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DEPARTMENT OF INFECTIOUS DISEASE

TITLE: A Phase 1/2 Trial of Leflunomide for the Treatment of Severe COVID-19 in Patients with Concurrent Malignancy

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Clinical Trial Protocol

A Phase 1/2 Trial of Leflunomide for the Treatment of Severe COVID-19 in Patients with a Concurrent Malignancy

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Short Title	Leflunomide for Severe COVID-19

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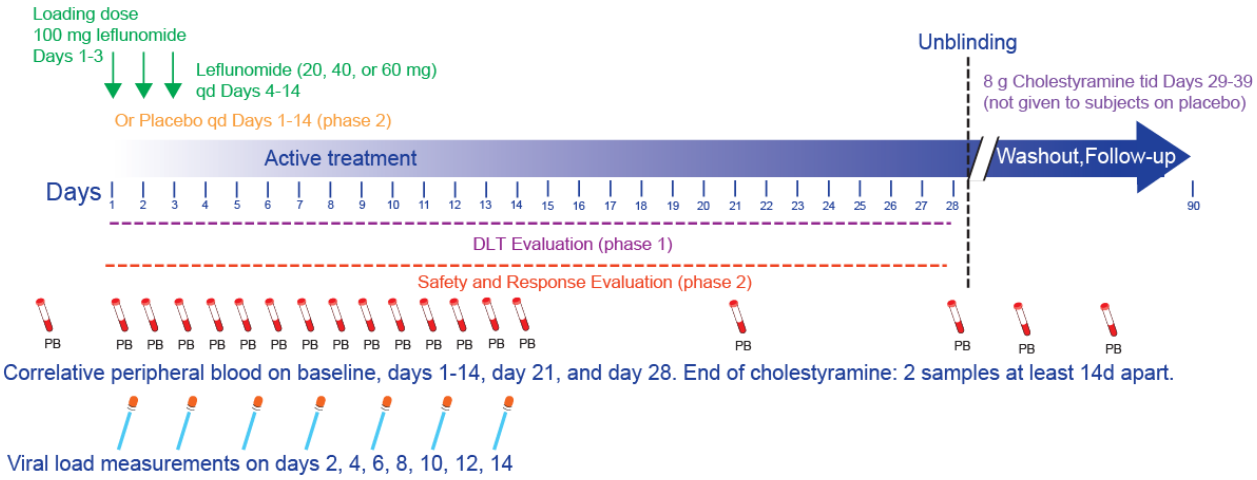
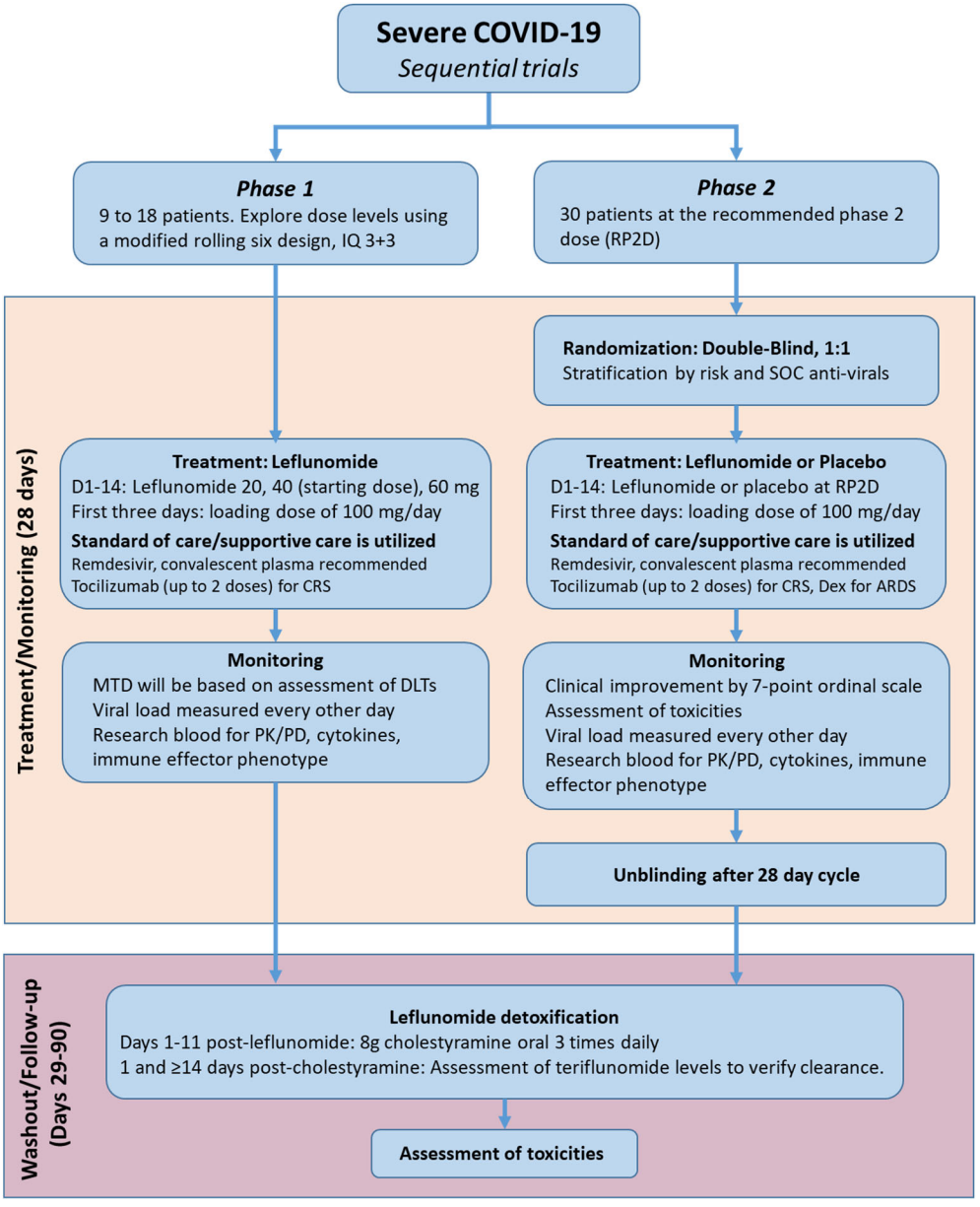
STUDY SCHEMAS

TREATMENT SCHEMA



STUDY DESIGN SCHEMA



PROTOCOL SYNOPSIS

Protocol Title

A phase 1/2 trial of leflunomide for treatment of severe COVID-19 in patients with a concurrent malignancy

Study Detail

Population/Indication(s):	SARS-CoV-2 infection/COVID-19 disease
Phase:	Phase 1: 20mg, 40mg, 60mg Phase 2: Randomization (1:1), with stratification
Sample Size:	Expected: 39 = 9 (Phase 1) and 15 per arm (Phase 2) Maximum: 48 = 18 (Phase 1); 15 per arm (Phase 2)
Estimated Accrual Duration:	Phase 1: 6 months Phase 2: 6-9 months
Estimated Study Duration	12-15 months
Participant Duration:	3 months
Participating Sites:	City of Hope, Duarte, CA University of Southern California (USC) Medical Center, Los Angeles, CA
Study Agents:	Leflunomide
Sponsor:	City of Hope

Rationale for this Study

Novel interventions are urgently needed to address the COVID-19 pandemic, especially for high-risk populations, including patients with cancer, who may be immunocompromised and/or have comorbid conditions. The potentially serious complications encountered by these patients present a challenge to clinical management and patient care, and therapies must be generated accordingly.

Leflunomide is an orally available dihydroorotate dehydrogenase (DHODH) inhibitor, impacting pyrimidine synthesis for DNA and RNA production, and has been in use for over 20 years with an excellent safety profile for the treatment of autoimmune diseases [1, 2]. It has known anti-viral activity and has been applied against CMV and BK infections in immunocompromised hosts [3, 4]. *In vitro* and *in vivo* experiments conducted in Wuhan, China demonstrated DHODH inhibitor activity against COVID-19, including teriflunomide, the active metabolite of leflunomide [5]. Our preliminary data also suggest that leflunomide significantly arrests viral RNA replication in cancer cells infected with a natural occurring RNA virus (reovirus) and impairs ex-vivo IL-6 expression in virally infected peripheral blood mononuclear cells (PBMCs). City of Hope has conducted a phase 1 trial of leflunomide in patients with relapsed/refractory multiple myeloma, demonstrating disease stabilization and a favorable safety profile at doses below 60 mg/day [6]. We have extensive experience in the clinical management, pharmacokinetic (PK) analysis, and correlative study of this repurposed agent. Leflunomide requires weekly monitoring for potential liver toxicity, and since COVID-19 itself can cause liver toxicities, we are proposing to begin the study with dose escalation, testing up to 3 doses of leflunomide to determine optimal pharmacokinetics and an acceptable toxicity profile in the setting of COVID-19 infection [5].

Here we propose an open-label phase 1 trial followed by a randomized, placebo-controlled, double-blind, phase 2 pilot trial to evaluate the safety and clinical activity of leflunomide added to standard-of-care (SOC), in patients with severe COVID-19 infection and a concurrent malignancy.

Treatment Description

Phase 1: Patients will be treated with the assigned dose of leflunomide for 14 days as part of a 28-day treatment cycle that may include current SOC COVID-19 agents and supportive care. Cholestryamine will be administered

starting on day 29 to promote teriflunomide clearance. Patients will receive a leflunomide loading dose of 100 mg po daily x 3 days followed by a daily dose of 20, 40 or 60 mg of po leflunomide for 11 days.

Randomized Phase 2: Patients will be treated as in the phase 1 portion at the recommended phase 2 dose, or they will receive placebo instead of leflunomide.

The first 7 days of leflunomide/placebo will be inpatient, with hospital discharge contingent on improvement of COVID symptoms to moderate severity or better and resolution of treatment-emergent adverse events to ≤ grade 1 or a return to baseline level(s). Subjects will receive a single cycle of treatment; there will be no intra-patient dose escalation. All participants will be followed for a minimum of 3 months from start of therapy or until resolution of any serious adverse events occurring during treatment, whichever occurs later. Up to 2 doses of tocilizumab may also be administered to patients with cytokine release syndrome (CRS) as supportive care.

Abbreviated Eligibility Criteria

Main Inclusion Criteria

- SARS-CoV-2 infection confirmed by RT-PCR within 4 days prior to enrollment
- COVID-19 disease baseline severity of Severe as defined by:
  - Symptoms suggestive of systemic illness with COVID-19, which could include fever, cough, sore throat, malaise, headache, muscle pain, gastrointestinal symptoms, or shortness of breath at rest, or respiratory distress
  - Clinical signs indicative of severe systemic illness with COVID-19, such as respiratory rate ≥30 per minute, heart rate ≥125 per minute, SpO2 ≤93% on room air at sea level or PaO2/FiO2 <300
- Active cancer requiring systemic treatment within the last 2 years.
- Subjects should not have received prior cancer treatment within specified intervals: radiotherapy – 1 to 2 weeks; chemotherapy or major surgery – 2 weeks; biologic therapy, CAR T or autologous transplant – 12 weeks, allogeneic transplant – 16 weeks
- Subjects should be be stable enough to forgo cancer therapy for the duration of active leflunomide treatment and until after the cholestyramine washout (~39 days).

Main Exclusion Criteria

- Patients with indolent local malignancies or pre-malignant conditions
- Requiring mechanical ventilation at enrollment / critical severity COVID
- Patients with pre-existing acute or chronic liver disease, or those with serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) ≥ 2 x ULN, or total bilirubin > 1.5 x ULN

Objectives

In patients with severe COVID-19 and active malignancy in the last 2 years who are receiving SOC treatment for COVID-19:

Primary Objectives

Phase 1 (Single-arm, Single-agent, Multi-Center)

- Assess the safety and tolerability of leflunomide when combined with COVID-19 SOC by evaluation of toxicities including: type, frequency, severity, attribution and duration.
- Determine the maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D) of leflunomide when given in combination with SOC.

Phase 2/Pilot (Two Arm, Randomized, Double-blind, Multi-Center):

- Evaluate the clinical activity of leflunomide plus SOC (Arm 1) and placebo plus SOC (Arm 2) on the basis of clinical improvement (response) rate in each treatment arm, as assessed by a 7-point ordinal scale.
- Evaluate the safety and tolerability of leflunomide plus SOC (Arm 1) and placebo plus SOC (Arm2).

Secondary Objectives

Phase 1

Evaluate the clinical activity of leflunomide when combined with SOC on the basis of clinical improvement (response) rate.

Phase 1 and Phase 2

- Estimate the following:
  - Time to clinical improvement (days);
  - Time to SpO_2 > 93% on room air;
  - Time to first negative SARS-CoV-2 polymerase chain reaction (PCR);
  - Duration of oxygen therapy (days);
  - Duration of hospitalization (days);
  - Duration of mechanical ventilation;
  - All cause mortality at Day 28.
- Measure trough plasma concentrations of metabolite teriflunomide during treatment and evaluate relationships between levels, pharmacodynamic biomarkers, concomitant medications, and outcomes.

Exploratory Objectives

- Investigate inflammatory response by measuring changes in circulating cytokines and immune effector cell phenotype before and after leflunomide treatment
- Assess the kinetics of viral replication through serial measurements of viral load by nasopharyngeal swab and tracheal aspirates (if on ventilator and can be safely obtained).

Study Design

Phase 1 Design (Single-arm, Single-agent, Multi-center):

Prior to formally initiating the multi-center, randomized, placebo-controlled, double-blind, phase 2 pilot, a multi-center, single-arm phase 1 trial will be conducted to assess the safety and tolerability of leflunomide when combined with COVID-19 SOC.

