RANDOMIZED PHASE IIA CLINICAL TRIAL OF CYCLOSPORINE FOR THE TREATMENT OF COVID-19(+) NON-ICU HOSPITAL INPATIENTS

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SCHEMA

Phase IIa clinical trial in which 75 non-ICU hospital inpatients will be randomized 2:1 to 7 days of an oral formulation of cyclosporine, Neoral (2.5mg/kg PO BID) + standard of care (SOC) or no Neoral + SOC. The primary endpoint is disease severity based on the World Health Organization (WHO) COVID Ordinal Outcomes Scale, on day 14. Secondary endpoints include safety and changes in serum inflammatory markers.

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1. OBJECTIVES

1.1 Primary Objectives

1.1.1 To assess the effect of a 7-day course of oral cyclosporine (Neoral) on clinical outcome using the World Health Organization (WHO) COVID Ordinal Clinical Outcomes Scale, on day 14.

1.2 Secondary Objectives

1.2.1 To establish the safety of Neoral in this patient population (adverse events).

1.2.2 To determine the effect of Neoral on serum inflammatory markers (CRP, d-dimer, ferritin, ANC, absolute lymphocyte count, WBC, PLT (daily while inpatient and including day 14 and 28 for those discharged).

1.2.3 To determine the effect Neoral on viral SARS-CoV2 PCR positivity from baseline (day 0 to -2) before receiving Neoral) to day 14, and from baseline to day 28.

1.2.4 To determine the effect of Neoral on survival (days 14 and 28)

1.2.5 To determine the effect of Neoral on disease improvement (alive and free of invasive or non-invasive mechanical ventilation; days 14 and 28).

1.2.6 To determine the effect of Neoral on proportion of those requiring invasive mechanical ventilation.

1.2.7 To determine the effect of Neoral on incidence of deep vein thrombosis.

1.2.8 To determine the effect of Neoral on proportion of patients discharged on day 28.

1.2.9 To determine the effect of Neoral on time to hospital discharge.

1.2.10 To determine the effect of Neoral on disease resolution (alive and discharged home without oxygen; days 14 and 28).

2. BACKGROUND

2.1 Study Disease(s)

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a novel coronavirus that causes coronavirus disease 2019 (COVID-19). Since initial detection of the virus, more than 10 million cases of COVID-19 have been confirmed worldwide, and COVID-19 is responsible for more than 505,500 deaths. The United States has seen over 2.5 million cases of COVID-19

and 126,000 deaths from this disease (as of June, 30, 2020, source: Johns Hopkins University). SARS-CoV-2 is efficiently transmitted from person-to-person and the World Health Organization (WHO) has declared coronavirus disease 2019 (COVID-19) to be a pandemic. COVID-19 primarily spreads through the respiratory tract, by droplets, respiratory secretions, and direct contact. Current data suggest an incubation period of 1–14 days, in most cases 3–7 days. The virus is highly transmissible in humans and causes severe problems especially in the elderly and people with underlying chronic diseases. COVID-19 patients typically present with specific, similar symptoms, such as fever, malaise, and cough. Most adults or children infected with SARS-CoV-2 have presented with mild flu-like symptoms, but a few patients are in critical condition and rapidly develop acute respiratory distress syndrome (ARDS), respiratory failure, multiple organ failure, and death. The case fatality rate increases with the severity of illness and can reach up to 49% in critically ill patients (Wu).

Unfortunately, specific and effective therapies for COVID-19 are highly limited. Recent evidence suggest that administration of the anti-viral agent, Remdesivir, to hospital inpatients with COVID-19 decreases time to recovery from 15 to 11 days and decreases mortality at 14 days from 11.9% to 7.1% (Beigel). A preliminary, unpublished analysis from a large, multicenter, randomized, open-label trial for hospitalized patients in the United Kingdom showed that patients who were randomized to receive dexamethasone had a reduced rate of mortality compared to those who received standard of care. This benefit was observed in patients with severe COVID-19 and was greatest in those who required mechanical ventilation at enrollment (RECOVERY Trial). These 2 agents are considered in the standard of care (**SOC**) for treating patients with COVID-19. However, additional therapies with larger effect sizes and that are administered at earlier stages to prevent progression to severe COVID-19 are critically needed.

2.1.1 IND Agent(s)

The PI has filedan IND with cross-reference letter from Novartis and has received a Safe to Proceed determination (IND #152065).

The rationale for the proposed starting dose is based on the standard renal transplantation dose, which has a time-honored profile of safety in this population. Please see United States Product Insert (**USPI**) for full detail on summaries of nonclinical and clinical studies, nonclinical and clinical pharmacokinetics, and major route of elimination (https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/050715s035,050716s038lbl.pdf).

2.2 Rationale

Severe COVID-19. Initial reports from cases identified between February 12 to March 16 in the United States (U.S.) show rates of hospitalization for COVID-19 to be 21-31%, intensive care unit (ICU) admissions to be 5- 12%, and fatality to be 2%-3% [CDC COVID-19 Response Team 2020]. High-risk groups for severe COVID-19 have been identified as the elderly population and

those with underlying comorbidities such as cardiovascular disease, liver disease, pulmonary disease, renal disease, and diabetes mellitus [Xu 2020; Huang 2020].

Severe COVID-19 results from a dysregulated hyperimmune state. Severe symptoms of COVID-19 are associated with a hyperimmune response referred to as a cytokine storm. In one study, all 41 patients with COVID-19 admitted to the hospital demonstrated elevated plasma levels of cytokines and chemokines compared with healthy volunteers that included IL -1, -1R, -7, -8, -9, -10, and basic FGF2, GCSF, GMCSF, IFN-γ, IP10, MCP1, M1P1A, MIP1B, PDGF, TNF-α, and VEGF. Patients admitted to the ICU had higher levels of IL-2, IL-7, IL-10, GCSF, IP10, MCP1, MIP1A, and TNF-α than patients who did not require ICU admission [Huang 2020]. Emerging data has shown that early rapid SARS-CoV-2 replication causes massive epithelial and endothelial cell death that initiates a cytokine storm and vascular leakage, causes pyroptosis in macrophages and lymphocytes, and results in exhaustion of T cells and NK cells [Yang 2020][Chen2020][Zheng 2020].

COVID-19 similarities to hemophagocytic lymphohistiocytosis (HLH). HLH is an under-recognized, hyperinflammatory syndrome characterized by a fulminant and fatal cytokine storm and multi-organ failure. In adults, HLH is most commonly triggered by viral infections and occurs in 3.7% to 4.3% cases of sepsis. Cardinal features include unremitting fever, cytopenias, and hyperferritinemia. Pulmonary involvement (including ARDS) occurs in ~50% of patients. Each of these clinical features and a highly overlapping cytokine profile is seen in severe COVID-19. This demonstrates that the clinical presentation and pathologic mechanisms of severe COVID-19 is similar to, or is HLH.

