CLINICAL TRIAL ON THE SAFETY AND EFFICACY OF REGADENOSON FOR MODERATE TO SEVERE COVID-19 ADULT PATIENTS.

IND149635 Application

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Volume 1: Detailed Draft Protocol Synopsis

Title: Clinical Trial on the Safety and Efficacy of Regadenoson for Moderate to Severe COVID-19 Adult Patients.

Short Title: Regadenoson Trial on COVID-19 Patients

Disease: Moderate to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), aka Coronavirus Disease 2019 (COVID-19).

Target Population: Moderate to Severe adult patients with COVID-19.

Phase of Development: Phase 2

Primary Objectives:

The primary objective of this study is to evaluate the safety, tolerability, and efficacy of Regadenoson in moderate to severe adults with COVID-19. **Moderate illness** is defined as individuals who have evidence of lower respiratory disease by clinical assessment or imaging and a saturation of oxygen (SpO2) >93% on room air at sea level. **Severe Illness** is defined as individuals who have respiratory frequency >30 breaths per minute, SpO2 $\leqslant$ 93% on room air at sea level, ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO2/FiO2) <300, or lung infiltrates >50%

Primary Endpoint:

Proportion of patients alive and free of respiratory failure through the 30-day trial. Respiratory failure is defined based on resource utilization requiring at least 1 of the following modalities:

a. Endotracheal intubation and mechanical ventilation

b. Oxygen delivered by high-flow nasal cannula (heated, humidified, oxygen delivered via reinforced nasal cannula at flow rates >20L/min with fraction of delivered oxygen ≥0.5)

c. Noninvasive positive pressure ventilation or CPAP

d. ECMO

Secondary Endpoints:

1. A 2-point improvement from baseline using the National Institutes of Allergy and Infectious Diseases (NIAID) 8-point ordinal scale for COVID-19 (see APPENDIX A). All-cause mortality at Day 30.

2. Proportion of participants with at least 2-point improvement at Day 15 and Day 30 using the NIAID 8-point ordinal scale.

3. ICU length of stay

4. Hospital length of stay

In addition: The data on 1) Physical Exam; 2) Clinical Laboratory Values 3) Arterial Blood Gas; and 4) Vital Signs, including heart rate, blood pressure and oxygen saturation, at the different time-points prior, during and post the study drug administration will be collected to evaluate the effects of Regadenoson on these patients. The data on the ventilator parameters, the length of stay on ventilator, and the length of stay in ICU and/or hospital will also be collected during the post follow up to evaluate the efficacy of Regadenoson in these moderate to severe Covid-19 patients.

Due to the mixture of the moderate and severe COVID-19 patients with various baseline severity levels, we will record pertinent patient information, such as age, sex, race, symptoms, signs, saturation of oxygen, medications, treatments, days of illness, comorbidities, chest X-ray imaging, and baseline severity (such as, on oxygen, on ventilator, on ECMO etc.) prior to Regadenoson infusion. We will also record whether each participated patient is treated with additional COVID-19 therapies. When we analyze the effects of Regadenoson on COVID-19, we can subgroup these patients and conduct primary efficacy analysis and propose appropriate methods of covariate adjustment.

Study Design:

This is a single center, randomized, phase 2 study to evaluate the safety, tolerability, and efficacy of Regadenoson for the treatment of moderate to severe adults with COVID-19. Patients will be followed up for 30 days from the start of study drug administration.

Estimated sample size: 40

Estimated accrual period: 18 months

Allocation: Randomized

Masking: Double-blind, placebo-controlled

Study Duration for each subject: 30 days

Study Treatments:

Regadenoson will be given intravenously as a 5μg/kg loading dose (a dose less than 400μg) over 30 minutes, followed by a continuous slow infusion (1.44μg/kg/hour) with

the use of a pediatric infusion pump for 6 hours. The saline will be used as placebo controls.

Eligibility Criteria

Inclusion Criteria:

1. 18 years of age or older
2. Laboratory-confirmed COVID-19+ by RT-PCR
3. Moderate to Severe COVID-19 patients according to FDA's COVID-19 treatment guideline on Management of Persons with COVID-19:
4. Verbal or written informed consent must be obtained from patient or LAR (Legally Authorized Representative) before any study procedure is performed.

Exclusion Criteria:

1. Pregnant or breastfeeding women
2. Subject has signs or symptoms of acute myocardial ischemia or has required a cardiac intervention within the past 90 days.
3. Subject with chronic cardiac conditions including non-vascularized coronary artery disease, heart failure, valvular disease, and cardiomyopathy.
4. Subject has an acute or chronic cardiac arrhythmia such as Sinoatrial (SA) or Atrioventricular (AV) Nodal Block/dysfunction, bradycardia, a permanent pacemaker, an internal defibrillator, or Atrial Fibrillation/Atrial Flutter requiring treatment or observation.
5. Subject has history of hypotension (sustained systolic blood pressure < 80 mmHg)
6. Subject has a history of severe hypertension not adequately controlled with anti-hypertensive medications (Systolic blood pressure ≥ 200 mmHg and/or Diastolic blood pressure ≥ 110 mmHg)
7. Subject has moderate or severe renal impairment as well as subject with end stage renal disease (defined as GFR < 60 mL/min/1.73 m2)
8. Subject has a history of clinically overt stroke within the past 3 years
9. Subject with a history of seizure disorder
10. Subject has pre-existing respiratory conditions, most notably asthma or chronic obstructive pulmonary disease or emphysema.
11. Subject with respiratory failure for greater than 72 hours. Defined as the continuous use of mechanical ventilation, HFNC >20L/min, CPAP, and/or ECMO. (CPAP use due to obstructive sleep apnea is acceptable).
12. Subject who is being treated with chronic anti-coagulation or anti-platelet therapy (prophylactic aspirin is acceptable)
13. Subject who is receiving or has received within 30 days any other investigational agents as part of a research study.

14. Subject who has received theophylline or aminophylline within 12 hours of study dosing
15. Subjects who are currently taking or have taken Persantine and/or Aggrenox within 5 days
16. Subjects who have any other clinical conditions that in the opinion of the investigator would make the subject unsuitable for the study.

Safety Assessments:

This study will be conducted in 2 cohorts. Our ongoing phase 1 trial with slow infusion of Regadenoson (1.44micrograms/kg/hour) in lung transplant patients study showed no obvious dose limiting toxicity. Safety assessments (vital signs, Pulse oximetry and Arterial Blood Gas, laboratory studies, electrocardiograms, etc.) will be performed on a schedule commensurate with the level of care and the identified potential risk of the study drug during Regadenoson infusion and 12 hours post infusion. Given the cardiac risk with Regadenoson, we will continue cardiac monitoring during the infusion of Regadenoson, and EKGs daily or as needed, and cardiac markers only if cardiac symptoms occur. To monitor potential platelet aggregation effects, labs (PT, PTT, INR, platelets, D-Dimer, and thromboelastography (TEG)) will be drawn prior to the infusion and on post infusion day 1. Patients who receive anti-clotting medications during Regadenoson infusion or within 6 hours of the end of infusion will be checked for clotting time and if necessary, anti-coagulant administration will be reduced or stopped. In addition, we will have a pregnancy test for patients who may have potential risk with a urine sample before study drug administration. Patients with a negative result will be enrolled. We will ensure that protections for women of childbearing potential align with International Council on Harmonization M3 R2 guidelines for highly effective methods of contraception.

In cohort 1, we will enroll patients to test the safety, tolerability and toxicity in moderate to severe COVID-19 patients. Initial subjects in cohort 1 will receive a loading dose of 5.0 µg/kg (not to exceed 400 µg) over 30 minutes followed by a low dose infusion of 1.44 µg/kg/hr. Toxicity of Regadenoson treatment will be graded by using the Common Terminology Criteria for Adverse Events (CTCAE) Criteria version 4.03 in this trial. If two dose limiting toxicities occur at a loading dose of 5.0 µg/kg then the loading dose will be halved to 2.5 µg/kg and enrollment will continue. If two dose limiting toxicities occur at a loading dose of 2.5 µg/kg then the Data and Safety Monitoring Board (DSMB) will convene to determine if the study should continue and omitting the loading dose may be considered. Alternatively, the DSMB will convene after 5 out of 5 patients or 5 out of 6 patients tolerate a given loading dose to make a determination on moving forward to cohort 2. Otherwise, the trial will be terminated. (please see the details in the Detailed DSMB Plan section)

The sickle cell trial and our phase 1 trial in lung transplant patients did not show any renal/hepatic toxicity. Because COVID-19 itself may cause renal injury, we will closely monitor the renal and hepatic function of these patients per their standard of care. To assess safety of each subject, we will tabulate sequentially the number of participants

who unexpectedly experience serious cardiovascular side effects or persistent (> 30 min) intolerable side effects. If at any point in the accrual, the posterior probability exceeds 90% that the risk of at least one serious side effect for participants in the Regadenoson arm is more than 10 percentage points greater than in the standard care arm, we will stop the trial.