Because the objective is to keep the dose-limiting toxicity (DLT) probability to low levels, a modified rolling-six design, the IQ 3+3 [7], will govern the dose escalation (de-escalation), as opposed to methods that calibrate to a DLT rate. Up to three doses of leflunomide will be considered: 20 mg/day (DL-1), 40 mg/day (DL1-starting dose), and 60 mg/day (DL2) as shown in **Table 1**. The treatment cycle length is 28 days; patients will receive only one cycle of treatment. There will be no dose escalation within a patient.

Table 1. Dose Levels

Dose Level	Leflunomide Dose*
-1	20 mg PO daily
(starting dose) **1**	40 mg PO daily
2	60 mg PO daily

*NOTE: Leflunomide is administered with a loading dose of 100 mg for the first three doses.

This phase 1 dose-escalation trial is designed to determine the MTD and RP2D. The RP2D of leflunomide will generally be the MTD, but it may be less than the MTD based on a review of available data. Extensive pharmacokinetic and pharmacodynamic studies are also incorporated into this protocol and will be helpful in determining the RP2D, along with clinical activity.

Phase 2 Pilot Design (Two-arm, Randomized, Double-blind, Multi-center):

The phase 2 portion will be a multi-center, randomized, placebo-controlled, double blind, phase 2 pilot trial of two treatments; Arm 1: leflunomide plus standard of care, Arm 2: placebo plus standard of care. The study design consists of randomization in a 1:1 ratio. The randomization will be stratified by COVID-19 risk (Lower: 1 additional risk factor; High: ≥2 additional risk factors) and concomitant SOC anti-viral treatment (SOC1: FDA approved/EUA COVID-19 anti-viral agent; SOC2: other/none) to limit imbalance between the two treatment arms. Risk factors considered -beyond the risk associated with cancer diagnosis: age (≥65), COPD, diabetes, hypertension,

cardiovascular disease, obesity, and time from most recent cancer related treatment to trial enrollment (≤ 3months) [8-11].

Our target difference in response rate is 20%. As designed, with 30 patients (15 patients per arm), this trial is powered to observe a superior response rate in the leflunomide arm if leflunomide is associated with a true increase in the response rate of 20%. If leflunomide is associated with a greater number of responses this will be considered evidence in support of a larger definitive study. However, given the rapidly changing landscape of COVID-19 treatment, additional factors may be considered when determining future investigation of leflunomide in addition to considerations for the variability in patient's status and SOC delivered not considered in the risk factors. Toxicity, feasibility, and overall survival will also guide further investigation of leflunomide.

Sample Size and Accrual Rate: In the phase 1 segment, assuming the highest dose tested is well tolerated, we expect to enroll and treat approximately 9-12 patients, with a maximum of approximately 18 patients (24 absolute maximum due to the rules of the IQ 3+3 design employed). The phase 2 segment is expected to treat 15 patients per arm, for an overall total of n=30 patients. Assuming 2-3 patient(s) are enrolled each month, across the two segments, accrual is expected to be completed in 12-15 months.

Primary Endpoints

Phase 1 Segment:

The primary endpoint is toxicity graded according to the NCI-CTCAEv5. DLT is defined in **Section 11.1.1**. MTD will be based on the assessment of DLT during the 28-day treatment period.

Phase 2 Segment:

- The primary endpoint is best reponse/clinical improvement attained by day 28 that is sustained for 2 days, defined as a ≥2-point improvement in clinical status from Day 1 on a 7-point ordinal scale.
- The co-primary endpoint is toxicity graded by NCI CTCAE v5. Unacceptable toxicity follows the same definition as DLT for the phase 1 segment.

Statistical Considerations

Patient demographic and baseline characteristics, including age, gender, medical history, and prior therapy, will be summarized using descriptive statistics. For continuous variables, descriptive statistics [number (n), mean, standard deviation, standard error, median (range)] will be provided. For categorical variables, patient counts and percentages will be provided.

Primary Safety Analysis: Observed toxicities will be summarized, for all dose levels of the phase 1 segment and for the phase 2 segment, in terms of type (organ affected or laboratory determination), severity, time of onset, duration, serum concentration of the active leflunomide metabolite, probable association with the study treatment and reversibility or outcome.

Primary Activity Analysis: The primary analysis set for the clinical activity analysis in the phase 2 segment is defined as the full analysis set, which will include all participants who (1) were randomized and (2) have received at least 1 dose of leflunomide/placebo. Participants will be grouped according to the treatment to which they were randomized. The response rate will be calculated as the percent of treated patients; the exact 95% confidence interval will be calculated for this estimate. Time to response and survival will be estimated using the product-limit method of Kaplan and Meier.

Pharmacokinetics: Total and free teriflunomide levels will be summarized at sampling time points. Descriptive statistics will be used to characterize possible inter-patient variability and relationship to dose, toxicity and response for future studies, including guidance with respect to RP2D selection.

Investigational Product Dosage and Administration

Leflunomide, 100 mg PO once daily as loading dose on Days 1-3, and thereafter at the assigned dose of 20, 40 or 60 mg/day on Days 4-14.

Clinical Observations and Special Monitoring to be Performed

- Safety assessments
- Clinical response assessments
- Correlative blood samples (for pharmacokinetics, circulating cytokines, immune effector cell phenotype)
- SARS-CoV-2 viral load assessment by PCR
- Patients must be carefully monitored for symptoms of respiratory failure, CRS, and liver failure, for which they should be transferred to the intensive care unit.

COVID SOC and Supportive Care

Institutional standard operating procedures will be utilized in addition to leflunomide to address viral symptoms, and these may change over time.Concomitant SOC medications should be considered for all participants as add-on standard of care, as available. A 5-day course of remdesivir is currently recommended. Convalescent plasma may be considered when remdesivir is not available or indicated. Recommended concomitant medications may change during the course of the protocol. Adverse events should be carefully monitored, with appropriate dose modifications as necessary.

Patients with Grade 3 CRS (CTCAE v5), or Grade 2 CRS with either baseline C-reactive protein (CRP) of >75 mg/L or rapid rise in CRP (≥ 50 mg/L in 1 day), will receive tocilizumab 8 mg/kg i.v. (maximum 800 mg) with an additional dose permitted after 12 hours if symptoms are not resolved to Grade 1.

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ABBREVIATIONS

Abbreviation	Meaning
AE	Adverse Event
ARDS	Acute Respiratory Distress Syndrome
AUC	Area Under the Curve
CFR	Code of Federal Regulations
COH	City of Hope
CRC	Clinical Research Coordinator
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DHODH	Dihydroorotate Dehydrogenase
DSMC	Data & Safety Monitoring Committee
EOT	End of Treatment
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GVHD	Graft Versus Host Disease
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ID	Infectious Disease
IDS	Investigational Drug Services
IND	Investigational New Drug
IRB	Institutional Review Board
MTD	Maximum Tolerated Dose
OIDRA	Office of IND Development and Regulatory Affairs
PBMC	Peripheral Blood Mononuclear Cells
PI	Principal Investigator
PK	Pharmacokinetic
PMT	Protocol Management Team
RA	Rheumatoid Arthritis
RBC	Red Blood Cell
RDV	Remdesivir
RP2D	Recommended Phase 2 Dose
SAE	Serious Adverse Event
SOC	Standard of Care
TRALI	Transfusion Related Acute Lung Injury
UT	Unacceptable toxicity

1.0 OBJECTIVES

In patients with severe COVID-19 and active malignancy in the last 2 years who are receiving standard-of-care (SOC) treatment for COVID-19:

Primary Objectives

Phase 1 (Single-arm)

- Assess the safety and tolerability of leflunomide when combined with COVID-19 SOC by evaluation of toxicities including: type, frequency, severity, attribution and duration.
- Determine the maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D) of leflunomide when given in combination with SOC.

Phase 2/Pilot (Two-arm, Randomized, Double-blind):

- Evaluate the clinical activity of leflunomide plus SOC (Arm 1) and placebo plus SOC (Arm 2) on the basis of clinical improvement (response) rate in each treatment arm, as assessed by a 7-point ordinal scale.
- Evaluate the safety and tolerability of leflunomide plus SOC (Arm 1) and placebo plus SOC (Arm2).

Secondary Objectives

Phase 1

Evaluate the clinical activity of leflunomide when combined with SOC on the basis of clinical improvement (response) rate.

Phase 1 and Phase 2

- Estimate the following:
  - Time to clinical improvement (days);
  - Time to SpO2 > 93% on room air (days);
  - Time to first negative SARS-CoV-2 polymerase chain reaction (PCR) (days);
  - Duration of oxygen therapy (days);
  - Duration of hospitalization (days);
  - Duration of mechanical ventilation;
  - All cause mortality at Day 28.
- Measure trough plasma concentrations of the active metabolite teriflunomide on days 1 through 14, day 21, and day 28, and evaluate relationships between teriflunomide levels and pharmacodynamic biomarkers (e.g., viral load, cytokines), response, safety, and concomitatnt medications.

Exploratory Objectives

- Investigate inflammatory response by measuring changes before and after leflunomide treatment in:
  - circulating cytokines (e.g., IL-6, IL-8, TNF-alpha, IL-12, IFNs and GM-CSF), and
  - immune effector cell phenotype associated with monocyte, T cell, and NK cell activation.
- Assess the kinetics of viral replication through serial measurements of viral load by nasopharyngeal swab and tracheal aspirates (if on ventilator and can be safely obtained).

2.0 BACKGROUND

2.1 Introduction/Rationale for Development

The disease known as coronavirus disease 2019 (COVID-19) has emerged as a threat to public health worldwide. The virus that is the causative pathogen, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is an enveloped virus with a single strand of RNA [12]. Its genome encodes four major structural proteins: spike,

envelope, membrane, and nucleocapsid. The spike protein plays a critical role by binding to human angiotensin-converting enzyme 2 (ACE2) [13]. The virus RNA is translated by the host cell to yield an RNA-dependent RNA polymerase that catalyzes its own replication [14]. The SARS-CoV-2 genome is therefore never reverse-transcribed to DNA. Two other coronaviruses causing disease of concern earlier this century, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), share sequence homology with SARS-CoV-2 [15]; in particular, multiple specific regions of SARS-CoV-2 have high homology to those of SARS-CoV [16].

SARS-CoV-2 is primarily spread through respiratory droplets or close contact. The disease, driven in part by asymptomatic transmission, has an incubation period of 1-14 days, but primarily between 3 and 7 days. The most common symptoms are fever, cough, and fatigue, followed by headache, nasal congestion, sore throat, myalgia, and arthralgia. Some patients exhibit gastrointestinal symptoms [17]. Although most cases are mild, patients may experience shortness of breath, which can progress to hypoxemia [18]. In 10-20% of patients with severe disease, acute respiratory distress syndrome (ARDS) develops, which is the chief cause of respiratory failure. Risk factors for the development of severe disease include advanced age, hypertension, diabetes, cardiovascular disease, and cerebrovascular disease [18]. The case fatality rate is difficult to estimate because of asymptomatic disease and experimental biases, but an approximation of 1% appears to be reasonable, therefore about ten times more fatal than influenza [19]. The comparatively high rate of mortality and morbidity associated with COVID-19, coupled with its rapid spread, threatens to overwhelm the capacity of health-care systems if uncontrolled.

Patients with a diagnosis of cancer, may be at increased risk of contracting COVID-19 and have a poorer prognosis upon becoming infected, likely a result of immunosuppression caused by cancer or by treatments including chemotherapy and radiation. Indeed, the available evidence suggests that patients with cancer are more likely to contract COVID-19 relative to the general population, and their symptoms tend to be more severe [20]. Immune system recovery may be slowed or impaired after cancer treatment, and as result even survivors of cancer are at heightened risk for COVID-19 and its complications.

A number of potential therapies worldwide have been considered for COVID-19, with many currently under clinical investigation. In parallel, efforts are proceeding to develop a vaccine. In May 2020, the FDA issued an emergency use authorization (EUA) for remdesivir, an RNA polymerase inhibitor, for the treatment of suspected or confirmed severe disease, on the basis of data showing an improvement in the time to recovery in hospitalized adults, in comparison to a placebo arm [21]. Meanwhile, despite the considerable number of vaccine programs, vaccine development is a lengthy process with a high rate of attrition [22]. An investigation of the safety and effectiveness of a potential drug with encouraging preclinical evidence of activity is an appropriate response to the current crisis.

2.2 Leflunomide

Leflunomide is a commercially available oral immunosuppressive agent that has been FDA approved since 1998 for the treatment of rheumatoid arthritis (RA) as a single agent or in combination with methotrexate. It has been used in over 300,000 patients worldwide for RA treatment. Leflunomide is generally well-tolerated and may be taken over a long period of time.

The in vitro and in vivo mechanisms of leflunomide are not completely defined. The primary clinical mechanism of action is inhibiting de novo pyrimidine synthesis by targeting dihydroorotate dehydrogenase (DHODH), and thus achieving an anti-proliferative effect in B- and T-lymphocytes. A secondary mechanism of action is inhibition of cytokine and growth factor receptor associated tyrosine kinase activity [23].

Leflunomide (the pro-drug) is rapidly converted to its active primary metabolite teriflunomide (A77 1726), which mediates leflunomide's pharmacologic activity. Teriflunomide serum concentrations show wide inter-patient variability; in one study, the mean serum teriflunomide concentration in patients receiving 20 mg/ daily was 42 mg/L with a range from 3-150 mg/L and a standard deviation of 35 mg/L [24]. Of note, the serum concentration of this metabolite was shown to predict RA response [24].