Cyclosporine (CSA) suppresses hyperimmune states. Calcineurin inhibitors such as CSA suppress the phosphatase activity of calcineurin, which results in decreased IL-2 production and IL-2 receptor expression. This interrupts a central pathway of T-cell activation and dampens T cell responses and their associated cytokine storms [Holzner 2019]. CSA is approved by the FDA for three indications including 1) prophylaxis of organ rejection in kidney, liver, and heart transplants, 2) treatment of severe active rheumatoid arthritis, and 3) treatment of adult severe recalcitrant plaque psoriasis. It is critical to consider whether dampening of T-cell responses using CSA would curtail the vigor of T-cell hyperactivity in COVID-19 disease and provide an opportunity for these patients to recover.

CSA for COVID-19. Proposed is the use of the calcineurin inhibitor, CSA, for the treatment of patients with COVID-19. This is based on: **1)** observations that COVID-19 disease is associated with a hyperimmune response very similar to HLH, for which treatment with CSA is effective and recommended [La Rosee 2019], **2)** COVID-19 is associated with dysregulated macrophage activation similar to macrophage activation syndrome (MAS) which is also therapeutically suppressed by CSA [Merad M 2020; Liddacoat A 2019], and **3)** in vitro studies demonstrating that CSA specifically inhibits the replication of coronaviruses including SARS-CoV-2 with a high degree of specificity [Dittmar 2020].

CSA specifically inhibits coronavirus replication. Coronaviruses are RNA viruses with large genomes that enter host cells through binding of its transmembrane spike protein with angiotensin-converting enzyme 2 (ACE2) receptors expressed by host target cells, which is the

same mechanism utilized by SARS-CoV (i.e., SARS) [Walls 2020; Fu 2020]. Cyclophilins appear to play a critical role in the replication of many viruses including coronaviruses, HIV, and hepatitis C virus [de Wilde 2018; Tanaka 2013; Baugh 2012]. Although the exact mechanisms are not yet well understood, in vitro studies suggest that the coronavirus' nonstructural protein (Nsp) and nucleocapsid protein bind to cyclophilins, and knockdown of cyclophilin expression results in near complete inhibition of coronavirus replication [de Wilde 2018; Baugh 2012]. These data show that viral protein binding to cyclophilins is an important step for successful coronavirus replication, and inhibition of this interaction by CSA prevents viral replication. An important study demonstrated that CSA dominantly inhibited replication of human coronavirus 229E (HCoV-229E), mouse hepatitis virus (MHV), and SARS, and that treatment with increasing doses of CSA caused a dose-dependent decrease in SARS-CoV replication in human cells in vitro without affecting cell viability [de Wilde 2011]. The same group demonstrated that CSA inhibited replication of MERS-CoV without affecting cell viability of mock-infected cells [de Wilde 2013]. An independent group demonstrated that increasing concentrations of CSA treatment of SARS-CoV-infected human cells resulted in a dose-dependent decrease in viral replication, and inhibited the replication of other coronaviruses, including human CoV-NL63, CoV-229E, feline CoV serotypes I and II, porcine transmissible gastroenteritis virus (TGEV), avian infection bronchitis virus (IBV), and two isolates of SARS-CoV (Frankfurt and Hongkong) [Pfefferle 2011]. Taken together, Tanaka et al (2013) proposed a dual mechanism in which (1) calcineurin inhibition by CSA inhibits the phosphorylation of NFAT-P, thus preventing the production of IL-2 and other proinflammatory cytokines, and (2) CSA inhibits viral replication of coronaviruses, likely through blockade of calcineurin, causing the inhibition of cyclophilins required for viral replication. Most recently, Dittmar et al., demonstrated that CSA is highly specific and effective at inhibiting SARS-CoV-2 replication in various human cells, via inhibition of Cyclophilin [Dittmar 2020].

Safety of CSA in COVID-19. Since April 2020 mounting evidence from various patient populations has strongly suggested that CSA can be used safely in patients with COVID-19. Simon et al, recently demonstrated that patients with "Immune Mediated Inflammatory Diseases" (IMID) on various immunosuppressive drugs related to CSA have a "significantly reduced incidence of SARS-CoV-2 infection" (Simon 2020). Di Lernia et al., reported no significant increase in the incidence or severity of COVID-19 disease in patients undergoing CSA therapy for Psoriasis, but rather suggested a potentially milder disease in these patients (Di Lernia 2020). Another study of over 4000 patients in Madrid, Spain by Heili-Fredas et al., demonstrated "a universal relationship between the use of Cyclosporine A and better outcomes" in patients with COVID-19 disease (Heili-Frades S 2020). In line with the findings from Spain, Cavagna et al. in Italy observed that transplant patients with ongoing Calcineurin Inhibitor therapy developed only mild symptoms of COVID-19 disease and concluded that "Calcineurin inhibitor-based immunosuppressive regimens appear safe" in COVID-19 disease and should not be discontinued (Cavagna 2020). These recent clinical outcomes data suggest that the use of CSA in patients with COVID-19 is safe and potentially effective.

2.3 Correlative Studies Background

Correlative studies in this protocol are included as secondary endpoints and are based on: 1) serum inflammatory markers used clinically as biomarkers to monitor the severity of COVID-19

(CRP, d-dimer, ferritin, ANC, absolute lymphocyte count, WBC, PLT), and 2) SARS-CoV-2 viral load by a clinical PCR test.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Laboratory-confirmed SARS-CoV-2 infection within the past 10 days.

3.1.2 Patients admitted to non-ICU hospital floors or in an emergency department awaiting admission to a non-ICU hospital bed.

3.1.3 WHO COVID Scale Score 4 (Oxygen by mask or nasal prongs) or WHO COVID Scale Score 5 (non-invasive ventilation or high-flow oxygen)

3.1.4 Age 18 to 90 years old.

3.1.5 ECOG performance status ≤2 (see Appendix A).

3.1.6 Patients receiving or who have received standard of care therapy for COVID-19 can be included. This includes Remdesivir, Dexamethasone (or other steroids), and convalescent plasma.

3.1.7 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Allergy and/or hypersensitivity to CSA.

3.2.2 GFR<30 mL/min.

3.2.3 ALT or AST >3X upper limits of normal.

3.2.4 Resistant hypertension (BP>140/90 mm Hg despite adherence to maximal doses of three antihypertensive agents).

3.2.5 Active bacterial or mycobacterial infection.

3.2.6 Pregnant and/or nursing patients.

3.2.7 Participation in a COVID-19 therapeutic drug trial.

3.2.8 Patients who have received or who are receiving anti-viral medications including hydroxychloroquine will not be excluded.

3.2.9 Patients with psychiatric illness/social situations that would limit compliance with study requirements.

3.2.10 Total cholesterol is < 100 (increased risk of seizure)

3.2.11 Concomitant dosing with Tacrolimus is a relative contraindication (increases overall immunosuppression and decrease seizure threshold

3.2.12 Concomitant malignancy is a relative contraindication (Neoral can increase susceptibility to development of neoplasia)

3.2.13 Inability to swallow oral medication

3.2.14 Treatment with immunomodulators or immunosuppressant drugs, including but not limited to IL-6 inhibitors, TNF inhibitors, anti-IL-1 agents, and JAK inhibitors.