Adverse Event (AE) Recording and Reporting:

All adverse events will be collected starting at time of consent through POD 30. Adverse events will be recorded on a case report form and will be assessed for seriousness, severity, attribution and expectedness by the site Principal Investigator. **A serious cardiovascular side effect is defined as a sustained change (> 5 minutes) in systolic blood pressure >30 mm Hg or systolic blood pressure <75 mm Hg, tachycardia >130 bpm, or bradycardia <40 bpm.**

The COVID-19 patient population has increased risk for vascular events and these events are monitored as part of standard of care. Standard of care will be followed, and diagnostics may include lab values and imaging studies. Additionally, PT, PTT, INR, platelets, and TEG labs will be drawn before and after the infusion and the study team will monitor the study participants by observing their clinical progress/course for any such clinically significant vascular events that occur during the study period. These events will report them as adverse events as part of good clinical research practice.

Reporting to the UMB HSR-IRB and DSMB:

The Principal Investigator or designee is responsible for reporting SAEs and unanticipated problems to the site IRB according to the participating site institutional guidelines. (Please see the revised additional information form details)

Reporting to the FDA:

The PI for the study is responsible for providing safety updates to the FDA per the guidelines. The reporting times refer to the time the study team received knowledge of the AE. Please see the attached Table 1 for details.

Sample Size and Statistical Analysis:

The study aims to maximize knowledge on the use of Regadenoson to treat COVID-19 in order to plan for a larger trial. Safety analysis will be prioritized to allow for the expansion to a multi-site trial. The study will enroll moderate and severely ill subjects to maximize enrollment at the single enrolling institution. Severity of illness and baseline respiratory failure will be delineated within the analysis to account for the variability in the patient population. This trial will provide estimates of outcome variability in the primary and secondary outcomes that will be valuable for planning a larger randomized trial. Additionally, in planning for a larger trial, exploratory analyses will adjust estimates of treatment differences by known risk factors. Although we recognize that these

analyses will be limited by the number of observed events, they will be useful for decisions on eligibility criteria or stratification factors in the larger trial. The analyses will be carried out on data from all randomized participants.

We will enroll 40 moderate to severe COVID-19 patients, which is defined in the inclusion criteria above. Following the recommendation of the FDA review of our IND application, the trial focuses on providing estimates of outcome variability in the primary and secondary outcomes that will be valuable for planning a larger randomized trial. Additionally, in planning for a larger trial, exploratory analyses will adjust estimates of treatment differences by known risk factors. Due to the mixture of the moderate to severe COVID-19 patients with various baseline severity levels, we will record information regarding each patient, such as age, sex, race, symptoms, vital signs, saturation of oxygen, medications, treatments, days of illness, comorbidities, chest X-ray imaging, and baseline severity (such as, whether on oxygen, on ventilator, or on ECMO etc.) prior to Regadenoson infusion. When we analyze the effects of Regadenoson on COVID-19 patients, we can do exploratory analyses within subgroups of these patients and conduct primary efficacy analysis and propose appropriate methods of covariate adjustment. The prevention and treatment of respiratory failure will be analyzed within appropriate subgroups. For each subject, we will clearly document the reason for hospital admission, document the standard of care followed for each subject, and if care decisions are made based on resource limitation.

The primary outcome is the proportion of patients alive and free of respiratory failure through the 30-day trial. The total sample size of 40 participants will be randomized equally between the usual care and Regadenoson groups, using a randomly permuted block design, with random block sizes of 2 and 4. This plan will be generated by the study statistician. Within each group, the primary outcome proportion can be estimated with a standard error of no more than 11 percentage points; the difference between the groups can be estimated with a standard error that is no more than 16 percentage points. These calculations are based on the most conservative estimate in terms of standard error, using a proportion of 50% in each group. If, as expected, the proportions in each group are greater, such as 80% in usual care and 90% in the Regadenoson group, the difference can be estimated with a standard error of 11 percentage points. While these are relatively large standard errors, they are adequate to meet the objective suggested by the IND review, providing estimates of outcome proportions to be used in a larger trial.

To assess safety, the DSMB statistician will tabulate sequentially the proportion of participants in each group who unexpectedly experience serious cardiovascular side effects or persistent (> 30 min) intolerable side effects, based on clinical experience with Regadenoson. Intolerable side effects include headache or dyspnea. If the lower bound of a one-sided 80% confidence interval for the difference in proportions exceeds 10%, we will pause the trial, examine the adverse event profiles, and consider modifying dosing. With this rule, if the true proportion of patients who experience serious side effects is 20% in each group, there is a 16.5% chance that the trial will be paused; if the

true proportion with Regadenoson is 40%, there is a 58% chance the study will be paused.

To plan for a future study, we will compute a 90% one-sided confidence interval (CI) for the difference in failure free survival (FFS) proportions at 30 days. The CI will use standard formulas for estimates and standard errors for the Kaplan-Meier estimate of the FFS distribution at a specific point in time (Klein et al, 2007). Censoring on the primary outcome will be unlikely since we will have follow-up at 30 days for FFS. For the secondary outcome of time to improvement as well as for the length of stay outcomes, survival time will be used as a competing risk (see Brock et al, 2011 and Harhay et al, 2019). Both of these papers recommend the use of cumulative incidence curves (CIC) for competing risks, as described by Kleinbaum and Klein, 2012). Group comparisons of the specific CIC can be made with the test of Grey (1988); with covariates, the method of Fine and Gray (1999) can be used to model the CIC.

Study Centers:

University of Maryland Medical Center, Baltimore, MD.

References:

Brock GN, Barnes C, Ramirez JA, Myers J. How to handle mortality when investigating length of hospital stay and time to clinical stability. BMC Med Res Methodol. 2011; 11:144. Published 2011 Oct 26. doi:10.1186/1471-2288-11-144. PMC3269825

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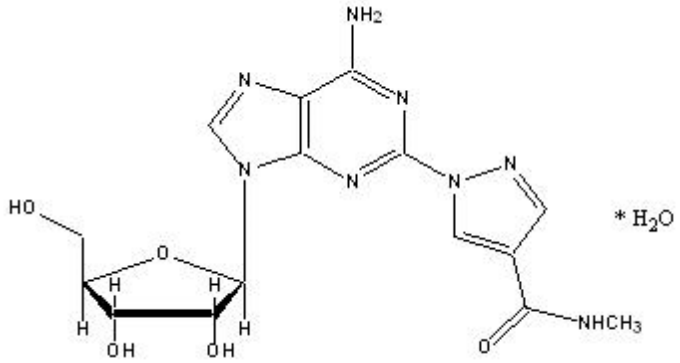
Volume 2: Detailed Information for IND 149635 Application

Drug Name: CVT3146, Generic Name: Regadenoson Injection, Trade Name: Lexiscan

A Description of The drug Substance: Regadenoson is an A2A adenosine receptor agonist that is a coronary vasodilator [see Clinical Pharmacology (12.1)]. Regadenoson is chemically described as adenosine, 2-[4- [(methylamino)carbonyl]-1H-pyrazol-1-yl]-, monohydrate. Its structural formula is:



The molecular formula for Regadenoson is C15H18N8O5 • H2O and its molecular weight is 408.37. LEXISCAN is a sterile, nonpyrogenic solution for intravenous injection. The solution is clear and colorless.

The Active Pharmaceutical Ingredient (API) and Formulation for Clinical Study: Each 1 mL in the 5 mL pre-filled syringe contains 0.084 mg of Regadenoson monohydrate, corresponding to 0.08 mg Regadenoson on an anhydrous basis, 10.9 mg dibasic sodium phosphate dihydrate or 8.7 mg dibasic sodium phosphate anhydrous, 5.4 mg monobasic sodium phosphate monohydrate, 150 mg propylene glycol, 1 mg edetate disodium dihydrate, and Water for Injection, with pH between 6.3 and 7.7.

Dosage Form, Formulation and Route of Administration: liquid, 400 micrograms/vial. The recommended dose of LEXISCAN is 5 mL (0.4 mg Regadenoson) administered as an intravenous injection within 10 seconds.

The Known or Suspected Mechanism of Action of The Drug: Regadenoson is a low affinity agonist (Ki ≈ 1.3 μM) for the A2A adenosine receptor, with at least10-fold lower affinity for the A1 adenosine receptor (Ki > 16.5 μM), and weak, if any, affinity for the A2B and A3 adenosine receptors. Activation of the A2A adenosine receptor by Regadenoson produces coronary vasodilation and increases coronary blood flow (CBF).