The FDA approved dose in adults for the treatment of RA is a loading dose of 100 mg orally (PO) once daily for 3 days, followed by a maintenance dose of 10-20 mg PO once daily. Leflunomide has been used at up to 40 mg/day in patients with Wegner's granulomatosis with a safety profile similar to the 20 mg/day dose used in RA [25, 26]. Doses up to 60 mg/day have been safely used for the treatment of allograft polyoma BK virus in renal allograft patients to achieve a targeted blood level of teriflunomide (A77 1726) of 50-100 micrograms/mL [27]; the targeted blood level was frequently achieved with a dose of 40 mg/day with no increase in toxicity reported [27].

In addition to studies that demonstrate the serum concentration of the metabolite may predict response, recent work demonstrates that polymorphisms in the gene encoding DHODH may be associated with leflunomide treatment outcome in RA patients [28, 29].

The long half-life of the metabolite (about 2 weeks) affects management of patients taking leflunomide. Without a loading dose, it is estimated that attainment of steady state concentrations would require nearly two months of dosing [30]. To eliminate the metabolite, a drug elimination procedure using cholestyramine is recommended. Cholestyramine, a bile acid sequestrant, fixes the metabolite, preventing reabsorption and expediting drug elimination. Without the drug elimination procedure, it may take up to 2 years to reach non-detectable plasma levels after stopping treatment with leflunomide [30].

2.3 City of Hope experience

We have extensive experience in the clinical management, pharmacokinetic (PK) analysis, and correlative studies of leflunomide. Guided by preclinical data indicating activity against multiple myeloma, we conducted and completed a phase 1 trial (clinicaltrials.gov: NCT#02509052). All patients had at least 3 prior therapies; heavily treated patients in this disease setting tend to have a number of co-morbidities. At dose levels 1 and 2 (20 mg, 40 mg), no dose-limiting toxicities (DLTs) were observed. At dose level 3 (60 mg), one patient experienced elevated alanine aminotransferase; an additional three patients were enrolled at this dose level without further DLTs. Overall, toxicities were infrequent and manageable. Nine out of 11 patients achieved stable disease (SD), two subjects experiencing SD for nearly one year or longer. The tolerable safety profile of leflunomide, combined with a potential disease stabilization, is motivating future studies of leflunomide, in combination with other MM drugs, or as an approach to delay progression of smoldering MM.

Plasma concentrations of total and free teriflunomide, the active metabolite of leflunomide, were available in all subjects. **Table 2.3.1** (below) summarizes the maximum total and free drug concentrations ($Cmax_{total}$ and $Cmax_{free}$) and cycle 1 total and free teriflunomide area under the concentration-time curves (AUC_{total} and AUC_{free}) observed in each patient. At the highest dose level tested (60 mg/day), the average $Cmax_{total}$ and $Cmax_{free}$ were 429±194 µM and 1.2±0.9 µM, respectively. Furthermore, the average AUC_{total} and AUC_{free} in patients at the highest dose were 5814±2620 µMxday and 11.3±7.6 µMxday, respectively. Consistent with previous reports of high plasma protein binding (i.e., >99.5%), free teriflunomide plasma concentrations were 0.18±0.07% of the total across all dose levels. As illustrated in **Figure 2.3.1**, the average plasma teriflunomide AUC_{free} and AUC_{total} values demonstrated dose-proportionality across the leflunomide dose levels tested. Free drug levels displayed similar dose-proportionality.

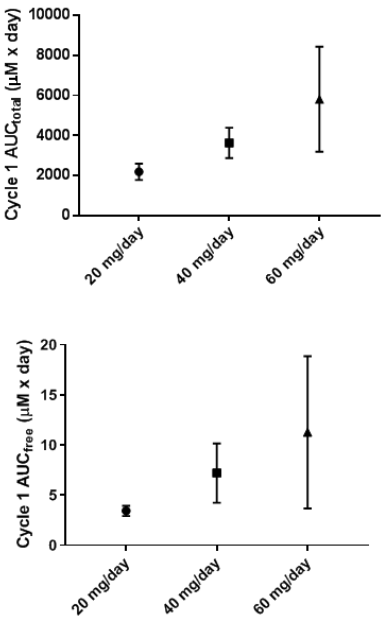


Figure 2.3.1. Average plasma AUC_{total} and AUC_{free} values.

We concurrently examined the anti-MM mechanism of leflunomide in vitro and in vivo systems and assessed its potential activity in combination with other MM drugs, expanding our experience with this agent [31].

Table 2.3.1. Teriflunomide plasma pharmacokinetic summary

Patient #	Dose (mg/day)	*$Cmax_{total}$ (µM)	$Cmax_{free}$ (µM)	^AUC_{total} (µM x day)	AUC_{free} (µM x day)	AUC_{free}/AUC_{total} (%)
1	20	138.3	0.29	2110.5	4.0	0.19
2	20	127.9	0.24	1839.0	3.3	0.18
3	20	212.8	0.27	2644.7	3.0	0.11
4	40	309.0	0.68	3325.7	5.8	0.17
5	40	334.0	0.73	3085.7	5.2	0.17
6	40	533.9	1.79	4501.2	10.6	0.23
7	60	523.0	0.83	8689.3	13.7	0.16
8	60	475.7	1.79	6660.2	25.1	0.38
9	60	695.2	2.75	3232.2	5.7	0.17
10	60	190.7	0.39	8728.9	11.3	0.13
11	60	673.4	0.95	4775.8	7.0	0.15
12	60	339.0	0.57	2798.3	4.9	0.17

*Cmax = maximum plasma concentrations, ^Area under the concentration-time curve from Cycle 1, Day 1 to Cycle 2, Day 1

2.4 Leflunomide as a potential agent against COVID-19

The development of agents to target a particular virus requires significant time and effort to address the specific pathophysiology that virus, and successes have been uncommon. Alternatively, host-targeting antiviral drugs may be appealing in that, by targeting the host machinery that the virus exploits, they may be applicable to a wide range of viruses and their strains. To this end, repurposed drugs are especially suitable, as many of the extensive preclinical validation and toxicity studies have already been performed. As a DHODH inhibitor impacting pyrimidine synthesis for DNA and RNA production, leflunomide may be worthy of study against SARS-CoV-2, an RNA-based virus. In particular, SARS-CoV-2 has a high content of uracil, one of the two nucleobases affected by leflunomide.

Researchers in China studied the preclinical anti-viral activity of leflunomide and other DHODH inhibitors against influenza, Ebola, and Zika viruses, as well as SARS-CoV-2 [5]. Cells were seeded and infected with a SARS-CoV-2 strain. Different concentrations of the drugs were added. After 24 hours, cell supernatants were harvested, and the number of viral copies was detected by RT-PCR. Terfilunomide exhibited significant suppression of viral replication in all tested samples. The researchers also found that viral growth was largely inhibited by DHODH -/- cells, and addition of uracil and cytosine (the pyrimidine bases) restored viral activity. DHODH inhibitors were further effective against influenza in a mouse model.

Our preliminary data at COH also suggest that leflunomide significantly arrests viral RNA replication in cancer cells infected with a natural occurring RNA virus (reovirus) and impairs ex-vivo IL-6 expression in virally infected peripheral blood mononuclear cells (PBMCs).

The antiviral activity of leflunomide aligns with the experience of the drug as an agent against cytomegalovirus (CMV) and polyoma BK virus infections in immunocompromised hosts [3, 4]. Reovirus serotype 3–dearing strain is a naturally occurring, ubiquitous, nonenveloped human reovirus with a genome that consists of 10 segments of double-stranded RNA (1). As with COVID-19, Reoviridae can infect human and animals and never passes through a DNA phase. It actively replicates its RNA genome and induces a strong anti-viral immune response and cytokine storm. The difference between the two is mainly in the envelope and the fact that coronaviruses need to synthetize the RNA negative strand before starting the genome transcription.

Using two different cell lines (MM.1S and U266), we confirmed that leflunomide's active form, teriflunomide, impairs viral RNA replication in myeloma plasma cells (**Figure 2.4**).

We also found that teriflunomide potentiates the anti-viral response (interferons) but impairs IL-6 production in peripheral blood cells obtained from 2 healthy donors. The experiment was repeated in biological duplicates using two different healthy donors with each measurement done in three technical replicates.

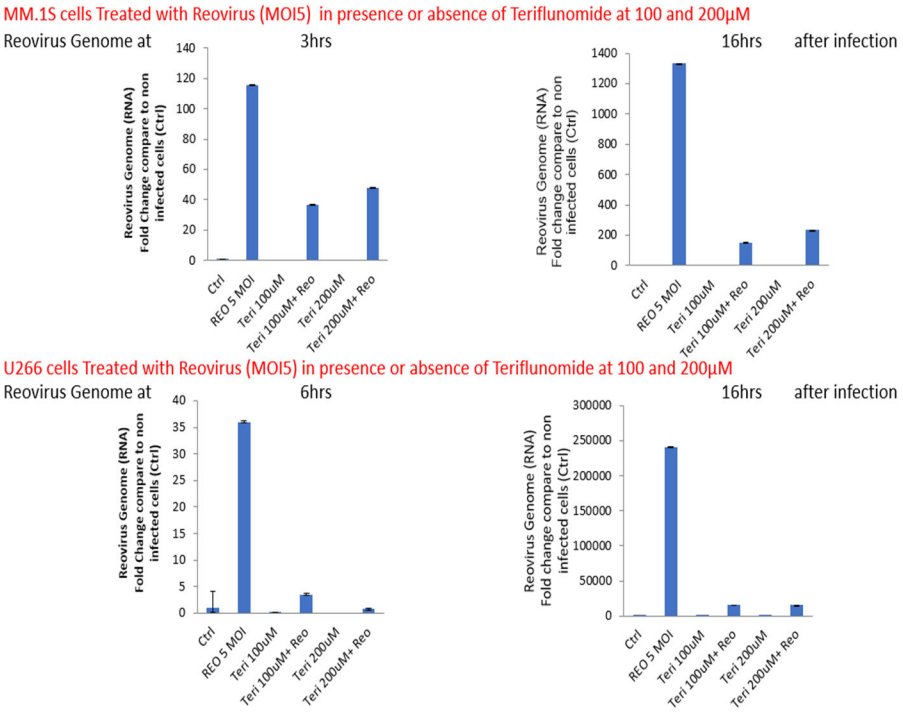


Figure 2.4. Teriflunomide impairs viral RNA replication in myeloma plasma cells. One of two duplicate experiments is shown.

2.5 Correlative studies

Leflunomide Pharmacokinetics: Teriflunomide PK data from our recently completed phase 1 trial in the 60 mg/d dosing group show that patients reach plasma levels > 40 µg/mL between days 5 and 10. In this trial we will carefully monitor plasma teriflunomide levels in real time, as some patients may be ventilated and require dispersed tablets given via a feeding tube, which carries the possibility that bioavailability differs from that of oral administration.

Analytical method: Total (i.e., protein bound) and free (i.e., unbound) teriflunomide plasma concentrations will be determined using a validated LC-MS/MS method available in the City of Hope Analytical Pharmacology Core Facility. The validated assay is based on a previously reported method [32] and has a wide dynamic concentration range of 10-4000 ng/mL from a starting plasma volume of 200 µL. Non-compartmental pharmacokinetic analyses of teriflunomide will determine the average steady-state teriflunomide trough concentrations (C_{trough}). C_{trough} results will be summarized within and between patients using means and standard deviations.

Plasma cytokine/chemokines: The Human Cytokine 30-Plex Antibody Bead Kit (Invitrogen) will be used to analyze plasma samples for 30 cytokines. The assay plate will be transferred to the Bio-plex HTF Luminex System instrument for analysis. Cytokine concentrations will be calculated using Bio-plex Manager 3.0 Software with a 5-parameter curve-fitting algorithm applied for standard curve calculations for duplicate samples.

Immune Cell Profiling: CyTOF analyses will be conducted on longitudinally collected PBMCs to assess changes in the immune cell profile during treatment. Briefly, 1-2x10^6 PBMCs will be incubated with our custom-made 37 surface cellular marker antibodies, which include the most clinically relevant immune exhaustion markers (TIM3, LAG3, TIGIT, PD1, PDL-1 and CD95) and two intracellular markers (anti-granzyme B and IL-2). Specifically, we will measure: **a)** CD3, CD4, CD8, CD127, CD27, CD69, HLA-DR, granzyme B, and CD25 surface receptors to quantify changes in T cell subpopulations during treatment; **b)** CD69 and CD40L to assess early CD4+ T cell activation; **c)** CD14+, CD11b+, CD15+ and HLADR low/negative to monitor variations in mo-MDSC populations; **d)** CD3 and CD8 surface markers to measure CD8+ expansion; and **e)** KLRG1 and CD38 expression to determine CD8+ activation, which is normally associated with enhanced innate immune activity.

2.6 Overview, rationale, and impact

2.6.1 Overview

We propose a multi-center, phase 1 dose-escalation trial of leflunomide plus SOC followed by a multi-center, randomized phase 2 pilot trial. We plan to study the safety and clinical activity of leflunomide when combined with COVID-19 SOC in patients with severe COVID-19 infection who have an active malignancy, a group especially vulnerable to the pandemic. In the phase 1 segment, up to three doses of leflunomide will be considered: 20 mg/day (DL-1), 40 mg/day (DL1-starting dose), and 60 mg/day (DL2). The phase 1 segment is designed to evaluate safety/tolerability and to determine the MTD and RP2D. The RP2D of leflunomide will generally be the MTD, but it may be less than the MTD based on a review of available data. Extensive pharmacokinetic and pharmacodynamic studies are also incorporated into this protocol and will be helpful in determining the RP2D. Once the RP2D has been determined, a small, randomized, proof-of-concept, placebo-controlled study, with 15 patients in each arm, will be conducted to augment the interpretation of the safety and clinical activity of leflunomide.

