Title:

Open-label, pharmacokinetic, pharmacodynamic, ascending dose safety lead-in followed by a single center, placebo-controlled, double-blind, adaptive, safety and efficacy, pilot study of Trans Sodium Crocetinate (TSC) in SARS-CoV-2 infected subjects

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The following Sponsor's signature confirms acceptance and approval of Protocol 100-303.

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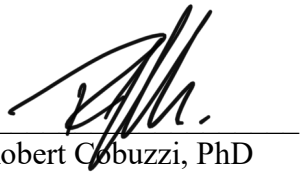

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PROTOCOL 100-303 SYNOPSIS

Title	Open-label, pharmacokinetic, pharmacodynamic, ascending dose, safety and tolerability lead-in followed by a single-center, randomized, placebo-controlled, double-blind, adaptive, safety and efficacy, pilot study of Trans Sodium Crocetinate (TSC) in SARS-CoV-2 Infected Subjects
Protocol #	100-303
Study Phase	1b/2b
Indication	Treatment of hypoxemia associated with respiratory SARS-CoV-2 infection
Study Design	Open-label, pharmacokinetic, pharmacodynamic, ascending dose, safety and tolerability lead-in followed by a single-center, randomized, placebo-controlled, double-blind, adaptive, safety and efficacy pilot
Study Population	Hospitalized patients with confirmed SARS-CoV-2 infection and hypoxemia, defined as blood oxygen saturation as measured by pulse oximetry (SpO2) < 94% on room air or requiring supplemental oxygen, WHO ordinal scale scores of 3 through 7 (exclusive of Extracorporeal Membrane Oxygenation (ECMO))
Number of Subjects	Lead-In - up to 36 subjects Randomized pilot - up to 200 subjects
Study Centers	The National Institute of Infectious Diseases, Bucharest, Romania
Objectives	**Lead-In PK/PD and Dose Selection** Determine the safety and tolerability of TSC when administered four times per day for up to 15 days for each of the up to six doses to be studied. Determine the relative degree of improvement by TSC dose in blood oxygenation following treatment with TSC as measured by the SpO2:FiO2 (S:F) ratio via recorded continuous pulse oximetry. For pharmacodynamic and pharmacokinetic purposes determine blood oxygenation as measured by arterial oxygen partial pressure (PaO2 in mmHg) to fractional inspired oxygen (FiO2), the PaO2:FiO2 (P:F) ratio or alternatively the S:F ratio following TSC administration at each dose level with matching PK blood sampling.

	Determine the optimum, safe and tolerable biologic dose of TSC among the up to six doses to be studied given four times per day (every 6 hours) for up to 15 days using the S:F ratio. **Randomized pilot** Determine the safety and efficacy of TSC administered at the selected optimum, safe and tolerable biologic dose four times per day (every 6 hours) for up to 15 days as compared to placebo.
Study Overview	**Lead-In PK/PD and Dose Selection** The lead-in study will enroll up to 36 subjects. Each TSC dose will be administered as an IV bolus injection to 6 unique subjects per dose level administered four times per day (every 6 hours) for up to 15 days (a minimum of 5 days). Up to six (6) TSC dose levels will be studied, at the discretion of the Sponsor. Subjects will be assigned to dose levels in ascending order. The dose range is as follows. • 0.25 mg/kg TSC + Standard of Care • 0.50 mg/kg TSC + Standard of Care • 1.00 mg/kg TSC + Standard of Care • 1.50 mg/kg TSC + Standard of Care • 2.00 mg/kg TSC + Standard of Care • 2.50 mg/kg TSC + Standard of Care TSC has been well tolerated in previous clinical studies at doses up 2.5 mg/kg based on safety, laboratory results, ECG findings, and vital signs. From these studies, the maximum tolerated dose was set at 2.5 mg/kg based on mild, transient, yellow visual disturbances seen in 2 subjects at a dose of 5 mg/kg. A dose of 1.5 mg/kg, which has a t ½ of 1.1 hours, has previously been identified from a peripheral arterial disease study (Mohler et al, 2011) as a target dose for longer duration efficacy studies. This study will utilize an ascending dose scheme, starting with a dose of 0.25 mg/kg. All TSC doses will be administered as an IV bolus injection every six hours for up to 15 days (a minimum of 5 days). Safety data from each cohort of subjects at a given dose level will be reviewed by the Safety Monitoring Committee (SMC), which will adjudicate Dose Limiting Toxicities (DLTs) and other safety data. The first two dose levels will be reviewed by the SMC in groups of 3 subjects. Subsequent doses will be reviewed in cohorts of 6 subjects. If there are 0 or 1 DLT among the 6 subjects in a given dose level, 6 subjects in the next higher dose level will be studied. The study will continue in this fashion seeking

an observed toxicity rate that is < 0.33 among 6 patients at any one dose level, or TSC at 1.5 mg/kg proves to be safe and tolerable. If a TSC dose higher than 1.5 mg/kg is warranted, and there are no safety signals, 6 patients may be studied at 2.0 mg/kg and 6 patients at 2.5 mg/kg.

The SMC will review the safety data of a given cohort of patients and approve or disapprove enrollment of the next cohort. For the first group of 3 subjects at 0.25 mg/kg, the SMC reviewed safety data of up to 15 days of treatment. For subsequent cohorts, the SMC will review safety data associated with the first 5 days of treatment. The table below illustrates the SMC review schedule:

# of Subjects per SMC Meeting	# of SMC Meetings per Dose Cohort	TSC Dose Cohort	Duration of Treatment Prior to SMC Meeting (days)
3	2	0.25 mg/kg	15 /5
3	2	0.50 mg/kg	5 / 5
6	1	1.00 mg.kg	5
6	1	1.50 mg/kg	5
6	1	2.00 mg/kg	5
6	1	2.50 mg/kg	5

At the completion of the lead-in subjects, the SMC will examine the resultant safety and blood oxygenation (S:F) data for all subjects and determine the optimum, safe and tolerable dose of TSC for use in the pilot study.

Dose Limiting Toxicity (DLT) is defined as any study drug related CTCAE grade 3 or 4 adverse event during the treatment period, with the exception of pulmonary events in the CTCAE that are known complications of SARS-CoV-2 infection: ARDS, Cough, Dyspnea, Hypoxia, Pneumonitis, Pulmonary Edema, Respiratory Failure, or Respiratory, Thoracic and Mediastinal disorders – Other. The SMC will apply clinical judgement in their review of adverse events (particularly abnormal laboratory results).

A Data Safety Monitoring Committee (DSMB) will examine the data for the randomized pilot.

	Randomized pilot The two arm, randomized pilot will enroll up to 200 subjects. TSC dosing will be at the selected optimum, safe and tolerable biologic dose with an active to placebo ratio of 2:1 toward providing the maximum potential benefit to subjects. If two doses of TSC are to be studied in the randomized pilot the active to placebo ratio will be 2:2:1. Randomization will be stratified by disease severity, age and presence of pre-specified comorbidities. The treatment arms are as follows. • TSC + Standard of Care • Placebo + Standard of Care Each TSC dose will be administered as an IV bolus injection 4 times per day (every 6 hours) for up to 15 days. Subjects randomized to placebo will receive an IV bolus injection of an equivalent volume by patient weight of Normal Saline four times per day (every 6 hours) for up to 15 days. All study drug administration will be performed by unblinded medical staff. Patients, investigators and care givers will not see the injection or injection site or be aware of randomization. Blood oxygenation will be measured via recorded continuous pulse oximetry and the S:F ratio calculated. Provided that an arterial line is established, serial arterial blood gas measurements will be collected and recorded ~ 2 minutes prior to TSC administration and at 1 minute, 10 minutes, 30 minutes, 1.5, 3, 6, 24, and 48 hours post the first TSC administration. The calculated P:F ratio for the same time points will be recorded. This procedure will occur only once per subject per TSC dose level at Day 1 only. Alternatively, the S:F ratio may be used as the measure of blood oxygenation with recording of the S:F ratio at the corresponding timepoints. Subjects will be assessed daily while hospitalized. Discharged subjects will be asked to attend study visits at day 29 and by telephone at day 60.

All subjects will undergo safety and efficacy assessments including laboratory assays, blood sampling on days 1 through day 15 (while hospitalized) and Day 29 if still hospitalized.

All subjects whether a part of the lead-in phase or randomized pilot will be assessed for survival, serious adverse events and adverse events on Day 29 and Day 60.

Clinical Support
At each study day, while hospitalized, the following measures of clinical support should be assessed and recorded.

- Hospitalization
- Oxygen requirement
- Prone positioning
- Use of remdesivir or other antivirals
- Use of corticosteroids
- Non-invasive mechanical ventilation (via mask)
- Mechanical ventilator requirement (via endotracheal tube or tracheostomy
- ECMO requirement

WHO Ordinal Scale
The WHO 9-point ordinal scale assessment will be the first assessment of the subject's clinical status each day recording the worst score for the previous day. (i.e. on day 3, the score for day 2 is recorded as day 2). The ordinal scale is as follows.

Patient State	Descriptor	Score
Uninfected	No clinical or virological evidence of infection	0
Ambulatory	No limitation of activities	1
	Limitation of activities	2
Hospitalized Mild Disease	Hospitalized, no oxygen therapy	3
	Oxygen by mask or nasal prongs	4

<table>
<tr><td></td><td>
<table>
<tr><td>Hospitalized Severe Disease</td><td>Non-invasive ventilation or high-flow oxygen</td><td>5</td></tr>
<tr><td></td><td>Intubation and mechanical ventilation</td><td>6</td></tr>
<tr><td></td><td>Ventilation + additional organ support – pressors, Renal Replacment Therapy (RRT), Extracorporeal Membrane Oxygentation (ECMO)</td><td>7</td></tr>
<tr><td>Dead</td><td>Death</td><td>8</td></tr>
</table>

Glasgow Coma Scale
The Glasgow Coma Score will be performed daily until hospital discharge and in accordance with the standards established by the Institute of Neurological Sciences NHS Greater Glasgow and Clyde. The Glasgow Coma Score will be calculated individualy and by addition of the total points selected under each of the three components including eye, verbal and motor.

Sequential Organ Failure Assessment (SOFA)
The Sequential Organ Failure Assessment (SOFA) Score is a mortality prediction score that is based on the degree of dysfunction of six organ systems. The score will be calculated on admission and every 24 hours until discharge using the worst parameters measured during the prior 24 hours.</td></tr>
<tr><td>Participant Duration</td><td>An individual subject will complete the study in about 60 days, from screening/baseline at day -1 or 1 to follow-up on Day 29 ±3 days. All subjects whether a part of the lead-in phase or randomized pilot will be assessed for survival, serious adverse events and adverse events by telephone on Day 60.</td></tr>
</table>

Endpoints	**Primary** **Lead-In PK/PD** Serious Adverse Events / Adverse Events (Dose Limiting Toxicity) **Randomized pilot** Time to recovery through Day 28, defined as time to achieve (and maintain through Day 28) a WHO ordinal COVID-19 severity scale score of 1, 2 or 3 with a minimum 1-point improvement from baseline. **Secondary** WHO Ordinal severity scale: • Proportion of subjects with WHO ordinal severity scale score of 6 or 7 at any time through Day 28 • Time to an improvement of one category (i.e., a 1-point improvement) from baseline • Change from baseline in WHO scale score at days 2, 4, 7, 10, 14 and 28, as a categorical improvement or worsening • Mean change in WHO ordinal severity scale score from baseline through days 2, 4, 7, 10, 14 and 28 Oxygenation: • Oxygenation free days in the first 28 days from start of therapy • Incidence and duration of new oxygen use during the trial • Proportion on mechanical ventilation, ECMO, noninvasive ventilation and high-flow nasal cannula oxygen delivery and return to room air or baseline oxygen requirement • Time to return to room air or baseline oxygen requirement • Days on extracorporeal membrane oxygenation (ECMO) • Blood oxygenation by recorded continuous pulse oximetry (SpO_2:FiO_2 ratio) • Blood oxygenation by serial arterial blood gas measurements collected prior to the first dose of TSC and at 1 minute, 10 minutes, 30 minutes, 1.5 hours, 3 hours and 6 hours post TSC administration by calculated PaO_2:FiO_2 ratios Mechanical Ventilation: • Ventilator free days in the first 28 days (to day 29).

	• Incidence and duration of new mechanical ventilation use during the trial
	
	Hospitalization
	• Hospital length of stay by Day 29
	• ICU length of stay by Day 29
	
	Mortality
	• 15-day mortality
	• 28-day mortality
	• All-cause mortality at day 29
	• In hospital mortality
	• Mortality at Day 60
	
	Other
	• Glasgow Coma Score
	• Sequential Organ Failure Assessment (SOFA) Score at baseline, 24 and 48 hours, Day 7, Day 15
	• 28-day vasopressor free days
	• Development of acute kidney injury (as defined by AKIN criteria)
	• 28-day new renal replacement therapy (RRT) free days (excluding patients on chronic HD)
	• Proportion of patients alive and free of respiratory failure by Day 28 defined as at least one of the following:
	  • Endotracheal intubation and mechanical ventilation
	  • Oxygen delivered by high-flow nasal cannula (heated, humidified oxygen delivered via reinforced nasal cannula at flow rates >20 L/min with fraction of delivered oxygen $\geq$ 0.5)
	  • Noninvasive positive pressure ventilation
	  • Extracorporeal membrane oxygenation
	  • Clinical diagnosis of respiratory failure with initiation of none of these measures only when clinical decision making is driven solely by resource limitation
	
	Safety
	• Cumulative incidence of serious adverse events (SAEs) to Day 60
	• Cumulative incidence of Grade 3 and 4 adverse events (AEs) to Day 60
	• Discontinuation or temporary suspension of study drug injections (for any reason).
	• Changes in white cell count, haemoglobin, platelets, creatinine, glucose, total bilirubin, ALT, and AST on days 1; 3, 5, 8, 11

	(while hospitalized); and Day 15 and 29 (if able to return to clinic or still hospitalized • Death • DVT/PE • Nervous system disorders • Respiratory (acute respiratory failure, cough, pneumonia) • Angina • Infections including sepsis • Injection site reactions • Drug hypersensitivity
Inclusion Criteria	1. Hospitalized subjects with confirmed SARS-CoV-2 infection and hypoxemia, defined as SpO2 < 94% on room air or requiring supplemental oxygen (inclusive of nasal cannula, high flow oxygen, non-invasive ventilation, and mechanical ventilation) 2. Laboratory-confirmed SARS-CoV-2 infection as determined by PCR, or other commercial or public health assay in any specimen within 7 days prior to enrollment. 3. WHO ordinal scale score of 3 through 7 (exclusive of ECMO) at baseline 4. Male or non-pregnant female adult ≥18 years of age at time of enrolment. 5. Subject (or legally authorized representative) provides written informed consent prior to initiation of any study procedures. 6. Understands and agrees to comply with planned study procedures. 7. Illness of any duration 8. Women of childbearing potential must have a negative blood pregnancy test at the screening/baseline visit (Day 1) and agree to use a double method of birth control through 30 days after the last dose of study drug.
Exclusion Criteria	1. Receiving extracorporeal membrane oxygenation (ECMO) at baseline 2. Severe organ dysfunction (SOFA score > 10) at enrollment 3. Patient or LAR unable to provide written informed consent 4. ALT/AST > 3 times the upper limit of normal or serum bilirubin > 1.5 times the upper limit of normal

	5. Estimated glomerular filtration rate (eGFR) by Modification of Diet in Renal Disease (MDRD) formula < 30 mL/min/1.73 m^2 or on dialysis 6. Pregnancy or breast feeding. 7. Anticipated transfer to another hospital which is not a study site within 72 hours. 8. Allergy to any study medication 9. Patient not expected to survive >24 hours, or likely to be discharged <24 hours per PI discretion.
Safety	Given the severity of illness in COVID-19, there are no pre-specified study stopping rules for safety. The Medical Monitor will regularly review blinded AE / SAE data. If there are a concerning number of unexpected AEs, the Safety Monitoring Committee (SMC) during the open-label lead-in phase and the Data Safety Monitoring Board (DSMB) during the randomized phase will be asked to review unblinded safety data in an ad hoc meeting Patient enrollment during the randomized phase will not stop awaiting DSMB review, though the DSMB may recommend temporary or permanent cessation of enrolment based on their safety reviews. The DSMB will review safety data during the randomized phase after every 50 subjects. Ad hoc reviews will be undertaken if there are other specific safety concerns. Adverse events will be graded and promptly recorded according to the revised National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 from the start of study drug administration through the Day 60 visit. Safety assessments will include physical exam, concomitant medication usage, vital signs, 12-lead ECG, and clinical chemistries, including CBC and urinalysis. Safety assessments will include the following: • Adverse events

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- Physical exam
- Vital signs
- 12-lead ECG
- Clinical laboratory

The above safety assessments will be made daily (except ECG) through hospital discharge and at day 29. Serious adverse events and adverse events through day 60 will be collected as part of the study database.
</td></tr>
<tr><td>PK/PD</td><td>

The pharmacokinetics and pharmacodynamics of TSC will be assessed in each subject concomitant with the <u>first dose</u> of TSC on Day 1, as follows:

<table>
<tr><td>Pre-dose (~ 2 minutes prior to injection)</td></tr>
<tr><td>1 minute post end of injection (±1 minute)</td></tr>
<tr><td>10 minutes (± 1 min)</td></tr>
<tr><td>30 minutes (± 1 min)</td></tr>
<tr><td>1.5 hours (± 2 min)</td></tr>
<tr><td>3 hours (± 5 min)</td></tr>
<tr><td>6 hours (within10 min before TSC dosing)</td></tr>
<tr><td>24 hours (within 1 hour before TSC dosing)</td></tr>
<tr><td>48 hours (within 2 hours before TSC dosing)</td></tr>
</table>

Provided that an arterial line is established, blood oxygenation data by serial arterial blood gas and the calculated PaO2:FiO2 ratios will form the means of a pharmacokinetic/pharmacodynamic assessment.

Blood oxygenation data by recorded continuous pulse oximetry (SpO2:FiO2 ratio) will serve as an alternate source in the absence of arterial blood gas data.
</td></tr>
<tr><td>DSMB</td><td>

A Safety Monitoring Committee (SMC) will be established to review the DLTs during the lead-in PK/PD dose selection phase for the purpose of determining the optimal safe and tolerable TSC dose for the randomized pilot.