A Summary of The Available Pharmacokinetic Information: In healthy subjects, the Regadenoson plasma concentration-time profile is multi-exponential in nature and best characterized by 3-compartment model. The maximal plasma concentration of Regadenoson is achieved within 1 to 4 minutes after injection of LEXISCAN and parallels the onset of the pharmacodynamic response. The half-life of this initial phase is approximately 2 to 4 minutes. An intermediate phase follows, with a half-life on average of 30 minutes coinciding with loss of the pharmacodynamic effect. The terminal

phase consists of a decline in plasma concentration with a half-life of approximately 2 hours [*see Clinical Pharmacology (12.2)*]. Within the dose range of 0.3–20 µg/kg in healthy subjects, clearance, terminal half-life or volume of distribution do not appear dependent upon the dose.

According to the Lexiscan label description, in a randomized, placebo-controlled trial of 504 patients (LEXISCAN n=334 and placebo n=170) with a diagnosis or risk factors for coronary artery disease and NKFK/DOQI Stage III or IV renal impairment (defined as GFR 15-59 mL/min/1.73 m2), no serious adverse events were reported through the 24-hour follow-up period.

Since the dose we will use in this trial is lower than the MPI trial, we anticipate that Regadenoson infusion may not impair renal function. Our phase I trial in lung transplant patients did not show any renal/hepatic toxicity. However, because COVID-19 itself may cause renal injury, plus standard of care treatments may also affect kidney function, these factors may impair renal or hepatic function.

The Renal and Hepatic Function Criteria:

The stages of Chronic Kidney Disease are classified as follows:

- Stage 1: Kidney damage with normal or increased GFR (>90 mL/min/1.73 m2)
- Stage 2: Mild reduction in GFR (60-89 mL/min/1.73 m2)
- Stage 3a: Moderate reduction in GFR (45-59 mL/min/1.73 m2)
- Stage 3b: Moderate reduction in GFR (30-44 mL/min/1.73 m2)
- Stage 4: Severe reduction in GFR (15-29 mL/min/1.73 m2)
- Stage 5: Kidney failure (GFR < 15 mL/min/1.73 m2 or dialysis)

or

- Acute kidney injury Grade scales:
- Grade 1: Creatinine level increase of >0.3 mg/dL; creatinine 1.5 - 2.0 x above baseline
- Grade 2: Creatinine 2 - 3 x above baseline
- Grade 3: Creatinine >3 x baseline or >4.0 mg/dL; hospitalization indicated
- Grade 4: Life-threatening consequences; dialysis indicated
- Grade 5: Death

The Child- Pugh classification is a means of assessing the severity of liver injury.

Score	1	2	3
bilirubin (micromol/l)	<34	34-50	>50

albumin (g/l)	>35	28-35	<28
PT (s prolonged)	<4	4-6	>6
encephalopathy	none	mild	marked
ascites	none	mild	marked

If there is primary biliary cirrhosis or sclerosing cholangitis, then bilirubin is classified as <68=1; 68-170=2; >170=3.
The individual scores are summed and then grouped as:

- <7 = A
- 7-9 = B
- >9 = C

If any participated patients developed renal or liver impairments as described above (Kidney: Stages 3 and 4 or Grade Scale>3, or Liver summed scores>B), we will stop the infusion of Regadenoson and take the following steps: All study activities will take place within the hospital setting with available physician, nurse, and facility support. Should a participant suffer any injury as a result of taking part in this research study, all needed facilities, emergency treatment and professional services will be available to them, just as they are to the community in general. Participants will be informed that there are no arrangements to provide free treatment of the injury or any other type of payment for the injury. These will be billed to the participant or his/her insurance and this information will be reviewed during the informed consent process. Any injuries will be reported to the clinical site PI. The PI contact information will be provided to the participant within the informed consent. The participant/LAR will also be informed that he/she (they) will not give up any legal rights by providing verbal consent or signing the consent form.

Data and Literature Supporting The Proposed Use of The Drug in COVID-19:

Activation of adenosine 2A receptors inhibits inflammation by directly targeting multiple inflammatory/immune cells. It is well established that the activity of inflammatory cells, including invariant natural killer T (iNKT), NKs, macrophages, DCs, monocytes, T-cells, platelets, and neutrophils, is inhibited by A2AR activation, resulting in reduced proinflammatory cytokines and decreased endothelial adhesion molecule expression during acute lung injury in different animal models (mouse/rat acute lung injury models, pre-clinical porcine lung transplantation model) [1-4]. A2AR agonist inhibited inflammatory/immune cells (such as iNKT cells and neutrophil) activation and infiltration into the lungs, Therefore, A2A agonists reduce acute lung injury through inhibiting proinflammatory cytokine releasing (including IL-6, TNFα, IFN-γ. etc) [5-9]. Our unpublished data showed A2A agonists also have been found to reduce lethality to cytokine storm associated with bacterial sepsis (Figures 1 & 2). Our phase I trial in lung transplant patients showed that low dosage, slow infusion of Regadenoson inhibited IL-6 production when compared with a patient without Regadenoson treatment (Figure 3). Regadenoson also decreased matrix metalloproteinase -9 (MMP-9) levels, but not MMP-2 level, when compared with control patient (Figure 4). We demonstrated

that MMP-9, but not MMP-2, was significantly elevated in the acute injured lungs when compared with sham operated lungs [10]. The recent publications showed that MMP-9 plays an important role in acute lung injury by increasing vascular permeability, promoting inflammatory cell infiltrating into the lungs and positively associating with IL-6, TNFα, and IL-8 [11-14]. In addition, our proteomic analysis data showed that Regadenoson treated lung transplantation patients increased tissue inhibitor of metalloproteinase -1 (TIMP-1) when compared with control patient at the same time points (Figure 5). TIMP-1 is an endogenous inhibitor of MMP-9. Therefore, Regadenoson not only down-regulated MMP-9, but also up-regulated it's inhibitor. The phase II trials of adult sickle cell patients showed that Regadenoson inhibited iNKT cells, which is a key immune cell involved in both innate and adaptive immune response [15]. The newly published data showed that respiratory failure from acute respiratory distress syndrome (ARDS) is the leading cause of mortality for severe COVID-19 patients. The severe COVID-19 patients might have a cytokine storm syndrome, as indicated by elevated levels of various proinflammatory cytokines, including IL-6, TNFα, IFN-γ and others [16-29]. These life-threatening cytokines impair pulmonary function. Therefore, there is an urgent need to identify and treat hyperinflammation using existing, approved therapies with proven safety profiles to address the immediate need to reduce the high 2-4% mortality of severe COVID-19 patients. We propose that Regadenoson be used for the treatment of patients with virally induced lung hyperinflammation. We predict that A2AR agonist treatment will decrease the pulmonary cytokine storm evoked by viruses, reduce lung inflammation and enhance pulmonary function. Based on our current phase 1 trial data and published evidence, we expect that Redadenoson may save lives of severe COVID-19 patients through several different mechanisms (inhibiting proinflammatory cytokine (IL-6) and MMP-9 and increasing TIMP-1).

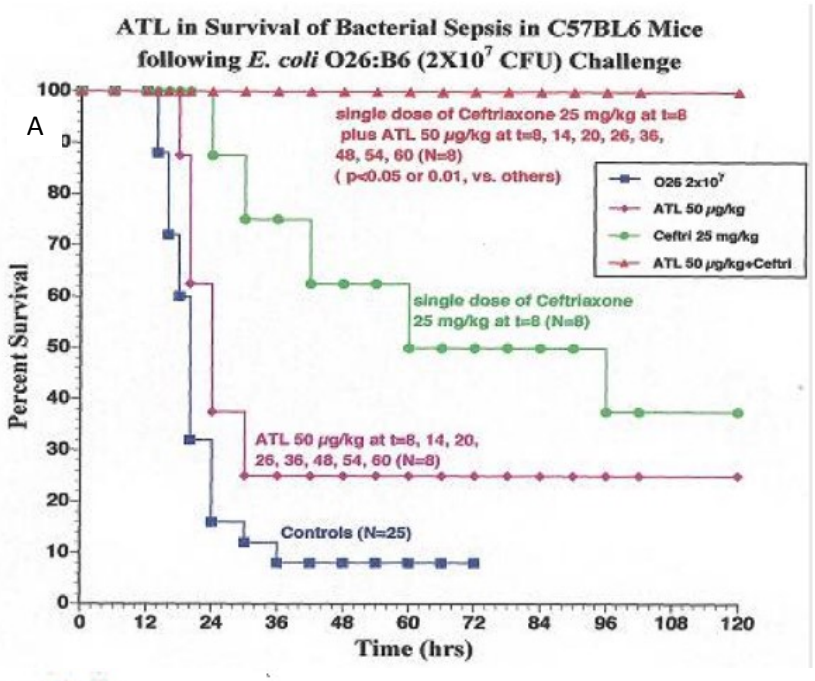


Figure 1. ATL-146e improves survival in a mouse *E. Coli* model of sepsis and is synergistic with Ceftriaxone (N=8). (In collaboration with Dr. Mike Scheld, UVa).