2.6.2 Rationale for eligibility criteria

Because the population of all patients with a history of malignancy is broad and heterogeneous, the interpretation of results would be difficult in the absence of placing limitations. Specifically, it would be challenging to include patients with early precursor states such as smoldering multiple myeloma, as well as patients with a distant history of cancer. It is therefore required that enrolled subjects must have had active cancer necessitating systemic treatment within the prior 2 years.

Additionally, subjects must have not been exposed to prior cancer treatments in intervals listed in the enrollment criteria (Section 3), to prevent potentially harmful interactions between leflunomide and the prior treatment. These intervals are common in cancer research. Local radiation may confer transient immunologic effects. The half-lives of chemotherapeutic drugs tend to not exceed 48 hours; therefore, 5 half-lives falls within a two-week interval. In contrast, antibodies may have a half-live of ~3 weeks, and biologic therapies may similarly be long-lasting. Stem cell transplantation is immunodepleting, and acute graft-versus-host-disease is a potential complication lasting up to 120 days.

2.6.3 Rationale for dose levels tested

Patients will receive a loading dose of leflunomide 100 mg daily x 3 days or placebo, followed by the assigned dose of leflunomide, or placebo, for 11 days. Patients will be followed for toxicity for 28 days following the last dose of leflunomide or placebo. Based on the pharmacokinetic (PK) data from our phase 1 dose escalation study in patients with multiple myeloma, 5 of 6 patients receiving a 60 mg daily dose of leflunomide achieved teriflunomide serum concentrations above 40 µg/mL compared to only 1 of 3 and 0 of 3 at doses of 40 mg and 20 mg daily, respectively, even though all patients received the same loading dose of 100 mg/d x 3. Furthermore, we estimate that serum teriflunomide levels above 40 µg/mL were reached between days 5 and 10 at a dose of 60 mg/day.[6] However, because of a dose-limiting toxicity (1 in 6 patients treated) and other adverse events observed at the 60 mg dose, we have selected 40 mg as the starting dose in this phase 1 trial to assure safety in the context of a COVID-19 infection and potential concomitant use of other agents such as remdesivir and tocilizumab. Although the standard dose of leflunomide, 20 mg daily, is FDA approved for continuous treatment of rheumatoid arthritis, leflunomide has been administered without an increase in the type, frequency or severity of adverse events at doses up to 40 mg/day in patients with Wegner's granulomatosis, and up to 60 mg/day in a subset of patients with polyoma BK neuropathy to achieve targeted therapeutic levels. The results of our prior PK studies also indicate that teriflunomide levels in patients receiving 40 mg daily will be above the IC90 of COVID-19 (100 µM or 27 µg/ml) reported in the study of Xiao et al[5]. Since we have previously demonstrated safety of daily leflunomide doses up to 60 mg in patients with multiple myeloma, we also plan to allow escalation to 60 mg to determine whether the pharmacokinetics are more favorable and the toxicity profile is acceptable in the setting of COVID-19 infection [5].

2.6.4 Impact

Leflunomide is a repurposed, oral drug with a favorable safety profile. Thus, it is well-positioned for rapid approval for treatment of COVID-19 patients with immunosuppression or cancer history, but ultimately all COVID-19 patients. Additionally, its low cost makes leflunomide a desirable option for use in resource-poor settings.

3.0 ELIGIBILITY CRITERIA

Patient MRN (COH Only)	Patient Initials (F, M, L):
Institution:	

Participants must meet all of the following criteria on screening examination to be eligible to participate in the study:

3.1 Inclusion Criteria

Informed Consent

__1. Documented informed consent of the participant and/or legally authorized representative.

Assent, when appropriate, will be obtained per institutional guidelines. Cognitively impaired subjects may enroll in the phase 2 portion if adequate psychosocial support is provided. See Section **17.6.4**.

Age Criteria

__2. Age: ≥ 18 years

Nature of Illness and Illness Related Criteria

__3. SARS-CoV-2 infection confirmed by a PCR-based test within 4 days prior to enrollment. See Section 4.1 for details.

__4. COVID-19 disease baseline severity of Severe according to FDA guidance [33], as defined by:

- Symptoms suggestive of severe systemic illness with COVID-19, which could include any symptom of fever, cough, sore throat, malaise, headache, muscle pain, gastrointestinal symptoms, or shortness of breath at rest, or respiratory distress
- Clinical signs indicative of severe systemic illness with COVID-19, such as respiratory rate ≥30 per minute, heart rate ≥125 per minute, SpO2 ≤93% on room air at sea level or PaO2/FiO2 <300

__5. Active cancer requiring systemic treatment within the last 2 years. Subjects should not have received the following therapies for their malignancy within the indicated time frames (See **Section 2.6** for rationale):

- Local radiation therapy within 2 weeks prior to enrollment. If the involved field is small (single nodal area), 7 days prior to enrollment is allowed.
- Chemotherapy within 2 weeks prior to enrollment
- Major surgery within 2 weeks prior to treatment
- Autologous hematopoietic stem cell infusion within 12 weeks prior to enrollment
- Antibody therapy, CAR T cells, or other biologic therapies within 12 weeks prior to enrollment
- Allogeneic hematopoietic stem cell infusion within 16 weeks prior to enrollment

These time frames should be considered the minimum allowed interval and may be longer per the judgment of the investigator.

__6. Adverse events related to prior cancer therapy must have recovered to ≤grade 1 or to baseline

__7. Subjects must be able to forgo systemic cancer therapy for ~39 days (14 days treatment/placebo + 14 days monitoring + ~ 11 days cholestyramine)

Clinical Laboratory and Organ Function Criteria (To be performed within 4 days prior to Day 1 of protocol therapy unless otherwise stated)

__8. ANC ≥ 500/mm^3	ANC:	Date:
__9. Platelets ≥ 25,000/mm^3	Plts:	Date:
__10. Total bilirubin ≤ 1.5 x ULN (unless has Gilbert's disease)	ULN: Bil:	Date:
__11. AST ≤ 2 x ULN	ULN:	Date:

	AST:	
__12. ALT ≤ 2 x ULN	ULN: ALT:	Date:
__13. Creatinine clearance of ≥ 60 mL/min per 24-hour urine test or the Cockcroft-Gault formula or $\text{CrCl (mL/min)} = \frac{(140\text{-age}) \times \text{actual body weight (kg)}}{72 \times \text{serum creatinine (mg/dL)}}$ (x 0.85 for females) **Or** $\text{CrCl (mL/min)} = \frac{(140\text{-age}) \times \text{actual body weight (kg)}}{0.8136 \times \text{serum creatinine (umol/L)}}$ (x 0.85 for females)	Serum Cr: Cr Clearance:	Date:

Contraception

__14. Agreement by females **and** males of childbearing potential* to use an effective method of birth control or abstain from heterosexual activity for the course of the study until teriflunomide levels are verified to be less than 0.02 mg/L (0.02 µg/mL) for patients given leflunomide (see **Section 5.9**), or until unblinding occurs for those given placebo. Contraception should also be used for the duration of administration of SOC drugs during this study for the duration recommended in the prescribing information.

* Childbearing potential is defined as not being surgically sterilized (men and women) or have not been free from menses for > 1 year (women only).

3.2 Exclusion Criteria

Other illnesses or conditions

__1. Evidence of acute respiratory distress syndrome (ARDS), defined by at least one of the following: endotracheal intubation and mechanical ventilation, oxygen delivered by high-flow nasal cannula (heated, humidified, oxygen delivered via reinforced nasal cannula at flow rates > 20 L/min with fraction of delivered oxygen ≥ 0.5), noninvasive positive pressure ventilation, ECMO, or clinical diagnosis of respiratory failure (i.e., clinical need for one of the preceding therapies, but preceding therapies not able to be administered in setting of resource limitation)

__2. Shock (defined by systolic blood pressure < 90 mm Hg, or diastolic blood pressure < 60 mm Hg or requiring vasopressors)

__3. Evidence of multi-organ dysfunction/failure

__4. Pre-existing acute or chronic liver disease

__5. Patients with indolent local malignancies or pre-malignant conditions including but not limited to:

- Smoldering multiple myeloma or monoclonal gammopathy of undetermined significance (MGUS)
- Basal or squamous cell carcinoma of the skin
- Carcinoma in situ of the cervix or breast
- Incidental histologic finding of prostate cancer (T1a or T1b using the TNM clinical staging system) or prostate cancer that is curative

__6. History of allergic reactions attributed to compounds of similar chemical or biologic composition to study agent

__7. Secondary bacterial, fungal, or viral infections that are not adequately controlled.

__8. Any other condition that would, in the Investigator's judgment, contraindicate the patient's participation in the clinical study due to safety concerns with clinical study procedures.

__9. *If HIV-positive*: CD4+ T cell count <200

__10. Positive for tuberculosis antigen (e.g., T-spot test)

__11. Presence of liver metastasis

__12. GI malignancies associated with malabsorption and inability to take cholestyramine

Concurrent medications

__13. Steroids, except for low-dose replacement or high-dose for management of acute symptoms such as ARDS.

__14. Any new immunosuppressive medication in the 4 weeks prior to enrollment, excepting agents used for treatment of COVID-19 that may also have immunosuppressive properties.

__15. Medications that are CYP1A2 inducers, CYP2C8 inhibitors, and vitamin K antagonists, if these medications are not approved by the investigator. CYP1A2 inducers, CYP2C8 inhibitors, and vitamin K antagonists that are approved by the investigator are allowed.

__16. Concurrent administration of live vaccines.

Noncompliance

__17. Prospective participants who, in the opinion of the investigator, may not be able to comply with all study procedures (including compliance issues related to feasibility/logistics).

Eligibility Confirmed* by (Choose as applicable):	**Print Name**	**Signature**	**Date**
☐ Site PI			
☐ Authorized study MD			
☐ Study Nurse			
☐ Study CRA/ CRC			
☐ Other:_____			
*Eligibility should be confirmed per institutional policies.			

4.0 PARTICIPANT ENROLLMENT

Note: Sites must meet activation requirements prior to enrolling participants.

4.1 Pre-Enrollment Informed Consent and Screening Procedures

Diagnostic or laboratory studies performed exclusively to determine eligibility will be done only after obtaining written informed consent. Studies or procedures that are performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values and/or to determine pre-eligibility, even if the studies were done before informed consent was obtained. The informed consent process is to be fully documented (see **Section 17.4**), and the prospective participant must receive a copy of the signed informed consent document. Screening procedures are listed in **Section 10.0** (Study Calendar).

The standard kit manufactured by DiaSorin will be used for initial PCR confirmation of COVID-19 positivity. In the event the kit becomes unavailable, an alternative assay may be used.

4.2 Participant Enrollment

4.2.1 COH DCC Availability and Contact Information

Eligible participants will be registered on the study centrally by the Central Registration/Data Coordinating Center (DCC) at City of Hope.

DCC staff are available **between the hours of 8.00 am and 5.00 pm PST, Monday through Friday (except holidays)**.

- Phone: (626) 218-7904
- E-mail: DCC@coh.org

4.2.2 Slot verification and reservation

Designated study staff should call or email the DCC to verify current slot availability, and to reserve a slot for a specific prospective subject (provide DCC with subject initials). Slots can only be held for a limited time (~1 week).

The DCC should be notified of cancellations of prospective participants holding slots as soon as possible.

4.2.3 Registration Process

To register a participant, the subsequent procedure is to be followed:

1. The study team (data manager/coordinator/research nurse) should contact the DCC via telephone or email to provide notification regarding the pending registration and communicate desired timeline of the registration, especially if it must be completed promptly to meet the registration window.
2. The data manager/coordinator/research nurse will email a copy of the following documents to the DCC:
    - Registration Cover Sheet (**Appendix A**)
    - Completed eligibility checklist (printed from **Section 3.0** of the protocol)
    - Source documentation to support eligibility criteria**
    - Signed Informed Consent
    - Signed subject's bill of Rights
    - Signed HIPAA authorization form (if separate from informed consent)

    ** It is **NOT** acceptable to submit emails as source documentation
3. After having received all transferred documentation, the DCC will complete the review of the documents to verify eligibility, working with the study team as needed to resolve any missing required source elements. A participant failing to meet all protocol eligibility requirements will not be registered.

4. Once eligibility is confirmed, DCC staff will send a Confirmation of Registration Form and signed Eligibility Checklist within 24 hours, including the participant study number and cohort assignment (dose level or expansion cohort) to:
    - The study team: Site Lead Investigator, treating physician/ sub-investigator, protocol nurse, CRC and pharmacy (as needed).
    - The COH Study PI and COH study team designees (including but not limited to study monitor(s) and statistician(s). The DCC will register non-COH patients in OnCore.
5. Upon receipt of the Confirmation of Registration email from the DCC, COH study team will register the patient in OnCore.

4.3 Randomization (Phase 2):

For subjects enrolled in the randomized phase 2 segment, the DCC staff will use a computer-generated randomization table, prepared by the trial Biostatistician, to assign subjects to receive either leflunomide plus standard of care (Arm 1) or placebo plus standard of care (Arm 2). The study design consists of randomization in a 1:1 ratio. The randomization will be stratified by COVID-19 risk (Lower: 1 additional risk factor; High: ≥2 additional risk factors) and concomitant SOC anti-viral treatment (SOC1: FDA approved/EUA COVID-19 anti-viral agent; SOC2: other/none) to limit imbalance between the two treatment arms. Risk factors considered -beyond the risk associated with cancer diagnosis: age (≥65), COPD, diabetes, hypertension, cardiovascular disease, obesity, and time from most recent cancer-related treatment to trial enrollment (≤ 3months) [8-11]. We will accrue 15 patients to each arm.