3.2.15 Investigational Antiviral agents

3.3 Inclusion of Women and Minorities

Women and minorities will be included in this study.

4. REGISTRATION PROCEDURES

IRB Approval

- IRB approval is pending at each of the 2 sites (BCM and BWH)

4.1 Patient Registration

To register a patient, the following documents will be completed by the research nurse or data manager and faxed and/or e-mailed to the Study Coordinator:

- Copy of required laboratory tests
- Signed patient consent form
- Eligibility Screening Worksheet

The research nurse or data manager at the participating site will then call and/or e-mail the Study Coordinator at each site to verify eligibility. To complete the registration process, each Coordinator will:

- assign a patient study number
- register the patient on the study
- randomize the patient to one arm or the other by entering data into OnCore
- fax or e-mail the patient study number and result of randomization to the participating site
- call the research nurse or data manager at the participating site and verbally confirm registration and randomization

4.2 General Guidelines

Following registration, patients should begin protocol treatment within 2 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

Additionally, we will record the number of days between the onset of symptoms and the initiation of treatment for each subject and we will include this information in the final study report.

4.3 Randomization

Patients will be randomized in a 2:1 ratio to receive either CSA or placebo (no CSA). Randomization will be open-label, using the permuted block method and will be stratified by age (<60 or >60 years), WHO severity (4 vs. 5), presence or absence of comorbidities (at least one of the following – hypertension, coronary artery disease, congestive heart failure, or diabetes). Randomization will be performed centrally at the Baylor College of Medicine by the Project Manager.

5. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

Biomarkers of disease severity will be:

1. Circulating cellular and protein inflammatory markers including CRP, d-dimer, ferritin, ANC, absolute lymphocyte count, WBC, and PLT.
2. SARS-CoV-2 positivity

5.1 Specimen Collection

Blood biomarkers will be drawn as described in **Table 1** (Schedule of Events).

1. Blood biomarkers are all standard hospital tests with standard blood draw collection protocols.
2. SARS-CoV-2 viral positivity will be determined from nasopharyngeal swabs using in-house hospital real-time RT-PCR by the Cepheid GeneXpert platform. Both nostrils will be used for virologic assessment. Please see the package insert for full detail.

(https://www.cepheid.com/Package%20Insert%20Files/Xpress-SARS-CoV-2/Xpert%20Xpress%20SARS-CoV-2%20Assay%20ENGLISH%20Package%20Insert%20302-3750%20Rev.%20C.pdf)

5.2 Specimen Procurement Kits and Scheduling

5.2.1 Specimen Procurement Kits

All specimens will be processed by site hospital laboratories using standard clinical use blood tubes or standard clinical SARS-COV-2 nasopharyngeal swabs.

5.2.2 Scheduling of Specimen Collections

Blood biomarkers will be drawn as described in the **Table 1** (Schedule of Events).

5.3 Specimen Collection.

5.3.1 Blood Collection. Described in **Table 1**(Schedule of Events).

5.3.2 SARS-CoV-2 positivity. Described in **Table 1** (Schedule of Events).

5.4 Biomarker Plan

According to the schedule described in **Table 1,** below, we will determine the effect of Neoral on circulating inflammatory markers including CRP, d-dimer, ferritin, ANC, absolute lymphocyte count, WBC, and PLT; and also on SARS-CoV-2 positivity using PCR.

6. TREATMENT AND/OR IMAGING PLAN

6.1 Agent Administration

Treatment will be administered on an inpatient basis. Reported adverse events and potential risks are described in Section 10. Appropriate dose modifications are described in Section 7

Arm A Regimen Description					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***
Neoral, Investigational, Generic Acceptable,	NA	2.5 mg/kg	PO	BID	7 days

N=50 Patients					

Arm B Regimen Description					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***
None*, N= 25 Patients	NA	NA	NA	NA	7 days

*Note: The PI has undertaken thorough discussions with the sponsor and generation of placebo capsules is not feasible for the timing of this study.

6.2 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of Neoral with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential for drug interactions. The study team should check a frequently-updated medical reference for a list of drugs to avoid or minimize use of (shown below). Patients in this study may receive Neoral in combination with other standard of care agents for COVID-19. These include Remdesivir and Dexamethasone. Adverse drug interactions between Neoral and these agents are not described but will be rigorously monitored.

*The data that follow are also found in the **USPI** for Neoral (*https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/050715s035,050716s038lbl.pdf):

*6.2.1 **Drug Interactions:** Effect of Drugs and Other Agents on Cyclosporine Pharmacokinetics and/or Safety*

6.2.1.1 Renal Impairment: All of the individual drugs cited below are well substantiated to interact with cyclosporine. In addition, concomitant non-steroidal anti-inflammatory drugs, particularly in the setting of dehydration, may potentiate renal dysfunction.

Drugs That May Potentiate Renal Dysfunction

Antibiotics	*Antineoplastics*	*Antifungals*	*Anti-inflammatory Drugs*	*Gastrointestinal Agents*	*Immunosuppressives*	*Other Drugs*
ciprofloxacin	melphalan	amphotericin B	azapropazon	cimetidine	tacrolimus	fibric acid derivatives (e.g., bezafibrate, fenofibrate)
gentamicin		ketoconazole	colchicine	ranitidine		methotrexate
tobramycin			diclofenac			
vancomycin			naproxen			
trimethoprim with sulfamethoxazole			sulindac			

During the concomitant use of a drug that may exhibit additive or synergistic renal impairment with cyclosporine, close monitoring of renal function (in particular serum creatinine) will be

performed. If a significant impairment of renal function occurs, the dosage of the co-administered drug should be reduced or an alternative treatment considered.

6.2.1.2 Cytochrome P450 System**:** Cyclosporine is extensively metabolized by CYP 3A isoenzymes, in particular CYP3A4, and is a substrate of the multidrug efflux transporter P-glycoprotein. Various agents are known to either increase or decrease plasma or whole blood concentrations of cyclosporine usually by inhibition or induction of CYP3A4 or P-glycoprotein transporter or both. Compounds that decrease cyclosporine absorption such as orlistat should be avoided. Appropriate Neoral dosage adjustment to achieve the desired cyclosporine concentrations is essential when drugs that significantly alter cyclosporine concentrations are used concomitantly.

6.2.1.3 HIV protease inhibitors (e.g., indinavir, nelfinavir, ritonavir, and saquinavir) are known to inhibit cytochrome P-450 3A and thus could potentially increase the concentrations of cyclosporine, however no formal studies of the interaction are available. Care should be exercised when these drugs are administered concomitantly.

6.2.1.4 Grapefruit, grapefruit juice, pomegranate, and orange juice affect metabolism, increasing blood concentrations of cyclosporine, thus should be avoided.