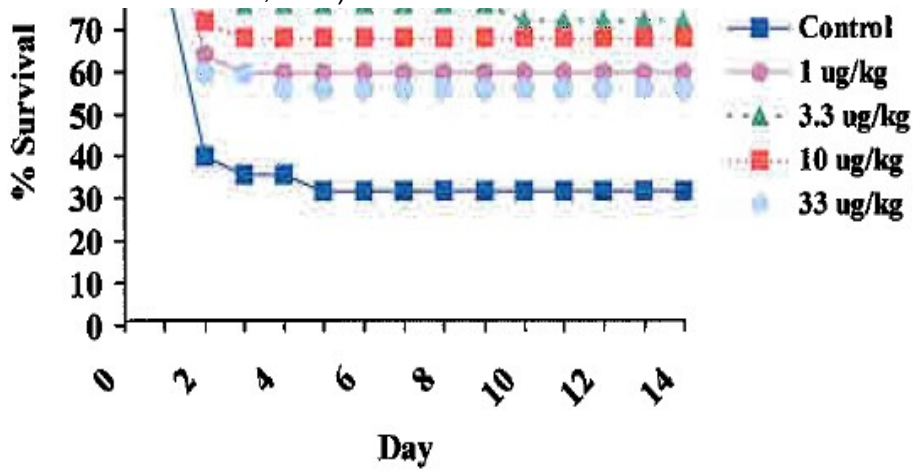


Figure 2. ATL-146e improves survival in a rat fibrin-thrombin clot model of gram-negative sepsis. Fisher 344 male rats (N=25) were treated with 5 mg/kg gentamicin twice daily 2 hours post infection and ATL-146e at 2, 8 12 and 20h post infection. (In collaboration with Dr. Peter DeMarsh,

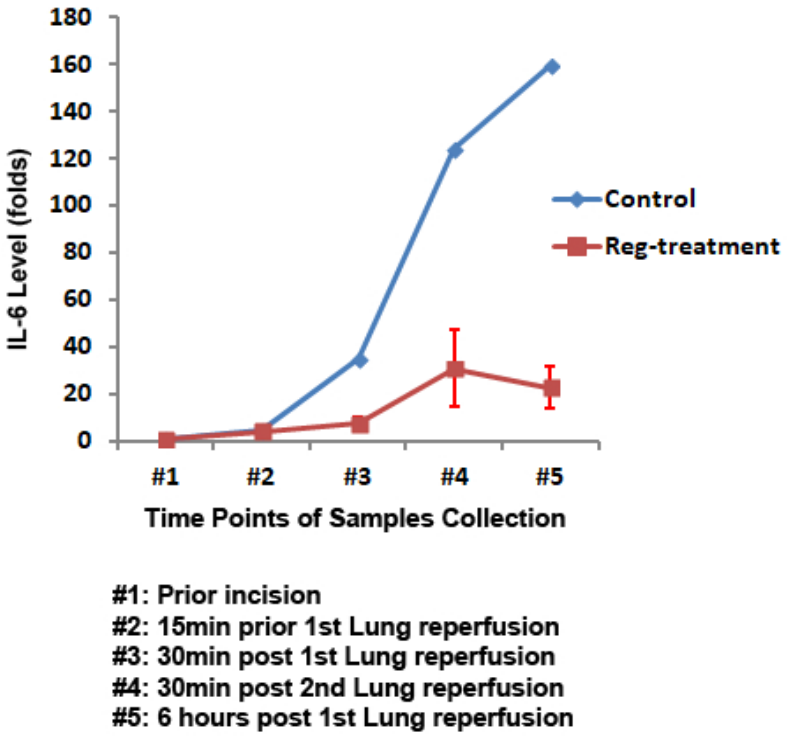


Figure 3. Regadenoson inhibits IL-6 releasing post lung transplantation. All the patients underwent double lung transplantation. The data are collected from 8 Regadenoson treated patients and 1 control patient. The data are normalized to prior Regadenoson infusion.

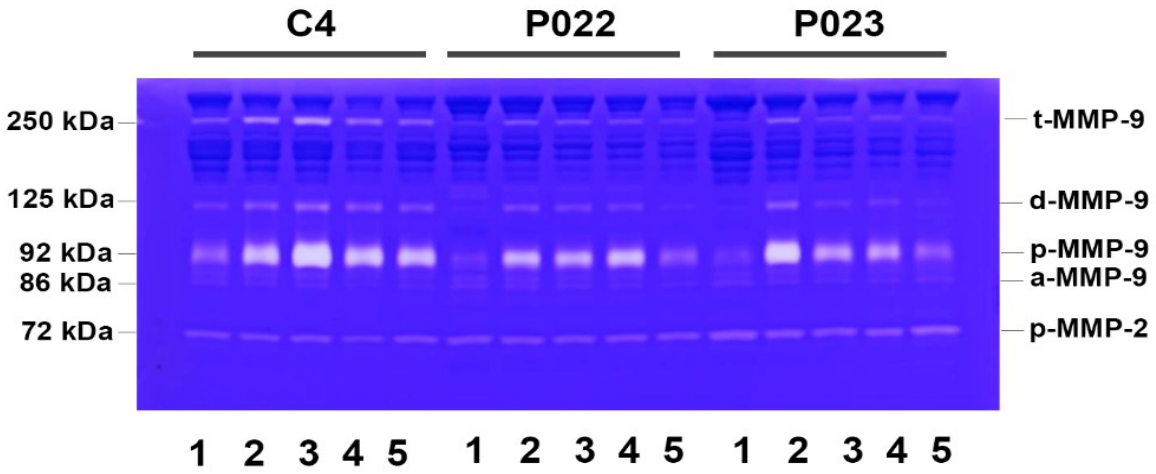


Figure 4. Plasma Levels of MMP-9 and MMP-2 in two Regadenoson infused patients (P022 &P023) and 1 control patient (C4) at different time points. 1. Prior incision, 2. 15min prior 1st Lung reperfusion, 3. 30min post 1st Lung reperfusion, 4. 30min post 2nd Lung reperfusion, 5. 6 hours post 1st Lung reperfusion. t-MMP-9, tetramer MMP-9; d-MMP-9, dimerized MMP-9 and TIMP-1, p-MMP-9, pro-MMP-9; a-MMP-9, active MMP-9; p-MMP-2, pro-MMP-2.

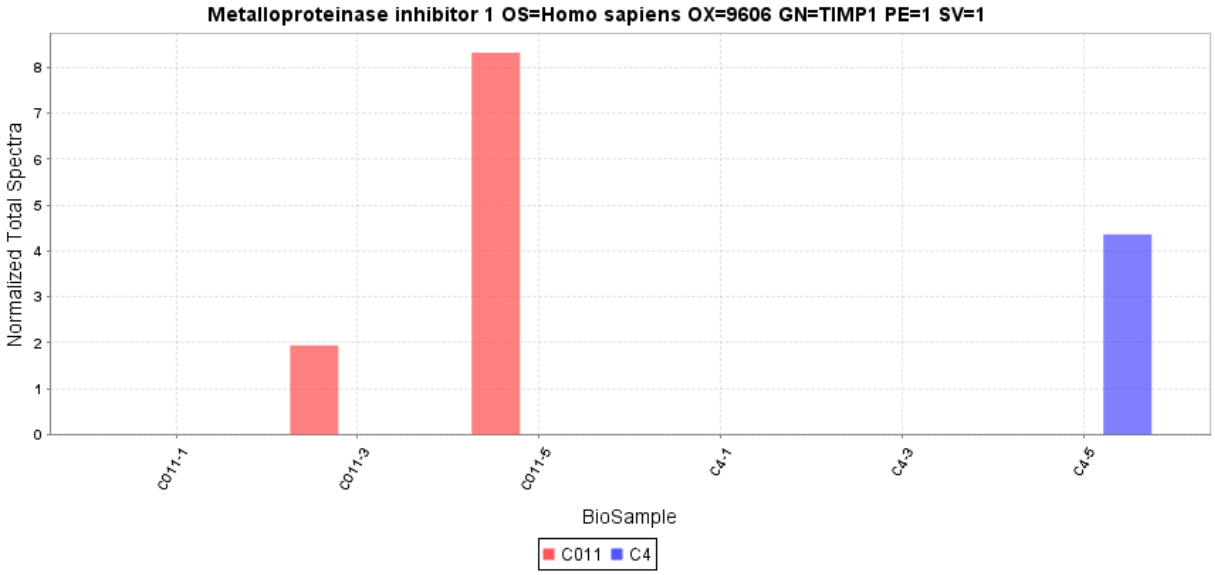


Figure 5. Tissue inhibitor of metalloproteinase -1(TIMP-1) protein in Regadenoson infused lung transplant patient (C011) and control patient (C4). TIMP-1 was detected with the LC-MS system consisted of a Thermo Electron Q Exactive HF mass spectrometer system. The data were analyzed by database searching using the Sequest search algorithm against a database of Uniprot Human. C011, Regadenoson infused patient; C4, non-Regadenoson treated patient. -1: Prior incision, -3: 30min post 1st Lung reperfusion, -5: 6 hours post 1st Lung reperfusion.