4.4 Unblinding Procedures

4.4.1 End of Treatment Unblinding

The following procedure should be followed for non-emergent unblinding at the end of the 28-day treatment cycle, or if a patient goes off-study prior to completion of the 28-day treatment cycle.

- Information regarding the rationale for de-blinding will be provided to the Site PI, the COH DCC, and the Study PI.
- The Site PI will communicate with Pharmacy and will provide authorization to unblind patient's treatment.

4.4.2 Emergency Unblinding

The COH IDS which will be authorized in an emergency to break the code for an individual participant and inform the responsible party at the corresponding site pharmacy. Emergency un-blinding will occur if a patient on this study develops a life-threatening toxicity or SAE and the participant's physician feels that it is in the patient's best interest to know the randomization status of the participant. Unblinding to leflunomide treatment allows use of cholestyramine for clearance of teriflunomide in cases where leflunomide-related toxicity has not been ruled out. In this very unlikely event, the PMT will determine if and how the unblinding should impact the participant's continued participation in the study or analysis of collection points post de-blinding. This plan will be provided to the IRB of record and the COH IRB and external DMC as per COH institutional requirements. The date and reason for unblinding must be noted in the medical record and captured in the eCRF.

4.5 Screen Failures and Registered Participants Who Do Not begin Study Treatment

Notify the DCC if the participant screen fails after registration or if the participant does not start treatment.

For non-COH sites, the reason for screen failure will be documented in the registration coversheet and submitted to the DCC.

Issues that would cause treatment delays should be discussed with the Principal Investigator.

5.0 TREATMENT PROGRAM

5.1 Treatment Program Overview

This trial has a phase 1 segment and a randomized phase 2 segment (leflunomide vs placebo) to test the safety and clinical activity of adding leflunomide to current SOC for COVID-19 in patients with concurrent malignancies. In addition to other approved or investigational COVID-19 therapies, oral lefluomide will be given for the first 14 days of a single 28-day treatment cycle. After completion of the treatment cycle, cholesyramine will be administered as part of the leflunomide washout; patients will be followed for 3 months from start of treatment.

5.2 Treatment Plan

Phase 1: Patients will receive a loading dose of leflunomide at 100 mg po qd x 3 days followed by the assigned dose of leflunomide (20 mg, 40 mg, or 60 mg) po qd for 11 days. Subjects will be monitored for AEs/DLTs for the full 28-day treatment cycle and up to 2 additional months thereafter. SOC drugs for COVID-19 may be given in addition to leflunomide during the study, with doses and timing documented.

Randomized Phase 2: Patients will be randomized to receive leflunomide at the RP2D identified in the phase 1 segment plus SOC or placebo in addition to SOC COVID-19 treatment. The assignment will be blinded to both the subjects and the treating clinicians. Subjects will be monitored for 28 days for unacceptable toxicity and response (clinical improvement) to treatment and followed for an additional 2 months for toxicity.

Cholestyramine: Cholestyramine will be given for at least 11 days starting on day 29 to washout plasma teriflunomide. All patients in phase 1 and those in phase 2 who upon unblinding are revealed to have received leflunomide will receive cholestyramine. See **Section 5.9** for details.

5.3 Agent Administration

5.3.1 Leflunomide administration

Leflunomide is administered with a loading dose of 100 mg per day for the first three doses (first three days), followed by the assigned dose (20 mg, 40 mg, or 60 mg) for 11 additional days.

The first 7 days of treatment/placebo will be inpatient, with hospital discharge contingent on improvement of COVID symptoms to moderate severity or better and resolution of treatment-related adverse events to ≤ grade 1 or a return to baseline level(s). (Exception: the patient requires further hospitalization because of the malignancy.)

On Day 1, all participants must have adequate baseline laboratory values collected within 4 days before initiating study therapy.

Participants will take each dose of leflunomide/placebo orally, once a day, at approximately the same time each day. It may be taken with or without food. If >8 hours have passed since the scheduled dose time, the missed dose will be skipped and not made up.

If necessary, tablets may be dispersed and administered via feeding tube.

Doses that are vomited will not be made up. Doses that are missed because of toxicity will not be made up.

5.3.2 Cholestyramine administration

Cholestyramine is not a study agent but is required for the elimination of the study agent leflunomide after completion of the 28-day treatment cycle, or immediately after discontinuation of leflunomide because of toxicities or withdrawal from study participation. See **Section 5.9** for cholestyramine administration details.

5.4 Assessments and Special Monitoring

For a detailed list of all study procedures including timing and windows, see **Section 10.0**.

- Safety assessments
- Clinical response assessments
- Correlative blood samples (circulating cytokines, changes in immune effector cell phenotype)
- SARS-CoV-2 viral load assessment by PCR
- Patients must be carefully monitored for symptoms of CRS, respiratory failure, and liver failure, for which they should be transferred to the intensive care unit
- Patients who are receiving both leflunomide and remdesivir are at increased risk of liver function abnormalities and must remain inpatient and be monitored daily. See Section 7.1 for discontinuation guidelines for leflunomide and Section 5.9.1 for cholestyramine detoxification, should patients experience dose-limiting or unacceptable liver toxicity.
- Patients will undergo daily sampling of blood levels for the active metabolite teraflunomide on days 1 through 14, day 21, and day 28 of treatment. After the 11th day of cholestyramine and again ≥14 days after first post-washout test, CLIA laboratory testing will be used to verify depletion of teriflunomide levels.

5.5 Duration of Therapy and Criteria for Removal from Protocol Therapy/Placebo

Discontinuation of leflunomide/placebo+SOC treatment does not equate to discontinuation from study participation, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine whether any change in participant management is needed.

Participants will receive protocol therapy (leflunomide/placebo) until one of the below criteria are met:

- Completed leflunomide/placebo (14 days)
- Participant is deemed intolerant to protocol therapy because of toxicity, despite dose modification/ delay
- General or specific changes in the patient's condition which render the patient unacceptable for further treatment in the judgment of the investigator
- Withdrawal of consent for further protocol therapy (Refer to Withdrawal Section from Ethical Considerations)

Once participants meet criteria for removal from protocol therapy, the participant should continue the remainder of the 28-day cycle, and then proceed to follow-up (**Section 5.10**). In the randomized phase 2 segment, once participants meet criteria for removal from protocol therapy/placebo and continue the remainder of the 28-day cycle, participants will be unblinded to their assignment by the study PI or a designee.

Documentation of the reason for discontinuing protocol therapy/placebo and the date effective should be made in the Electronic Health Record/medical record and appropriate eCRF. The COH DCC and the Study PI should be promptly notified of the change in participant status.

The COH DCC and the Study PI should be promptly notified of the change in participant status.

5.6 Duration of Study Participation

Study participation may conclude when any of the following occur:

- Completion of study activities (treatment and 2 months of follow-up after protocol treatment)
- Withdrawal of consent (Refer to Withdrawal Section from Ethical Considerations)
- Participant is lost to follow-up. All attempts to contact the participant must be documented.
- At the discretion of the investigator for safety, behavioral, study termination or administrative reasons

Documentation of the reason for discontinuing study participation and the date effective should be made in the Electronic Health Record/medical record and appropriate eCRF. The COH DCC should be promptly notified of the change in participant status.

The COH DCC and the Study PI should be promptly notified of the change in participant status.

5.7 Concomitant Therapies/Medications

Include all investigational antivirals (if any) that were used and their time of administration, by patient in the study report to the FDA.

5.7.1 Allowed concomitant medications

FDA approved, EUA and investigational/off-label agents will be allowed as SOC since there are no highly effective treatments for COVID-19. At the time of protocol submission, only remdesivir has an EUA from the FDA. Additional agents may become FDA approved or available under EUA during the course of this trial, and the protocol will be amended to include descriptions of side effects and potential interactions with leflunomide; however, they will be allowed prior to amendment.

5.7.1.1 Remdesivir

Recommendation: Recommended for severe disease

Availability: Emergency Use Authorization (EUA): As of May 19, 2020, City of Hope has been selected to receive emergency remdesivir (RDV). Use will be determined on a case by case basis. ID approval is required for use. Supply is limited.

Dosing and Pharmacology: 200 mg IV x 1 IV followed by 100 mg IV once daily for 4 days

Adverse events:

- Infusion-related reactions
  - If signs and symptoms of a clinically significant reaction occur, immediately discontinue administration of RDV and initiate appropriate treatment.
  - RDV is contraindicated in patients with known hypersensitivity to RDV.
- Elevated transaminases
  - RDV should not be initiated in patients with ALT >5x upper limit of normal (ULN) at baseline
  - RDV should be discontinued in patients who develop ALT ≥ 5x ULN during treatment or any ALT elevation accompanied by signs or symptoms of liver inflammation, increasing conjugated bilirubin, alkaline phosphatase, or INR.
  - RDV may be re-started when ALT is < 5x ULN.

Pharmacologic Considerations:

- Renal Insufficiency: Pharmacokinetics of RDV have not been evaluated in patients with renal impairment. No dose adjustment required for patients with eGFR ≥ 30 ml/min. RDV is not recommended with eGFR < 30 ml/min.
- Hepatic insufficiency: It is unknown if dosage adjustment is needed in patients with hepatic impairment.
- Drug interactions (*in vitro):* RDV is a substrate for CYP2C8, CYP2D6, and CYP3A4 and is a substrate for OATP1B1 and P-gp transporters. RDV is an inhibitor of CYP3A4. The clinical relevance of these in vitro data is unknown. **Note:** teriflunomide is an inhibitor of CYP2C8 in vivo, and remdesivir is a CYP2C8 substrate. However, according to National Institutes of Health guidelines [34], co-administration of remdesivir with an inhibitor of CYP2C8 is not expected to have a significant impact on remdesivir concentrations.

5.7.1.2 Convalescent plasma

Recommendation: Convalescent plasma is recommended for patients for whom remdesivir is not available or indicated. City of Hope is participating in an open-label expanded access protocol program available for patients who may benefit.

Adverse events: TRALI, allergic/anaphylactic reactions, RBC allo-immunization, hemolytic transfusion reaction

Please refer to **Appendix B** for the consideration of other therapies.

5.7.1.3 Investigational or off-label agents for COVID therapy

Due to the life-threatening nature of the disease and the lack of effective and available treatments, concomitant use of off-label or investigational products prescribed for the treatment of COVID-19 is allowed according to institutional standard operating procedures (SOPs). The agents listed in **Appendix B** have been used for COVID-19 by other institutions, but are not recommended for concomitant use in this trial.

Tocilizumab is in a special category in this trial, due to its potential to increase the metabolism of CYP450 substrates, including leflunomide. Due to this potential interaction, tocilizumab will not be used in this trial as an off-label anti-COVID therapy upfront, but instead will be used only as supportive care in patients with CRS (see **Section 5.8.1**), where its potential additional risk to increase levels of teriflunomide is more justified.

If concomitant therapy must be added or changed, including over-the-counter medications or alternative therapies, the reason and name of the agent/therapy should be recorded in the eCRF and documented in the Electronic Health Record/medical record. The timing, dosing, and duration of these treatments should be collected.

5.7.2 Cautionary: medications with potential teriflunomide interactions

The following medications are not necessarily disallowed, but monitoring of drug interactions is mandated:

- Drugs that are substrates of CYP2C8 and OAT3 transporters: Monitor patients because teriflunomide may increase exposure of these drugs. **Note:** teriflunomide is an inhibitor of CYP2C8 in vivo, and remdesivir is a CYP2C8 substrate. However, according to National Institutes of Health guidelines [34], co-administration of remdesivir with an inhibitor of CYP2C8 is not expected to have a significant impact on remdesivir concentrations.
- Teriflunomide may increase exposure of ethinylestradiol and levonorgestrel. Choose an appropriate oral contraceptive.
- Drugs metabolized by CYP1A2: Monitor patients because teriflunomide may decrease exposure of these drugs.
- Warfarin: Monitor INR as teriflunomide may decrease INR.
- Drugs metabolized by BCRP and OATP1B1/B3 transporters: Monitor patients because teriflunomide may increase exposure of these drugs.
- Rosuvastatin: The dose of rosuvastatin should not exceed 10 mg once daily in patients taking leflunomide
- Medications that are CYP1A2 inducers, CYP2C8 inhibitors and vitamin K antagonists.

5.7.3 Prohibited medications

Systemic steroids, except for low-dose replacement or high-dose for management of acute symptoms such as ARDS.

Any new immunosuppressive medication in the 4 weeks prior to enrollment, excepting agents used for treatment of COVID-19 that may also have immunosuppressive properties.

Concurrent administration of live vaccines.

5.7.4 Non-recommended therapies

Because hydroxychloroquine carries a risk of cardiac and other toxicities, without a proven therapeutic benefit, it should not be co-administered with leflunomide. See **Appendix B** for a list of other therapies not recommended for co-administration with leflunomide in this study.

5.8 Supportive care

With the exception of prohibited therapies (refer to **Section 5.7.2**), participants should receive prophylactic or supportive treatment as clinically indicated per institutional policies.

5.8.1 Tocilizumab

Recommendation: Tocilizumab is recommended for treatment of Cytokine Release Syndrome (CRS) associated with COVID-19, on the basis of the following criteria. If the patient does not meet below criteria, an evaluation and approval from the PI or an Infectious Disease physician must be obtained.

Eligibility Criteria:	• Virologic diagnosis of SARS-CoV-2 infection
	• Oxygen saturation at rest in ambient air ≤ 94% or requiring oxygen therapy or mechanical ventilation either non-invasive or invasive (intubated)
	• Abnormal Chest X-Ray/CT findings that are new compared to baseline imaging
	• IL-6 elevation (see below) *
	• CRS grade 3 or CRS grade 2 and CRP ≥ 75 mg/L or increase by >50 mg/L between 2 tests

Dosing: 8 mg/kg (max 800 mg) based on actual body weight. One additional dose may be given after 12 hours if symptoms worsen or there is no clinical improvement.