A Data Safety Monitoring Board (DSMB) will review the efficacy endpoints, safety profile and potential sample size re-estimation during the trial and and generally safeguard the interests of study participants. The details regarding the functioning of the SMC and DMC will be outlined in their individual charters.
</td></tr>
</table>

Statistical	Time to recovery through Day 28 is the primary efficacy endpoint for the randomized part of the study. Subjects enter the study with a WHO COVID-19 ordinal severity scale score of 3, 4 or 5. To meet the definition of recovery, a subject must achieve a WHO severity score of 1, 2 or 3 and have an improvement of at least 1 point, maintained through the Day 28. Time to recovery will calculated from day of randomization to day of recovery. Subjects who have not recovered by Day 29 evaluation will be censored at Day 28. A stratified log-rank test will be used to test the primary endpoint of time to recovery. The key secondary endpoints in order of importance are Hospital length of stay by Day 29, Proportion of subjects with WHO severity score of 6 or 7 at any time through Day 28, and All-cause mortality at Day 29. A hierarchical approach to the testing of these endpoints will be performed if the primary efficacy endpoint is statistically significant. **Sample Size Considerations** A total of 171 patients (114 in TSC arm and 57 in placebo arm) are needed to have 85% power with a two-sided alpha of 0.05 to detect an improvement in median time to recovery from 12 days to 7 days (HR=1.71). A total of 138 events (recovered subjects) are required for the analysis. Assuming a 10% dropout rate, a total of 190 subjects need to be randomized. A blinded review of the primary endpoint will be performed after approximately 50 subjects to assess the assumptions regarding the primary endpoint, due to uncertainties about standard of care and the clinical course of the disease. An unblinded interim analysis of the primary endpoint will be conducted by the DSMB after approximately 95 randomized subjects have enrolled (50% of randomized subjects). A Lan-DeMets spending function with O'Brien-Fleming boundaries will be used to control for an overall two-sided type-I error rate of 0.05. Conditional power will also be reviewed and the DSMB may recommend a change in sample size or to stop the study for futility.

1 INTRODUCTION

1.1 Study Rationale

COVID-19 is a respiratory disease caused by a novel coronavirus (SARS-CoV-2) associated with substantial morbidity and mortality. This clinical trial is designed to evaluate the safety and efficacy of a novel agent, trans sodium crocetinate (TSC), to improve oxygenation in SARS-CoV-2 infected patients with hypoxemia as a means of mitigating the unfortunate progression to acute respiratory distress syndrome (ARDS) and systemic organ injury.

1.1.1 Summary Background

TSC is a novel drug developed to re-oxygenate hypoxic tissue. TSC appears to act via a novel mechanism of action involving improving diffusivity of oxygen in blood plasma. Thus, it is based on physical-chemical principles, unlike most drugs which are based on biochemistry based mechanisms.

TSC was first made for a project between the University of Virginia and the Division of Battlefield Casualties of the United States Office of Naval Research (ONR). Blood loss is responsible for a significant proportion of battlefield casualties, and ONR supported the development of TSC to the limit of their capabilities, which included considerable preclinical studies plus a Phase 1 clinical trial for safety and dosing in TSC-treated normal subjects. At this point, Diffusion Pharmaceuticals took over the manufacture of TSC as well as the oversight of Phase 2 clinical trials, including the treatment of Peripheral Artery Disease (PAD) and as an adjunct to radiation therapy for glioblastoma multiforme (GBM).

The PAD trial established a dose response for TSC IV administration in humans (Mohler Vasc Med 2011), and established a dose of 0.25 mg/kg that was also used in the Phase 2 GBM trial (Gainer Journal of Neursurg 2017). There have been few serious adverse events attributed to TSC in ~150 TSC-treated patients to date. Follow-up preclinical studies in the internal laboratories of Diffusion Pharmaceuticals have also established that TSC is beneficial when given daily for 2-5 days per week, but is statistically significantly better if dosed 3 days per week. Such studies have been done for different animal types under different experimental conditions, usually given MWF.

Several preclinical studies support the biological plausibility of TSC as a potential therapeutic for patients suffering ARDS and systemic hypoxemia due to SARS-CoV2 infection. These include studies in animal models of hemorrhagic shock (Giassi Shock 2002) and acute lung injury (Gainer Pulm Pharm & Therapeutics 2005) – SEE SECTION 1.4.

1.2 TSC as a Therapeutic

TSC (Disodium 2,6,11,15 Tetramethyl-hexadeca 2E,4E,6E,8E,10E,12E,14E-heptaene-1,16-diotate) is a bipolar synthetic carotenoid having the chemical formula $Na_2C_{20}H_{24}O_4$, and a molecular weight of 372. The Chemical Abstracts registry number is 64603-92-5. The chemical structure is shown in Figure 1. Carotenoids are a diverse group of highly colored nonpolar, monopolar, and bipolar polyenes (conjugated double-bonded carbon chain backbone). Crystals and concentrated solutions of TSC are orange-red, but in dilute solution, TSC is yellow.

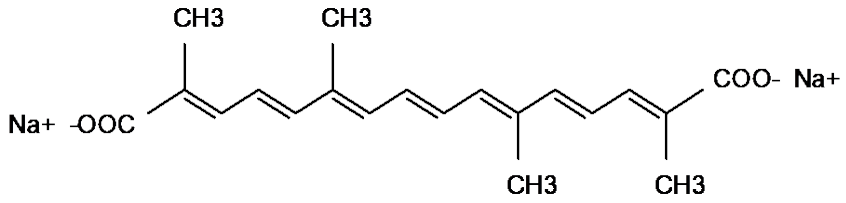

Figure 1. Trans Sodium Crocetinate

The carbon chain structure of TSC is identical to the naturally occurring dicarboxylic acid carotenoid, crocetin. However, crocetin and TSC differ in that crocetin is comprised of a mixture of isomers. TSC is a synthetically produced sodium salt of the single trans isomer. TSC has been the subject of investigation in 5 clinical trials to date (Diffusion Protocols 100-001, 100-201, 100-202, 100-301, 100-206).

Based on in vitro and computer modeling studies, it has been proposed that TSC works by altering the molecular arrangement of the water molecules which constitute the bulk of blood plasma, thereby creating a more ordered water structure (Laidig et al., 1998; Stennett et al., 2006). This altered water structure is less dense, reducing resistance to oxygen diffusion and allowing a more rapid movement of oxygen through the plasma. More oxygen can thereby reach oxygen-deprived tissue (Giassi et al., 2003). In vitro studies have shown that TSC also increases the diffusion of glucose in a manner similar to oxygen (Stennett et al., 2006).

TSC administered intravenously does not appear to significantly affect oxygen levels in normal animals, but TSC increases oxygen levels in hypoxic animal models. Healthy animals breathing normal ventilated air show little effect on brain PO2 after TSC administration, suggesting that TSC does not significantly affect oxygen tissue levels in normal animals (Okonkwo et al., 2003).

When TSC is administered to animals ventilated with 100% oxygen, the animals show a significant increase in brain PO2 levels over a saline control. It is hypothesized that a sufficient oxygen concentration gradient must be established in order to significantly increase the diffusion of oxygen with TSC. This gradient appears to be present if the

tissue is hypoxic, however, it can also be produced with hyper-oxygenation (such as 100% O2). Such a gradient is not present under normal conditions.

1.3 Preclinical Experience

1.3.1 Animal Pharmacokinetics

Pharmacokinetic (PK) studies of TSC have been conducted in rats and dogs. TSC has a relatively short half-life after a single intravenous injection. The half-life of TSC in the rat after a single IV bolus injection of 5, 15, 45, or 100 mg/kg varied from 20 minutes to 2 hours. Clearance varied from 50 to 300 mL/hr/kg. These data are shown below in Table 1 for both the low and high doses used in both 14- and 90-day studies. In addition, the volume of distribution varied from 60 to 140 mL/kg.

Table 1. Rat and Dog PK Half-Life and Clearance of TSC Dosed Once Daily for 14 Days and 90 Days

	Half-Life		**Clearance**	
Species	**Low Dose**	**High Dose**	**Low Dose**	**High Dose**
Rat 14 Day	5 mg/kg/day ~0.4 hr	100 mg/kg/day ~2 hr	5 mg/kg/day 300 ml/hr/kg	100 mg/kg/day 70 ml/hr/kg
Rat 90 Day	5 mg/kg/day	45 mg/kg/day	5 mg/kg/day	45 mg/kg/day
	~0.4	~1.4	233 ml/hr/kg	59 ml/hr/kg
Dog 14 Day	2.6 mg/kg/day ~2 hr	50 mg/kg/day 4 hr	2.6 mg/kg/day 70 ml/hr/kg	50 mg/kg/day 40 ml/hr/kg
Dog 90 Day	2.6 mg/kg/day	50 mg/kg/day	2.6 mg/kg/day	50 mg/kg/day
	8 hr*	6 hr	115 ml/hr/kg	52 ml/hr/kg

*Half-lives for male dogs were 4, 1.91, 0.924 and 48.4 hours, and 4, 1.65, 0.0389 and 2.35 hours for female dogs.

The half-life of TSC in dogs after a single IV bolus injection of 2.6, 5, 25, or 50 mg/kg varied from 2 to 6 hours. The half-life of 8 hr for the low-dose in the 90-day study is an average of values (listed below the table) that included an apparent erroneous value of 48.4 hours. Clearance varied from 40 to 115 mL/hr/kg. Again, these data are included in Table 1. In addition, the volume of distribution varied from 160 to 270 mL/kg.

The PK parameters in rats and dogs in the 14-day and 90-day repeat-dose toxicology studies did not vary significantly alter daily injections of TSC compared to a single dose of TSC in rats and dogs. The pharmacokinetics in rats and dogs appears to be non-linear

with greater than dose proportional increase for both Cmax and AUC. The half-life increases with dose in rats and dogs. The volume of distribution remained reasonably similar from low to high dose in rats and dogs, and did not exceed the total body weight for either species, indicating that TSC was not highly distributed into the tissues of rats and dogs after intravenous dosing.

1.3.2 Safety Pharmacology

Cardiovascular safety was tested in dogs. Central nervous system and pulmonary safety studies were conducted in rats. Single doses of 0.1, 1, or 10 mg/kg were tested in all safety pharmacology studies. No effects were observed in the cardiovascular studies when assessed by body temperature, blood pressure, heart rate, and QRS, RR, PR, and QT intervals. The no observable effect level (NOEL) was at least 10 mg/kg. TSC did not have any adverse effects on pulmonary function as measured by respiratory rate, tidal volume, or minute volume. Neurobehavioral studies did not show any behavioral changes suggesting neurotoxicity. The no observable adverse effect level (NOAEL) was at least 10 mg/kg in the pulmonary studies.

1.3.3 Animal Toxicology

Toxicology studies up to 14 days in one study and up to 90 days in another study were conducted in rats and dogs by the IV route of administration at maximum technically feasible doses based on limits of solubility and a clinically relevant dosing regimen. In both the 14-day and 90-day rat studies, animals received a daily IV dose of TSC at levels of 5, 15, 45, or 100 mg/kg/day. In the 14-day and 90-day dog studies, animals received a daily IV dose of TSC at levels of 2.6, 25, or 50 mg/kg/day.

In the 14-day study, TSC did not result in any mortality. In both species, TSC produced dose-dependent yellow discoloration of urine, hair, skin, and eyes. The discoloration generally disappeared after a 7-day recovery period indicating that the effects were reversible. The discoloration was not considered adverse to the health of the animals and was considered related to the color of TSC, which is yellow at dilute concentrations. Tissue discoloration appears to be a class effect of carotenoids because of their color in solution. When given in sufficient doses to humans, carotenoids are known to color tissues and particularly contribute to a yellow cast of the skin (Alaluf et al., 2002; Micozzi et al., 1988).

In the 90-day rat study, animals received a daily intravenous dose of TSC at 5, 15, or 45 mg/kg/day. There were no adverse effects noted following daily intravenous dosing of TSC for 90 days in Sprague Dawley rats, so the NOAEL for this study is considered to be 45 mg/kg/day. The observations of yellow discolored hair and skin continued through the recovery period for both males and females at 45 mg/kg/day, with the incidence remaining constant for discolored hair while the incidence of discolored skin reduced in incidence with time. The observations of discolored skin/hair are, again, attributed to the

color of the test article, and since neither the discoloration nor sparse hair were associated with any impact on the health of the animals they were not considered adverse.

In the 90-day dog study, animals received a daily intravenous dose of TSC of 2.6, 25 or 50 mg/kg/day. The NOAEL in dog was considered to be 25 mg/kg/day for the females due to the effects on body weight and euthanasia of 2 females at this level. The NOAEL is considered to be 50 mg/kg/day for the males as there were no adverse effects noted for the males during the course of the study. Yellow discoloration of the body (minimal to severe) that occurred for males and females at ≥ 25 mg/kg/day was generally dose-related in incidence and severity and was attributed to the color of the test article. The yellow discoloration was not considered adverse, due to a general lack of microscopic correlates in most animals and an absence of clear toxicity associated with the color change.

1.3.4 Hemocompatibility

In vitro rat, dog, and human blood plasma and serum compatibility studies indicated that TSC was compatible with blood from each of these species since there was no evidence of coagulation or precipitation. Hemocompatibility studies showed evidence of hemolysis in rat and dog blood, but not in human blood. In animals, a small reduction (5 to 10%) in erythrocytes, hematocrit, and hemoglobin were found in the rat and dog 2-week studies in the highest dose groups tested; 100 mg/kg/day and 50 mg/kg/day, respectively in the rat and dog. In addition, a 46 to 56% reduction in reticulocytes was noted at 50 mg/kg/day in the dog study. The increased bilirubin, when combined with an increase in urea nitrogen and the changes in the values of red cell variables described, may suggest a decreased red blood cell lifespan with impaired regeneration at the dose of 50 mg/kg/day in dogs. The magnitude of effect on hematological variables was small and the findings were reversible.

Consistent with the in vitro hemocompatibility results in human blood, there was no evidence of hemolysis or hemocompatibility issues in a Phase 1 study in 30 healthy human volunteers given a single IV bolus dose of TSC at a rate of 15 ml/min up to a maximum dose tested of 5 mg/kg. There were no laboratory reports of hemolysis or hemocompatibility issues in the Phase 1/2 study in 40 PAD subjects who received multiple doses of 0.25 mg/kg to 2.0 mg/kg TSC with samples collected at multiple time points.

1.3.5 Intravenous Irritation

An IV irritation study was conducted in rabbits. Using the marginal ear vein of rabbits, treatment with TSC resulted in very slight erythema in only 2 of 9 animals. When administered perivascularly, TSC was considered to induce slight- to well-defined erythema and slight edema, with effects persisting for several days.

1.3.6 Genotoxicity

TSC was negative in the in vitro Ames assay and the L5178Y mouse lymphoma cells, in both studies in the presence and absence of metabolic activation.

1.3.7 Reproductive Toxicology

The International Conference on Harmonisation (ICH) Stages C-D (Segment II) nonclinical reproductive toxicology studies in rats and rabbits using TSC as the formulated drug product administered intravenously to both species have been completed. For the rat Segment II study, doses of 0 (0.9% saline), 25, 50, or 100 mg/kg/day TSC were administered intravenously in the tail vein. The data demonstrated that the maternal NOAEL of TSC was 25 mg/kg/day. The 50 and 100 mg/kg/day TSC dosages caused reductions in body weight gain, and the 100 mg/kg/day TSC dosage also reduced food consumption. Injection site reactions occurred at all dosages; the severity of these reactions at 50 and 100 mg/kg/day resulted in the early sacrifice of 2 and 1 rats at these dosages, respectively. The developmental NOAEL was 50 mg/kg/day. The 100 mg/kg/day TSC dosage reduced fetal weight and increased the number of skeletal variations. Based on these data from the rat ICH Stages C-D (Segment II) reproductive toxicology study, TSC should not be identified as a developmental toxicant.

In the rabbit Segment II study, doses of 0 (0.9% saline), 25, 50, or 75 mg/kg/day TSC using the formulated drug product were administered intravenously. The maternal NOAEL of TSC was 50 mg/kg/day. The 75 mg/kg/day TSC dosage caused reductions in body weight gain and feed consumption. The developmental NOAEL was greater than 75 mg/kg/day since no effects were observed at the highest dosage tested. There were no adverse effects on embryo-fetal development as evaluated in this study. Based on these data from the ICH Stages C-D (Segment II) study in rabbits, TSC should not be identified as a developmental toxicant.

1.3.8 Absorption, Distribution, Metabolism, and Excretion

The results from in vitro assessment of reaction phenotyping (enzyme identification) of human CYP450 enzymes by TSC indicate that TSC is not metabolized in large amounts by liver microsomal CYP450 enzymes. Four in vitro metabolism studies within the full profile of absorption, distribution, metabolism and excretion (ADME) studies have been completed. TSC has been evaluated in the following metabolism studies: Cytochrome (CYP) P450 enzyme inhibition and induction studies, reaction phenotyping for CYP P450 enzyme identification, and metabolic stability of TSC in hepatocytes from multiple species. The in vitro studies testing the potential impact of TSC on liver direct enzyme inhibition in human microsomes showed no marked concentration-related increases at concentrations up to 40 μM in all tests done. TSC did not have an effect on the time-dependent inhibition of the standard battery of agents tested and is not likely a mechanism-based inhibitor of these isoforms. No significant induction responses (≥ 40% of adjusted positive control) of CYP1A2, CYP2B6, and CYP3A4 enzyme activity were

observed with any of the concentrations of TSC examined in any of the human donor hepatocyte preparations. In addition, TSC treatment resulted in no marked CYP1A2, CYP2B6, and CYP3A4 mRNA induction. The results from these studies suggest a low potential for drug-drug interactions with TSC due to enzyme inhibition or enzyme induction of CYP1A2, CYP2B6, and CYP3A4 at the concentrations examined. A study was done to determine the in vitro metabolic stability of TSC in rat, dog, rabbit, and human cryopreserved hepatocytes. The metabolic stability was evaluated based on analysis of the disappearance of TSC as a function of time. The results from this multi-species metabolic stability study in cryopreserved hepatocytes suggest that TSC is not significantly (> 35%) metabolized via the particular CYP-mediated pathways present in the hepatocytes tested. Ultimately, drug interactions will be dependent on multiple metabolism and PK factors encountered in vivo.

The in vivo ADME of TSC was also investigated using [^{14}C]-labelled Trans Sodium Crocetinate ([^{14}C]-TSC) in the rat. Pharmacokinetic, excretion/balance and tissue distribution experiments were conducted and evaluated from samples collected following single oral and intravenous administration of [^{14}C]-TSC combined with non-radioactive TSC for a total of 50 mg/kg doses to rats.

Maximum mean plasma total radioactivity concentrations (C_{max}) were observed at 0.5 hours (T_{max}) post oral dose administration. Radioactivity/concentrations declined slowly thereafter with an apparent half-life of approximately 18 hours. Systemic exposure to the total radioactivity (AUC_{0-48}) was calculated to be 1700 µg equivalents·h/g (µg eq./g). Mean plasma concentrations (radioactivity) were 8 µg eq./g at 48 hours post dose.

Maximum mean whole blood total radioactivity concentrations were measured at 0.5 hours post oral dose administration. Concentrations declined slowly thereafter with an apparent half-life of 25 hours. Systemic exposure to the total radioactivity (AUC_{0-48}) was 1230 µg eq·h/g. Mean whole blood concentrations/radioactivity equated to 7 µg eq./g at 48 hours post dose.

Pharmacokinetics
The mean pharmacokinetic parameters correlated with sample radioactivity obtained in whole blood and plasma following a single oral gavage dose of [^{14}C]-TSC to male rats at 50 mg/kg, is displayed in Table 2.