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FDA-Approved Labeling and Existing IND for Regadenoson:

Regadenoson, the proposed drug in this proposal, is FDA approved agent for 1) Radionuclide myocardial perfusion imaging, 2) Phase II clinical trial in adult sickle cell disease patient (above reference #15), 3) Our phase I clinical trial in lung transplantation (IND# 062862, above reference #30). In the trial 1), Regadenoson (400 micrograms) was given intravenously within 30 seconds. In trials 2) and 3), Regadenoson was administrated with low dose, slow infusion (1.44micrograms/kg/hour) for up to 12 hours. No dose limiting toxicity was observed in these trials. Therefore, we anticipate the dose we are going to use in these moderate to severe COVID-19 patients in this proposal will be safe. The labeling of Regadenoson is attached.

Results For In Vitro and In Vivo Toxicology: Regadenoson was negative in the Ames bacterial mutation assay, chromosomal aberration assay in Chinese hamster ovary (CHO) cells, and mouse bone marrow micronucleus assay. Long-term animal studies have not been conducted to evaluate LEXISCAN's carcinogenic potential or potential effects on fertility. Minimal cardiomyopathy (myocyte necrosis and inflammation) was observed in rats following single-dose administration of Regadenoson. Increased incidence of minimal cardiomyopathy was observed on day 2 in males at doses of 0.08, 0.2 and 0.8 mg/kg (1/5, 2/5, and 5/5) and in females (2/5) at 0.8 mg/kg. In a separate study in male rats, the mean arterial pressure was decreased by 30 to 50% of baseline values for up to 90 minutes at Regadenoson doses of 0.2 and 0.8 mg/kg, respectively. No cardiomyopathy was noted in rats sacrificed 15 days following single administration of Regadenoson. The mechanism of the cardiomyopathy induced by Regadenoson was not elucidated in this study but was associated with the hypotensive effects of

Regadenoson. Profound hypotension induced by vasoactive drugs is known to cause cardiomyopathy in rats.

Brief Assay Descriptions: The blood samples will be collected at different timepoints prior to, during, and after the Regadenoson infusion. Cytokines, biomarkers, and immune cell levels will be analyzed. The plasma level of Regadenoson in the participants will be measured according to our phase I trial in lung transplantation. The samples (plasma) will be precipitated (150 µL) using 1350 µL of acetonitrile. Samples will be vortexed of 15 seconds and spun for 10 minutes at max speed on a benchtop microfuge. 1400 µL of supernatant will be removed and dried. The samples will be reconstituted in 30 µL of 50% methanol/0.1% formic acid – water. The LC-MS system consists of a Thermo Electron Orbitrap ID-X mass spectrometer with a HESI source interfaced to a Thermo Accucore Vanquish C18 1.5um, 2.1 x 100mm column. 10 µL of the extract will be injected and the compounds eluted from the column by a methanol/0.1% formic acid gradient at a flow rate of 250 µL/min over 0.12 hours (0.25 hours total time). The nanospray ion source will be operated at 3.2 kV. The sample will be analyzed by MS, MS/MS and PRM quantification.

A Detailed Justification for The Proposed Dose, Dosing range, Number of Doses, and Dose Interval: Regadenoson will be given intravenously as 5 µg/kg loading dose over 30 mins (to avoid unpleasant side effects sometimes associated with the rapid bolus injection of Regadenoson), followed by a continuous slow infusion (1.44micrograms/kg/hour) with the use of a pediatric infusion pump for 6 hours. The initial high dose (5 µg/kg) infusion is based on: Regadenoson is a FDA approved drug for myocardial perfusion imaging with a bolus dosing (400 micrograms, in 10 seconds). The initial dose (5 µg/kg) is lower than the MPI dose (400 µg). And it will be given slower as a bolus over 30 mins. The low dosage (1.44micrograms/kg/hour) slow infusion is based on the Sickle cell disease phase II clinical trial and our clinical phase I trial in lung transplant patients. Both trials have demonstrated that low dosage infusion(1.44micrograms/kg/hour) is safe with no dose limiting toxicities up to 12 hours (above reference #30). A loading dose is needed in the present study to get effective drug concentrations to the patients as soon as possible after diagnosis of cytokine storm syndrome. We will keep Aminophylline, a competitive adenosine receptor antagonist, on hand as a rescue medication for rare side effects of Regadenoson, such as bronchoconstriction. Aminophylline has been used to terminate persistent pharmacodynamic effects. In such case, aminophylline may be administered in doses ranging from 50 mg to 250 mg by slow intravenous injection (50 mg to 100 mg over 30–60 seconds).

Trial Timeline

Timeline: Please see the attached for the detailed (Table 2) projected timeline for each patient. A loading dose is needed in the present study to get effective drug concentrations to the patients as soon as possible after diagnosis of cytokine storm syndrome in hospitalized COVID-19 patients. We propose to treat a total of 40 moderate to severe COVID-19 patients in this protocol. In this trial, the discontinuation from study drug is defined as any subject discontinuing Regadenoson treatment or discontinuing from the study post drug treatment for any reasons (such as, COVID-19

caused mortality, comorbidity caused serious injury). Because the study involves a single Regadenoson treatment with a duration less than 7 hours, the possibility of discontinuation from the study drug is very low. Withdraw from the study is defined as any subject or his/her LAR willingly withdrawing from the clinical trial for any reasons. The collected data from discontinued subjects may also be used in the outcome analysis.

Our Retention and Follow Up Plans: Since study participation is 30 days, we anticipate that most participating subjects will remain either as an inpatient or will follow up frequently after COVID19 diagnosis and recovery. Patients will be monitored by study personnel according to the Post Infusion Procedures. We will try to minimize missing data in this trial by taking the following steps: We will encourage patients who discontinue therapy to remain in the study and to continue follow-up for all key safety and efficacy outcomes. Due to the pandemic, we will follow-up with these patients virtually via phone for all required timepoints post Regadenoson infusion that occur after discharge. We will record vital status for all patients. We will also take the following actions (1) the protocol and informed consent forms clearly differentiate treatment discontinuation from study withdrawal; (2) site investigators will be trained on the importance of retention and steps to prevent missing data; (3) the consent forms include a statement educating patients about the continued scientific importance of their data even if they discontinue study treatment early; and (4) take steps to be able to ascertain vital status in all randomized patients (e.g., with a vital records search).

Infusion of Regadenoson will be added to current standard of care. We recognize that standard of care may change as the study progresses and these issues will be addressed as they arise. If not completed as standard of care, CRP will be collected at screening and on day 1,

The End of Study: We anticipate the Regadenoson treatment will 1) enhance lung function by reducing hypoxia (as measured by pulse oximetry, ventilator parameters, ABGs as clinically indicated, and CXR findings), and 2) increase survival. At the end of this study, we will perform primary efficacy analyses and survival analyses. If this statistical analysis proves that Regadenoson treatment enhanced lung function, shortened the length of staying in ICU and/or hospital, and increased survival when compared with the randomized placebo-control group, we will 1) pursue a large scale multiple sites phase III trial for the efficacy and long term safety of Regadenoson in moderate to severe COVID-19 patients, 2) will explore the mechanisms of its efficacy by measuring levels of pro- and anti- inflammatory cytokines (such as IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12, TNFα, IFN-γ etc) and levels of enzymes and their inhibitors (MMP-2, MMP-9, MPO, neutrophil elastase, TIMP-1, TIMP-2, etc) and activation of inflammatory/immune cells (such as macrophages, neutrophils, monocytes, invariant

NKT cells, etc).