1. Drugs That Increase Cyclosporine Concentrations

Calcium Channel Blockers	*Antifungals*	*Antibiotics*	*Glucocorticoids*	*Other Drugs*
diltiazem	fluconazole	azithromycin	methylprednisolone	Allopurinol
nicardipine	itraconazole	clarithromycin		Amiodarone
verapamil	ketoconazole	erythromycin		Bromocriptine
	voriconazole	quinupristin/ dalfopristin		colchicine
				danazol
				imatinib
				metoclopramide
				nefazodone
				oral contraceptives

6.2.1.5 Drugs/dietary supplements that decrease cyclosporine concentrations include the following agents:

6.2.1.5.1 Bosentan: Coadministration of bosentan (250 to 1000 mg every 12 hours based on tolerability) and cyclosporine (300 mg every 12 hours for 2 days then dosing to achieve a Cmin of 200 to 250 ng/mL) for 7 days in healthy subjects resulted in decreases in the cyclosporine mean dose-normalized AUC, Cmax, and trough concentration of approximately 50%, 30%, and 60%, respectively, compared to when cyclosporine was given alone (See also Effect of Cyclosporine on the Pharmacokinetics and/or Safety of Other Drugs or Agents). Coadministration of cyclosporine with bosentan should be avoided.

6.2.1.5.2 Boceprevir: Coadministration of boceprevir (800 mg three times daily for 7 days) and cyclosporine (100 mg single dose) in healthy subjects resulted in increases in

the mean AUC and Cmax of cyclosporine approximately 2.7-fold and 2-fold, respectively, compared to when cyclosporine was given alone.

6.2.1.5.3 Telaprevir: Coadministration of telaprevir (750 mg every 8 hours for 11 days) with cyclosporine (10 mg on day 8) in healthy subjects resulted in increases in the mean dose-normalized AUC and Cmax of cyclosporine approximately 4.5-fold and 1.3-fold, respectively, compared to when cyclosporine (100 mg single dose) was given alone.

6.2.1.5.4 St. John's Wort: There have been reports of a serious drug interaction between cyclosporine and the herbal dietary supplement St. John's Wort. This interaction has been reported to produce a marked reduction in the blood concentrations of cyclosporine, resulting in subtherapeutic levels, rejection of transplanted organs, and graft loss.

6.2.1.5.5 Rifabutin: Rifabutin is known to increase the metabolism of other drugs metabolized by the cytochrome P-450 system. The interaction between rifabutin and cyclosporine has not been studied. Strict precaution should be taken when these two drugs are administered concomitantly.

2. Drugs/Dietary Supplements That Decrease Cyclosporine Concentrations

Antibiotics	*Anticonvulsants*	*Other Drugs/Dietary Supplements*	
nafcillin	carbamazepine	bosentan	St. John's Wort
rifampin	oxcarbazepine	octreotide	
	phenobarbital	orlistat	
	phenytoin	sulfinpyrazone	
		terbinafine	
		ticlopidine	

6.2.2 ***Drug Interactions:*** *Effect of Cyclosporine on the Pharmacokinetics and/or Safety of Other Drugs or Agents.*

6.2.2.1 Cyclosporine is an inhibitor of CYP3A4 and of multiple drug efflux transporters (e.g., P-glycoprotein) and may increase plasma concentrations of comedications that are substrates of CYP3A4, P-glycoprotein or organic anion transporter proteins.

6.2.2.2 Cyclosporine may reduce the clearance of digoxin, colchicine, prednisolone, HMG-CoA reductase inhibitors (statins), and, aliskiren, bosentan, dabigatran, repaglinide, NSAIDs, sirolimus, etoposide, and other drugs.

6.2.2.3 Digoxin: Severe digitalis toxicity has been seen within days of starting cyclosporine in several patients taking digoxin. If digoxin is used concurrently with cyclosporine, serum digoxin concentrations should be monitored.

6.2.2.4 Colchicine: There are reports on the potential of cyclosporine to enhance the toxic effects of colchicine such as myopathy and neuropathy, especially in patients with renal dysfunction. Concomitant administration of cyclosporine and colchicine results in significant increases in colchicine plasma concentrations. If colchicine is used

concurrently with cyclosporine, a reduction in the dosage of colchicine is recommended.

6.2.2.5 HMG-CoA reductase inhibitors (statins): Literature and postmarketing cases of myotoxicity, including muscle pain and weakness, myositis, and rhabdomyolysis, have been reported with concomitant administration of cyclosporine with lovastatin, simvastatin, atorvastatin, pravastatin, and, rarely fluvastatin. When concurrently administered with cyclosporine, the dosage of these statins should be reduced according to label recommendations. Statin therapy needs to be temporarily withheld or discontinued in patients with signs and symptoms of myopathy or those with risk factors predisposing to severe renal injury, including renal failure, secondary to rhabdomyolysis.

6.2.2.6 Repaglinide: Cyclosporine may increase the plasma concentrations of repaglinide and thereby increase the risk of hypoglycemia. In 12 healthy male subjects who received two doses of 100 mg cyclosporine capsule orally 12 hours apart with a single dose of 0.25 mg repaglinide tablet (one-half of a 0.5mg tablet) orally 13 hours after the cyclosporine initial dose, the repaglinide mean Cmax and AUC were increased 1.8 fold (range: 0.6 to –3.7 fold) and 2.4 fold (range 1.2 to 5.3 fold), respectively. Close monitoring of blood glucose level is advisable for a patient taking cyclosporine and repaglinide concomitantly.

6.2.2.7 Ambrisentan: Coadministration of ambrisentan (5 mg daily) and cyclosporine (100 to 150 mg twice daily initially, then dosing to achieve Cmin 150 to 200 ng/mL) for 8 days in healthy subjects resulted in mean increases in ambrisentan AUC and Cmax of approximately 2-fold and 1.5–fold, respectively, compared to ambrisentan alone. When coadministering ambrisentan with cyclosporine, the ambrisentan dose should not be titrated to the recommended maximum daily dose

6.2.2.8 Anthracycline antibiotics: High doses of cyclosporine (e.g., at starting intravenous dose of 16 mg/kg/day) may increase the exposure to anthracycline antibiotics (e.g., doxorubicin, mitoxantrone, daunorubicin) in cancer patients.

6.2.2.9 Aliskiren: Cyclosporine alters the pharmacokinetics of aliskiren, a substrate of P-glycoprotein and CYP3A4. In 14 healthy subjects who received concomitantly single doses of cyclosporine (200 mg) and reduced dose aliskiren (75 mg), the mean Cmax of aliskiren was increased by approximately 2.5-fold (90% CI: 1.96 to 3.17) and the mean AUC by approximately 4.3 fold (90% CI: 3.52 to 5.21), compared to when these subjects received aliskiren alone. The concomitant administration of aliskiren with cyclosporine prolonged the median aliskiren elimination half-life (26 hours versus 43 to 45 hours) and the Tmax (0.5 hours versus 1.5 to 2.0 hours). The mean AUC and Cmax of cyclosporine were comparable to reported literature values. Coadministration of cyclosporine and aliskiren in these subjects also resulted in an increase in the number and/or intensity of adverse events, mainly headache, hot flush, nausea, vomiting, and somnolence. The coadministration of cyclosporine with aliskiren is not recommended.