Table 2. Mean Pharmacokinetic Parameters Correlated with Sample Radioactivity in Whole Blood and Plasma

Parameter	Male Rats (50 mg/kg)	
	Whole Blood	Plasma
$C_{max\ (measured)}$ (µg eq./g)	196	254
$C_{max\ (extrapolated\ to\ zero\ time)}$ (µg eq./g)	223	279
$T_{max\ (measured)}$ (hours)	0.5	0.5
$T_{½}$ (hours)	25.10	17.78
$AUC_{(0-48)}$ (µg eq·h/g)	1228.53	1675.27
$AUC_{(0-inf)}$ (µg eq·h/g)	1508.4	1861.25

Excretion balance
Excretion of radioactivity was followed through 168 hours after a single intravenous administration of [^{14}C]-TSC. The main route of elimination was through feces as metabolites with a mean recovery of approximately 50% of the dose administered. Expired air accounted for another 23% for each animal representing the next largest route of recovery. Urine accounted for a smaller proportion of radioactivity (approximately 6 % per each animal) with remaining quantitated concentrations of radioactivity measured in the carcass and cage washings. The mean total recovery of administered radioactivity was 83%. A summary of the relative amounts of recovered radioactivity through 168 hour post dose period following single intravenous administration of [^{14}C]-TSC at 50 mg/kg, is shown in Table 3.

Table 3. Recovered Radioactivity through 168 hour Post-dose

Sample	% Recovery Male (50 mg/kg) (n = 3)
Urine	5.90
Faeces	49.47
Expired Air Trap 1	10.43
Expired Air Trap 2	12.87
Cage Wash	0.05
Carcass	4.29
Total	83.01

Normally, the minimum target for radioactive recovery is 90%. The incomplete recovery may be explained by the fact that a large proportion of the radioactivity was eliminated in the first 24 hours via expired air. This could potentially be attributed to volatile components (i.e. CO_2 based metabolites) not being trapped in the expired air trapping solutions.

Tissue distribution
Following a single intravenous dose of [^{14}C]-TSC at 50 mg/kg to male Sprague Dawley rats, distribution of radioactivity was quick and widespread. The highest concentrations of radioactivity at early time points were observed in the gastro-intestinal tract contents, implicating biliary excretion as a route of elimination. Peak concentrations in remaining tissues generally occurred at 1 hour post dose, though some tissues (brain, spinal cord and some secretory glands) achieved peak concentrations at later times.

Elimination of radioactivity from tissues was generally slow; at 168 hours post dose, radioactivity remained quantifiable in most of the tissues analyzed, with greatest concentrations associated with the liver, skin, glandular tissue and brown fat.

Tissue: blood ratios indicate that up to 2 hours post dose, only the small intestine contents contained consistently greater concentrations of radioactivity than levels observed in blood. Six (6) hours post dosing and beyond indicated secretory glands had equal to or greater than the corresponding blood concentrations. Distribution of radioactivity in pigmented animals followed a similar pattern as non-pigmented animals, with no evidence of affinity for melanin-containing tissues observed.

1.4 TSC in Model of Hemorrhagic Shock

The objective of this study was to determine if TSC could enhance survival in rats with severe hemorrhage in a time-dependent fashion, mirroring a battlefield scenario where there is often a treatment delay prior to resuscitation (Giassi et al. Shock 2002). A constant-volume hemorrhage protocol was used in Male Sprague-Dawley rats. In the first set of experiments, the protocol involved removing 60% of the estimated blood volume, and 20 minutes elapsed before TSC (or saline) was first injected. TSC injections were repeated four times, every 10 minutes. After an additional 30 minutes, fluid resuscitation with saline was initiated. Blood pH, base excess, and lactate levels were monitored for 90 minutes after hemorrhage, prior to the point of initiation of fluid resuscitation. Possible liver damage was assessed 24 hours later by measurement of enzymes.

Blood pressure decreased by 40-45% of its baseline value following hemorrhage, with a commensurate increase in heart rate. Following the 20 min delay and initial TSC administration, blood pressure rose immediately by around 10-15 mmHg, but there was no change in response to saline injections. This improvement declined over 10 min and TSC was injected again with a similar increase in blood pressure, with no change in the saline group. This discrepancy in blood pressure improvement between TSC and saline was reproduced at each of the serial five injections (Figure 2).

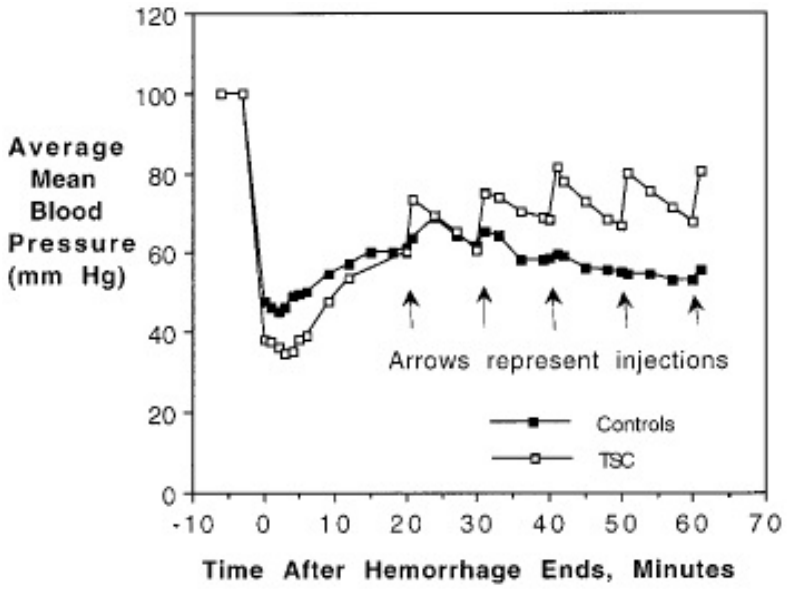

Figure 2. Effect of TSC On Blood Pressure After Hemorrhage When Therapy Is Delayed 20 Minutes

Similarly, a significant decrease in heart rate was noted following TSC injection compared to saline, and with each serial injection following the 20 min delay from hemorrhage (Figure 3).

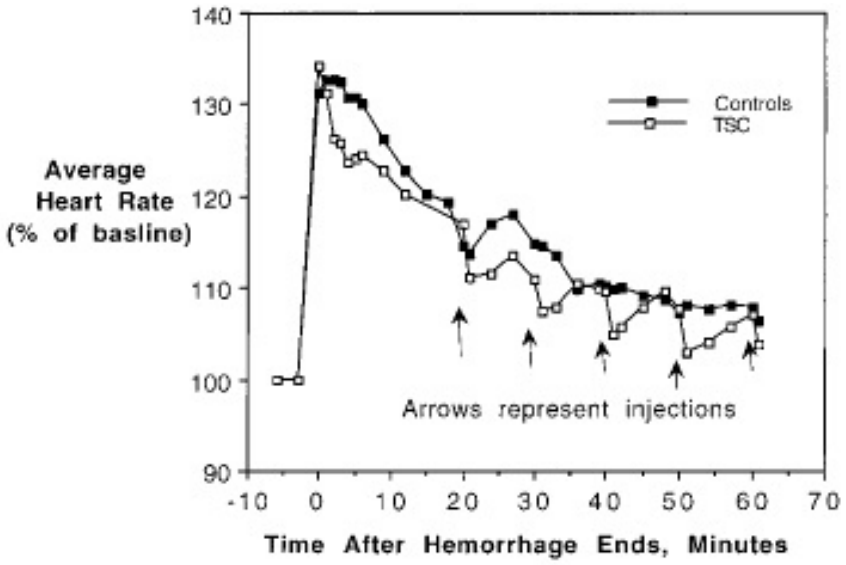

Figure 3. Effect of TSC on Heart Rate After Hemorrhage When Therapy Is Delayed 20 Minutes

Blood parameters of systemic hypoxia were taken at baseline, 15 min post-hemorrhage prior to injection, and 90 min post-hemorrhage following TSC (vs. saline) injection. TSC resulted in significant improvement in blood pH and base deficit compared saline, and demonstrated non-significant improvements in PO2, PCo2, lactate, and bicarbonate (Table 4).

Table 4. Blood Parameters of Systemic Hypoxia Following Hemorrhagic Shock – Baseline, Post-Hemorrhage, and Following TSC vs. Saline Injection

	Baseline (before hemorrhage)		15 minutes post-hem. (before injection)		90 minutes post-hem. (before infusion)		
Measurement	Control	TSC	Control	TSC	Control	TSC	p
PO_2 (mmHg)	98 ± 5	100 ± 5	129 ± 6	126 ± 11	130 ± 12	135 ± 7	NS
PCO_2 (mmHg)	37.2 ± 5.5	36.2 ± 4.3	24.3 ± 4.7	27.5 ± 3.3	30.2 ± 6.3	29.2 ± 5.6	NS
pH	7.42 ± 0.03	7.42 ± 0.03	7.23 ± 0.02	7.24 ± 0.04	7.33 ± 0.03	7.41 ± 0.07	0.04
Base Deficit (mmoles/liter)	−0.9 ± 1.8	−1.4 ± 1.4	14.6 ± 2.2	13.1 ± 2.9	8.1 ± 2.1	4.3 ± 1.5	0.01
Lactate (mmoles/liter)	0.98 ± 0.08	0.87 ± 0.10	5.6 ± 1.0	5.1 ± 1.4	3.5 ± 2.1	2.6 ± 1.1	NS
Bicarbonate (mmoles/liter)	24.5 ± 2.5	25.1 ± 1.3	10.4 ± 2.4	12.0 ± 2.5	15.9 ± 2.9	18.4 ± 1.5	NS

Liver injury was assessed by serum levels of glutamate-oxalacetic transaminase (GOT) and glutamate-pyruvate transaminase (GPT) (i.e. aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) before hemorrhage and 24 hours later. At the 24 hour

mark post-hemorrhage, liver enzymes doubled in the saline group but remained stable in the TSC group ($p<0.05$).

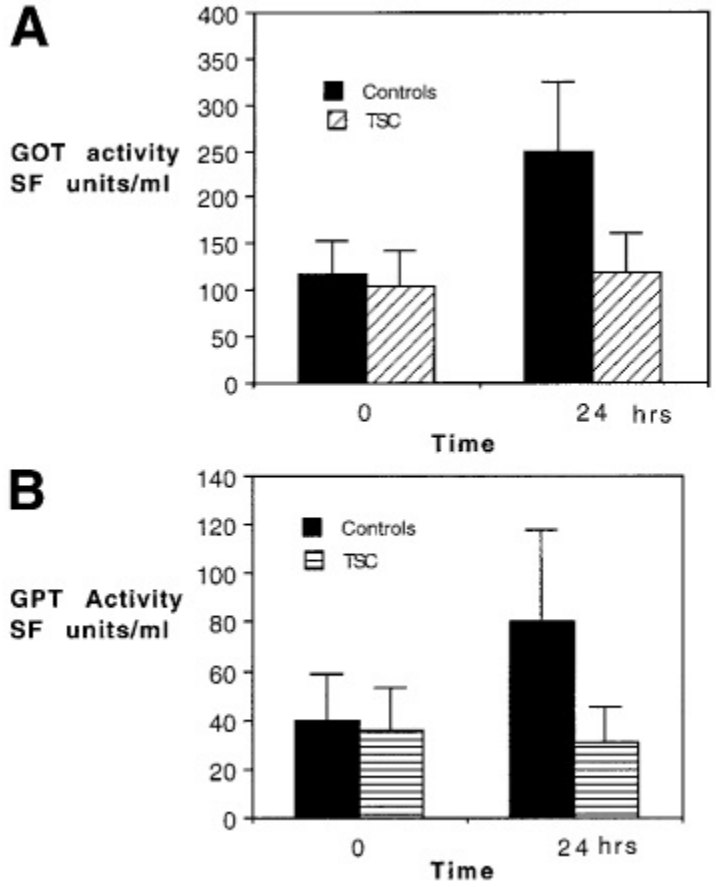

Figure 4. Liver Transaminase Activity At Baseline and 24-Hours Post-Hemorrhagic Shock in TSC vs. Saline Treated Groups [A – aspartate aminotransferase (AST), B – alanine aminotransferase (ALT)]

In a second experiment, a smaller hemorrhage (10% blood volume) was incurred ten minutes after the initial severe hemorrhage (60% blood volume). TSC (vs. saline) was given immediately following the second hemorrhage and every ten minutes for a 60-minute period. No subsequent fluid resuscitation was provided. In the saline group, almost all of the animals died within 50 minutes, whereas the majority treated with TSC survived.

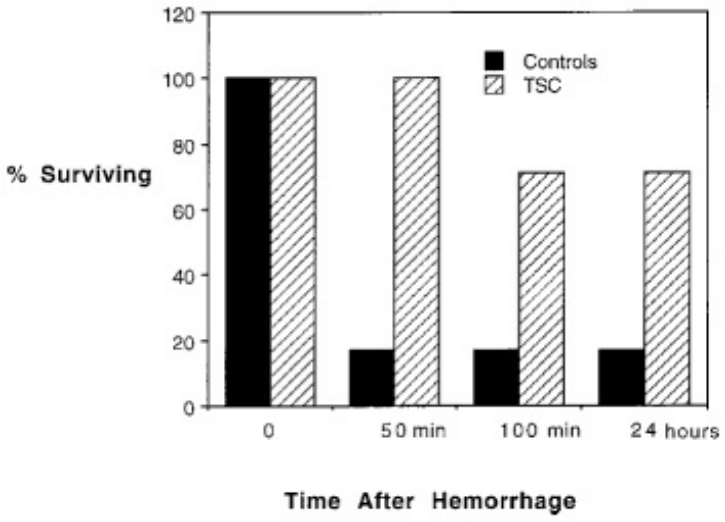

Figure 5. Survival with TSC vs. Saline Following Second Hemorrhage

These sets of experiments in rats with hemorrhagic shock suggest that TSC has a measurable effect on markers of systemic hypoxia, including normalization of acidosis and acute liver injury. TSC's effect on correcting hypotension and tachycardia following severe hemorrhage also supports the hypothesis that TSC enhances oxygenation and systemic oxygen transport.

1.5 TSC in Model of Acute Lung Injury

The objective of this experiment was to determine if TSC improves arterial oxygenation in a model of acute lung injury (Gainer et al. Pulm Pharma & Therapeutics 2005). Male, Sprague-Dawley rats were injected with oleic acid, which produces pulmonary hemorrhage and edema resulting in severe hypoxemia and mirroring ARDS. Following one hour after injection, the average arterial pO2 was 59 ± 8 mmHg. Animals were then treated with either TSC or sterile water with a bolus injection repeated every 10 min over an hour. Arterial blood gas (ABG) measures were then recorded over a 3-hour period. Following TSC treatment, arterial pO2 improved immediately and remained statistically higher than controls throughout the treatment period and up to 30 min after treatment ended. Values remained higher at the 3-hour mark but were not statistically significant.

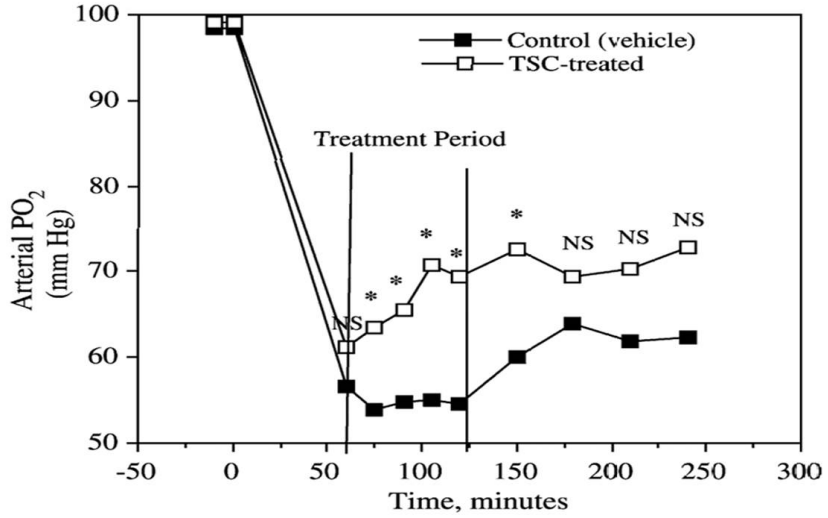

Figure 6. Arterial pO2 Following Oleic Acid Injection, TSC vs. Control

Concomitantly, pCO2 levels decreased immediately following TSC treatment and remained lower than controls from the treatment period up to the 3-hour mark.

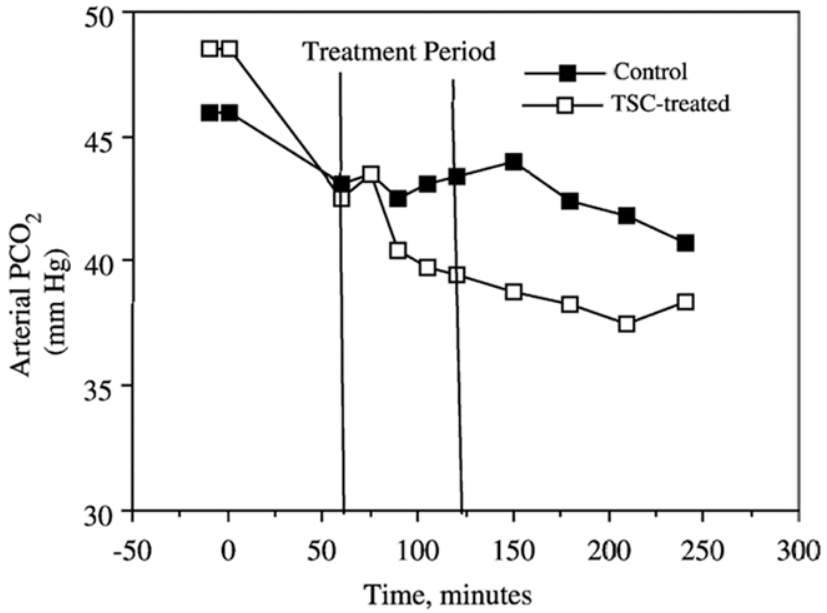

Figure 7. Arterial pCO2 Following Oleic Acid Injection, TSC vs. Control

These data further support the hypothesis that TSC improves blood oxygenation in hypoxic conditions, in this case an acute lung injury with pulmonary hemorrhage, edema,

and inflammation modeling ARDS. By enhancing diffusivity of oxygen through plasma, not only does TSC seem to enhance systemic oxygenation of tissues, but it may also affect passage of oxygen from the alveoli to erythrocytes to enhance the oxygen carrying capacity of blood in the setting of acute lung injury and ARDS.