Adverse events: See section 6.3

5.8.2 Anticoagulation

Recommendation

- Thromboprophylaxis with LMWH (heparin with renal failure or fondaparinux if history of heparin-induced thrombocytopenia) is recommended in all hospitalized patients with COVID19 infection [35, 36]. Abnormal coagulation tests are common in COVID19 patients and in the absence of active bleeding, a standard prophylactic anticoagulation dose is advised, unless platelet count is less than 25 x 10^9/L, or fibrinogen less than 0.5 g/L [37] .

Dosing/monitoring

- Monitor platelet count, PT/aPTT or anti-Xa, D-dimer, and fibrinogen daily.
- Therapeutic anticoagulation is not required without evidence of active thrombosis (e.g. VTE, atrial fibrillation, or presence of mechanical valve).
    - If patient is already on therapeutic anticoagulation for VTE or atrial fibrillation, this therapy should be continued but may need to be held if platelet count is less than 30-50 x 10^9/L or if the fibrinogen is less than 1.0 g/L.
    - Patients with central venous catheters, arterial lines, on CRRT with extracorporeal circuits, on ECMO, or those with frequent clotting of the devices, increasing intensity of anticoagulation can be considered.
- Confirmed or suspected VTE should be started on therapeutic anticoagulation.
- Post-discharge thromboprophylaxis recommendations: hospitalized patients with acute medical illness, including COVID19 patients are at increased risk for VTE after discharge for up to 90 days; therefore, extended thromboprophylaxis after discharge should be considered with a regulatory-

approved regimen, e.g betrixaban 160 mg on day 1, followed by 80 mg once daily for 35-42 days; or rivaroxaban 10 mg daily for 31-39 days. Individual patient's risks of bleeding and benefits of thromboprophylaxis should be considered.[38-40]

- In a patient who is actively bleeding, transfuse platelets if the platelet count is less than 50 x 10^9/L, give plasma (4 units) if the INR is above 1.8 and give fibrinogen concentrate (4 grams) or cryoprecipitate (10 units) if the fibrinogen level is less than 1.5 g/L.

For questions on COVID19- related coagulopathy and anticoagulation, please contact the COH Department of Hematology.

5.8.3 Immune globulin

Recommendation: Routine use is not recommended for COVID-19. Refer to F.008.05 and IVIG Pharmacy Policy for indications for use and dosing.

5.8.4 Corticosteroids

Recommendation: Avoid in mild and moderate COVID-19 given the uncertainty of optimal timing of steroid therapy and potential for corticosteroids to worsen disease severity and lead to secondary infections in the immunocompromised population. Use of dexamethasone in patients with severe COVID-19 should be considered as part of the supportive care regimen for patients with ARDS on a case-by-case basis with an ICU specialist. Corticosteroids should be continued for indications based on underlying medical conditions (i.e. asthma, COPD, GVHD etc.)

5.8.5 HMG-CoA Reductase Inhibitors

Recommendation: Patients who are receiving a statin for the treatment or prevention of cardiovascular disease should continue therapy. Ensure that high risk primary prevention (for ASCVD) patients are on guideline-directed statin therapy. The NIH recommends against use of statins for the treatment of COVID-19 except in the context of a clinical trial.[12]

5.8.6 Renin angiotensin aldosterone receptor (RAAS) antagonists

Recommendation: Continue treatment with renin-angiotensin-aldosterone system (RAAS) antagonists in those who are currently prescribed such agents. Abrupt withdrawal of RAAS inhibitors in high-risk patients (e.g., heart failure patients, patients with prior myocardial infarction) may lead to clinical instability and adverse health outcomes. For COVID patients who develop acute kidney injury, hypotension, or other contraindications, recommend discontinuation.

5.8.7 Vitamin D

Recommendation: Recommend supplementation in patients with vitamin D deficiencies. Supplemented patients should be monitored to ensure their circulating vitamin D levels are normalized and sustained.

Dosing:

Vitamin D level 20-30 ng/ml: 50,000 IU daily for 1 week then 1,000 – 2,000 IU daily.

Vitamin D level < 20 ng/ml: 50,000 IU daily for 2 weeks then 1, 000 – 2,000 IU daily.

5.8.8 Zinc

Recommendation: The routine use of zinc sulfate is not recommended in patients with COVID-19. Zinc may be indicated in patients at risk for zinc deficiency (i.e. malnourished, chronic diarrhea, total parental nutrition, poor gut absorption and/or diet). Consult nutrition for further recommendations.

5.9 Follow-Up

All participants will enter follow-up after completing the 28-day cycle. All participants will be followed until resolution or stabilization of any reportable AEs occurring during treatment. See **Section 10** for a list of all assessments and windows.

Follow-up comprises the following:

- **Safety Follow-up** - 90 days post-initiation of protocol therapy.
- **Leflunomide Detoxification** – 29 days post-initiation of protocol therapy (**Section 5.9.1**)

Note: the period for safety follow-up will be extended until stabilization or resolution for all reportable AEs (per the agreement of the Study PI) and accompanying follow-up safety report.

5.9.1 Leflunomide Detoxification

Participants who have completed the 28-daytreatment/monitoring period will undergo oral administration of cholestyramine 8 grams oral suspension three times daily for 11 days, starting 29 days post-initiation of protocol therapy. *Phase 1*: If the participant discontinues leflunomide because of study withdrawal or toxicities that are at least possibly related to leflunomide, cholestyramine should be given immediately after leflunomide discontinuation. *Phase 2*: If the participant discontinues leflunomide or placebo because of study withdrawal or toxicities that are at least possibly related to leflunomide, the participant should become unblinded to the treatment assignment (see **Section 4.4**). Only participants who were given leflunomide should be given cholestyramine. Liver function tests should be performed weekly during the course of cholestyramine administration.

It will not be a deviation if an administration of cholestyramine is delayed or if the 11 days are not consecutive. Cholestyramine administration may be adjusted per the discretion of the treating investigator. After the treatment with cholestyramine, CLIA laboratory testing will be performed to verify plasma levels less than 0.02 mg/L or 0.02µg/mL by two separate tests at least 14 days apart. If plasma levels are higher than 0.02 mg/L or 0.02µg/mL, additional cholestyramine treatment will be considered. See **Section 10** for a tabular view of cholestyramine administration and metabolite testing windows and days of administration. The amount of additional cholestyramine is at the discretion of the treating physician and should take into account the remaining plasma level of teriflunomide, with consultation with Dr. Timothy Synold.

Note: Cholestyramine can affect the absorption of other oral medications. Such medications should be taken 1 hour before the first administration or per other guidance in the package insert. Watch for bleeding abnormalities due to vitamin K deficiency (using PT/INR, see **Section 10**), for which intravenous vitamin K may be administered.

6.0 ANTICIPATED ADVERSE EVENT LIST

6.1 Leflunomide

Per the package insert for leflunomide, the expected toxicities for leflunomide follow, where the asterisk (*) signifies a common event (10%-30% of patients), unmarked items are infrequent events (1-3% of patients), a double asterisk (**) signifies a rare but possibly serious event, and a triple asterisk (***) signifies an event that may become serious and occurs in up to 10% of patients:

Blood and lymphatic system: anemia (including iron deficiency anemia), ecchymosis, pancytopenia**, agranulocytosis**, neutropenia**, thrombocytopenia**, leukopenia**

Cardiac: angina pectoris, migraine, palpitation, tachycardia, vasodilatation

Endocrine: diabetes mellitus, hyperthyroidism

Eye: blurred vision, cataract, conjunctivitis, eye disorder

Gastrointestinal: diarrhea*, abdominal pain, dyspepsia, nausea, vomiting, oral ulceration, anorexia, pancreatitis**, constipation, esophagitis, flatulence, gastritis, gingivitis, melena, oral moniliasis, pharyngitis, salivary gland enlarged, stomatitis (or aphthous stomatitis), tooth disorder

Hepatobillary: cirrhosis**, hepatitis**, hepatic failure**, acute hepatic necrosis**, cholelithiasis**, cholestasis**, elevated hepatic enzymes (primarily ALT and AST)

Immune system: allergic reactions, anaphylactoid reactions**

Infections: respiratory infection*, infections (including bronchitis, rhinitis, sinusitis, pharyngitis, pneumonia, and urinary tract infections, oral or vaginal candidiasis, herpes simplex, herpes zoster, and fungal dermatitis), opportunistic and/or severe infections** including sepsis, that may be fatal (especially Pneumocystis jiroveci pneumonia, tuberculosis, aspergillosis)

Metabolism and nutrition: hyperglycemia, creatine phosphokinase increased, hyperlipidemia

Musculoskeletal and connective tissue: arthrosis, bone necrosis, bone pain, bursitis, muscle cramps, myalgia, tendon rupture

Neoplasms: secondary malignancy**, cyst

Nervous system: headache*, peripheral neuropathy* (including peripheral numbness, tingling, burning, severe pain, cold sensation in the distal extremities, or extremity weakness), paresthesias, taste perversion (dysgeusia), anxiety, depression, dry mouth, insomnia, neuralgia, neuritis, sleep disorder, sweating increased, vertigo, migraine

Renal and urinary: albuminuria, cystitis, dysuria, hematuria, hypophosphaturia, hyperuricemia, increased urinary frequency

Reproductive: menstrual irregularity/disorder, menstrual disorder, vaginal moniliasis vaginal moniliasis, prostate disorder

Respiratory: interstitial lung disease** (sometimes fatal), interstitial pneumonitis**, pulmonary fibrosis**, asthma, dyspnea, epistaxis, lung disorder

Skin and subcutaneous tissue: maculopapular rash*, dry skin*, alopecia*, hair discoloration*, Stevens-Johnson syndrome**, toxic epidermal necrolysis**, erythema multiforme**, cutaneous lupus erythematosus**, acne, contact dermatitis, fungal dermatitis, hematoma, nail disorder, skin discoloration, skin disorder, skin nodule, subcutaneous nodule, skin ulcer

Vascular: varicose veins, hypertension***, vasculitis, cutaneous necrotizing vasculitis**

Miscellaneous: weight loss*, leg cramps*, jaundice**, allergy related angioedema**, fever, peripheral edema, hernia, neck pain, pelvic pain, pain, abscess, malaise

6.2 Cholestyramine

Per the package insert for cholestyramine, the expected toxicities for cholestyramine follow, where the double asterisk (**) signifies a common event, and a single asterisk (*) signifies a less common event, and remaining unmarked items are less likely:

Blood and lymphatic system: anemia, ecchymosis, prolonged prothrombin time, hypoprothrombinemia

Ear and labyrinth: tinnitus, vertigo

Eye: uveitis

Gastrointestinal: constipation**, abdominal pain*, anorexia*, nausea*, vomiting*, diarrhea*, flatulence*, diarrhea*, eructation*, steatorrhea*, diverticulitis, bleeding from known duodenal ulcer, dysphagia, gastrointestinal hemorrhage, hemorrhoidal bleeding, hiccups, intestinal obstruction (rare), melena,

pancreatitis, rectal pain, tongue irritation, tooth enamel damage (dental erosion), dental bleeding, dental caries, dental discoloration

Hepatobiliary: abnormal hepatic function tests, biliary colic, gallbladder calcification,

Immunologic: hypersensitivity reaction

Metabolism: hyperchloremic metabolic acidosis

Musculoskeletal and connective tissue: arthralgia, arthritis, backache, myalgia, osteoporosis associated with vitamin D deficiency

Nervous system: anxiety, dizziness, drowsiness, dysgeusia, fatigue, headache, neuralgia, paresthesia, syncope

Respiratory: asthma dyspnea and wheezing associated with hypersensitivity reaction

Renal and urinary: hematuria, dysuria, burnt odor to urine, diuresis

Skin and subcutaneous tissue: perianal skin irritation, skin irritation, skin rash, urticaria

Miscellaneous: adenopathy, increased libido, edema, bleeding tendencies due to vitamin K deficiency, vitamin deficiency (A, D, E, K), weight loss, weight gain, hematoma, hemorrhage, femoral nerve pain

6.3 Tocilizumab (ACTEMRA®)

A complete description of anticipated toxicities can be found in the ACTEMRA® Full Prescribing Information.

6.3.1 Common Adverse Reactions

Most common adverse reactions (incidence ≥ 5%): upper respiratory tract infections, nasopharyngitis, headache, hypertension, increased ALT.

6.3.2 Warnings and Precautions

Serious Infections: Serious and sometimes fatal infections due to bacterial, mycobacterial, invasive fungal, viral, protozoal, or other opportunistic pathogens have been reported in patients receiving immunosuppressive agents including ACTEMRA for rheumatoid arthritis. The most common serious infections included pneumonia, urinary tract infection, cellulitis, herpes zoster, gastroenteritis, diverticulitis, sepsis and bacterial arthritis. Among opportunistic infections, tuberculosis, cryptococcus, aspergillosis, candidiasis, and pneumocystosis were reported with tocilizumab. Other serious infections, not reported in clinical studies, may also occur (e.g., histoplasmosis, coccidioidomycosis, listeriosis). Patients have presented with disseminated rather than localized disease, and were often taking concomitant immunosuppressants such as methotrexate or corticosteroids which in addition to rheumatoid arthritis may predispose them to infections.