6.2.2.10 Bosentan: In healthy subjects, coadministration of bosentan and cyclosporine resulted in time-dependent mean increases in dose-normalized bosentan trough concentrations (i.e., approximately 21-fold on day 1 and 2 fold on day 8 (steady state)) compared to

when bosentan was given alone as a single dose on day 1. Coadministration of cyclosporine with bosentan should be avoided.

6.2.2.11 Dabigatran: The effect of cyclosporine on dabigatran concentrations had not been formally studied. Concomitant administration of dabigatran and cyclosporine may result in increased plasma dabigatran concentrations due to the P-gp inhibitory activity of cyclosporine. Coadministration of cyclosporine with dabigatran should be avoided.

6.2.2.12 Potassium-Sparing Diuretics: Cyclosporine should not be used with potassium-sparing diuretics because hyperkalemia can occur. Caution is also required when cyclosporine is coadministered with potassium-sparing drugs (e.g., angiotensin converting enzyme inhibitors, angiotensin II receptor antagonists), potassium-containing drugs as well as in patients on a potassium rich diet. Control of potassium levels in these situations is advisable.

6.2.2.13 Nonsteroidal Anti-inflammatory Drug (NSAID) Interactions: Clinical status and serum creatinine should be closely monitored when cyclosporine is used with NSAIDs in rheumatoid arthritis patients. Pharmacodynamic interactions have been reported to occur between cyclosporine and both naproxen and sulindac, in that concomitant use is associated with additive decreases in renal function, as determined by 99mTc-diethylenetriaminepentaacetic acid (DTPA) and (p-aminohippuric acid) PAH clearances. Although concomitant administration of diclofenac does not affect blood concentrations of cyclosporine, it has been associated with approximate doubling of diclofenac blood concentrations and occasional reports of reversible decreases in renal function. Consequently, the dose of diclofenac should be in the lower end of the therapeutic range.

6.2.2.14 Methotrexate Interaction: Preliminary data indicate that when methotrexate and cyclosporine were coadministered to rheumatoid arthritis patients (N=20), methotrexate concentrations (AUCs) were increased approximately 30% and the concentrations (AUCs) of its metabolite, 7-hydroxy methotrexate, were decreased by approximately 80%. The clinical significance of this interaction is not known. Cyclosporine concentrations do not appear to have been altered (N=6).

6.2.2.15 Sirolimus: Elevations in serum creatinine were observed in studies using sirolimus in combination with full-dose cyclosporine. This effect is often reversible with cyclosporine dose reduction. Simultaneous coadministration of cyclosporine significantly increases blood levels of sirolimus. To minimize increases in sirolimus concentrations, it is recommended that sirolimus be given 4 hours after cyclosporine administration.

6.2.2.16 Nifedipine: Frequent gingival hyperplasia when nifedipine is given concurrently with cyclosporine has been reported. The concomitant use of nifedipine should be avoided in patients in whom gingival hyperplasia develops as a side effect of cyclosporine.

6.2.2.17 Methylprednisolone: Convulsions when high dose methylprednisolone is given concurrently with cyclosporine have been reported.

6.2.2.18 Other Immunosuppressive Drugs and Agents: Psoriasis patients receiving other immunosuppressive agents or radiation therapy (including PUVA and UVB) should

not receive concurrent cyclosporine because of the possibility of excessive immunosuppression.

6.2.3 ***Effect of Cyclosporine on the Efficacy of Live Vaccines***: During treatment with cyclosporine, vaccination may be less effective. The use of live vaccines should be avoided.

6.3 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue for 7 days or until one of the following criteria applies:

- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Patient non-compliance
- Termination of the study by sponsor
- The drug manufacturer can no longer provide the study agent

The reason(s) for protocol therapy discontinuation, the reason(s) for study removal, and the corresponding dates must be documented in the Case Report Form (CRF).

6.4 Duration of Follow-Up

Patients will be followed for 60 days after beginning the 7. day course of neoral or until death, whichever occurs first. Please refer to the schedule of events (**Table 1**), below. Patients experiencing adverse event(s) that require cessation of Neoral will be followed until resolution or stabilization of the adverse event.

6.5 Missing Data

To minimize missing data, we will differentiate treatment discontinuation from study withdrawal. Study withdrawal will be considered as withdrawal of consent or loss of follow-up.

7. DOSING DELAYS/DOSE MODIFICATIONS

7.1.1 The dose of Neoral is 2.5mg/kg PO BID, based on the renal transplant dose, and Neoral will be administered for 7 days.

7.1.2 A blood cyclosporine trough level will be obtained on day 3 to avoid toxicity due to high concentrations. If this level is greater than 250 ng/ml, the Neoral dose will be decreased by 25-50%. If the level is 250 ng/ml or less, no modifications will be made.

7.1.3 Cyclosporine trough levels will be also obtained on day 6 and day 9. If this level is greater than 250 ng/ml on day 6 the Neoral dose will be decreased by 25-50% for the rest of the study period (7 days).

8. STUDY CALENDAR

Table 1 Schedule of Study Events (Part 1)

	Screening	Baseline	Treatment[1]							Follow-up[2]		
Visit			1	2	3	4	5	6	7	8	9	10
Study Day	-21 to -1	-2 to 0	1	2	3	4	5	6	7	8	9	10
Written Informed Consent	X											
Assign Screening Number	X											
Complete Medical History	X											
Demographics	X											
Inclusion/Exclusion Criteria Review	X											
WHO COVID-19 Severity Scale	X	X	X	X	X	X	X	X	X	X	X	X
Assign Enrollment Number		X										
Complete Physical Examination	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs (BP, HR, RR, Temp, S_pO_2)	X	X	X	X	X	X	X	X	X	X	X	X
FIO_2 Assessment	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy Test for Female Patients	X											
Cyclosporine Administration			X	X	X	X	X	X	X			
12-Lead ECG	X								X			X
Chest X-ray	X								X			X
BMP	X	X	X	X	X	X	X	X	X	X	X	X
LFTs	X	X	X	X	X	X	X	X	X	X	X	X
CBC	X	X	X	X	X	X	X	X	X	X	X	X
ANC	X	X	X	X	X	X	X	X	X	X	X	X
Absolute Lymphocyte Count	X	X	X	X	X	X	X	X	X	X	X	X
Total Cholesterol	X	X										
Ferritin	X	X	X	X	X	X	X	X	X	X	X	X
D-Dimer	X	X	X	X	X	X	X	X	X	X	X	X
CRP	X	X	X	X	X	X	X	X	X	X	X	X
LDH	X	X	X	X	X	X	X	X	X	X	X	X
Magnesium	X	X	X	X	X	X	X	X	X	X	X	X
Phosphorus	X	X	X	X	X	X	X	X	X	X	X	X
PT/PTT	X	X							X			
SARS-CoV-2 PCR	X											
AE Assessment	----------Continuous Monitoring from Signing of the Informed Consent----------►											
Concomitant Medications/Therapies	----------Continuous Monitoring from Signing of the Informed Consent----------►											

ANC =Absolute neutrophil count; BMP =basic metabolic panel; BP = blood pressure; CBC = complete blood count; CRP = c-reactive protein; ECG = Electrocardiogram; FIO_2 = oxygen requirement; HR = heart rate; LDH = lactate dehydrogenase; LFTs = liver function tests; PT = prothrombin time; PTT = partial thromboplastin time; RR = respiratory rate; S_pO_2 = oxygen saturation; WHO = World Health Organization.