1.6 Experience in Humans

Phase 1 - Safety

The safety and tolerability of the lyophilized injectable formulation of TSC in humans has been evaluated in a randomized, double-blinded, placebo-controlled Phase 1 trial in 40 normal healthy subjects (Diffusion Pharmaceuticals Clinical Study Report 100-001, 2008). Tolerability and PK were assessed after a single intravenous dose of 0.1, 0.5, 1, 2.5, and 5 mg/kg (6 subjects per TSC dose) through a 1 month follow-up visit after a single IV bolus injection of 20 mg/ml TSC. A total of 30 subjects received TSC and 10 received placebo. A single IV dose of TSC was very well tolerated and the maximum tolerated dose was 2.5 mg/kg based on mild, transient, yellow visual field disturbances seen in 2 subjects at a dose of 5 mg/kg. There were no clinically significant dose-dependent changes in laboratory parameters, vitals, or ECG up to the maximum dose evaluated of 5 mg/kg. There were no serious adverse events (SAEs) or deaths and no subject withdrew from the study.

Phase 2

Peripheral Artery Disease

A randomized, double-blinded, placebo-controlled Phase 1/2 study in 48 subjects has been conducted to evaluate the safety, PK, and dose response of TSC in subjects with symptomatic peripheral artery disease (PAD) and claudication (Diffusion Pharmaceuticals Clinical Study Report 100-301, 2010; Mohler et al., 2011). A total of 40 subjects received TSC and 8 subjects received placebo. Overall, multiple dosing by IV injection of TSC from 0.25 mg/kg to 2.00 mg/kg in elderly subjects with PAD was well tolerated when compared to placebo. The distribution of adverse events (AEs) observed in the study did not suggest predominance in any dosing arm compared to placebo. No treatment-emergent or dose limiting toxicity was discovered in this study and no subject withdrew due to an AE. The 3 AEs reported by the principal investigators (PIs) as related to TSC were single incidents during dosing of mild to moderate burning or irritation at the injection site, and a yellow pigmented streaking at the injection site at the time of dosing; all resolved without treatment. These were likely associated with the IV line catheter placement or dosing technique, since reconstituted TSC has a yellow color. One subject receiving TSC at 2.00 mg/kg reported a single episode of a mild visual disturbance of “spots” that completely resolved without treatment. The PI evaluated this event as not related to TSC. The Sponsor determined this event was not a suspected adverse reaction, since it was not yellow in nature as was observed in the Phase 1 study and did not re-occur with repeated exposure to TSC. There were no AEs considered by the PI or Sponsor to be associated to the highest TSC dose (2.00 mg/kg).

In the 48 PAD subjects enrolled in Protocol 100-301, there were a total of 4 SAEs in TSC-treated subjects, including 1 death which occurred after the protocol-defined reporting period. There were 2 SAEs, including 1 death, reported in the placebo group. All of the SAEs were unexpected and considered unrelated to blinded study medication by the PI and Sponsor with the exception of the SAE of deep vein thrombosis (DVT). The DVT occurred in a subject who was randomized to 0.50 mg/kg TSC, and it was considered possibly related by the PI to blinded study medication. Although the DVT was an unexpected AE, the Sponsor determined this event was not a suspected adverse reaction since there was no evidence to suggest a causal relationship. While a causal attribution to study medication cannot be excluded, neither the PI nor the Sponsor can offer a probable pathophysiological mechanism by which TSC could result in a DVT. Study blind was not broken for any of these AEs.

The efficacy and dose response of TSC was evaluated in Protocol 100-301 using objective measurements from exercise treadmill tests in subjects with PAD and intermittent claudication symptoms. The change in claudication onset time (COT) showed a significant increase in the 0.25 mg/kg TSC dosing arm after Dose 1 and Dose 5 as well as lesser signals of improvement at TSC doses 1.25 mg/kg to 1.75 mg/kg. Notable signs of clinical benefit were observed after Dose 1 and Dose 5 of TSC above 1.0 mg/kg for both increased peak walking time (PWT) and COT on the exercise treadmill test, as well as subject-perceived increases in walking distance from the Modified Walking Improvement Questionnaire survey.

The PK profile of TSC from Protocol 100-301 in the elderly PAD subject population after multiple doses was similar to the PK results observed in the Phase 1 study in normal healthy subjects. TSC was eliminated quickly and the PK profile of TSC appears to be nonlinear after IV doses of 0.25 to 2.0 mg/kg in PAD subjects. The mean elimination half-life (t½) increased with dose and at doses greater than 1 mg/kg, t½ appeared to fluctuate in the 1.5 hour range. For the higher doses, 0.75 to 2.00 mg/kg, plasma concentrations appeared to decay at the same rate. Due to the short half-life of TSC relative to the 24-hour dosing interval, essentially 12.5 times the longest mean t½, and since only 4 subjects had a pre-dose plasma TSC concentration that was ≥ 10 ng/ml limit of quantitation (LOQ) and < 0.15% of corresponding maximal plasma concentration (C_{max}), the Dose 3 PK data may be viewed as if a single dose.

Glioblastoma Multiforme (GBM)
Diffusion Pharmaceuticals completed a phase 2 trial with a phase 1 safety lead-in (Diffusion Pharmaceuticals, Clinical Study Report 100-202, 2015) that evaluated the safety, tolerability, PK profile, efficacy, progression-free survival (PFS), quality of life (QOL), and overall survival (OS) in adults with GBM who are treated with TSC in addition to the standard of care (SOC) consisting of radiation therapy (RT) and temozolomide. The Phase 1 safety lead-in portion of the protocol incorporated a TSC dose escalation approach. The Phase 2 study portion enrolled 56 subjects at the

recommended dose based on the safety lead-in study results. A previously conducted study by Stupp et al (2005) was used as a historical control.

During the conduct of Phase 1, monitoring for dose-limiting toxicity (DLT) events was performed. A Safety Monitoring Committee (SMC) evaluated safety data from the Phase 1 cohort and recommended a dosage regimen for Phase 2.

Safety and efficacy variables were evaluated at scheduled time points over 2 years after administration of study treatment. Investigators and independent reviewers assessed tumors and reported on tumor status. Tumor scans were subsequently analyzed by a single, central group, Biomedical Medical Systems.

The SOC treatment regimen, administered concomitantly with TSC treatment in both phases of the study, included RT and temozolomide, which were administered in a manner similar to that utilized in the Stupp et al (2005), which was used as a historical control study. Treatment with RT began within 5 weeks after tumor resection surgery or definitive biopsy. The RT treatment regimen included 5 sessions per week for 6 weeks (30 sessions total). Temozolomide 75 mg/m^2 was taken orally (capsule formulation) daily for 6 weeks (ie, 42 days) concomitantly with RT.

- Phase 1: 3 weeks of TSC .25mg/kg with concomitant RT and temozolomide; TSC administered 45 to 60 minutes before RT (9 doses total).
- Phase 2: 6 weeks of TSC .25 mg/kg with concomitant RT and temozolomide, TSC administered 45 to 60 minutes before RT (18 doses total).

After completion of administration of the study treatment, efficacy and safety evaluations were continued for a total of 2 years (24 months).

Overall survival (modified Intent-to-Treat [mITT] population; TSC 18 doses group)
- Two years after initiation of treatment with TSC, 35 subjects (62.5%) had died while 21 subjects (37.5%) remained alive. The probability (Kaplan-Meier analysis) of OS was 71.2% after 1 year and 36.3% after 2 years.
- The mean standard deviation (SD) OS duration was 16.31 (7.313) months, and the median (95% confidence interval [CI]) OS duration (using a Kaplan-Meier analysis) was 16.3 months (13.27, 23.66).

Progression-free survival (mITT population; TSC 18 doses group)
- The median (95% CI) PFS duration (using a Kaplan-Meier analysis) was 3.3 (3.15, 5.16) months. This may be a misleading value as many tumors that initially increased in size later decreased in size (see below).

Tumor size (mITT population; TSC 18 doses group)

- A mean change from Baseline of 28.7% in tumor size (sum of tumor area) was seen at Week 10. However, at the next time point (Week 18) mean tumor size was smaller, and subsequently mean tumor size remained smaller than at Baseline and continued to progressively decrease throughout the remainder of the 2-year treatment evaluation period. At the 1-year time point, mean tumor size in surviving subjects was approximately half of the mean tumor size at Baseline, and by the 2-year time point, 11 subjects had a 100% reduction in tumor size, indicating essentially complete disappearance of the GBM tumor.
- Of 14 subjects who had a complete resection before Baseline, 6 were alive at 2 years and showed no tumor present.

Quality of life and performance measures

- Mean and median scores for the European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30 and Karnofsky Performance Score (KPS) remained fairly constant over time, with subscale mean scores suggesting continued relatively good function and global health and generally low levels of symptoms.

Safety

A total of 56 subjects (94.9%) had a treatment emergent adverse event (TEAE), most of whom had more than one TEAE. The most frequently occurring TEAEs included fatigue (40.7%), alopecia (35.6%), nausea (27.1%), and constipation (27.1%). Most TEAEs were categorized as either common terminology for adverse events (CTCAE) Grade 1 (mild) or 2 (moderate); 10 subjects (16.9%) had a Grade 3 (severe) TEAE and 4 subjects (6.8%) had a total of 6 Grade 4 (life-threatening) TEAEs (ie, brain oedema, neutropenic sepsis, tooth abscess, pulmonary embolism, febrile neutropenia, drug hypersensitivity). There were no CTCAE Grade 5 (death related to AE) TEAEs.

A total of 24 TEAEs in 12 subjects (20.3%) were assessed as related to TSC. The most frequently reported TSC-related AEs were fatigue (5 events in 4 subjects [6.8%]) and headache (3 events in 2 subjects [3.4%]).

Eleven subjects (18.6%) had a total of 19 SAEs. The most frequently reported SAEs were hydrocephalus, pulmonary embolism, and muscular weakness, each of which occurred in 2 subjects (3.4%). All other SAEs occurred in 1 subject each. None of the SAEs were considered related to TSC.

No subject had an AE that resulted in death, and no subject had an AE that resulted in discontinuation of TSC.

Overall, diastolic and systolic blood pressure and pulse rate mean values over time showed variability, but no concerning patterns of clinically meaningful TSC-related changes from Baseline were discerned for evaluated vital signs, ECGs, or clinical

laboratory tests.

In summary, TSC 0.25 mg/kg administered as an IV bolus 3 times per week for 6 consecutive weeks (18 doses total) as concomitant treatment along with SOC treatments of RT and temozolomide was well tolerated in the subjects in this study with newly diagnosed GBM, and no safety findings were identified that would preclude further clinical development for the indication of treatment of GBM.

Acute Ischemic Stroke
TSC is currently being studied in a Phase 2, randomized, placebo-controlled trial of ambulance-based patients being transported to the hospital with suspected acute stroke (https://clinicaltrials.gov/ct2/show/NCT03763929). While this trial is early in the enrollment period, several patients have been enrolled at the time of this writing with no significant safety concerns reported thus far.

2 STUDY METHODS

2.1 Rationale

In December 2019, a cluster of viral pneumonias of unknown etiology developed in Wuhan, China garnering the attention of the international medical community. By January 11, 2020 China released the genetic sequence of the culprit novel coronavirus. This outbreak quickly garnered the attention of the World Health Organization, who declared the outbreak a public health emergency of international concern by January 30, 2020 and finally a pandemic by March 11, 2020. This novel coronavirus has been designated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the disease caused by this virus has been designated Coronavirus Disease 2019 (COVID-19). As of July 19, 2020, there were over 14.2 million confirmed cases and 599,000 deaths worldwide, more than 3.6 million cases and 136,000 deaths in the U.S., and 36,000 cases and 2,000 deaths in Romania. There are a number of therapies under investigation for the treatment of coronaviruses.

Patients suffering from moderate to severe COVID-19 disease present with abnormal chest radiography indicating bilateral and peripheral ground glass and consolidative opacities, with many progressing to acute respiratory distress syndrome (ARDS). These patients often go on to require transfer to an intensive care unit, mechanical ventilatory support, and possibly extra-corporeal membrane oxygenation (ECMO). COVID-19 leading to severe ARDS is associated with a high degree of morbidity and mortality. Prior to developing ARDS, COVID-19 patients may experience a period of so-called *silent hypoxemia*, consisting of observable hypoxemia by oxygen saturation (SaO2) measurements, but showing minimal outward signs of respiratory distress.

As has been demonstrated in laboratory testing and preclinical models, TSC works by a novel mechanism of action to enhance diffusivity of oxygen by altering the structure of water molecules and decreasing the density of plasma (Gainer Exp Opin Invest Drugs 2008). By enhancing oxygen diffusivity, TSC facilitates increased oxygen delivery to tissues in the setting of hypoxia where the diffusion gradient is heightened. Conformingly, TSC also seems to enhance tissue oxygenation in the setting of increased oxygen supply (Okonkwo J Neurosurg 2003). Preclinical models not only suggest an effect of TSC on systemic hypoxia and end organ injury, but also the likely property of increasing blood oxygenation in the setting of acute lung injury (i.e. enhanced diffusivity of oxygen from the alveoli to the blood in the setting of pulmonary disease). Models of hemorrhagic shock further suggest an ameliorative effect of TSC on multiorgan injury due to systemic hypoxia and a resultant improvement in mortality.

Moreover, TSC is well tolerated in humans with no significant safety concerns in early human trials, including phase 2 trials in patients with peripheral arterial disease, glioblastoma, and early enrollment in an acute stroke trial. Additionally, TSC is a stable compound, and easily stored and delivered through reconstitution and intravenous bolus injection.

Given the urgent demand for novel therapeutics in patients with COVID-19, we feel there is ample biological support for an early phase trial of TSC to improve oxygenation and outcomes in SARS-CoV-2 infected patients with hypoxemia at risk for acute respiratory distress syndrome.

2.2 Study Overview

The 100-303 trial is composed of an open-label, pharmacokinetic, pharmacodynamic, ascending dose, safety and tolerability lead-in study to a single-center, randomized, placebo-controlled, double-blind, adaptive, safety and efficacy pilot study of TSC in SARS-CoV-2 infected patients with hypoxemia. The study includes assessment of blood oxygenation via continuous pulse-oximetry (SpO2) with calculation of the SpO2:FiO2 ratios (S:F ratio). The lead-in phase as well as the randomized phase also include serial blood oxygenation measurements by arterial blood gas (ABG) measurements or SpO2 by pulse oximetry on Day 1 prior to TSC administration and at 1 minute, 10 minutes, 30 minutes, 3, 6, 24, and 48 hours with matching pharmacokinetic blood sampling.

2.3 Study Objectives

Lead-In PK/PD and Dose Selection

1. Determine the safety and tolerability of TSC when administered four times per day (every 6 hours) for up to 15 days (a minimum of 5 days) for each of the up to six doses to be studied.

2. Determine the relative degree of improvement by TSC dose in blood oxygenation following treatment with TSC as measured by the SpO2:FiO2 (S:F) ratio via continuous pulse oximetry.
3. For pharmacodynamic and pharmacokinetic purposes determine blood oxygenation as measured by arterial oxygen partial pressure (PaO2 in mmHg) to fractional inspired oxygen (FiO2), the PaO2:FiO2 (P:F) ratio or S:F ratio following the TSC administration at each dose level with matching PK blood sampling.
4. Determine the optimum, safe and tolerable biologic dose of TSC among the up to six doses to be studied given four times per day (every 6 hours) for up to 15 days using the S:F ratio.

Randomized Pilot

1. Determine the safety and efficacy of TSC administered at the optimum, safe and tolerable biologic dose four times per day (every 6 hours) for up to 15 days as compared to placebo
2. Demonstrate that TSC is not associated with an increased occurrence of serious adverse events in COVID-19 patients. The study endpoint analysis will compare the frequency of SAEs in the TSC and placebo groups.
3. Demonstrate that treatment with TSC is not associated with increases in any organ-specific classes of serious adverse events or increased mortality.

2.3.1 Primary Endpoints

Lead-In PK/PD

- Serious adverse events / Adverse events (Dose Limiting Toxicity)

Randomized pilot

- Time to recovery through Day 28, defined as time to achieve (and maintain through Day 28) a WHO ordinal severity scale score of 1, 2 or 3 with a minimum 1-point improvement from baseline

2.3.2 Secondary Endpoints

Pharmacokinetics/Pharmacodynamics (PK/PD):

The pharmacokinetics and pharmacodynamics of TSC will be assessed via an ascending dose lead-in prior to initiating the randomized trial. Up to 36 subjects will be studied at up to 6 doses between 0.25 mg/kg and 2.5 mg/kg administered

as an IV bolus injection 4 times per day (every 6 hours) for up to 15 days. Subjects will be assigned (not randomized between treatments) at baseline. Blood samples for pharmacokinetic purposes will be collected in each subject concomitant with the first dose of TSC on Day 1, as described in Section 5.8.

WHO ordinal severity scale score:

- Proportion of subjects with WHO ordinal severity scale score of 6 or 7 at any time through Day 28
- Time to an improvement of one category (i.e., a 1-point improvement) from baseline
- Change from baseline in WHO ordinal severity scale score at days 2, 4, 7, 10, 14 and 28, as a categorical improvement or worsening
- Mean change in WHO ordinal severity scale score from baseline through days 2, 4, 7, 10, 14 and 28

Oxygenation:

- Oxygenation free days in the first 28 days from start of therapy
- Incidence and duration of new oxygen use during the trial
- Proportion on mechanical ventilation, ECMO, noninvasive ventilation and high-flow nasal cannula oxygen delivery and return to room air or baseline oxygen requirement
- Time to return to room air or baseline oxygen requirement
- Days on extracorporeal membrane oxygenation (ECMO)
- Blood oxygenation by recorded continuous pulse oximetry (SpO2:FiO2 ratio)
- Blood oxygenation by serial arterial blood gas measurements collected at 1 minute, 10 minutes, 30 minutes, 1.5 hours, 3 hours and 6 hours by calculated PaO2:FiO2 ratio
- Durability of blood oxygenation via SpO2:FiO2 ratios

Mechanical Ventilation:

- Ventilator free days in the first 28 days (to day 29).
- Incidence and duration of new mechanical ventilation use during the trial

Hospitalization

- Hospital length of stay by Day 29
- ICU length of stay by Day 29

Mortality

- 15-day mortality
- 28-day mortality
- All-cause mortality at day 29
- In hospital mortality

- Mortality at Day 60

Other

- Glasgow Coma Score
- Sequential Organ Failure Assessment (SOFA) Score at baseline, 24 and 48 hours, Day 7, Day 15
- 28-day vasopressor free days
- Development of acute kidney injury (as defined by AKIN criteria)
- 28-day new renal replacement therapy (RRT) free days (excluding patients on chronic HD)
- Proportion of patients alive and free of respiratory failure by Day 28 defined as at least one of the following:
  - Endotracheal intubation and mechanical ventilation
  - Oxygen delivered by high-flow nasal cannula (heated, humidified oxygen delivered via reinforced nasal cannula at flow rates >20 L/min with fraction of delivered oxygen $\geq$ 0.5)
  - Noninvasive positive pressure ventilation
  - Extracorporeal membrane oxygenation
  - Clinical diagnosis of respiratory failure with initiation of none of these measures only when clinical decision making is driven solely by resource limitation

Safety

- Cumulative incidence of serious adverse events (SAEs) to Day 60
- Cumulative incidence of Grade 3 and 4 adverse events (AEs) to Day 60
- Discontinuation or temporary suspension of study drug injections (for any reason).
- Changes in white cell count, haemoglobin, platelets, creatinine, glucose, total bilirubin, ALT, and AST from day 1 through day 15 (while hospitalized); andday 29 (if able to return to clinic or still hospitalized)
- Death
- DVT/PE
- Nervous system disorders
- Respiratory (acute respiratory failure, cough, pneumonia)
- Angina
- Infections including sepsis
- Injection site reactions
- Drug hypersensitivity

2.4 Dosing Regimen

Lead-In PK/PD

The lead-in study will enroll up to 36 subjects. Each TSC dose will be administered as an IV bolus injection to 6 unique subjects per dose level administered four times per day (every 6 hours) for up to 15 days. Up to six TSC dose levels will be studied. Subjects will be assigned to dose levels in ascending order. The dose range is as follows.