Tocilizumab should not be administered in patients with an active infection with the exception of SARS-CoV-2, including localized infections. The risks and benefits of treatment should be considered prior to initiating tocilizumab in patients:

- with chronic or recurrent infection;
- who have been exposed to tuberculosis;
- with a history of serious or an opportunistic infection;
- who have resided or travelled in areas of endemic tuberculosis or endemic mycoses; or
- with underlying conditions that may predispose them to infection.

Patients should be closely monitored for the development of signs and symptoms of infection during and after treatment with tocilizumab, as signs and symptoms of acute inflammation may be lessened due to suppression of the acute phase reactants.
Tocilizumab should be interrupted if a patient develops a serious second infection, an opportunistic infection, or sepsis. A patient who develops a new infection during treatment with tocilizumab should undergo a prompt and

complete diagnostic workup appropriate for an immunocompromised patient, appropriate antimicrobial therapy should be initiated, and the patient should be closely monitored.

Treatment with tocilizumab is not recommended in patients with active hepatic disease or hepatic impairment.

Live vaccines should not be given concurrently with tocilizumab as clinical safety has not been established.

Patients should be monitored for the following:

- tuberculosis
- viral reactivation
- gastrointestinal perforations as complications of diverticulitis.
- neutropenia
- thrombocytopenia
- transaminase elevation
- increases in lipid parameters such as total cholesterol, triglycerides, ldl cholesterol, and/or hdl cholesterol.
- immunosuppression
- hypersensitivity reactions
- demyelinating disorders

7.0 DOSE DELAY / MODIFICATION GUIDELINES

7.1 Dose Modifications of Leflunomide

7.1.1 General Information

a. The study will use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5 to grade toxicities. A copy of this version can be downloaded from:

http://evs.nci.nih.gov/ftp1/CTCAE/About.html

b. Intra-patient dose escalation is never permitted in this study. Rules for dose modification are found in **Table 7.1.2**.

c. Baseline values are from the last values obtained prior to treatment.

7.1.2 Dose Modifications

The tables below detail the specific dose modifications for toxicities on single-agent leflunomide and are to be used in agreement with the information in **Section 6**.

Table 7.1.2 Dose Modifications for Leflunomide Treatment

Adverse Event	Treatment modification
Hematological Toxicities:	
Febrile Neutropenia Grade 3 (ANC: <1.0 x10^9/L with a single temperature of >38.3 ˚C or a sustained temperature ≥ 38.0˚C for more than one hour.	Permanently discontinue study agent. *Exception*: If participants have pre-existing grade 3 febrile neutropenia associated with underlying hematologic malignancy and not expected to improve over the next 14 days, and/or pre-existing fever due to COVID, the drug may be continued. If lower-grade neutropenia progresses to grade 3 febrile neutropenia, leflunomide should be discontinued.
Febrile Neutropenia Grade 4 Life threatening consequences; urgent intervention needed.	Permanently discontinue study agent. *Exception*: If participants have pre-existing grade 4 febrile neutropenia associated with underlying hematologic malignancy and not expected to improve over the next 14 days, and/or pre-existing fever due to COVID, the drug may be continued. If lower-grade neutropenia progresses to grade 4 febrile neutropenia, leflunomide should be discontinued.

Neutropenia (ANC) Grade 4 (<0.5x 10^9/L)	Permanently discontinue study agent. *Exception*: If participants have pre-existing grade 4 neutropenia associated with underlying hematologic malignancy and not expected to improve over the next 14 days, the drug may be continued. If lower-grade neutropenia progresses to grade 4, leflunomide should be discontinued.
Thrombocytopenia Grade 3 with bleeding (25 – <50 x 10^9/L)	Permanently discontinue study agent. *Exception*: If participants have pre-existing grade 3 thrombocytopenia with bleeding associated with underlying hematologic malignancy and not expected to improve over the next 14 days, the drug may be continued. If lower-severity thrombocytopenia progresses to grade 3 thrombocytopenia with bleeding, leflunomide should be discontinued.
Thrombocytopenia Grade 4 (<25 x 10^9/L)	Permanently discontinue study agent. *Exception*: If participants have pre-existing grade 4 thrombocytopenia associated with underlying hematologic malignancy and not expected to improve over the next 14 days, the drug may be continued. If lower-severity thrombocytopenia progresses to grade 4 thrombocytopenia, leflunomide should be discontinued.
Gastrointestinal	
Diarrhea Grade 3 (7-9 stools/day > baseline)	Hold study agent and provide optimal anti-diarrheal therapy. *First Occurrence:* If resolves to ≤ grade 1 in ≤ 7 days, resume at pre-hold dose. Otherwise, permanently discontinue study agent. *Recurrence:* Permanently discontinue study agent.
Diarrhea Grade 4 (≥10 stools/day > baseline)	Hold study agent and provide optimal anti-diarrheal therapy. If does not resolve to Grade 3 within 48 hours, permanently discontinue study agent. If resolves to Grade 3 within 48 hours, then: *First Occurrence:* Hold until resolution to ≤ grade 1. If resolved in ≤ 7 days, resume at pre-hold dose. Otherwise, permanently discontinue study agent. *Recurrence:* Permanently discontinue study agent.
Vomiting or Nausea Grade 3	Hold study agent and provide optimal supportive care/therapy. *First Occurrence:* If resolves to ≤ grade 1 in ≤ 7 days, resume at pre-hold dose. Otherwise, permanently discontinue study agent. *Recurrence:* Permanently discontinue study agent.
Vomiting Grade 4	Hold study agent and provide optimal supportive care/therapy. If does not resolve to Grade 3 within 48 hours, permanently discontinue study agent. If resolves to Grade 3 within 48 hours, then: *First Occurrence:* Hold until resolution to ≤ grade 1. If resolved in ≤ 7 days, resume at pre-hold dose. Otherwise, permanently discontinue study agent. *Recurrence:* Permanently discontinue study agent.
Hepatic investigations	

ALT (SGPT) or AST (SGOT) >3.0 - 5.0 x ULN or TBili >1.5 - ≥ 3.0 x ULN	*First Occurrence:* Hold until resolution to < 3.0 x ULN for ALT or AST or < 2.0 for TBili. If does not resolve within 14 days, permanently discontinue study agent. *Recurrence:* Permanently discontinue study agent.
ALT (SGPT) or AST (SGOT) > 5.0 x ULN or TBili > 3.0 x ULN	Permanently discontinue study agent.
Electrolyte/metabolic toxicity	
Electrolyte/metabolic Grade 3	Provide supportive care. Maintain study agent per investigator discretion. If alteration persists despite aggressive replacement therapy, then dose reduce or permanently discontinue (per investigator discretion with PI consultation).
Electrolyte/metabolic Grade 4	Provide supportive care. Maintain study agent per investigator discretion. If resolves to Grade 1 or baseline within 48: for first event, maintain at pre-hold dose; for recurrent event, dose reduce or permanently discontinue (per investigator discretion with PI consultation). If does not resolve to Grade 1 or baseline within 48 hours, permanently discontinue study agent.
Other unspecified Non-Hem toxicities considered related to leflunomide	
Grade 3	*First Occurrence:* Hold until resolution to ≤ Grade 2. Resume at pre-hold dose if resolved within 7 days. If does not resolve within 7 days, permanently discontinue study agent. *Recurrence:* Permanently discontinue study agent.
Grade 4	Permanently discontinue study agent.
Other unspecified Non-Hem Toxicities considered UNRELATED to study agent	
Other unspecified events of any grade considered unlikely to be related or not related to study agents.	Maintain treatment with study agents. Interruption of study agent or dose de-escalation is permitted if the investigator consults with the Principal Investigator to determine that this is in the best interest of the participant.

7.2 Dose Reduction Steps

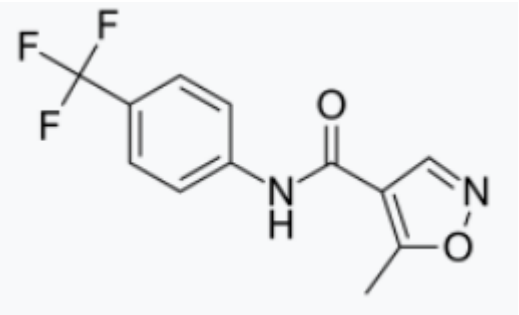
Leflunomide/placebo dose reduction steps: If the current dose is 60 mg (3 pills/capsules), the dose should be reduced to 40 mg (2 pills/capsules). If the current dose is 40 mg (2 pills/capsules), the dose should be reduced to 20 mg (1 pill/capsule). If the current dose is 20 mg, leflunomide should be discontinued.

8.0 AGENT INFORMATION

8.1 Leflunomide

Leflunomide is an FDA approved agent for the treatment of rheumatoid arthritis. Please refer to the Package Insert for additional details not provided in this section.

8.1.1 Description



The chemical name for leflunomide is N-(4'-trifluoromethylphenyl)-5-methylisoxazole-4-carboxamide. It has a molecular formula $C_{12}H_9F_3N_2O_2$, a molecular weight of 270.2. Leflunomide is a pyrimidine synthesis inhibitor.

8.1.2 Mode of action

Leflunomide is an isoxazole immunomodulatory agent which inhibits dihydroorotate dehydrogenase (an enzyme involved in de novo pyrimidine synthesis) and has antiproliferative activity. Several in vivo and in vitro experimental models have demonstrated an anti-inflammatory effect.

8.1.3 Pharmacology

Following oral administration, leflunomide is metabolized to an active metabolite teriflunomide (A77 1726) which is responsible for essentially all of its activity *in vivo*. Following oral administration, peak levels of the active metabolite occurred between 6 to 12 hours after dosing. Due to the very long half-life of teriflunomide (18-19 days), a loading dose of 100 mg for 3 days was used in clinical studies to facilitate the rapid attainment of steady-state levels of teriflunomide. Without a loading dose, it is estimated that attainment of steady-state plasma concentrations would require nearly two months of dosing. Biliary recycling is a major contributor to the long elimination half-life of teriflunomide. *In vitro* inhibition studies in human liver microsomes suggest that cytochrome P450 (CYP) 1A2, 2C19 and 3A4 are involved in leflunomide metabolism. The active metabolite of leflunomide is eliminated slowly from the plasma. Use of a drug elimination procedure (cholestyramine or activated charcoal) is used to reduce the drug concentration more rapidly after stopping leflunomide therapy.

8.1.4 Storage and stability

Leflunomide tablets should be stored at 25°C (77°F); excursions are permitted to 15–30°C (59–86°F). Protect from light.

8.1.5 Preparation

Leflunomide is commercially available for oral administration as tablets containing 10, 20, or 100 mg of active drug. In the randomized phase 2 arm, only 20 mg tablets should be used and will be over-encapsulated, so that treatment is indistinguishable from placebo.

8.1.6 Agent administration

Leflunomide is administered orally. See **Section 5.3.1** for administration details.

8.1.7 Availability and supply

Tablets in 10 and 20 mg strengths are packaged in bottles of 30 pills per bottle. Leflunomide will be provided to patients by the study as an investigational agent. A commercial supply will be purchased by the study and distributed directly by the supplier to each site as an investigational agent.

8.2 Cholestyramine

Cholestyramine is not the study agent being evaluated for clinical activity, but is required for the elimination of the study agent leflunomide. Cholestyramine is FDA approved for hypercholesterolemia. Cholestyramine use is indicated in the leflunomide package insert to eliminate the leflunomide active metabolite teraflunomide**.**

8.2.1 Description

Cholestyramine is a strong anion exchange resin comprised of a quaternary ammonium group attached to an inert styrene-divinylbenzene copolymer. Cholestyramine removes bile acids from the body by forming insoluble complexes with bile acids in the intestine, which are then excreted in the feces.

8.2.2 Other names

Questran®, Questran Light, Cholybar, Olestyr

8.2.3 Mode of action

Cholestyramine resin adsorbs and combines with the bile acids in the intestine to form an insoluble complex which is excreted in the feces. This results in a partial removal of bile acids from the enterohepatic circulation by preventing their absorption.

8.2.4 Storage and stability

Store between 20°-25°C (68°-77°F). [See USP Controlled Room Temperature]. Excursions permitted to 15°-30°C (59°-86°F).

8.2.5 Agent administration

Cholestyramine should be administered orally after being prepared per package insert (powder diluted in fluid). See **Section 5.9.1** for study administration details.

8.2.6 Availability and supply

Cholestyramine will be provided to participants by the study. A commercial supply will be purchased by the study and distributed directly to each site by the supplier as an investigational agent.

8.3 Tocilizumab (ACTEMRA®)

8.3.1 Description:

Tocilizumab is not the study agent being evaluated for clinical activity, but is required as supportive care for patients experiencing moderate to severe CRS. Tocilizumab is approved for adults and pediatric patients 2 years of age and older with rheumatoid arthritis or chimeric antigen receptor (CAR) T cell-induced cytokine release syndrome.

Tocilizumab binds specifically to both soluble and membrane-bound IL-6 receptors (sIL-6R and mIL-6R), and has been shown to inhibit IL-6-mediated signaling through these receptors. IL-6 is a pleiotropic pro-inflammatory cytokine produced by a variety of cell types including T- and B-cells, lymphocytes, monocytes and fibroblasts. IL-6 has been shown to be involved in diverse physiological processes such as T-cell activation, induction of immunoglobulin secretion, initiation of hepatic acute phase protein synthesis, and stimulation of hematopoietic precursor cell proliferation and differentiation. IL-6 is also produced by synovial and endothelial cells leading to local production of IL-6 in joints affected by inflammatory processes such as rheumatoid arthritis.