Volume 3: Detailed DSMB Plan (Regadenoson trial on COVID-19)

Introduction

This study will utilize a Data Safety Monitoring Board to be requested through the National Institute of Health. The DSMB will follow the guidelines as described in the NIH issued policy on data and safety monitoring (http://www.nhlbi.nih.gov/research/funding/human-subjects/data-safety-monitoring-policy). Safety reports will include summary tables describing patient enrollment, baseline characteristics of patients, adverse events, serious adverse events and any other relevant data requested by the board. The DSMB may pause or halt the trial at any time based on data from this or other trials. The DSMB will determine the frequency of their meetings and establish a charter for this trial prior to the enrollment of the first subject in this study.

Responsibilities of the DSMB

Protections Against Risk and Loss of Confidentiality

All research interventions will be administered by trained healthcare professionals at each site. Resuscitation equipment and trained personnel will be immediately available during the infusion procedure.

The federal regulation under the Health Insurance Portability and Accountability Act (HIPPA) governs the protection of individual identifiable health information. The University of Maryland Baltimore Institutional Review Board for Health Sciences Research serves as the Privacy Board and oversees institutional compliance with and interpretation of the HIPPA Privacy Rule. Institutional policies contain best practices for activities such as secure email and written documentation regarding individual patients and provides resources for clinical staff and investigators on safeguards to protect patient rights. Only approved investigators and research staff will have access to the data for this study. Institutional Data Protections Standards will be followed for collection and storage of the research data which contains Protected Health Information (PHI). HIPPA identifiers will be destroyed with the data after all retention requirements have been met.

Monitoring Plan

Study site monitoring will be conducted by the University of Maryland, Baltimore Office of Accountability and Compliance (UMB OAC). For instances of remote monitoring, participating institutions will be required to forward de-identified copies of subjects' medical record and source documents to the UMB OAC to aid in source documentation verification. A complete documentation of the monitoring plan is found in the full Site Monitoring Plan.

STUDY CONDUCT AND ETHICAL CONSIDERATIONS

This study will be conducted in accordance with the standards of Good Clinical Practice (GCP), all applicable federal, state, and local laws, and in accord with the ethical principles that originated in the Declaration of Helsinki. The PI will ensure that staff are trained and carry out the study in accord with the protocol specifications. The PI will ensure that all study site personnel are aware that the study protocol and all data generated are confidential and should not be disclosed to third parties (with the exception of local and national regulatory bodies which require access for oversight purposes). As part of our routine in clinical research at the University of Maryland, we collect all disparity data and report it to the IRB on an annual basis during the continuing review process. The study may not begin at each site until IRB approval has been obtained.

All COVID-19 clinical research studies at the medical center are overseen by the institution's COVID-19 task force committee that helps organize the screening of COVID-19 patients for study enrollment. They have instituted a daily research huddle to facilitate this process and direct potential study subjects to the most appropriate clinical trials. All active studies are invited to this meeting where the newly admitted/diagnosed COVID-19 patients are discussed and presented for consideration of enrollment in clinical trials. This group of clinicians and investigators has been conducting this meeting since the Spring 2020 and thus far it has been a very successful mechanism by which patients are enrolled into clinical trials in a fair and clinically relevant approach. We will have a representative from our study team attend this meeting each day and do not anticipate any barriers or conflicts related to competing clinical trials.

Consent Forms and the Consenting Process

Consent forms will be written in accord with 21 CFR 50 and will be reviewed and approved by each institutions IRB prior to use. Subjects or legally authorized representatives (LARs) should be educated separately about the risks associated with COVID-19. Informed consent will be obtained from each participant or LAR prior to conducting any study-specific procedures.

Since COVID19 positive patients are in isolation rooms, discussion of the study is more complicated.

If the patient is alert and not intubated, the consent form could be presented to the patient in 2 ways; either physically by the nurse or electronically (patient can view via iPad). The PI will then call into the patient room and go over the study with the patient and answer any questions they may have.

The patient will be evaluated to determine if they have the capacity to consent to the study. This evaluation will be documented. If the patient is intubated or otherwise incapacitated, the legally authorized representative (LAR) will be identified and their relationship to the patient documented. PI will call the LAR to discuss the study. The research staff will send the consent form electronically to the LAR, and the consent

process will occur over the phone, since there may be a no visitor policy in place for COVID patients. Verbal consent will be accepted.

Maintenance of Study Documents

Signed consent forms and other research records will be retained in a confidential manner. Record retention will be in accord with 21 CFR 312.62 and HIPAA regulations.

Organization and Interactions

Communication with DSMB members will be primarily through the NHLBI Program Office. It is expected that study investigators will not communicate with DSMB members about the study directly, except when making presentations or responding to questions at DSMB meetings or during conference calls.

This charter may be sent, if requested, to an IRB or an outside party under a formal FOIA inquiry.

DSMB Members and NHLBI Program Staff

DSMB members and their expertise are listed in Appendix A. NHLBI Program Staff involved in the study and their responsibilities are listed in Appendix B. Consistent with NHLBI policy, each DSMB is assigned an Executive Secretary (ES) to provide an unbiased staff interface between the DSMB members and other meeting participants, especially during closed and executive sessions. The ES is responsible for assuring the accuracy and timely transmission of the final recommendations and DSMB minutes.

Roles of NHLBI staff in DSMB Meetings

- NHLBI program staff members involved in the day-to-day conduct of the study may attend the open sessions of DSMB meetings. These Program staff may attend portions of a closed session as needed, but not when post-randomization outcome data by treatment group will be discussed.
- NHLBI's Office of Biostatistics Research has assigned a statistician to this DSMB. The NHLBI study statistician will also serve as a resource to the DSMB as needed.
- The NHLBI ES will be a federal employee with appropriate expertise and training who has no other involvement in the conduct of the trial and does not report directly to the lead program official.
- The ES is the only NHLBI staff member who can routinely be in the executive session. The DSMB can opt to have an executive session without the ES, but then they will be responsible for minutes for that portion of the meeting. The DSMB can request to have other staff members attend the executive session to provide additional information as needed.
- The NHLBI ES and statistician are expected to report issues of substantive concern to the NHLBI Division Director responsible for the trial. The NHLBI

Division Director will communicate with the Office of the Director, NHLBI. Under special circumstances, and with the concurrence of the DSMB Chair, the Division leadership and Director and Deputy Director of the NHLBI may see unmasked data presented at DSMB meetings.

- There may be occasions when it is appropriate for new staff not involved in the study to attend a DSMB meeting as a training opportunity. This will be discussed with the DSMB Chair before the meeting; the new staff member(s) would attend only the portions of the meeting outlined in the first bullet above.

Scheduling, Timing, and Organization of Meetings

DSMB meetings are usually held by conference call, or in the Washington, DC area when an in-person meeting is required.

The purposes of the first meeting are to:

- Convey NHLBI's expectations for DSMB operations
- Review this Charter
- Provide an overview of study activities
- Review and accept or make recommendations to the protocol, consent and the analysis plan before approving these documents to be sent to the IRB.

Enrollment in the study cannot begin until the DSMB's recommendations for approval has been accepted by the Director, NHLBI, and approval has been obtained from the IRB.

Meetings will be scheduled based on enrollment. They will occur after the completion of cohort 1 as described in the safety assessment section. Meetings will also be scheduled after enrollment of 15, 25, and 40 subjects, with additional meetings or conference calls scheduled as needed to review SAEs or other issues. Meetings and conference calls will be scheduled by the CCC in collaboration with the NHLBI Program Office.

The agenda for DSMB meetings and calls will be drafted by the DSMB chair in consultation with NHLBI staff. The ES will finalize the agenda after consultation with the DSMB Chair. The agenda and meeting materials should be distributed by the NHLBI two weeks before each meeting or call.

At the time that the agenda is sent out, and again at the beginning of the meeting, the ES will ask all DSMB members to state whether they have developed any new conflicts of interest since the last formal annual report to NHLBI. If a new conflict is reported, the Chair and staff will determine if the conflict limits the ability of the DSMB member to participate in the discussion.

The DSMB will review adverse event data, other safety data, quality and completeness of study data, and enrollment data at each meeting to ensure proper trial conduct. Study personnel should provide any new literature particularly pertinent to the trial,

along with their recommendation as to whether it affects the trial conduct or design. The DSMB will review the informed consent form when it reviews the protocol. The DSMB will review the consent periodically and/or as needed and consider whether the consent form requires revision in light of any new findings or amendments. At intervals, if included in the statistical analysis plan, the DSMB will also review formal interim analyses of the primary end point.

In addition to regular meetings, it may be necessary to convene the DSMB urgently on an *ad hoc* basis to discuss new data or other information that raises questions about equipoise, safety, or anything else in the trial.

It is expected that all DSMB members will attend every meeting and conference call. However, it is recognized that this may not always be possible. A quorum for voting is half of the standing members plus one. The Board may wish to decide if particular expertise is needed within the quorum for a particular meeting. All standing Monitoring Board members are voting members. The Board may decide in advance whether *ad hoc* members can vote.