- 0.25 mg/kg TSC
- 0.50 mg/kg TSC
- 1.00 mg/kg TSC
- 1.50 mg/kg TSC
- 2.00 mg/kg TSC
- 2.50 mg/kg TSC

TSC has been well tolerated in previous clinical studies at doses up 2.5 mg/kg based on safety, laboratory results, ECG findings, and vital signs. From these studies, the maximum tolerated dose was set at 2.5 mg/kg based on mild, transient, and yellow visual disturbances seen in 2 subjects at a dose of 5 mg/kg. No subjects have withdrawn from any study as a result of an AE related to TSC. A dose of 1.5 mg/kg has previously been identified from a peripheral arterial disease study (Mohler et al, 2011) as a target dose for longer duration efficacy studies.

This study will utilize an ascending dose scheme, starting with a dose of 0.25 mg/kg. All TSC doses will be administered as an IV bolus injection every six hours for up to 15 days (a minimum of 5 days). Safety data from each cohort of subjects at a given dose level will be reviewed by the Safety Monitoring Committee (SMC), which will adjudicate Dose Limiting Toxicities (DLTs) and other safety data. The first two dose levels will be reviewed by the SMC in groups of 3 subjects. Subsequent doses will be reviewed in cohorts of 6 subjects. If there are 0 or 1 DLT among the 6 subjects in a given dose level, 6 subjects in the next higher dose level will be studied. The study will continue in this fashion seeking an observed toxicity rate that is < 0.33 among 6 patients at any one dose level, or TSC at 1.5 mg/kg proves to be safe and tolerable. If a TSC dose higher than 1.5 mg/kg is warranted, and there are no safety signals, 6 patients may be studied at 2.0 mg/kg and 6 patients at 2.5 mg/kg.

The SMC will review the safety data of a given cohort of patients and approve or disapprove enrollment of the next cohort. For the first group of 3 subjects at 0.25 mg/kg, the SMC reviewed the safety data of up to 15 days of treatment. For subsequent cohorts, the SMC will review safety data associated with the first 5 days of treatment. The table below illustrates the SMC review schedule:

# of Subjects per SMC Meeting	# of SMC Meetings per Dose Cohort	TSC Dose Cohort	Duration of Treatment Prior to Meeting (days)
3	2	0.25 mg/kg	15 /5
3	2	0.50 mg/kg	5 / 5
6	1	1.00 mg.kg	5
6	1	1.50 mg/kg	5
6	1	2.00 mg/kg	5
6	1	2.50 mg/kg	5

Following SMC review after subjects complete the initial 5 days of treatment they will continue at their assigned TSC dose for up to 15 days.

At the completion of the lead-in the SMC will examine the safety and blood oxygenation (S:F) data for all subjects and determine the optimum, safe and tolerable dose of TSC for use in the pilot study.

Randomized pilot

The two arm, randomized pilot will enroll up to 200 subjects. TSC dosing will be at the selected optimum, safe and tolerable biologic dose with an active to placebo ratio of 2:1 or 2:2:1 if two TSC doses are to be studied. The treatment arms are as follows.

- TSC + Standard of Care
- Placebo +Standard of Care

Each TSC dose will be administered as an IV bolus injection 4 times per day (every 6 hours) for up to 15 days.

Subjects randomized to placebo will receive an IV bolus injection of Normal Saline at a volume which is matched to the volume that they would receive if they were receiving TSC, 4 times per day (every 6 hours) for up to 15 days.

All study drug administration will be performed by unblinded medical staff. Patients, investigators and care givers will not see the injection or injection site nor be aware of randomization.

Blood oxygenation will be measured via recorded continuous pulse oximetry and the S:F ratio calculated.

For both the lead-in phase and randomized phase, provided that an arterial line is established, serial arterial blood gas measurements will be collected and recorded prior to TSC administration and at 1 minute, 10 minutes, 30 minutes, 3, 6, 24, and 48 hours and the P:F ratio calculated, but only once per subject per TSC dose level. Alternatively, the S:F ratio will be used.

Subjects will be assessed daily while hospitalized. Discharged subjects will be asked to attend study visits at Days 29 and 60. All subjects whether a part of the lead-in phase or randomized pilot will be assessed for survival, serious adverse events and adverse events by requested return to the clinic on Day 60.

All subjects will undergo safety and efficacy assessments including laboratory assays, blood sampling on day 1 through day 15 (while hospitalized) and day 29 by return clinic visit or if still hospitalized.

Clinical Support
For both the lead-in phase and randomized phase, at each study day, while hospitalized, the following measures of clinical support should be assessed and recorded.

- Hospitalization
- Oxygen requirement
- Prone positioning
- Use of remdesivir or other antivirals
- Use of corticosteroids
- Non-invasive mechanical ventilation (via mask)
- Mechanical ventilator requirement (via endotracheal tube or tracheostomy)
- Extra Corporeal Membrane Oxygenation (ECMO) requirement

WHO Ordinal Severity Scale Score

For both the lead-in phase and randomized phase, the WHO 9-point ordinal scale assessment will the first assessment of the subject's clinical status each day recording the worst score for the previous day (i.e. on day 3, the score for day 2 is recorded as day 2). The ordinal scale is as follows:

Patient State	Descriptor	Score
Uninfected	No clinical or virological evidence of infection	0
Ambulatory	No limitation of activities	1

	Limitation of activities	2
Hospitalized Mild Disease	Hospitalized, no oxygen therapy	3
	Oxygen by mask or nasal prongs	4
Hospitalized Severe Disease	Non-invasive ventilation or high-flow oxygen	5
	Intubation and mechanical ventilation	6
	Ventilation + additional organ support – pressors, Renal Replacement Therapy (RRT), Extracorporeal Membrane Oxygenation (ECMO)	7
Dead	Death	8

2.5 Study Population

For both the lead-in phase and randomized phase, the study population will consist of hospitalized patients with confirmed SARS-CoV-2 infection and hypoxemia, defined as SpO2 < 94% on room air or requiring supplemental oxygen, WHO ordinal scale 3 through 7 (exclusive of ECMO), and further characterized by inclusion and exclusion criteria of this protocol.

For the randomized phase, the population to be analyzed is the Intention-to-Treat (ITT) dataset (i.e., all randomized participants). We do not anticipate subject dropout for the primary outcome, and there will be no 'drop-in' of usual care participants receiving TSC. The safety dataset will include all randomized participants.

2.5.1 Inclusion Criteria

1. Hospitalized subjects with confirmed SARS-CoV-2 infection and hypoxemia, defined as SpO2 < 94% on room air or requiring supplemental oxygen (inclusive of nasal cannula, high flow oxygen, non-invasive ventilation, and mechanical ventilation)
2. Laboratory-confirmed SARS-CoV-2 infection as determined by rtPCR, or other commercial or public health assay in any specimen within 7 days prior to enrollment.
3. WHO ordinal scale score of 3 through 7 (exclusive of ECMO) at baseline
4. Male or non-pregnant female adult ≥18 years of age at time of enrollment.
5. Subject (or legally authorized representative) provides written informed consent prior to initiation of any study procedures.
6. Understands and agrees to comply with planned study procedures.
7. Illness of any duration
8. Women of childbearing potential must have a negative blood pregnancy test at the screening/baseline visit and agree to use two forms of birth control through 30 days after the last dose of study drug.

2.5.2 Exclusion Criteria

1. Receiving extracorporeal membrane oxygenation (ECMO) at baseline
2. Severe organ dysfunction (SOFA score > 10) at enrollment
3. Patient or LAR unable to provide written informed consent
4. ALT/AST > 3 times the upper limit of normal or serum bilirubin > 1.5 times the upper limit of normal
5. Estimated glomerular filtration rate (eGFR) by Modification of Diet in Renal Disease (MDRD) formula < 30 mL/min/1.73 m^2 or on dialysis
6. Pregnancy or breast feeding.
7. Anticipated transfer to another hospital which is not a study site within 72 hours.
8. Allergy to any study medication

9. Patient not expected to survive >24 hours, or likely to be discharged <24 hours per PI discretion.

2.6 Enrollment and Consent

A physician-investigator or study coordinator will interview and examine the subject in the hospital. The physician-investigator will discuss inclusion in the clinical investigation, the details of the investigation, and other information contained in the informed consent document; and that the subject's participation in the study may be discontinued at any time without penalty or loss of benefits to which the subject is otherwise entitled.

2.7 Randomization

Subjects participating in the open-label, PK/PD, ascending dose, safety and tolerability lead-in will be assigned to treatment with TSC (not randomized) in ascending dose fashion.

Subjects participating in the single-center, randomized, placebo-controlled, double-blind, adaptive, safety and efficacy pilot will be randomized to the selected optimum, safe and tolerable biologic dose of TSC or placebo with an active to placebo ratio of 2:1 or 2:2:1 if two TSC doses are to be studied. Randomization to treatment will be stratified by the following factors assessed at randomization:

- Disease severity (WHO ordinal scale 3 vs 4 or 5)
- Age at enrollment (<60 years vs ≥60 years of age)
- Comorbidities (presence of any of the following: hypertension, diabetes or immune-related disease vs absence of all)

The identified person administering study drug will be unblinded given the orange-red coloration of the reconstituted TSC solution compared to Normal Saline placebo. Unblinded personnel will play no role in making patient assessments.

The lead-in PK/PD dose selection phase is not randomized whereas the follow-on pilot study is randomized. Both phases require four times a day (every 6 hour) dosing for up to 15 days. The lead-in phase will study up to 36 subjects and the randomized pilot will study up to 200 subjects.

Investigational Medicinal Product (IMP) will be delivered to the investigational pharmacy as labeled vials in bulk. The IMP will composed of Trans Sodium Crocetinate (TSC) and Normal Saline (Placebo), both of which will be appropriately labeled in accordance with all applicable Romanian regulations.

The investigational pharmacy is expected to manage the randomization of IMP in accordance with the supplied randomization scheme. Randomization will be accomplished using an electronic IRT system.

The investigational pharmacy is expected to prepare and dispense the correct IMP for administration to each patient four times per day (every 6 hours) for up to 15 days.

The investigational pharmacy will prepare each dose and dispense same to the identified unblinded medical staff who are trained and assigned to administer IMP to each patient.

TSC can only be reconstituted with Sterile Water. Sterile Water will not be supplied but will be provided by the investigational pharmacy.

The lead-in PK/PD phase requires preparation of IMP (TSC) and dispensing to an identified, trained Research Nurse or other trained assigned medical staff who will administer IMP to patients.

The randomized pilot requires preparation of IMP (either TSC or placebo) in accordance with a randomized scheme (via an IRT system) and dispensing to an unblinded Research Nurse or other trained assigned medical staff who will administer IMP to the patient.

Because TSC is highly colorized, the unblinded study team (pharmacist and administering personnel) will take specific steps to ensure that the subjects and blinded study personnel are not made aware of treatment assignment during the randomized phase of the trial.

- Prior to release from the pharmacy, the pharmacist will package the prepared syringe(s) in foil that masks the contents (i.e. cannot see through the foil). The study drug should not be removed from the foil at the time of administration to the subject.
- The medical staff assigned to administer the foil wrapped IMP will do so using a shroud to insure that the patient is blinded to treatment. The shroud may be made of any material and be of any size as long as it sufficiently blocks the patient's view of the injection site and injection syringe at the time of IMP administration. The shroud need not be in place at times other than during the time of study drug injection.

When documenting IMP administration in the case report form, the medical staff who administers the IMP will document volume given, without revealing treatment assignment. This will allow study personnel carrying out assessments, and the blinded study monitor, to remain blinded to treatment assignment.

In similar fashion, the sponsor has contracted with two Clinical Research Associates (CRAs) who will monitor various aspects of the 100-303 trial. One of the CRAs will

remain blinded to study treatment and the other will be unblinded to study treatment. The unblinded CRA will be assigned to monitor investigational pharmacy procedures and particularly the randomization of IMP to patients in accordance with the randomization scheme (via IRT).

2.7.1 Criteria for Early Withdrawal

The following events are considered sufficient reason to discontinue a subject from the study:

- Subjects are free to withdraw from the study at any time, for any reason, and without prejudice.
- The subject experiences an adverse event (AE) that in the investigator's opinion precludes continued participation.
- The subject incurs a significant protocol violation that constitutes a safety hazard or significantly confounds the interpretation of the data from that subject.
- At the PI's request.
- The study is terminated.

2.7.2 Screening/Baseline Failures

After the screening/baseline evaluations have been completed, the investigator or designee is to review the inclusion/exclusion criteria and determine the subject's eligibility for the study.

Only the reason for ineligibility will be collected on screen failures. Subjects who are found to be ineligible will be told the reason for ineligibility.

Individuals who do not meet the criteria for participation in this study (screen/baseline failure) because of an abnormal laboratory finding may be rescreened once.

2.7.3 Subjects Lost to Follow-up

Given that only hospitalized subjects will be enrolled drop outs are not anticipated. The investigator will attempt to contact any subject that is lost to follow-up or fails to return by the latest follow-up time point in order to evaluate the reason the subject has not returned and to obtain follow-up safety information. All attempts to contact the subject should be documented.

2.7.4 Recruitment

It is anticipated that patients with COVID-19 will present to participating hospitals, and that no other efforts to recruit potential subjects are needed. Recruitment efforts may also include dissemination of information about this trial to other medical professionals / hospitals. Patients that are confirmed to have SARS-CoV-2 will be assessed for eligibility. Screening will begin with a brief discussion with study staff. Some will be

excluded based on demographic data and medical history i.e. pregnant, < 18 years of age, renal failure, etc. Information about the study will be presented to potential subjects (or legally authorized representative) and questions will be asked to determine potential eligibility. Screening procedures can begin only after informed consent is obtained.

2.7.5 Retention

Participating subjects will be reminded of subsequent visits.

2.7.6 Costs

There is no cost to subjects for the research tests, procedures/evaluations and study drug while taking part in this trial. Procedures and treatment for clinical care including costs associated with hospital stay may be billed to the subject, subject's insurance or third party, if and as appropriate.

2.7.7 Replacement of Subjects

Subjects who have received TSC or placebo will not be replaced. The analysis will be conducted on an intention-to-treat basis.

2.7.7.1 Study Drug Injection Halting

For an individual subject, an individual study drug injection must be stopped if they have a suspected drug-related event of hypersensitivity (Grade 2 or higher) during the injection.

Subjects who have an IV injection stopped for a safety related issued will not continue with dosing.

2.7.8 Study Halting for Safety

Given severity of illness in COVID-19, there are no pre-specified stopping rules. Instead there will be close oversight by the Medical Monitor and frequent SMC/DSMB reviews for safety. Treatment should be stopped if a patient is found to be pregnant after randomization

2.7.9 Withdrawal from Randomized Treatment or from the Study

Patients are free to withdraw from participation in the study at any time upon request, without any consequence. Patients should be listed as having withdrawn consent only when they no longer wish to participate in the study and no longer authorize the Investigators to make efforts to continue to obtain their outcome data. Every effort should be made to encourage patients to remain in the study for the duration of their planned outcome assessments. Patients should be educated on the continued scientific importance of their data, even if they discontinue study drug. In the case of a patients becoming lost to follow-up, attempts to contact the patient should be made and documented in the patient's medical records.

2.7.10 Discontinuation of Study Drug

A patient in this clinical study may discontinue study drug for any of the following reasons:

- Patient requests to discontinue study drug
- Occurrence of any medical condition or circumstance that exposes the patient to substantial risk and/or does not allow the patient to adhere to the requirements of the protocol
- Any serious adverse event (SAE), clinically significant adverse event, severe laboratory abnormality, intercurrent illness, or other medical condition that indicates to the Investigator that continued participation is not in the best interest of the patient
- Patient fails to comply with protocol requirements or study-related procedures

Unless the patient withdraws consent, those who discontinue study drug early should remain in the study for further acquisition of endpoint measurements. The reason for patient discontinuation of study drug should be documented in the case report form.

2.8 Study Drug

2.8.1 Trans sodium Crocetinate (TSC)

Trans sodium crocetinate (TSC) will be administered intravenously as an IV bolus. The concentration of the reconstituted drug is 20 mg/mL.

2.8.1.1 TSC Packaging

Study drug will be supplied as a sterile lyophilized powder in 5 mL vials and will be in bulk cardboard boxes stored at room temperature (15 to 30° Celsius or 59 to 86° Fahrenheit) under secure conditions with limited access. Each vial will contain:

	5 mL Vial
Trans Sodium Crocetinate	40 mg
Gamma cyclodextrin	160 mg
Glycine USP	7.5 mg
Mannitol USP	46 mg

The label will contain appropriate labeling in accordance with all relevant Romanian labeling regulations.

2.8.1.2 Reconstitution of TSC

The 5 mL vial will be reconstituted with 2 mL of Sterile Water for Injection (USP). TSC should **not** be reconstituted with any other diluent other than Sterile Water for Injection. The lyophilized powder should rapidly reconstitute to a clear deep orange-red solution. Each reconstituted vial will contain: 20 mg/mL TSC, 8% gamma cyclodextrin, 50 mM glycine, 2.3% mannitol at an approximate pH of 8.0 - 8.2.

TSC is dosed based on the patient's baseline weight, obtained on the day of screening, on a milligram per kilogram basis. Several vials may be needed depending on the patient's weight. Reconstitution of multiple vials will be carried out per institutional Standard Operating Procedures relative to preparation of IV injectable drugs.

The reconstituted test article will be prepared separately for each subject at room temperature (15 to 30°C or 59 to 86°F). TSC must be administered within 4 hours of reconstitution and any remaining TSC in the vial should not be used for further dosing of the same subject or another subject. Reconstituted TSC should **not** be diluted prior to administration.

The sponsor will supply separately, a sufficient quantity of TSC for use in the lead-in phase and the randomized pilot phase.

2.8.1.3 Placebo

For the randomized phase, subjects randomized to placebo will receive an IV bolus injection of Normal Saline at a volume which is matched to the volume that they would receive if they were receiving TSC, four times per day (every 6 hours) for up to 15 days.