[1]Treatment assessments will be performed every 24±6 hours. Treatment is Neoral for 7 days and will discontinued on the day of discharge if the patient is discharged before the 7d course is completed. If the patient is discharged before 7 days, the patient will not undergo any assessments until the first outpatient visit.

[2]Follow up will be performed every 24±6 hours while inpatient. Once patient is discharged, follow up will be at days 14±3 days, 28±3 days, and 60±3 days either by in person clinic visit or telemedicine visit.

Table 1 Schedule of Study Events (Part 2)

	Follow-up[2]											
Visit	11	12	13	14	15-27	28	60					
Study Day	11	12	13	14	15-27	28	60					
Written Informed Consent												
Assign Screening Number												
Complete Medical History												
Demographics												
Inclusion/Exclusion Criteria Review												
WHO COVID-19 Severity Scale	X	X	X	X	X	X	X					
Assign Enrollment Number												
Complete Physical Examination	X	X	X	X	X	X						
Vital Signs (BP, HR, RR, Temp, S_pO_2)	X	X	X	X	X	X						
FIO_2 Assessment	X	X	X	X	X	X						
Pregnancy Test for Female Patients												
Cyclosporine Administration												
12-Lead ECG												
Chest X-ray												
BMP	X	X	X	X	X	X						
LFTs	X	X	X	X	X	X						
CBC	X	X	X	X	X	X						
ANC	X	X	X	X	X	X						
Absolute Lymphocyte Count	X	X	X	X	X	X						
Ferritin	X	X	X	X	X	X						
D-Dimer	X	X	X	X	X	X						
CRP	X	X	X	X	X	X						
LDH	X	X	X	X	X	X						
Magnesium	X	X	X	X	X	X						
Phosphorus	X	X	X	X	X	X						
PT/PTT	X	X	X	X	X	X						
Cyclosporine Level[3]												
SARS-CoV-2 PCR				X		X	X					
AE Assessment	----------Continuous Monitoring from Signing of the Informed Consent----------►											
Concomitant Medications/Therapies	----------Continuous Monitoring from Signing of the Informed Consent----------►											

ANC =Absolute neutrophil count; BMP =basic metabolic panel; BP = blood pressure; CBC = complete blood count; CRP = c-reactive protein; ECG = Electrocardiogram; FIO_2 = oxygen requirement; HR = heart rate; LDH = lactate dehydrogenase; LFTs = liver function tests; PT = prothrombin time; PTT = partial thromboplastin time; RR = respiratory rate; S_pO_2 = oxygen saturation; WHO = World Health Organization.

[1]Treatment assessments will be performed every 24±6 hours. Treatment is Neoral for 7 days and will begin as an inpatient and will be discontinued if the patient is discharged before the 7d course is completed. If the patient is discharged before 7 days, the patient will not undergo any assessments until the first outpatient visit.

[2]Follow up will be performed every 24±6 hours while inpatient. Once patient is discharged, follow up will be at days 14±3 days, 28±3 days, and 60±3 days either by in person clinic visit or telemedicine visit.

[3]Cyclosporine trough levels will be checked on day 3. If >250 mg/ml, the dose will be decreased by 25-50%. Additional levels will be at the discretion of treating physicians but will be performed on day 6 and day 9 if the participant is an inpatient.

9. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated Neoral can be found in Section 10.1.

9.1 IND Agent

9.1.1 Neoral

Availability

Neoral is an investigational agent supplied to investigators by Novartis.

9.1.2 Agent Ordering and Agent Accountability

9.1.2.1 Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, dispensing and final disposition of Neoral including, strength, formulation and ordering investigator on this protocol.

9.1.3 Investigator Brochure Availability

The sponsor does not have an investigator brochure for Neoral, however the USPI will be available to all investigators (https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/050715s035,050716s038lbl.pdf)

10. MEASUREMENT OF EFFECT

The primary outcome of this study is the WHO COVID-19 clinical severity scale on day 14. Please, see the Table 2.

Table 2. WHO COVID-19 Ordinal Outcomes Scale

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

11. STATISTICAL CONSIDERATIONS

This is a multicenter, open-label randomized phase IIa trial study to assess the effect and safety of Neoral (CSA) for treatment of COVID-19 (+) non-ICU hospital inpatients.

11.1 Primary Endpoint and Analytic Plan

The primary endpoint is the clinical outcome based on the World Health Organization (WHO) COVID Ordinal Clinical Improvement Scale (Table 2) on day 14. Wilcoxon rank sum test will be used to compare the ordinal scale between groups on day 14.

11.2 Sample Size /Justification

We expect to see higher grades of WHO COVID ordinal clinical scale in control group compared to the CSA treatment group, and propose to use a 1 to 2 randomization in favor of CSA group.

The total sample size of 75 evaluable participants will provide 81% power to reject the null hypothesis that the grade in control group is less than or equal to the CSA group with a 10% one-sided significance level when the true effect side, as measured by the probability that

Control grade is greater than CSA grade, is 0.65 or greater. Sample size and/or power for the various scenarios were calculated using nQuery 8 (StatSols Version 8.5.2.0).

The following provides the rationale for the alternate hypothesis effect size. We have assumed that all participants will be starting in category 4. Based on review of hospital data, about one third of such patients end-up in the ICU and about half of those patients will die. Of the two thirds that do not go to the ICU, most will be discharged in 2 weeks or less. We have therefore hypothesized that at 2 weeks following enrollment, the control group will be distributed as shown in the table 3.

We have hypothesized several scenarios to estimate the sample size needed to detect a CSA effect with reasonable power. We expect the effect of CSA will be to reduce ICU admissions, or to shorten time in the ICU and reduce deaths, so that the fraction in categories 6, 7, and 8 on Day 14 will be reduced and less ill patients will go home earlier, increasing the fraction of 1 and 2's on day 14.

Since the outcome is ordinal, a Wilcoxon rank sum test is used to control (no CSA) and CSA on day 14. The one-tailed null hypothesis is that the distribution of WHO scores is the same in two groups or worse in CSA, and the alternative is that the distribution of WHO scores in the CSA group is shifted lower. The effect size (P1=Pr(Control>CSA)) is the probability that a randomly selected observation from the control group has a score greater than one from CSA group. The null value for P1 is 0.5 and the closer P1 to 1, the bigger the effect.

