic BP	Point Value
• <=90	+3
• 91-100	+2
• 101-110	1
• 111-219	0
• >=220	+3
Level of Consciousness	Point Value
• Alert	0
• Not Alert (V, P or U)	3
Total score	