In similar fashion to active drug a sufficient quantity of labeled placebo (Normal Saline), supplied by the sponsor will be set aside in the pharmacy for sole use in this study.

Study coordinators will maintain an accountability log, ensuring study drug (both active and placebo) is accounted for from the time of administration in a subject, return, or destruction. Drug accountability tasks may be delegated to a pharmacist or other appropriately trained party at the site.

The dispensing records should include the subject's initials, subject number, the number of vials of active and placebo and date dispensed for each subject.

2.9 Concomitant Therapy

Subjects enrolled in this trial may receive any conventional treatment at the discretion of their attending physicians.

Therapy with antivirals including remdesivir or lopinavir/ritonavir (Kaletra) or other therapeutic agents (e.g. corticosteroids) prior to enrollment in this trial are permitted.

If the local standard of care per written policies or guidelines (i.e., not just an individual clinician decision) includes remdesivir, lopinavir/ritonavir (Kaletra) or other agents, then continuing these during the study is permitted, but may require additional safety monitoring by the site.

All medications taken in the 7 days prior to the first dose of study drug will be captured in the eCRF. After the first dose of study drug, concomitant medications to be captured in the eCRF will be vasopressors and any medication given to specifically target COVID-19 (e.g. antivirals and corticosteroids).

Additionally, there should be plans on how the concomitant drugs are stopped for transaminase elevations, and prior to the thresholds for remdesivir dose modification.

2.10 Schedule of Events

A charted schedule of events is included in Appendix 9.1.

2.10.1 Screening/Baseline Evaluation (Day -1/Day1)

Information commonly collected and recorded in source documents at the time of hospital admission for hypoxemia associated with SARS-CoV-2 infection can be used to qualify a subject for the study and hence, need not be repeated.

Following completion of the informed consent process the study coordinator will record the following from source documents or perform the following.

- Demography
- Medical History
- Vital signs (heart rate, blood pressure, respiratory rate, temperature)
- Full physical examination
- 12-Lead ECG
- Oxygen saturation (with calculated SpO2:FiO2) by recorded continuous pulse oximetry
- Arterial blood gas measurement (with calculated PaO2:FiO2), if monitored
- WHO 9-point ordinal severity scale score
- Glasgow Coma Score (per supplied NHS Greater Glasgow & Clyde)
- Sequential Organ Failure Assessment (SOFA)

Laboratory data that will be captured at baseline includes:

- Complete blood count (CBC)
- Basic metabolic panel (BMP)
- Creatine kinase (CK), creatine kinase muscle-brain (CK-MB), glutamate dehydrogenase (GLDH), troponin

- Liver function test (LFT) or hepatic panel
- Coagulation panel
- Serology panel
- Urinalysis
- Laboratory confirmation of SARS-CoV-2 infection
- Blood pregnancy test for women of child bearing potential
- SARS-CoV-2 IgM and IgG antibodies
- SARS-CoV-2 viral load
- Immunological assessments (Section 9.6 Laboratory Parameters)

Imaging data at baseline will include:

- Chest X-Ray (CT Scan)

Subjects meeting all inclusion/exclusion criteria and who have completed the informed consent process may be enrolled in the study.

The unblinded bedside nurse and physician who were delegated, may then perform the following:

- Schedule all PK blood sample collection times from pre-dose through 48 hours
- Vital signs taken prior to and after every dose of study drug
- Collect pre-dose PK blood sample (time zero ~ 2 minutes prior to study drug administration)
- Administer study drug (active or placebo) by IV bolus injection
- Record the end of injection time (hour/minutes using appropriate timepiece)
- Perform the PK blood sample collections as follows
  - Pre-dose PK blood sample collection (~ 2 minutes prior to study drug)
  - 1 minute post the end of injection (± 1 minute)
  - 10 minutes post the end of injection (±1 minute)
  - 30 minutes post the end of injection (±1 minute)
  - 1.5 hours post end of injection (± 2 minutes)
  - 3.0 hours post end of injection (± 5 minutes)
  - 6.0 hours post end of injection (within 10 minutes before TSC dosing)
- Record oxygen saturation (SpO_2) from the continuous recorded pulse oximetry at the same timepoints
  - Pre-dose PK blood sample collection (~ 2 minutes prior to study drug)
  - 1 minute post the end of injection (± 1 minute)
  - 10 minutes post the end of injection (±1 minute)
  - 30 minutes post the end of injection (±1 minute)
  - 1.5 hours post end of injection (± 2 minutes)
  - 3.0 hours post end of injection (± 5 minutes)
  - 6.0 hours post end of injection (within 10 minutes before TSC dosing)

- Calculate and record the SpO2:FiO2 ratio for each time point
- Alternatively record oxygenation by arterial blood gas measurements (PaO2), if ABG is monitored, at the same timepoints

2.10.1.1 Additional PK blood sample collections

- 24 hours post end of initial injection (within 1 hour before TSC dosing)
- 48 hours post end of initial injection (within 2 hours before TSC dosing)

2.10.1.2 To be performed from Day 2 to the earlier of hospital discharge or Day 29

- Vital signs will be taken prior to and after every dose of study drug
- A targeted physical exam will conducted each day while the patient is hospitalized.
- A 12-lead ECG will be performed at Days 5, 14, 21 and 28
- Following the laboratory confirmation of SARS-CoV-2 infection at the Screening/Baseline (Day -1/Day1) visit, SARS-CoV-2 IgM and IgG antibodies and SARS-CoV-2 viral load will be assessed at Days 7, 14, 21, and 28 (see Time and Events Schedule 9.1)
- Oxygen saturation (SpO2:FiO2) by continuous recorded pulse oximetry daily
- Arterial blood gas measurements (PaO2:FiO2), if ABG monitored
- Non-invasive mechanical ventilation (via mask); in liters/minute, if any, or Mechanical ventilator requirement (via endotracheal tube or tracheostomy); current ventilatory settings, if applicable, daily
- WHO ordinal severity scale score, daily
- Glasgow Coma Score (per supplied NHS Greater Glasgow & Clyde), daily
- Sequential Organ Failure Assessment (SOFA), daily
- AKIN classification, daily

2.10.1.3 To be performed on Days 2 through Day 15, and 29 (and additional days when hospitalized)

- Laboratory data
    - Complete blood count (CBC)
    - Basic metabolic panel (BMP)
    - Liver function test (LFT) or hepatic panel
    - Coagulation panel (Coag)
    - Immunological assessments (Imm) (Section 9.6 Laboratory Parameters)
    - Urinalysis

All subjects whether a part of the lead-in phase or randomized pilot will be assessed for survival, serious adverse events and adverse events by telephone on Day 60 (± 10 days).

2.10.2 Assessments at the time of hospital discharge or Day 29

The following assessments will be recorded in the case report form by review of the source documents for each subject at the time of hospital discharge or Day 29.

WHO 9-point Ordinal Severity Scale Score:

- Time to an improvement of one category from admission on the WHO ordinal scale
- Subject clinical status on the WHO ordinal scale at days 2, 4, 7, 10, 14 and 28
- Mean change in the ranking on an ordinal scale from baseline to days 2, 4, 7, 10, 14 and 28

Oxygenation:

- Oxygenation free days in the first 28 days (to day 29)
- Incidence and duration of new oxygen use during the trial
- Proportion on mechanical ventilation, ECMO, noninvasive ventilation and high-flow nasal cannula oxygen delivery and return to room air or baseline oxygen requirement
- Time to return to room air or baseline oxygen requirement
- Days on extracorporeal membrane oxygenation (ECMO)
- Blood oxygenation by recorded continuous pulse oximetry (SpO_2:FiO_2 ratio)
- Blood oxygenation by serial arterial blood gas measurements collected prior to the first dose of TSC and at 1 minute, 10 minutes, 30 minutes, 1.5 hours, 3 hours and 6 hours post TSC administration by calculated PaO_2:FiO_2 ratios

Mechanical Ventilation:

- Ventilator free days in the first 28 days (to day 29).
- Incidence and duration of new mechanical ventilation use during the trial

Hospitalization

- Hospital length of stay by Day 29
- ICU length of stay by Day 29

Mortality

- 15-day mortality
- 28-day mortality
- All-cause mortality at Day 29
- In hospital mortality

- Mortality by Day 60

Other

- Glasgow Coma Score
- Sequential Organ Failure Assessment (SOFA) Score at baseline, 24 and 48 hours, Day 7, Day 15
- 28-day vasopressor free days
- Development of acute kidney injury (as defined by AKIN criteria)
- 28-day new renal replacement therapy (RRT) free days (excluding patients on chronic HD)
- Proportion of patients alive and free of respiratory failure by Day 28 defined as at least one of the following:
  - Endotracheal intubation and mechanical ventilation
  - Oxygen delivered by high-flow nasal cannula (heated, humidified oxygen delivered via reinforced nasal cannula at flow rates >20 l?min with fraction of delivered oxygen $\geq$ 0.5)
  - Noninvasive positive pressure ventilation
  - Extracorporeal membrane oxygenation
  - Clinical diagnosis of respiratory failure with initiation of none of these measures only when clinical decision making is driven solely by resource limitation

Safety

- Serious adverse events (SAEs) through Day 60
- Grade 3 and 4 adverse events (AEs) through Day 60
- Discontinuation or temporary suspension of study drug injections (for any reason).
- Changes in white cell count, haemoglobin, platelets, creatinine, glucose, total bilirubin, ALT, and AST from day 1 through day 15 (while hospitalized); and day 29 (if able to return to clinic or still hospitalized)
- Death
- DVT/PE
- Nervous system disorders
- Respiratory (acute respiratory failure, cough, pneumonia)
- Angina
- Infections including sepsis
- Injection site reactions
- Drug hypersensitivity

3 ADVERSE EVENT REPORTING

3.1 Adverse Events

An AE is any untoward medical occurrence associated with the use of the drug in humans, whether or not considered drug related. An AE is any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. This includes changes in anatomical, physiological, or metabolic functions as indicated by physical examination signs, symptoms, and/or laboratory changes or medical occurrence which develops or worsens while enrolled in this study regardless of whether the event is considered related to the investigational drug. Enrolled is defined as a subject that is assigned to treatment and is dosed.

A suspected adverse reaction means any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the AE. The sponsor will evaluate the available evidence and make a judgment about the likelihood that the drug actually caused the AE.

Abnormal laboratory values or test results not related to baseline disease constitute AEs if they are deemed clinically significant by the PI, and induce clinical signs or symptoms or require therapy. Abnormal laboratory values or test results that are related to baseline disease should be reported as AEs only if judged by the PI to be more severe than expected. If judged to be more severe than expected, they will be considered clinically significant.

Disease progression in the medical opinion of the investigator and/or disease-related morbidity will not be considered an AE.

3.1.1 Serious Adverse Events

AEs will be categorized as serious and non-serious and unexpected or not.

Serious adverse events (SAEs) are AEs that pose a threat to the subject's life or functioning based on the following outcome/actions regardless of whether the event is considered related to the investigational drug:

- Results in death
- Is life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Other events, based on medical judgment, which jeopardize the subject and require medical/surgical intervention to prevent one of the outcomes above.

3.1.2 Unexpected

An unexpected adverse event is any adverse event where the nature, specificity or frequency of the event is not consistent with either: 1) the known or foreseeable risk associated with the procedures involved in the research that are described in the protocol or Investigator's Brochure; or 2) the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

3.1.3 Unanticipated Problem

An unanticipatied problem is an event or outcome that meets all of the following criteria: 1) unexpected; 2) related or possibly related to participation in the research; and 3) places subjects or others at a greater risk of harm than was previously known or recognized.

3.1.4 Adverse Event Reporting Requirements

It is the responsibility of the investigator to document all AEs occurring during this investigation. All AEs will be reported to the sponsor via the CRF. Each subject will be evaluated for the development of AEs. All AEs occurring after administration of study medication must be recorded in the subject's CRF, regardless of whether or not they are considered drug-related. The nature of each experience, date and time of onset, outcome, frequency, intensity, action taken with respect to dosage, whether it was serious or non-serious, and relationship to treatment should be documented. Signs and symptoms should be grouped into a single diagnosis and reported as a single AE when appropriate.

AEs should be documented in terms of a medical diagnosis(es) when possible. AEs not previously documented at Baseline as pre-existing conditions will be recorded as medical history.

3.1.5 Expedited Reporting Requirements for Unanticipated Problem SAEs

Investigator's Responsibility

It is the responsibility of the investigator to document and report to the sponsor in expedited fashion all SAEs that are Unanticipated Problems, including:

Within 10 working days:

Any SAE which meets the following criteria:

- Unexpected
- Related or possibly related to the research participation

Within 3 working days:

Any SAE with fatal outcome that meets the following criteria:

- Unexpected
- Related or possibly related to the research participation

Sponsor's Responsibility

SAEs will be reported to the appropriate regulatory authorities per the Sponsor Regulatory Reporting Requirements Matrix maintained by Drug Safety Navigator (DSN). For expedited cases, DSN will finalize the required documents on or before Day 7 or Day 15, as applicable, per country /site requirements as outlined in the Reporting Matrix.

3.1.6 Data and Safety Monitoring Board / Safety Monitoring Committee

A Safety Monitoring Committee (SMC) will be established to review the DLTs during the lead-in phase for the purpose of determining the optimal safe and tolerable TSC dose for the randomized pilot.

Dose Limiting Toxicity (DLT) is defined as any study drug related grade 3 or 4 adverse event during the treatment period, with the exception of pulmonary events in the CTCAE that are known complications of SaARS-CoV-2 infection: ARDS, Cough, Dyspnea, Hypoxia, Pneumonitis, Pulmonary Edema, Respiratory Failure, or Respiratory, Thoracic and Mediastinal disorders – Other. The SMC will apply clinical judgement in their review of adverse events (particularly abnormal laboratory results).

The Data Safety Monitoring Board (DSMB) will review the efficacy endpoints, safety profile and potential sample size re-estimation during the trial and generally safeguard the interests of study participants.

The details regarding the functioning of the SMC and DSMB will be outlined in their individual charters.

Given the severity of illness in COVID-19, there are no pre-specified study stopping rules for safety.

The Medical Monitor will review all AE/SAE data in unblinded fashion during the lead-in phase and AE / SAE data in blinded fashion in the randomized phase. If there are a concerning number of unexpected AEs, the Safety Monitoring Committee (SMC) during the lead-in phase and the Data Safety Monitoring Board DSMB) during the randomized phase will be asked to review unblinded safety data in an ad hoc meeting

Patient enrollment during the randomized phase of the study will not stop awaiting DSMB review, though the DSMB may recommend temporary or permanent cessation of enrolment based on their safety reviews.

The DSMB will review safety data during the randomized phase after every 50 subjects. Ad hoc reviews will be undertaken if there are other specific safety concerns.

3.2 Research Related Injuries

For any potential research related injury, the site PI or designee will assess the subject. Study personnel will try to reduce, control, and treat any complications from this study. Immediate medical treatment may be provided by the participating study site, such as giving emergency medications to stop immediate allergic reactions.

As needed, referrals to appropriate health care facilities will be provided to the subject. The site PI should then determine if an injury occurred as a direct result of the tests or treatments that are done for this trial.

If it is determined by the participating site PI that an injury occurred to a subject as a direct result of the tests or treatments that are done for this trial, then referrals to appropriate health care facilities will be provided to the subject.

4 DATA MANAGEMENT

4.1 Collection of Study Data

All data will be collected on a standardized case report form designed for this study. All clinical data will be recorded on the case report forms,

Additional clinical information including concomitant medications, complications of therapy, and a narrative clinical summary of serious adverse events will be included.

4.2 Data Management and Quality Assurance

Study data will be collected and managed using an electronic data capture tool selected by the sponsor. The EDC tool will be a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration.

5 STATISTICAL METHODS

This study is intended to allow for two types of adaptations: 1) blinded confirmation or modification of the primary endpoint and 2) ability to re-estimate/increase the sample size of the randomized pilot part of the study. A brief summary is provided here for blinded confirmation of the primary endpoint and in Section 5.1 for the sample size re-estimation. Details will be described in the statistical analysis plan.

Blinded endpoint confirmation or modification

The current plan is to evaluate the primary endpoint of time to recovery by Day 29. Because there is uncertainty about the clinical course and potential different outcomes according to baseline disease severity and changes in standard of care for COVID-19, the primary endpoint may be modified after a blinded evaluation of recovery rates based on the WHO 9-point Ordinal Scale. This will occur after approximately 50 participants have completed through day 29, by the DSMB, without knowledge of treatment assignment. Analyses will be evaluated by baseline severity and other factors (eg, baseline S:F ratio).

5.1 Statistical Hypotheses

The primary outcome of time to recovery is a time-to-event endpoint based on the WHO 9-point Ordinal COVID-19 severity scale (refer to Analysis of the Primary Efficacy Endpoint). Time to recovery will be analyzed using a stratified log-rank test. Stratified analysis will be based on stratification factors used for randomization (refer to Randomization). The null hypothesis being tested is whether the distribution of time to recovery is the same for the placebo and TSC treatment arms.

5.2 Sample Size Determination

Time to recovery by Day 29 is the primary endpoint for the randomized pilot. A total of 171 patients (114 in TSC arm and 57 in placebo arm) are needed to have 85% power with a two-sided alpha of 0.05 to detect an improvement in median time to recovery from 12 days to 7 days (HR=1.71). A total of 138 events (recovered subjects) are required for the analysis. Assuming a 10% dropout rate, a total of 190 subjects need to be randomized.

A blinded review of the primary endpoint will be performed by the DSMB after approximately 50 subjects have been randomized to assess the assumptions regarding the primary endpoint, due to uncertainties about standard of care and the clinical course of the disease.

5.3 Unblinded Interim Analysis

An unblinded interim analysis of the primary endpoint will be conducted after approximately 95 randomized of subjects have completed the Day 29 follow-up evaluation (50% of randomized subjects). A Lan-DeMets spending function with O'Brien-Fleming boundaries will be used to control for an overall two-sided type-I error rate of 0.05.

Conditional power will also be presented to the DSMB. Conditional power estimates the probability of obtaining a statistically significant result by the end of the trial given the data accumulated to date. If conditional power is less than 20% under the original trial assumptions, consideration should be given to stopping the trial. If conditional power is

between 20% and 85%, consideration should be given to a re-estimation of the sample size necessary to have sufficient power for final analysis.

5.4 Statistical Analyses

5.4.1 General Approach

The randomized pilot period is a double-blind, placebo-controlled randomized trial testing a superiority hypothesis with a two-sided type I error rate of 0.05. Secondary hypotheses have been ordered according to relative importance. These will be described according to the appropriate summary statistics (e.g., proportions for categorical data, means with 95% confidence intervals for continuous data, median for time-to-event data).

A statistical analysis plan (SAP) will be developed prior to unblinding of study and database lock.