A sample size of 25 in the control group and 50 in the CSA group will have 81% power to reject the null hypothesis (based on the 8 observable category probabilities for each group shown in table 3) in favor of the alternative hypothesis that an observation in the control group has a higher score on day 14 than an observation in the CSA group using a Wilcoxon (Mann-Whitney) rank-sum test with the type 1 error of 10% one-sided significance level.

Table 3. Scenarios and associated sample size and power.

		At Accrual	Control	CSA Scenario 1 ~50% benefit	CSA Scenario 2 ~40% benefit	CSA Scenario 3 ~30% benefit
8	Death	0	17%	8%	10%	12%
7	Hospitalized, vented, pressors	0	8%	4%	5%	5%
6	Hospitalized w invasive ventilation	0	8%	4%	5%	6%
5	Hospitalized non-invasive ventilation	0	5%	2%	3%	3%
4	Hospitalized on O2	100%	5%	2%	3%	3%
3	Hospitalized not on O2	0	10%	2%	4%	7%
2	Not hospitalized with symptoms	0	24%	39%	35%	32%
1	Not hospitalized with no symptoms	0	23%	39%	35%	32%
Effect Size (P1=Pr(Control>CSA)				0.65	0.61	0.59
Power & sample size (α=10%, 1-tailed)						
	Power with N=25 control, 50 CSA			81%	62%	48%
	Sample size for ~80% power; Ctl:CSA			25:50	45:90	75:150

11.3 Stratification Factors

Randomization will be stratified by 1) age (<60 or ≥60 years), 2) WHO severity (4 vs. 5), and 3) presence or absence of comorbidities (at least one of the following- hypertension, coronary artery disease, congestive heart failure, or diabetes) with total of up to 8 strata and employ permutated block size of 3.

11.4 Populations for Analysis

11.4.1 Safety population

All patients will be evaluable for toxicity from the time of their first treatment with Neoral and throughout the study period. In this study, patients will receive 7 days of Neoral and incidence of Neoral-associated toxicity events (see 11.5) and all AEs will be assessed daily.

11.4.2 Intent to treat (ITT) population

All randomized patients in the study will be assessed for response to treatment, even if there are major protocol treatment deviations. Each patient will be assigned one of the WHO COVID scale categories outlined in **Table 2**. Primary and secondary objectives will be analyzed.

11.4.3 Modified intent to treat (MITT) population

Primary and secondary objectives will be analyzed in all randomized patients who complete 7 days of Neoral.

11.4.4 Per-protocol analysis population

Sub-analyses may then be performed on the basis of a subset of ITT population, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, *etc.*). However, these sub-analyses

may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.

11.5 Analysis of Secondary Endpoints

The secondary objectives are mostly concerned with the safety and effects of Neoral (CSA) in this patient population. The secondary endpoints include Neoral-associated toxicity events, serum inflammatory markers (CRP, d-dimer, ferritin, ANC, absolute lymphocyte count, WBC, PLT (daily while inpatient and including day 14 and 28 for those discharged), Viral load (days 14 and 28), vital status (days 14 and 28), disease improvement (days 14 and 28), proportion of those requiring invasive mechanical ventilation, incidence of deep vein thrombosis, proportion of patients discharged on day 28, time to hospital discharge, and disease resolution (days 14 and 28).

For safety, Neoral-associated toxicity events include nephrotoxicity, resistant hypertension, hepatotoxicity, signs of CNS toxicity, or superinfection. AE's and SAE's by CTCAE v5 will be summarized descriptively with frequencies and rates. Rates and 95% CI's of grade 3 and above AE's will be reported.

Serum inflammatory markers are viewed as interval scales and after appropriate normalizing transformation (if needed), will be analyzed using general linear mixed models to account for within patient dependencies to allow comparisons between groups.

Viral positivity between groups at days 14 and days 28 will be tested by Wilcoxon rank sum test.

Survival analysis methods will be used to describe overall survival experience, to estimate survival at selected time points, and to compare groups. Cumulative incidence analysis methods will be used if there are competing risks. The same approach will be used with disease improvement (alive and free invasive or non-invasive mechanical ventilation) and disease resolution (alive and discharged home without oxygen).

Fisher's exact test will be used to compare rates of need for invasive mechanical ventilation bench marked to a particular time, and ever need. If required, this endpoint will include deaths before intubation in the rare cases in which this occurs.

In exploratory analyses, we will use logistic regression to model ventilator need as a function of treatment group and other baseline characteristics. Survival analysis methods will be used to analyze time to mechanical ventilation, with censoring at last follow-up for those not needing ventilation.

Day 28 discharge will be trichotomized as death prior to day 28, discharge by day 28, hospitalized after day 28. Groups will be compared using Chi square test for independence.

Survival analysis methods will be used with death prior to discharge considered as a competing risk.

11.6 Stopping Rules

There is not planned interim analysis for efficacy.

11.6.1 Death possibly, probably to definitely related to CSA: Any death in the Neoral group that is assessed as at least possibly related to Neoral will result in a pause to study accrual and a review be the DSMB.

11.6.2 Targeted Toxicities: Other expected toxicities will be continuously monitored using the method of Ivanova et al [Ivanova, A., Qaqish, B.F., and Schell, M.J. (2005). Continuous toxicity monitoring in phase II trials in oncology. Biometrics 61: 540-545.]. The method generates a Pocock-type boundary for repeated testing for toxicity. This is a Pocock-type stopping boundary that yields the probability of crossing the boundary at most 5% when the rate of grade 3 or greater target toxicity is equal to the acceptable rate of 20%. The method will stop the trial early with 40% and 86% probability if the toxicity rate is 30% or 40%, respectively.

11.6.3 Data Safety Monitoring Board (DSMB): The conduct of this study will be overseen by a DSMB of 3 experts who are not participating in this study. The DSMB will be responsible for recommendations to the Principal Investigator regarding possible trial closure and/or early reporting of the study. The study team (with the exception of the study statistician) will not have access to the summary outcome data until released by the DSMB.

Table 4. Toxicity monitoring boundaries. The following table will be used. Targeted toxicities include renal dysfunction, hypertension and liver toxicity. The trial will be paused and reported to the DSMB if the number of patients with grade 3 or greater AE's for any of the targeted toxicities is equal to or exceeds b_n out of n patients with completed follow-up (Day 7).

Number of Patients, *n*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Boundary, b_n	-	-	3	4	4	5	5	5	6	6	6	7	7	7	8	8	8	9	9	9
Number of Patients, *n*	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Boundary, b_n	10	10	10	11	11	11	11	12	12	12	13	13	13	13	14	14	14	14	15	15
Number of Patients, *n*	41	42	43	44	45	46	47	48	49	50										
Boundary, b_n	15	16	16	16	16	17	17	17	17	18										

12. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting will be undertaken for 1) Neoral-associated toxicity events including nephrotoxicity, resistant hypertension, hepatotoxicity, signs of CNS toxicity, or new bacterial/mycobacterial infection; as well as 2) other AEs.