Discussion of Confidential Material

DSMB meetings and calls will be organized into open, closed, and executive sessions.

- During the **open sessions**, information will be presented to the DSMB by the principal investigator or staff at UMB, study investigators, and NHLBI staff as appropriate, with time for discussion.

- During the **closed sessions**, the DSMB**,** UVA statistician, and NHLBI statistician will discuss confidential data from the study, including information on efficacy and safety. NHLBI's expectation is that the DSMB will review unmasked data. If the closed session occurs on a conference call, steps will be taken to ensure that only the appropriate participants are on the call, and to invite others to re-join the call only at the conclusion of the closed session.

- The DSMB may hold an **executive session** in which only the DSMB members are present. The NHLBI ES may attend the executive session at the invitation of the DSMB Chair. If the ES does not attend the executive session, the DSMB Chair will be responsible for summarizing the DSMB's discussion and recommendations to the ES.

Voting on recommendations will follow Robert's Rules of Order.

At the conclusion of the closed and executive sessions, the DSMB chair may provide a summary of the preliminary recommendations to the lead investigators and masked NHLBI staff to provide an opportunity for study investigators, the PI, and NHLBI to ask

questions to clarify the recommendations. Recommendations that would unmask results, such as a recommendation to close a study prematurely, should not be disclosed until approved by NHLBI leadership. The meeting is then adjourned.

Reports of DSMB Deliberations

- Formal minutes: The NHLBI ES is responsible for preparation and transmission of the formal DSMB minutes to the Director of the applicable Division within 14 calendar days of each meeting or call. Minutes will document whether there is conflict of interest on the part of Board members and will summarize the key points of the discussion and debate, requests for additional information, response of the investigators to previous recommendations, and the recommendations from the current meeting.
- Following division review, the minutes are sent to:

    - DSMB Chair, who approves them on behalf of the DSMB
    - Division of Lung Diseases Director, NHLBI, for final Institute approval

- Once the Division of Lung Diseases Director, NHLBI has approved the minutes, they are considered final.
- Recommendations of the Board are sent to the primary study investigator(s) and may be included in the materials for the subsequent DSMB meeting.
- Reports to IRB: The NHLBI program office will prepare a Summary Report and submit it to primary study investigators within 30 calendar days of each meeting. Primary study investigators will forward the Summary Report to the IRB.
- If the DSMB does not identify any safety or other protocol-related concerns, the Summary Report will state that:

    - a review of outcome data, adverse events, and information relating to study
      performance (e.g., data timeliness, completeness, and quality) across all centers took place on a given date
    - the observed frequency of adverse events did not exceed what was expected and indicated in the informed consent;
    - a review of recent literature relevant to the research took place, and;
    - the DSMB recommended that the study continue without modification of the protocol or informed consent

- If the DSMB does identify concerns, the NHLBI staff will distribute, as soon as feasible, preferably within 7 calendar days of the DSMB meeting, the Summary Report as outlined above, outlining the concerns and the basis for any recommendations that the DSMB has made in response to the concerns. Adverse event reporting will be consistent with NHLBI policy.

Reports to the DSMB

For each meeting, unblinded analyses and reports will be prepared by the DSMB statistician as this is a double blinded study and the PI and study staff will not have access to the data required to prepare these reports. Reports will be prepared according to the DSMB's **recommendations** regarding which data should be presented and the format of the data presentation.

Statistical Monitoring Guidelines

The DSMB will review the adequacy of the statistical monitoring plan. The final plan, whether part of a research protocol or separate document, will be maintained as an appendix to this Charter. The DSMB should discuss the statistical monitoring procedures that will be followed to guide recommendations about termination or continuation of the trial. These procedures could include guidelines for termination due to safety reasons.

ADVERSE EVENTS AND REPORTING

This section defines the types of safety data that will be collected under this protocol and outlines the procedures for appropriately collecting, recording and reporting those data.

Definitions

Adverse Event

For the purposes of this protocol, an adverse event will be defined as any adverse medical change (i.e., de novo or increased severity in a preexisting condition) from the subject's baseline condition, that occurs during the course of the clinical study, after receiving Regadenoson infusion through Day 30, whether considered device related or not and meeting the protocol requirements.

Anticipated symptoms caused by the COVID-19 infection and standard care are expected to occur between time of Regadenoson infusion and Day 30. The following are examples of anticipated events caused by the COVID-19 infection and standard care are medications not considered AE's:

- Fever
- Incubation related inflammation, sour throat
- Shortness of breath
- Pneumonia
- Renal insufficiencies secondary to medications
- Sleep issues (insomnia)
- Gastrointestinal discomfort (i.e. constipation, decreased appetite, diarrhea)
- Pulmonary observations (i.e. wheezing, cough)
- Neurological observations (i.e. hallucinations, confusion)
- Renal observations (i.e. urinary tract infections, urinary retention)

- There may be additional expected events not listed which may be documented in the patient's medical record by the investigator.

The events above are not considered adverse events for this study and will not be recorded.

Serious Adverse Event

- A Serious Adverse Event (SAE) is one that:
- Leads to death;
- Leads to serious deterioration in the health of the study patient that:
    - Results in life-threatening illness or injury;
    - Results in a permanent impairment of a body structure or a body function;
    - Requires in patient hospitalization or prolongation of existing hospitalization;
    - Results in medical or surgical intervention to prevent permanent impairment to body structure or a body function;
- Results in congenital anomaly/birth defect
- Significant medical event.

NOTE: A planned hospitalization for a pre-existing condition, or anticipated procedures such as routine transplant related biopsy or elective surgery will not be considered serious adverse events.

Note that seriousness and severity are separate concepts. The term "severe" refers to the intensity of a specific event; a severe event may be of minor medical significance (e.g., a severe leg cramp). The term "serious" is based on an outcome or action criteria that are usually associated with events that pose a threat to the patient's life or functioning. An event that is mild in severity is serious if it leads to one of the outcomes defined above.

Unanticipated problem involving risks to subjects or others (UP) - Any incident, experience or outcome that meets all the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given:
- The research procedures that are described in the protocol related-documents, such the IRB-approved research protocol and the informed consent form document and,
- The characteristics of the subject population being studied;
- Related or possibly related to the subject participation in research; and

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

Grading of Adverse Events – Events will be graded by using the Common Terminology Criteria for Adverse Events (CTCAE) Criteria version 4.03.

Attribution Assessment

Attribution – the determination and documentation of whether an adverse event is related to Regadenoson infusion.

Attribution Categories:

1. Not Related – Event clearly related to other factors (e.g., clinical state, other therapies; concomitant drugs)
2. Possibly Related – Sequence of event is compatible with device, or procedure, but could have been produced by other factors
3. Probably Related - Sequence of event is compatible with device, or procedure and cannot be explained by other factors without much doubt
4. Definitely Related - Sequence of event is compatible with device or procedure and beyond doubt cannot be explained by other factors

Severity

The degree of severity of the AE to the subject's health will be documented on the AE CRF.

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.

Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.

Grade 4 Life-threatening consequences; urgent intervention indicated.

Grade 5 Death related to AE.

Unanticipated Adverse Device Effect (UADE)

A UADE is any serious adverse effect on health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan (e.g., ICF, Study Protocol, Instructions for Use (IFU), publications, etc.) or any other

unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Adverse Event Recording

All adverse events will be collected starting at time of infusion through POD 30. Events that meet the definition of "anticipated" (see section 8.1.1) will not be recorded as adverse events unless the event meets the criteria of a Serious Adverse Event. Adverse events should be recorded on a case report form and will be assessed for seriousness, severity, attribution and expectedness by the site Principal Investigator.

Reporting Requirements

Expedited Reporting

The following events require **expedited reporting**, i.e., notification to the study monitoring team within 24 hours and DSMB and NHLBI within at least 7 calendar days of learning of the event's occurrence:

- All Serious Adverse Events that are at least possibly related/associated with the study device.
- All unanticipated problems and unanticipated adverse device effects that occur within 30 days of Regadenoson infusion.
- All (fatal) events within 30 days of Regadenoson infusion.

Efforts should be made to report expedited events to the DSMB and NHLBI as soon as possible but within at least 7 days. All other events will be recorded in the electronic data capture system.

Events that require expedited reporting must be reported by email or telephone within 24 hours to:

Office of Accountability and Compliance

University of Maryland, Baltimore

410-706-2281

oac@umaryland.edu

Expedited reports will also be submitted to the DSMB and NHLBI (see Table 4) within 7 calendar days of learning of the event's occurrence.