5.4.2 Populations for Analysis

The primary analysis will be based on an intention-to-treat population, including all randomized subjects analyzed by assigned treatment group. Similarly, safety analyses will be based on a safety population consisting of all subjects who received at least one infusion of TSC or placebo and will be analyzed by actual treatment received. A Per-protocol population will include all treated subjects who do not have major protocol deviations that could impact the analysis of the primary endpoint. The Per-protocol population will be defined based on medical review prior to database lock and unblinding.

5.4.3 Analysis of the Primary Efficacy Endpoint

Time to recovery through Day 28 is the primary efficacy endpoint for the randomized part of the study. Subjects enter the study with a WHO COVID-19 ordinal severity scale score of 3, 4 or 5. To meet the definition of recovery, a subject must achieve a WHO severity score of 1, 2 or 3 and have an improvement of at least 1 point, maintained through the Day 28. In other words, subjects who enter the study with a baseline WHO scale score of 3 and improve to (and maintain to the Day 29 evaluation) a score of 1 or 2 have met the definition of recovery. Subjects who enter the study with a baseline WHO scale score of 4 or 5 and improve to (and maintain to Day 29 evaluation) a score of 1, 2 or 3 have met the definition of recovery. Time to recovery will counted from day of randomization to day of recovery (date of recovery – date of randomization + 1). Subjects who have not recovered by Day 29 evaluation will be censored at Day 28. Subjects who recover after Day 28 will be censored at Day 28. Subjects who die or discontinue the study prior to the Day 29 evaluation will be censored on their death date or date of last evaluation of WHO severity scale, respectively. Note that the evaluation that is performed on Day 29 is an assessment of the subject's WHO severity score of the previous day (Day 28).

A stratified log-rank test will be used to test the primary endpoint of time to recovery, using the randomization stratification factors. Median and 95% confidence intervals will be summarized using Kaplan-Meier methods. Kaplan-Meier curves will also be presented. A stratified Cox model will be used to estimate the hazard ratio.

Sensitivity analyses of the primary endpoint will be performed, including an unstratified log-rank test in the ITT population, stratified and unstratified log-rank tests for the Per-protocol population, time to recovery from start of study therapy and modifications to censoring rules. Additional sensitivity analyses may be specified in the SAP.

5.4.4 Analysis of Secondary Endpoints

The key secondary endpoints in order of importance are:

- Hospital length of stay through Day 28
- Proportion of subjects with WHO severity score of 6 or 7 at any time through Day 28
- All-cause mortality through Day 28

A hierarchical approach to the testing of these endpoints will be performed if the primary efficacy endpoint is statistically significant. Details of the analysis of the key secondary endpoints will be described in the SAP.

In general, secondary endpoints will be analyzed descriptively, as follow.

1. Time-to-event endpoints by treatment will be summarized with Kaplan-Meier curves, median with quartiles and 95% confidence bounds.
2. Categorical and response endpoints will be summarized by proportions and 95% confidence bounds.
3. Incidence data will be summarized as a percent with 95% confidence intervals.

Missing data procedures will be described in the SAP.

5.5 Subgroup Analysis

Subgroup analyses for the primary and key secondary endpoints will evaluate the treatment effect across the following subgroups: severity of symptoms prior to enrollment, comorbidities, age and sex. A forest plot will display confidence intervals across subgroups. Additional subgroups may be specified in the SAP.

5.6 Safety Analysis

No formal statistical hypothesis testing will be performed with regard to safety variables. The safety assessments will include the collection of adverse events, physical exam, vital signs, 12-lead ECG, and clinical laboratory.

5.6.1 Adverse Events

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be used for AE reporting. Adverse events will be coded using the MedDRA coding dictionary. Summaries of treatment emergent adverse events will be reported overall, by severity, by seriousness and by relationship to study drug. Incidence rates will be reported by body system, organ class and preferred term by treatment arm. Progression of disease including death will not be considered an AE.

The investigator and any designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or the study, or that caused the participant to discontinue the study treatment.

5.6.2 Time Period and Frequency for Collecting AE and SAE Information

All AEs will be collected from the start of study drug administration through the Day 60 visit.

Medical occurrences that begin before the start of study treatment but after obtaining informed consent will be recorded as pre-existing conditions on the Medical History section of the case report form (CRF).

All SAEs will be recorded and reported to the sponsor or designee within 24 hours. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AE or SAE information from former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment or study participation, the investigator must promptly notify the sponsor.

5.6.3 Method of Detecting AE and SAE

Care will be taken not to introduce bias when detecting an AE and/or SAE. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrence.

5.6.4 Follow-up of AE and SAE

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs, and non-serious AEs of special interest will be followed until resolution, stabilization, until the event is otherwise explained, or the participant is lost to follow-up.

5.6.5 Regulatory Reporting Requirements for SAE

Prompt notification by the investigator to the sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study treatment under clinical investigation are met.

The sponsor has a legal responsibility to notify both the local regulatory authority and other relevant regulatory agencies about the safety of a study treatment under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.

Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.

An investigator who receives an investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAE) from the sponsor will file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

5.6.6 Pregnancy

All female participants of childbearing potential must have a blood pregnancy test prior to receiving experimental treatment under this protocol and that test must be negative.

Details of all pregnancies in female participants and, female partners of male participants will be collected after the start of study treatment and until 3 months after treatment discontinuation. If a pregnancy is reported, the investigator should inform Sponsor or designee within 24 hours of learning of the pregnancy. Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered an SAE and should be reported to the sponsor.

A woman is of non-reproductive potential if the woman meets any of the following conditions:

- At least 2 years post-menopausal
- Hysterectomy
- Bilateral oophorectomy
- Tubal ligation

5.6.7 Acceptable Methods of Birth Control

Women of reproductive potential must use a double method of birth control through 30 days after the last dose of study drug. Subjects must use two of the following acceptable methods of birth control as applicable:

- Oral, transdermal, or implanted hormonal contraceptives at a stable dose for at least 2 months prior to randomization
- Intrauterine device
- Diaphragm with spermicide
- Male condom
- Abstinence

The concomitant medication and/or medical history documentation including that recorded on the eCRF for females of reproductive potential should support contraceptive usage, as applicable.

5.6.8 Physical Examination

A full physical examination will be performed at the Screening/Baseline (Day 1) visit. Thereafter a targeted physical exam will conducted each day while the patient is hospitalized. Abnormal physical examination findings will be reported on the medical history or adverse event CRFs. Height and body weight at baseline will be summarized.

5.6.9 Vital Signs

Vital signs will be taken at the Screening/Baseline (Day- 1/Day 1) visit and prior to and after every dose of study drug. Vital signs will be summarized at each time point as well as change from baseline to each time point.

5.6.10 Clinical Safety Laboratory Assessments

Local laboratories will be used for all clinical laboratory assessments. Laboratory results will be classified according to NCI-CTCAE, Version 5.0. Laboratory results not corresponding to an NCI-CTCAE term will not be graded. Incidences of laboratory abnormalities will be summarized by worst grade on study. Laboratory values will be summarized descriptively at each time point as well as the change from baseline to each time point. Abnormal laboratory findings will be flagged in the data listings.

The investigator must review the laboratory reports, document this review, and record any clinically significant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those which are assessed as not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.

All laboratory tests with values considered abnormal and clinically significant during participation in the study or within 30 days after the last dose of study treatment should be repeated until the values return to normal, baseline or are no longer of concern. If such values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified and the sponsor notified.

5.7 Other Assessments

Study procedures are specified in the protocol. A study physician licensed to make medical diagnoses and listed will be responsible for all trial-related medical decisions.

- Physical examination: A symptom-directed (targeted) physical examination will be performed to evaluate for any possible adverse events.

- Clinical laboratory evaluations:
  - Fasting is not required before collection of laboratory samples.
  - Blood will be collected at the time points indicated in the protocol.
  - This testing will be performed in real time.

- ECGs; A 12-lead ECG will be collected at the Screening/Baseline (Day -1/Day 1) visit and at Days 5, 14, 21 and 28

Table 5 Venepuncture Volumes
(lead-in and randomized phases separately)

	Screening /Baseline		
Day +/- Window	**1**	**2 to 15**	**29 ± 3**
CBC, BMP, LFT, Coag, Imm	24.5 mL	24.5 mL each day x 14 days	24.5 mL
Blood for PK (9 x 6mL)	54 mL		
Antibodies and viral load	8 mL	16 mL (Days 7, 14)	8 mL
Total volume	86.5 mL	359 mL	32.5 mL
Total all study days			478 mL

5.7.1 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

If a physiologic parameter, e.g., vital signs, or laboratory value is outside of range, then the measurement may be repeated once if, in the judgment of the investigator, the abnormality is the result of an acute, short-term, rapidly reversible condition or was an error.

A physiologic parameter may also be repeated if there is a technical problem with the measurement caused by malfunctioning, or an inappropriate measuring device (i.e., inappropriate-sized BP cuff).

5.8 Pharmacokinetics

The pharmacokinetics and pharmacodynamics of TSC will be assessed in each subject concomitant with the first dose of TSC on Day 1, as follows:

Pre-dose (~ 2 min prior to injection)
1 min post end of injection (± 1 minute)
10 min (± 1 min)
30 min (± 1 min)
1.5 hours (± 2 min)
3 hours (± 5 min)
6 hours (within 10 min before TSC dosing)
24 hours (within 1 hour before TSC dosing)
48 hours (within 2 hours before TSC dosing)

Provided that an arterial line is established, blood oxygenation data by serial arterial blood gas measurement taken at baseline and before the first dose of TSC and at 1 minute, 10 minutes, 30 minutes, 1.5 hours, 3 hours, 6 hours, 24 hours, and 48 hours and the calculated PaO_2:FiO_2 ratios will form the means of a pharmacokinetic/pharmacodynamic assessment. Blood oxygenation data by recorded continuous pulse oximetry (SpO_2:FiO_2 ratio) will serve as an alternate source if PaO_2:FiO_2 data is unavailable.

The Safety Monitoring Committee (SMC) will be tasked with examining the resultant safety data after all up to six dose groups have completed 5 days treatment. The SMC will also have access to the blood oxygenation data from which a decision can be made about the optimum, safe and tolerable biologic dose of TSC. PK blood sampling data will not be available to assist in that decision but will appear later.

The SMC will recommend an acceptable TSC dose for the single-center, placebo-controlled, double-blind, randomized, safety and efficacy pilot.

5.8.1 Pharmacokinetic Procedures

Blood samples of approximately 6 mL will be collected at each timepoint for measurement of plasma concentrations of TSC.

Blood should be collected from the opposite arm from that used for the TSC dose. Although the preference is to continue to collect from the opposite arm, the injected arm may be used to collect the PK sample after the 30-minute time point if limited venous access occurs in the opposite arm.

The actual date and time will be recorded by 24-hour clock time noting the hour and minute of the end of the TSC infusion.

The same digital clock will be consistently used to record the actual hour and minute of the start of each blood specimen collection.

Each sodium heparinized vacutainer blood collection tube will be clearly labeled with the patient identifier number, date and time point of the specimen collection.

1. About 6 mL samples of blood will be collected in a sodium heparinized vacutainer tube and the blood will be centrifuged to separate plasma.
2. The plasma will be separated into two approximately equal volumes in separate tubes. The plasma should be approximately 1.5 mL in each tube.
3. Protocol-specific instructions will be provided to the clinical site in a lab manual.
4. The plasma samples will be frozen at -70°C. One aliquot will be shipped to the bioanalytical lab and the other stored at -70°C as a back-up.
5. Samples will be used to evaluate the PK of TSC.
6. Samples should be sent to the bioanalytical laboratory identified in the PK Laboratory Manual and per the instructions contained there.
7. PK samples that are remaining after all PK analyses have been completed, may be used for additional analysis. Participant confidentiality will be maintained.

5.8.2 Pharmacokinetic Analysis

At the conclusion of the trial all of the available pharmacokinetic data will be analyzed to:

- To evaluate the effect of intrinsic and extrinsic factors (e.g. age, gender, disease severity) on the PK of TSC
- For exploratory exposure-response purposes

The pharmacokinetic analysis will be described in an addendum to the clinical study report and will be presented separately from the main clinical study report (CSR).

6 KEY ASSESSMENTS

6.1 WHO Ordinal Severity Scale

The WHO 9-point Ordinal Scale assessment will be performed as a first assessment of the subject's clinical status each study day until hospital discharge. Each day, the worst score for the previous day will be recorded (i.e. on day 3, the score for day 2 is recorded as day 2). The ordinal scale is as follows.

Patient State	Descriptor	Score
Uninfected	No clinical or virological evidence of infection	0
Ambulatory	No limitation of activities	1
	Limitation of activities	2
Hospitalized Mild Disease	Hospitalized, no oxygen therapy	3
	Oxygen by mask or nasal prongs	4
Hospitalized Severe Disease	Non-invasive ventilation or high-flow oxygen	5
	Intubation and mechanical ventilation	6
	Ventilation + additional organ support – pressors, Renal Replacement Therapy (RRT), Extracorporeal Membrane Oxygenation (ECMO)	7
Dead	Death	8

The WHO Ordinal Severity Scale to be used in the 100-303 study is provided in Appendix 9.2.

6.2 Glasgow Coma Score (GCS)

The Glasgow Coma Score will be performed daily until hospital discharge and in accordance with the standards established by the Institute of Neurological Sciences NHS Greater Glasgow and Clyde. The Glasgow Coma Score will be calculated by addition of the total points selected under each of the three components including eye, verbal and motor. The score for eye opening, verbal response and best motor response will be recorded individualy as well as the total score, daily. The procedure as well as the template for recording the Glasgow Coma Scale is described at the web site www.glasgowcomascale.org. The Glasgow Coma Scale to be used in the 100-303 study is provided in Appendix 9.4.

6.3 Sequential Organ Failure Assessment (SOFA)

The Sequential Organ Failure Assessment (SOFA) Score is a mortality prediction score that is based on the degree of dysfunction of six organ systems. The score is calculated on admission and every 24 hours until hospital discharge using the worst parameters measured during the prior 24 hours. Individual system scores and total will be recorded. SaO2/FiO2 data may be substituted when PaO2/FiO2 data is unavailable. The SOFA score to be used in the 100-303 study is provided in Appendix 9.5.

6.4 Confirmation of SARS-CoV-2 Infection

This trial requires that subjects be confirmed and documented positive for SARS-Co-V-2 by RT-PCR. Nasopharynx, throat, lower respisratory, blood or stool samples are acceptable. Diagnosis of COVID-19 requires detection of SARS-CoV-2 RNA by reverse transcription polymerase chain reaction (RT-PCR). Detection of SARS-CoV-2 viral RNA is better from nasopharynx samples compared to throat samples. Lower respiratory samples may have better yield than upper respiratory samples. SARS-CoV-2 RNA has also been detected in stool and blood. Detection of SARS-CoV-2 RNA in blood may be a marker of severe illness. Viral RNA shedding may persist over longer periods among older people and those who had severe illness requiring hospitalization. (median range of viral shedding among hospitalized patients 12–20 days).

The incubation period for COVID-19 is thought to extend to 14 days, with a median time of 4-5 days from exposure to symptoms onset. One study reported that 97.5% of people with COVID-19 who develop symptoms will do so within 11.5 days of SARS-CoV-2 infection.

Following the laboratory confirmation of SARS-CoV-2 infection at the Screening/Baseline (Day -1/Day 1) visit, SARS-CoV-2 IgM and IgG antibodies and

SARS-CoV-2 viral load will be assessed at Days 7, 14, 21, and 28 (see Time and Events Schedule 9.1)

7 ADMINISTRATIVE AND SPECIAL PROCEDURES

7.1 Protocol Amendments

Any changes to the protocol will be made in the form of an amendment. Unless the changes are designed to eliminate an apparent immediate hazard to subjects, both the Sponsor and the governing IRB must grant approval of the amendment before any changes may be implemented in study conduct.

7.2 Investigator Responsibilities

The investigator has overall responsibility for the conduct of the study at his/her site. The investigator is responsible for ensuring that study staff has suitable qualifications, training, and authorization to perform any delegated study tasks. The investigator is responsible for the care of the subjects throughout this study. The investigator/authorized person will monitor the subjects for the occurrence of AEs throughout their participation in this study.

7.2.1 Compliance with Protocol

The investigator is responsible for the conduct of the study. No alterations or changes in this protocol will be permitted without written approval from the Sponsor and the IRB unless the amendment is necessary to reduce immediate risk to trial participants. The investigator should document and report to the Sponsor all deviations from the protocol.

7.2.2 Good Clinical Practice (GCP)

The clinical trial will be conducted and monitored in accordance with GCP standards.

7.2.2.1 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of trial subjects are protected, that the reported trial data are accurate, complete, and verifiable. Clinical Monitoring also ensures conduct of the trial is in compliance with the currently approved protocol/ amendment(s), ICH, GCP, applicable regulatory requirement(s) and sponsor requirements. Clinical monitoring will also verify that any critical study procedures are completed following specific instructions in the protocol.

Monitoring for this study will be performed by qualified and trained Clinical Research Associates so contracted by the sponsor. Details of clinical site monitoring are documented in a clinical monitoring plan (CMP). The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports. Monitoring visits will include, but are not limited to, review of regulatory files,

accountability records, CRFs, ICFs, medical and laboratory reports, record storage, training records, and protocol and GCP compliance. Site monitors will have access to each participating site, study personnel, and all study documentation according to the sponsor approved clinical monitoring plan. Study monitors will meet with site PI, sub-Investigators and Study Coordinators to discuss any problems and outstanding issues and will document site visit findings and discussions.

Study Coordinators will fill out a screening log for all study sites that documents all COVID-19 subjects admitted to the hospital meeting inclusion criteria for enrollment. For admitted COVID-19 patients meeting inclusion criteria who are not enrolled in the trial, the reason for exclusion will be recorded.

Monitoring will be performed by a Study Monitor, either via remote or in-person monitoring. They will review the case report forms and compare the data entered to the subject's medical record (source documents). If remote monitoring is necessary the Study Coordinator will collect the appropriate source documents, copy and redact all PHI information and then scan and send them to the Study Monitor for review. Any inadequacies or errors will be reviewed with the PI at the site. Subsequently, on-site case report form monitoring visits (if in-person visits are cleared by the hospital) will be performed at regular intervals as appropriate. Otherwise remote monitoring procedures will continue.

Site investigators or Study Coordinators will inform the sponsor of every subject enrollment on the same or next business day, by electronic data entry, email, or fax.

7.2.3 Source Documentation

Source data are all information in original records (and certified copies of original records) of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH, GCP, regulatory, and institutional requirements. Data recorded in the CRF derived from source documents should be consistent with the data recorded on the source documents.

Interview of subjects is sufficient for obtaining medical history. Solicitation of medical records from the subject's primary care provider is not required.

7.2.3.1 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, any process that is noted in the protocol or GCP requirements or any critical study procedures with specific instructions in ancillary documents referenced in the protocol.

The noncompliance may be either on the part of the subject, the investigator, or the study site staff. Following a deviation(s), corrective actions should be developed by the site and implemented promptly. All individual protocol deviations will be addressed in subject study records.