Safety duration is at 60 days and we will follow up all AEs and mortality at day 60.

12.1 Comprehensive Adverse Events and Potential Risks List(s) (CAEPRs)

12.1.1.1 Adverse Event List(s) for Neoral. For a comprehensive list of adverse events, please see the product insert: (https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/050715s035,050716s038lbl.pdf).

12.1.1.2 Most Common Adverse Events. Cyclosporine is FDA approved for indications of: 1) Kidney, Liver, and Heart Transplantation 2) Rheumatoid Arthritis, and 3) Psoriasis.

a. In Transplant, the principal adverse reactions of cyclosporine therapy are renal dysfunction, tremor, hirsutism, hypertension, and gum hyperplasia.
b. In Rheumatoid Arthritis, the principal are renal dysfunction (See WARNINGS), hypertension (See PRECAUTIONS), headache, gastrointestinal disturbances, and hirsutism/hypertrichosis.
c. In Psoriasis, principal adverse reactions associated with the use of cyclosporine are renal dysfunction, headache, hypertension, hypertriglyceridemia, hirsutism/hypertrichosis, paresthesia or hyperesthesia, influenza-like symptoms, nausea/vomiting, diarrhea, abdominal discomfort, lethargy, and musculoskeletal or joint pain.

12.1.1.3 Taken together, and based on data from >1000 patients available in the USPI, the most common adverse events of Neoral that may be encountered in this study are: ***Renal dysfunction, hypertension, hirsutism, tremor, headache, nausea, and abdominal pain.***

12.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

12.3 Expedited Adverse Event Reporting

12.3.1 Expedited Reporting Guidelines

Note: A death on study requires <u>both</u> routine and expedited reporting, regardless of causality as long as the death occurred within 30 days after the last administration of the investigational agent. Attribution to treatment or other cause must be provided.

Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent [1]

<u>FDA</u> REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **<u>MUST</u>** immediately report to the sponsor **<u>ANY</u>** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64). These will be reported to the <u>FDA</u> and to the <u>Sponsor</u>.

An adverse event is considered serious if it results in **<u>ANY</u>** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

<u>ALL</u> <u>SERIOUS</u> adverse events that meet the above criteria MUST be immediately reported to the <u>Sponsor</u> via electronic submission within 7 calendar days of learning of the AE.

12.4 Routine Adverse Event Reporting

All Adverse Events must be reported in routine study data submissions.

13. STUDY OVERSIGHT AND DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 10 (Adverse Events: List and Reporting Requirements).

13.1 Study Oversight

This protocol is monitored at several levels, as described in this section. The Protocol Principal Investigator is responsible for monitoring the conduct and progress of the clinical trial, including the ongoing review of accrual, patient-specific clinical and laboratory data, and routine and serious adverse events; reporting of expedited adverse events; and accumulation of reported adverse events from other trials testing the same drug(s). The Protocol Principal Investigator and statistician have access to the data required for this at all times.

There are 2 sites in this protocol. The lead site is Baylor College of Medicine. The PI from Baylor College of Medicine (Burt) and the PI from the second site, Brigham and Women's

Hospital (Goldberg) will have a conference at least monthly to review enrollment, AEs, and administrative elements of this protocol.

All Study Investigators at participating sites who register/enroll patients on a given protocol are responsible for timely submission of data via the mechanism described elsewhere in this section. All studies are also reviewed in accordance with the enrolling institution's data safety monitoring plan.

13.2 Data Reporting

13.2.1 Method

Each of the 2 hospital sites will have a dedicated monitor. These individuals, in collaboration with the study monitor, will submit cumulative protocol- and patient-specific data at least every two weeks to a central Encore database. This reporting consists of Patient Demographics, Baseline Abnormalities, On/Off Treatment/Study Status, Treatment/Course/Dosing information, Adverse Events, Late Adverse Events, and Response data as applicable. On-site audits will be conducted two times annually (one annual site visit and one data audit).

13.2.2 Responsibility for Data Submission

It is the responsibility of the PI(s) at the site to ensure that all investigators at both sites understand the procedures for data submission and that protocol specified data are submitted accurately and in a timely manner to the CTMS via the electronic data capture system. Data are to be submitted to this system on a real-time basis, but no less than once every 2 weeks. Metrics for timeliness will be followed and assessed on a quarterly basis. For the purpose of Institutional Performance Monitoring, data will be considered delinquent if it is greater than 4 weeks past due.

13.3 Multicenter Guidelines

The Principal Investigator/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports to all participating institutions for submission to their individual IRBs for action as required.

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APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX B FORMULA TO ESTIMATE RENAL FUNCTION USING SERUM CREATININE

Formulas to estimate renal function using serum creatinine provided by the NCI's Investigational Drug Steering Committee (IDSC) Pharmacological Task Force in table below.

1. Estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (Levey *et al.*, 2009).

Formulae:

Race and Sex	Serum Creatinine (SCr), *µmol/L (mg/dL)*	Equation
Black		
Female	≤62 (≤0.7)	$GFR = 166 \times (SCr/0.7)^{-0.329} \times (0.993)^{Age}$
	>62 (>0.7)	$GFR = 166 \times (SCr/0.7)^{-1.209} \times (0.993)^{Age}$
Male	≤80 (≤0.9)	$GFR = 163 \times (SCr/0.9)^{-0.411} \times (0.993)^{Age}$
	>80 (>0.9)	$GFR = 163 \times (SCr/0.9)^{-1.209} \times (0.993)^{Age}$
White or other		
Female	≤62 (≤0.7)	$GFR = 144 \times (SCr/0.7)^{-0.329} \times (0.993)^{Age}$
	>62 (>0.7)	$GFR = 144 \times (SCr/0.7)^{-1.209} \times (0.993)^{Age}$
Male	≤80 (≤0.9)	$GFR = 141 \times (SCr/0.9)^{-0.411} \times (0.993)^{Age}$
	>80 (>0.9)	$GFR = 141 \times (SCr/0.9)^{-1.209} \times (0.993)^{Age}$

SCr in mg/dL; Output is in mL/min/1.73 m^2 and needs no further conversions.

2. eGFR using the Modification of Diet in Renal Disease (MDRD) Study (Levey *et al*., 2006).

$175 \times SCr^{-1.154} \times age^{-0.203} \times 0.742$ (if female) $\times$ 1.212 (if black)
Output is in mL/min/1.73 m^2 and needs no further conversions.

3. Estimated creatinine clearance (ClCr) by the Cockcroft-Gault (C-G) equation (Cockcroft and Gault, 1976).

$$CLcr\ (mL/min) = \frac{[140 - age\ (years)] \times weight\ (kg)}{72 \times serum\ creatinine\ (mg\ /\ dL)} \{\times 0.85\ for\ female\ patients\}$$

Followed by conversion to a value normalized to 1.73 m^2 with the patient's body surface area (BSA).

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