Reporting to the UMB HSR-IRB and DSMB

The Principal Investigator or designee is responsible for reporting SAEs and unanticipated problems to the site IRB according to the participating site institutional guidelines.

Table 4. DSMB and NHLBI Reporting Requirements

DSMB and NHLBI			
Type of Event	**To whom will it be reported:**	**Time Frame for Reporting**	**How reported?**
All Adverse Events	DSMB and NHLBI	30 calendar days prior to scheduled DSMB meetings	DSMB report will include listing of all Adverse Events
Reportable SAE's and Unanticipated Problems	DSMB and NHLBI	7 calendar days of knowledge of the event	Report to Designated DSMB member and NHLBI
DSMB Reports	IRB	15 calendar days of the study team receiving the report	Copy of DSMB report

Reporting to the FDA

The Sponsor for the study is responsible for providing safety updates to the FDA per the following guidelines (Table 5). The reporting times refer to the time the study team received knowledge of the AE.

Table 5. FDA Reporting Requirements

UMB PI HELD IDE			
Type of Event	**To whom will it be reported:**	**Time Frame for Reporting**	**How reported?**
Life-threatening and/or fatal unexpected events related or possibly related to the use of the investigational agent.	FDA	Within 7 calendar days of the study team learning of the event	Form FDA 3500A (MedWatch) or narrative
Serious, unexpected and related or possibly related adverse events	FDA	Within 15 calendar days after the study team receives knowledge of the event	Form FDA 3500A (MedWatch) or narrative

Unanticipated Adverse Device Effects	FDA	Within 10 working days of the study team learning of the event	Form FDA 3500A (MedWatch) or narrative
All adverse events	FDA	Annually	IDE annual report

Appendix A

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Appendix B

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Volume 4: Appendix

APPENDIX A: NIAID 8-Point Ordinal Scale for COVID-19

The ordinal scale is an assessment of the clinical status at the first assessment of a given study day. The scale is as follows:

1. Death;
2. Hospitalized, on invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO);
3. Hospitalized, on non-invasive ventilation or high flow oxygen devices;
4. Hospitalized, requiring supplemental oxygen;
5. Hospitalized, not requiring supplemental oxygen, requiring ongoing medical care (COVID-19 or otherwise);
6. Hospitalized, not requiring supplemental oxygen, and no longer requires ongoing medical care (e.g., patient still admitted for isolation purposes only).
7. Not hospitalized, limitation on activities;
8. Not hospitalized, no limitations on activities.

Table 1, Detailed Grading Scales of Toxicity of Regadenoson

	CTCAE v4.0 Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
1	Acute coronary syndrome	-	Symptomatic, progressive angina; cardiac enzymes normal; hemodynamically stable	Symptomatic, unstable angina and/or acute myocardial infarction, cardiac enzymes abnormal, hemodynamically stable	Symptomatic, unstable angina and/or acute myocardial infarction, cardiac enzymes abnormal, hemodynamically unstable	Death
2	Atrial fibrillation	Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Symptomatic and incompletely controlled medically, or controlled with device (e.g., pacemaker), or ablation	Life-threatening consequences; urgent intervention indicated	Death
3	Atrial flutter	Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Symptomatic and incompletely controlled medically, or controlled with device (e.g., pacemaker), or ablation	Life-threatening consequences; urgent intervention indicated	Death
4	Atrioventricular block complete	-	Non-urgent intervention indicated	Symptomatic and incompletely controlled medically, or controlled with device (e.g., pacemaker)	Life-threatening consequences; urgent intervention indicated	Death
5	Atrioventricular block first degree	Asymptomatic, intervention not indicated	Non-urgent intervention indicated	-	-	-
6	Cardiac arrest	-	-	-	Life-threatening consequences; urgent intervention indicated	Death
7	Heart failure	Asymptomatic with	Symptoms with mild to	Severe with symptoms at rest	Life-threatening consequences;	Death

		laboratory (e.g., BNP [B-Natriuretic Peptide]) or cardiac imaging abnormalities	moderate activity or exertion	or with minimal activity or exertion; intervention indicated	urgent intervention indicated (e.g., continuous IV therapy or mechanical hemodynamic support)	
8	Mobitz (type) II atrioventricular block	Asymptomatic, intervention not indicated	Symptomatic; medical intervention indicated	Symptomatic and incompletely controlled medically, or controlled with device (e.g., pacemaker)	Life-threatening consequences; urgent intervention indicated	Death
9	Mobitz type I	Asymptomatic, intervention not indicated	Symptomatic; medical intervention indicated	Symptomatic and incompletely controlled medically, or controlled with device (e.g., pacemaker)	Life-threatening consequences; urgent intervention indicated	Death
10	Myocardial infarction	-	Asymptomatic and cardiac enzymes minimally abnormal and no evidence of ischemic ECG changes	Severe symptoms; cardiac enzymes abnormal; hemodynamically stable; ECG changes consistent with infarction	Life-threatening consequences; hemodynamically unstable	Death
11	Ventricular arrhythmia	Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Medical intervention indicated	Life-threatening consequences; hemodynamic compromise; urgent intervention indicated	Death
12	Ventricular fibrillation	-	-	-	Life-threatening consequences; hemodynamic compromise; urgent intervention indicated	Death
13	Cardiac disorders -	Asymptomatic or mild	Moderate; minimal, local or	Severe or medically significant but not	Life-threatening consequences;	Death

	Other, specify	symptoms; clinical or diagnostic observations only; intervention not indicated	noninvasive intervention indicated; limiting age-appropriate instrumental ADL	immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	urgent intervention indicated	
14	Electrocardiogram QT corrected interval prolonged	QTc 450 - 480 ms	QTc 481 - 500 ms	QTc >= 501 ms on at least two separate ECGs	QTc >= 501 or >60 ms change from baseline and Torsade de pointes or polymorphic ventricular tachycardia or signs/symptoms of serious arrhythmia	-
15	Headache	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL	-	-
16	Stroke	Asymptomatic or mild neurologic deficit; radiographic findings only	Moderate neurologic deficit	Severe neurologic deficit	Life-threatening consequences; urgent intervention indicated	Death
17	Acute kidney injury	Creatinine level increase of >0.3 mg/dL; creatinine 1.5 - 2.0 x above baseline	Creatinine 2 - 3 x above baseline	Creatinine >3 x baseline or >4.0 mg/dL; hospitalization indicated	Life-threatening consequences; dialysis indicated	Death
18	Bronchial obstruction	Asymptomatic; clinical or diagnostic observations only; interventio	Symptomatic (e.g., mild wheezing); endoscopic evaluation indicated; radiographic	Shortness of breath with stridor; endoscopic intervention indicated (e.g., laser, stent placement)	Life-threatening respiratory or hemodynamic compromise; intubation or urgent	Death

		n not indicated	evidence of atelectasis/lobar collapse; medical management indicated (e.g., steroids, bronchodilators)		intervention indicated	
19	Bronchospasm	Mild symptoms; intervention not indicated	Symptomatic; medical intervention indicated; limiting instrumental ADL	Limiting self care ADL; oxygen saturation decreased	Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated	Death

Table 2: Detailed Projected Timeline for Each Subject:

	Screening	Treatment/follow-up				
Observations	D-3 to D1	D1	D3	D7 ± 1	D15 ± 2	D30 ± 2
Confirm COVID-19 results	X					
Medical History	X					
Informed Consent	X					
Confirm eligibility	X					
Collect demographic data	X					
Pregnancy Test	X[1]					
Randomization	X[2]					
Study Product administration		X				
AE assessments		X	X	X	X	X
Physical Examination[3]	X	X	X*	X*	X*	X*
Laboratory testing[4]	X	X	X*	X*	X*	X*
Arterial blood gas[5]	X	X	X*	X*	X*	X*
Chest imaging	X	X*	X*	X*	X*	X*
Cardiac monitoring/EKG	X	X	X*	X*	X*	X*
Vital/Respiratory Status	X	X	X	X	X	X
Research labs and storage[6]	X	X				

Notes:
Day 1 assessments are to occur within 24 hours of infusion end.
* Not required, but to be recorded if available.
1 If female and of childbearing age.
2 If enrolled in the randomization arm.
3 Record available medications and vital signs including heart rate, blood pressure, oxygen saturation, respiratory rate
4 Laboratory tests includes CBC with differential count, chemistries with creatinine, ALT, AST, total bilirubin, ESR, LDH, CK when available. PT, PTT, INR, TEG, CRP, and D-Dimer are required prior to infusion and on post infusion day 1.
5 Required at baseline and D1 if arterial line present.
6 For open enrollment and Randomized subjects: blood samples will be collected prior to infusion, immediately after the load dose, 4 hours into the low dose infusion, and post infusion day 1.