It is the responsibility of the site PI and personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations must be promptly reported per the protocol deviation reporting procedures. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site PI and personnel are responsible for knowing and adhering to their IRB requirements. A completed copy of the Protocol Deviation Form must be maintained in the Regulatory File, as well as in the subject's chart if the deviation is subject specific.

7.2.4 Institutional Review Board Review and Approval

It is the responsibility of the investigator to communicate with the IRB. The IRB will be functioning in accordance with current regulations. A copy of the IRB's unconditional written approval of the protocol and the informed consent will be obtained and provided prior to the initial supply of test article and start of the trial. All protocol amendments will be submitted to the IRB and approval sought prior to implementation, unless the amendment is necessary to reduce immediate risk to trial participants. The IRB will be informed of any new safety information as it becomes available. The investigator will provide reports to the IRB as requested, but at least annually, and after the trial is complete.

7.2.5 Informed Consent

The investigator is responsible for ensuring that a current informed consent is obtained from each subject or the subject's LAR. Subjects will be informed in simple terms and all questions answered about the objectives, procedures, and risks of study participation both verbally and in writing in accordance with current regulations. A signed, dated, IRB-approved written informed consent form will be obtained prior to any study-specific procedure.

7.3 Quality Assurance Audits and Regulatory Inspections

The Sponsor, its designee, or a regulatory authority may audit the study. Study-related documents must be made available for auditing purposes.

The investigator is advised to contact the Sponsor immediately if a regulator contacts the site inquiring or requesting to inspect and/or audit the investigative study site and/or this clinical trial.

7.4 Disclosure and Confidentiality

By signing the protocol, the investigator agrees to keep all information provided by the Sponsor or its designee in strict confidence and to request similar confidentiality from his/her staff and the IRB. Study documents provided by the Sponsor (protocols, investigators' brochure, CRFs/eCRFs, and other material) will be stored appropriately to ensure their confidentiality. Data generated by this study will be considered confidential by the investigator except to the extent that is included in a publication as provided in the investigator's respective contractual agreements. A description of this clinical trial will be available on http://www.ClinicalTrials.gov.

7.5 Changes in Study Personnel

If there is a change of any personnel listed on the FDA form 1572, a new form reflecting the change must be completed and forwarded to the Sponsor or its designee including, when applicable, any new staff member's signed and dated curriculum vitae, current medical license (as appropriate), and signed financial disclosure statement.

7.6 Study Record Retention

Study related records, including the regulatory file, test article accountability records, consent forms, subject source documents and electronic records should be maintained for a period of 2 years following the date a marketing application is approved for the investigational product for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and the regulatory authority is notified. These documents should be retained for a longer period, however, if required by local policies or regulations. No records will be destroyed without the written consent of the sponsor. Consent forms with specimen retention linked to identifiable specimens will be maintained for as long as the specimens remain in identifiable format, and a minimum of three years after use of the identifiable specimens.

7.7 Publication and Data Sharing Policy

The investigators will remain blinded (randomized phase) to any results and the study data will only be released if the trial were either stopped on the basis of a recommendation from the DSMB or had reached its targeted number of endpoints or participant follow-up

Reporting and publication processes will follow applicable laws and guidelines and insure that the design and results of the trial are reported in an accurate and complete manner. Author lists and contributorship statements will accurately reflect all substantial intellectual contributions to the research and be in accordance with the policies associated with the journals where published.

8 REFERENCES

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9 APPENDICES

9.1 Schedule of Events (applies to the lead-in AND the randomized pilot)

Lead In		**PK Collection Intervals**										**Day 2 through Day 15, and 29 (and additional days when hospitalized)**	**Day 60 (± 10 days)**
	Screen/ Baseline/ Day -1 /Day 1	**~2 min Pre Study Drug**	**Study Drug Admin (Day 1)**	**1 min**	**10 min**	**30 min**	**1.5 Hr**	**3 Hr**	**6 Hr**	**24 Hr**	**48 Hr**		
Informed Consent	X												
Inclusion/Exclusion criteria	X												
SARS-CoV-2 test	X[a]											Days 7,14,21,28	
Demographics/Medical history	X												
Physical Examination	X[b]											Daily	
Height(cm)	X												
Body Weight (Kg)	X												
Vital signs	X[c]	X										Daily, and prior to and after every dose	
12-lead ECG	X											Days 5,14,21,28	
Supplemental O^2(L/min)	X											Daily	
SpO2 (record pulse oximetry) and SpO2/FiO2 ratio	X[d]	X		X	X	X	X	X	X	X	X	Daily	
Blood sampling for lab tests	X[e]											Daily	
Blood pregnancy test	X												
Urinalysis	X											Daily	
WHO ordinal scale	X											Daily	
Chest Imaging	X												
Confirm eligibility	X												
Assignment for Lead-In	X												
Administer Study Drug (TSC) **			X**									TSC **q 6h	
Blood specimen collection for PK		X		X	X	X	X	X	X*	X*	X*		
Concomitant Medications / Therapies	X											Daily	
AKIN Classification[f]												Daily	
Glasgow Coma Score	X											Daily	
SOFA	X											Daily	
Serious Adverse Event/Adverse Events												Daily[g]	X

Randomized Pilot													
	Screen/ Baseline	**~2 min Pre Study Drug**	**Study Drug Admin**	**1 min**	**10 min**	**30 min**	**1.5 Hr**	**3 Hr**	**6 Hr**	**24 Hr**	**48 Hr**	**Day 2 through Day 15, and 29 (and additional days when hospitalized)**	**Day 60 + 10 days**
Informed Consent	X												
Inclusion/Exclusion criteria	X												
SARS-CoV-2 test	X[a]											Days 7,14,21,28	
Demographics/Medical history	X												
Physical Examination[b]	X											Daily	
Height(cm)	X												
Body Weight (Kg)	X												
Vital signs	X[c]	X										Daily, and prior to and after every dose	
12-lead ECG	X											Days 5,14,21,28	
Supplemental O2(L/min)	X											Daily	
SpO2(record pulse oximetry)and SpO2/FiO2 ratio	X[d]	X		X	X	X	X	X	X	X	X	Daily	
Blood sampling for lab tests	X[e]											Daily	
Blood pregnancy test	X												
Urinalysis	X											Daily	
WHO ordinal scale	X											Daily	
Chest Imaging	X												
Confirm eligibility	X												
Assignment for Lead-In	X												
Administer Study Drug TSC or Placebo **			X**									TSC or Placebo**q 6h	
Blood specimen collection for PK		X		X	X	X	X	X	X*	X*	X*		
Concomitant Medications / Therapies	X											Daily	
AKIN Classification[f]												Daily	
Glasgow Coma Score	X											Daily	
SOFA	X											Daily	
Serious Adverse Event /Adverse Events												Daily[g]	X

* PK blood sample at 6, 24, and 48 hours must precede the administration of study drug

** Study drug (TSC in the Lead-In phase and TSC or placebo in the randomized phase) will be administered every 6h starting on Day 1 and up to 15 days (a minimum of 5 days).

[a.] Following the laboratory confirmation of SARS-CoV-2 infection at the Screening/Baseline (Day -1/ Day 1) visit, SARS-CoV-2 IgM and IgG antibodies and SARS-CoV-2 viral load will be assessed at Days 7, 14, 21, and 28

[b.] Full physical examination at screening/baseline visit and a targeted physical exam each day while the patient is hospitalized

[c.] Vital signs includes heart rate, blood pressure, respiratory rate, temperature.

[d.] PaO2 and PaO2/FiO2 ratio, will be recorded at the same timepoints, if ABG is monitored

[e.] Blood sampling for lab tests: hematology,chemistry, coagulation (PT/INR, a PTT, D-Dimer) and immunological assessments will be performed each day while the patient is hospitalized; serology and enzymes only at screening visit; (see section 9.6 Laboratory Parameters)

[f.] AKIN classification will be perform daily, except Screening/Baseline(Day -1/Day 1) visit.

[g] Adverse events to be collected from the start of study drug administration

9.2 WHO Ordinal Severity Scale

The WHO 9-point Ordinal Scale assessment will be performed as a first assessment of the subject's clinical status each study day until hospital discharge. Each day, the worst score for the previous day will be recorded (i.e. on day 3, the score for day 2 is recorded as day 2). The ordinal scale is as follows:

Patient State	Descriptor	Score
Uninfected	No clinical or virological evidence of infection	0
Ambulatory	No limitation of activities	1
	Limitation of activities	2
Hospitalized Mild Disease	Hospitalized, no oxygen therapy	3
	Oxygen by mask or nasal prongs	4
Hospitalized Severe Disease	Non-invasive ventilation or high-flow oxygen	5
	Intubation and mechanical ventilation	6
	Ventilation + additional organ support – pressors, RRT, ECMO	7
Dead	Death	8

9.3 Glasgow Coma Scale

GLASGOW COMA SCALE : Do it this way

GCS EYES VERBAL MOTOR

Institute of Neurological Sciences NHS Greater Glasgow and Clyde

CHECK	OBSERVE	STIMULATE	RATE
For factors Interfering with communication, ability to respond and other injuries	Eye opening , content of speech and movements of right and left sides	Sound: spoken or shouted request Physical: Pressure on finger tip, trapezius or supraorbital notch	Assign according to highest response observed

Eye opening

Criterion	Observed	Rating	Score
Open before stimulus	✔	Spontaneous	4
After spoken or shouted request	✔	To sound	3
After finger tip stimulus	✔	To pressure	2
No opening at any time, no interfering factor	✔	None	1
Closed by local factor	✔	Non testable	NT

Verbal response

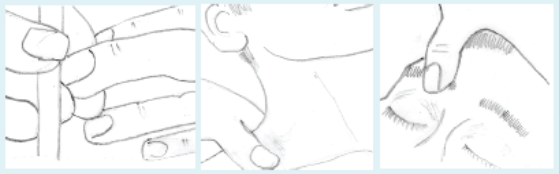
Criterion	Observed	Rating	Score
Correctly gives name, place and date	✔	Orientated	5
Not orientated but communication coherently	✔	Confused	4
Intelligible single words	✔	Words	3
Only moans / groans	✔	Sounds	2
No audible response, no interfering factor	✔	None	1
Factor interferring with communication	✔	Non testable	NT

Best motor response

Criterion	Observed	Rating	Score
Obey 2-part request	✔	Obeys commands	6
Brings hand above clavicle to stimulus on head neck	✔	Localising	5
Bends arm at elbow rapidly but features not predominantly abnormal	✔	Normal flexion	4
Bends arm at elbow, features clearly predominantly abnormal	✔	Abnormal flexion	3
Extends arm at elbow	✔	Extension	2
No movement in arms / legs, no interfering factor	✔	None	1
Paralysed or other limiting factor	✔	Non testable	NT

Sites For Physical Stimulation

Finger tip pressure | Trapezius Pinch | Supraorbital notch



Features of Flexion Responses

Modified with permission from Van Der Naalt 2004
Ned Tijdschr Geneeskd

Abnormal Flexion
Slow Sterotyped
Arm across chest
Forearm rotates
Thumb clenched
Leg extends

Normal flexion
Rapid
Variable
Arm away from body

For further information and video demonstration visit www.glasgowcomascale.org

9.4 SOFA Score

Respiratory system

PaO_2/FiO_2 [mmHg (kPa)] **SaO_2/FiO_2 [mmHg]** **reference: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2703722/**	**SOFA score**
≥ 400 (53.3); **SaO_2/FiO_2 [mmHg] > 301**	0
< 400 (53.3) **SaO_2/FiO_2 [mmHg] 221 - 301**	+1
< 300 (40) **SaO_2/FiO_2 [mmHg] 142 - 220**	+2
< 200 (26.7) **and** mechanically ventilated **SaO_2/FiO_2 [mmHg] 67 - 141**	+3
< 100 (13.3) **and** mechanically ventilated **SaO_2/FiO_2 [mmHg] <67**	+4

Nervous system

Glasgow coma scale	SOFA score
15	0
13–14	+1
10–12	+2
6–9	+3
< 6	+4

Cardiovascular system

Mean arterial pressure OR administration of vasopressors required	SOFA score
MAP ≥ 70 mmHg	0
MAP < 70 mmHg	+1
dopamine ≤ 5 µg/kg/min or dobutamine (any dose)	+2
dopamine > 5 µg/kg/min OR epinephrine ≤ 0.1 µg/kg/min OR norepinephrine ≤ 0.1 µg/kg/min	+3
dopamine > 15 µg/kg/min OR epinephrine > 0.1 µg/kg/min OR norepinephrine > 0.1 µg/kg/min	+4

Liver

Bilirubin (mg/dl) [µmol/L]	SOFA score
< 1.2 [< 20]	0
1.2–1.9 [20-32]	+1
2.0–5.9 [33-101]	+2
6.0–11.9 [102-204]	+3
> 12.0 [> 204]	+4

Coagulation

Platelets×10^3/µl	SOFA score
≥ 150	0
< 150	+1
< 100	+2
< 50	+3
< 20	+4

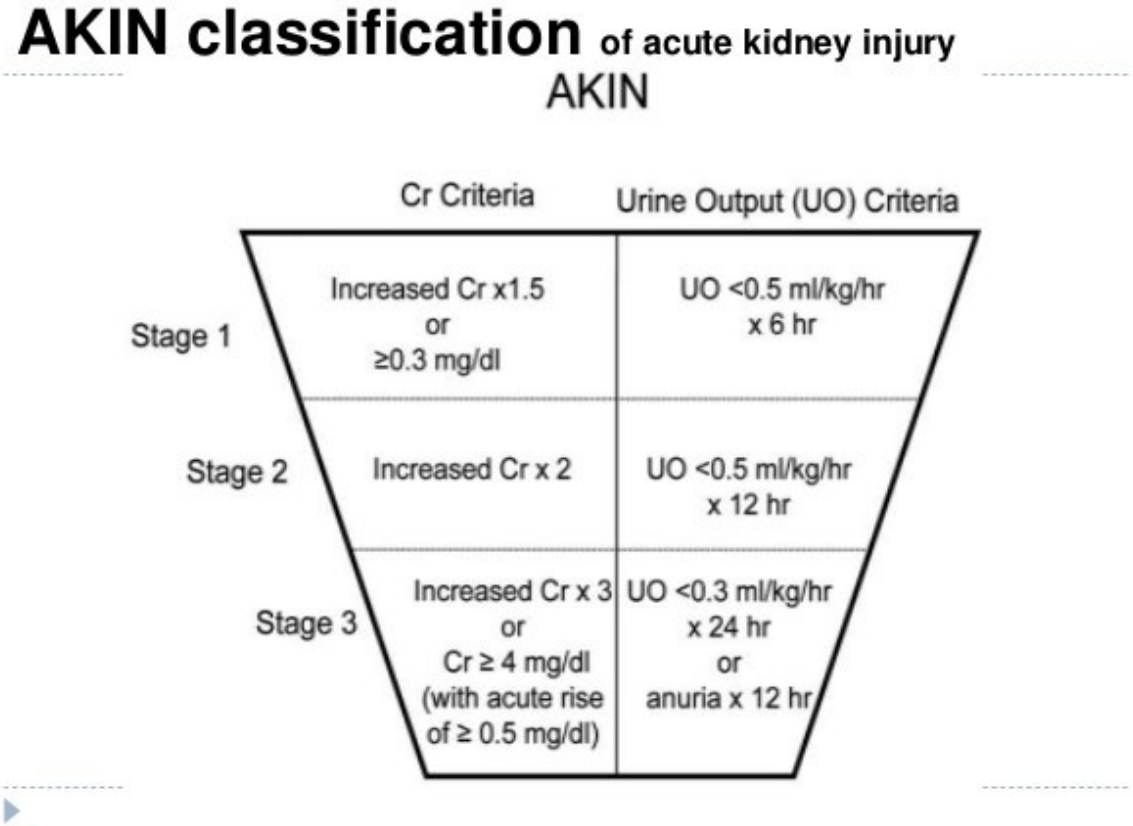
Kidneys

Creatinine (mg/dl) [µmol/L] (or urine output)	SOFA score
< 1.2 [< 110]	0
1.2–1.9 [110-170]	+1
2.0–3.4 [171-299]	+2
3.5–4.9 [300-440] (or < 500 ml/d)	+3
> 5.0 [> 440] (or < 200 ml/d)	+4
Total SOFA score	

9.5 Laboratory Parameters

rtPCR	Confirmation of SARS-CoV-2 infection, SARS-CoV-2 viral load, SARS-IgM, IgG antibodies
Haematology (CBC):	Erythrocytes*, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), haemoglobin, haematocrit, platelets*, leukocytes*, neutrophils, segmented neutrophile granulocytes, rod-shaped neutrophile granulocytes, eosinophils, basophils, lymphocytes, monocytes, large unidentifiable cells (LUC)
Serum chemistry (BMP):	alkaline phosphatase (AP), lipase, glutamic oxalacetic transaminase (GOT/ASAT), glutamic pyruvic transaminase (GPT/ALAT), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), glucose, total bilirubin, uric acid, iron, ferritin, sodium, potassium, chloride, calcium, creatinine, urea, total protein, albumin
Enzymes:	Creatine kinase (CK), creatine kinase muscle-brain (CK-MB), glutamate dehydrogenase (GLDH), troponin
Pregnancy test	blood pregnancy test in women of child bearing potential
Immunological parameters (Imm):	IL-6, IL-10, CRP, IFN-gamma, TNF-alpha
Coagulation:	prothrombin time (PT, Quick test), INR value (International Normalised Ratio), activated partial thromboplastin time (APTT), d-dimer
Serology:	markers of viral hepatitis (HBs-AG (hepatitis B virus surface antigen) and anti HCV-AB (anti hepatitis C virus antibodies)), HIV infection (anti-HIV-AB 1+2 (anti human immunodeficiency virus antibodies) including p24Ag)
Blood gas analysis	pO2, pCO2
Urine:	pH, specific gravity, semi quantitative analysis of leukocytes, protein, glucose, ketone bodies, bilirubin, nitrites, urobilinogen, blood and sediment,

9.6 AKIN Classification



9.7 Investigator Signature Page

DOCUMENT TYPE:	Clinical protocol
DOCUMENT NUMBER:	100-303
COMPOUND:	Trans Sodium Crocetinate
STUDY TITLE:	Open-label, pharmacokinetic, pharmacodynamic, ascending dose safety lead-in followed by a single-center, placebo-controlled, double-blind, adaptive, safety and efficacy, pilot study of Trans Sodium Crocetinate (TSC) in SARS-CoV-2 infected subjects
CLINICAL PHASE:	1b/2b
INDICATION:	Treatment of hypoxemia associated with respiratory SARS-CoV-2 infection
IND NUMBER:	EudraCT: 2020-002369-32
SPONSOR:	Diffusion Pharmaceuticals Inc. 1317 Carlton Avenue, Suite 200 Charlottesville, VA 22902
DOCUMENT VERSION/STATUS:	Version 7.0
DOCUMENT RELEASE/AMENDMENT DATE:	November 30, 2020

This acknowledges receipt of the above protocol.

Investigator Name: _____ (Print)

Investigator Signature: _____ Signature Date: _____