Recent clinical responses in the field of adoptive cellular immunotherapy related to the effective tumor targeting by engineered T cell therapy have been associated with acute cytokine release syndrome (CRS) which often requires intensive care to manage [41]. In one case of CRS, the use of steroids did not abrogate CRS, though this was subsequently controlled by the anti-cytokine antibodies[41] implying that the antibodies not only provide an adequate alternative to steroids but that they are in fact likely a superior alternative. Furthermore, with the avoidance of steroids, these patients have been able to go on and have good responses to therapy. Although this anti-cytokine agent is associated with immune suppression when dosed repeatedly in the context of disease like arthritis or psoriasis there is little evidence to suggest risk in this on-off dosing context where the immediate benefits may well be life-saving [41].

8.3.2 Toxicology:

Clinical toxicology: The most common adverse reactions (incidence ≥ 5%) for tocilizumab are: upper respiratory tract infections, nasopharyngitis, headache, hypertension, increased ALT. Serious and sometimes fatal infections due to bacterial, mycobacterial, invasive fungal, viral, protozoal, or other opportunistic pathogens have been reported in patients receiving immunosuppressive agents including tocilizumab for rheumatoid arthritis. For a

more extensive listing of expected adverse effects in humans, see **Section 6.3** or the ACTEMRA Full Prescribing information.

Non-clinical carcinogenesis/mutagenesis: No long-term animal studies have been performed to establish the carcinogenicity potential of tocilizumab. The literature indicates that the IL-6 pathway can mediate anti-tumor responses by promoting increased immune cell surveillance of the tumor microenvironment. However, available published evidence also supports that IL-6 signaling through the IL-6 receptor may be involved in pathways that lead to tumorigenesis. The malignancy risk in humans from an antibody that disrupts signaling through the IL-6 receptor, such as tocilizumab, is presently unknown.

Non-clinical impairment of fertility: Fertility and reproductive performance were unaffected in male and female mice that received a murine analogue of tocilizumab administered by the intravenous route at a dose of 50 mg/kg every three days.

8.3.3 Pharmacology

Inhibition of IL-6 signaling in RA patients treated with tocilizumab may restore CYP450 activities to higher levels than those in the absence of tocilizumab leading to increased metabolism of drugs that are CYP450 substrates. In vitro studies showed that tocilizumab has the potential to affect expression of multiple CYP enzymes including CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP3A4.

8.3.4 Handling, Storage, Dispensing and Disposal

Tocilizumab will be used per COH institutional SOPs (https://cityofhope.my.salesforce.com/069d0000001W24A) especially in the management of severe CRS when (C-Reactive Protein) CRP is >/=20mg/dl.[42]

Due to the 1-2 day turn-around time reasonably expected for cytokine results, it is reasonable to consider administration of tocilizumab based on clinical evidence of CRS, and before having the cytokine data in hand.

9.0 CORRELATIVE/ SPECIAL STUDIES

Refer to Section 2.0 for background information.

9.1 Viral Load Testing

Viral load will be measured every 2 days by PCR of nasopharyngeal swab and tracheal aspirates if on ventilator (if safely obtainable) to assess the kinetics of viral replication on days 2, 4, 6, 8, 10, 12, and 14.

Nasopharyngeal swabs should be obtained from the left and right nostril and pooled into the same collection tube. If only one nostril can be sampled, it should always be the same nostril (i.e., always the left or always the right) throughout the trial. The method of collection must follow City of Hope standard operating procedures.

Samples should be double-bagged, labelled appropriately as COVID-19-related specimens, and hand delivered to avoid loss of samples or spillage. Pneumatic tubes should not be used. Samples will be sent to TGen for PCR testing. The RT-PCR protocol is listed in **Appendix C**.

9.2 Other Research Sample Collection and Dispensation

Peripheral blood will be collected from all participants. All samples will be collected in 4 ml EDTA-containing purple-top tubes:

	Baseline	D1-3	D4	D5-7	D8	D9-13	D14	D21	D28
Synold lab	1x*	1x	1x	1x	1x***	1x***	1x***	1x	1x
Pichiorri lab	4x**		4x		4x		4x	4x	4x

*At each timepoint, one tube will be filled for the Synold laboratory.

**At each timepoint, four tubes will be filled for the Pichiorri laboratory.

***If outpatient, from Days 8-14, twice a week, at least 3 days apart.

Blood collection and delivery

1. Each tube should be labeled with the study IRB number, research patient number (RPN), and sample collection time point.
2. Put the vials in a -80°C freezer for 48 hours. For longer-term storage, move them to a nitrogen tank.

9.3 Pharmacokinetics

All participants will undergo serial blood sampling to evaluate the steady-state systemic exposure to teriflunomide (A77 1726) the active metabolite of leflunomide. Pharmacokinetic sampling will consist of trough level monitoring throughout the entire period of active treatment with leflunomide.

The PK samples will be analyzed in batch at the end of the phase 1 and phase 2 segments, but may be analyzed separately in individual patients with SAEs suspected to be due to teriflunomide levels, or in the event that levels need to be monitored in real time.

9.3.1 Specimen Collection

At the indicated timepoints above, venous blood will be collected into one 4-mL purple-top (EDTA) tube. Patients will be instructed not to take their leflunomide dose until after they have had their blood drawn. Blood samples will be kept on ice until prompt delivery to the Hematopoietic Tissue Bank (see **Sections 9.6 and 9.7**).

9.3.2 Initial specimen processing and storage

Specimen processing and storage will occur at the APCF. Plasma is separated from whole blood by centrifugation at 1500 x g (within 1 hour). Plasma will then be transferred to appropriately labeled polypropylene tubes and stored at < -70°C until analysis. Each sample will be labeled with the participant's name, medical record number, date of collection, and actual sample collection time.

9.3.3 Analytical Method

Total and free teriflunomide (A77 1726) plasma concentrations will determined in the Analytical Pharmacology Core Facility (APCF) at the City of Hope using a validated LC-MS/MS method. The method is based on a previously reported assay that has been validated over a wide dynamic concentration range of 10-4000 ng/mL from a starting plasma volume of 200 µl [32].

9.3.4 Pharmacokinetic Data Analysis

Non-compartmental pharmacokinetic analyses of teriflunomide (A77 1726) will be used to determine the average steady-state total and free teriflunomide trough concentration (C_{trough}). C_{trough} results will be summarized within and between participants using means and standard deviations.

9.4 Pharmacodynamics

9.4.1 Specimen Collection

Venous blood will be collected into 4-mL purple-top (EDTA) tubes as indicated above.

9.4.2 Initial specimen processing and storage

Any blood remaining in the three 4-mL purple-top tubes used to prepare plasma for PK above will be diluted 1:1 with Hank's Balanced Salt Solution ("HBSS", Irvine Scientific, Cat. 9228 or equivalent) and combined in a sterile 50 ml conical centrifuge tube. PBMC will then be isolated from the combined whole blood sample by Ficoll-gradient separation as described below;

- Allow Accuspin-Histopaque tubes ("Accuspin", Sigma Cat. A6929 or A0561, for 12 or 100 tubes, respectively) and HBSS to warm to room temperature. Place a Mr. Frosty container in the refrigerator and prepare the freezing media by adding 10% DMSO to fetal calf serum and chill at 4˚C or on ice.
- Prepare Accuspin tubes by centrifuging at 1000 x g for 1 minute at room temperature (RT) with brakes on. Each tube can process up to 20 mLs of whole blood; prepare the appropriate amount of tubes necessary. After centrifugation, the Histopaque reagent should be below the barrier of the tube. Add 5 mL of HBSS to the Accuspin tube. Add up to 20 mls of whole blood to each Accuspin tube until all the blood has been distributed.
- Centrifuge the blood sample at 800 x g for 15 minutes at RT with brakes on LOW. After centrifugation, three layers should be visible above the barrier of the tube: the plasma layer at the top, a cloudy layer in the middle where the PBMC are, and a clear Histopaque reagent layer right below. Using a pipette, remove the upper plasma layer to within 2 cm of the cloudy interphase. Carefully pipette the cloudy PBMC interphase and transfer to a sterile 50 mL centrifuge tube.
- Add HBSS up to the 45 mL mark in the centrifuge tube with the PBMC and spin at 400 x g for 10 minutes at RT with brakes on. Decant the supernatant and loosen the cell pellet before adding HBSS to the 45-mL mark again for a second wash. Centrifuge at 300 x g for 10 minutes at RT with brakes on. Decant the supernatant, loosen the cell pellet and then add a known volume of HBSS to resuspend the cells for counting. Mix the cell suspension up and down with a pipette several times before removing a small aliquot for cell count.
- Centrifuge the cell suspension one final time at 300 x g for 10 minutes at RT with brakes on. PBMC should be frozen down at 0.5 – 1 x 10^7 cells/vial. Determine the volume of freezing media (fetal calf serum with 10% DMSO) needed to give a 1 x 10^7 cell/mL suspension. After the last centrifugation is complete, discard supernatant and loosen the cell pellet before adding freezing media slowly, a small volume at a time with mixing in between (vortex at low speed). Aliquot 0.5 - 1 mL of the final cell suspension into individually labeled cryovials. Transfer the cryovials into Mr. Frosty and store at -80˚C. Twenty-four hours later, cryovials will be transferred to liquid nitrogen tanks for long-term storage.

9.4.3 Analytical Method

The intracellular levels of dihydroorotate, orotate, and nucleotide pools will be performed using liquid chromatography tandem mass spectrometry as previously described[43, 44]. Expression of cell surface markers of lymphocyte activation (CD25 and CD134) will be measured by flow cytometry as previously described[45].

9.5 CyTOF analysis

1-2x10^6 PBMCs will be incubated with our custom-made 37 surface cellular marker antibodies, which include the most clinically relevant immune exhaustion markers (TIM3, LAG3, TGIT, PD1, PDL-1 and CD95) and two intracellular markers (Anti-Granzyme B and IL-2). Samples will be analyzed on a CyTOF C5 system; data will be collected in an FCS file format and normalized using CyTOF Software 6.5.358 for Stand-Alone Processing Workstations (Fluidigm). Cell ID intercalator and cisplatin will be used to discriminate single nucleated cells and live cells. FCS files will be up-loaded into Cytobank software for further manual gating and spanning-tree progression analysis of density-normalized events (SPADE). Cell populations will be identified using dimension reduction methods, data visualization, and clustering. We will compare the results of several data analysis methods, including but not limited to, principal component analysis, t-SNE, UMAP, and a suite of clustering algorithms. CyTOF analysis will also be conducted on longitudinally collected peripheral blood to assess changes in the immune profile during treatment. Specifically, we will measure: **a)** CD3, CD4, CD8, CD127, CD27, CD69, HLA-DR, granzyme B, and CD25 surface receptors to quantify changes in T cell subpopulations during treatment; **b)** CD69 and CD40L to assess early CD4+ T cell activation; **c)** CD14+, CD11b+, CD15+ and HLADR low/negative to monitor variations in mo-MDSC populations; **d)** CD3 and CD8 surface markers to measure CD8+ expansion; and **e)** KLRG1 and CD38 expression to determine CD8+ activation, which is normally associated with enhanced innate immune activity.

9.6 Sample delivery within City of Hope

Frozen samples should be transferred to the Hematopoietic Tissue Bank (HTB), City of Hope. A notification of a shipment should be made at least 24 hours in advance (Tinisha McDonald, tmcdonald@coh.org), in the event that arranging for a delegate is necessary. A message from the HTB will be sent to the Synold lab (tsynold@coh.org) or the Pichiorri lab (fpichiorri@coh.org), as applicable, informing that the sample is ready to be picked up. A member of the respective labs will pick up the sample.

9.7 Sample delivery from outside City of Hope

Blood should be delivered to the Hematopoietic Tissue Repository:

Tinisha McDonald
City of Hope
1500 East Duarte Rd.
Duarte, CA 91010-3000
Kaplan CRB (158)
E-mail: tmcdonald@coh.org

Samples shipped from outside COH should be sent overnight at 4°C with a refrigerated cool pack. **Please note that samples should only be sent from outside sites on Monday-Thursday for receipt Tuesday-Friday.** An e-mail notification should be sent to recipients 24 hours prior so that a designee of the recipient can be made available if necessary.

9.8 Biorepository of specimens

- Samples from patients consenting to biobanking for possible future studies not related to this investigation will be stored indefinitely in the City of Hope Hematopoietic Tissue Repository. Patients may withdraw consent to biobanking at any time, and accordingly the specimens will be destroyed with a bleach solution.
- This research will be conducted in compliance with federal and state of California requirements relating to protected health information (PHI).
- All future research studies involving banked samples from this trial will be approved by City of Hope IRB.

All samples will be coded prior to submission to research laboratories. The coded identifier will be the City of Hope research patient number (RPN), which is devoid of direct participant identifiers. The key to the code is maintained in a secure environment.

10.0 STUDY CALENDAR

	Screening	Treatment Cycle									Follow up					
Treatment/ Assessment	**Day -4 to -1[1]**	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8-14**	**D15-28**	**D29-D39**	**D40**	**D42 (±5d)**	**D56 (±5d)**	**D70 (±5d)**	**D90 (±10d)**
Informed Consent[2]	X															
Eligibility criteria	X															
Registration[3]	X															
Medical history[4]	X															
Concomitant meds[5]	X	X	X	X	X	X	X	X	X	X	X	X	X[5]			
Vital signs[6]	X	X	X	X	X	X	X	X	X[7]	X[7]						
Adverse event evaluation[8]		X	X	X	X	X	X	X	X	X			X[9]	X[9]	X[9]	X[9]
COVID-19 diagnostic test	X															
O_2 saturation	X															
Electrocardiogram (ECG)[10]	X															
Complete Blood Count (CBC)	X	X	X	X	X	X	X	X	X[7]	X[7]		X				
Comprehensive Metabolic Panel (CMP)[11]	X	X	X	X	X	X	X	X	X[7]	X[7]	X[12]	X		X		X
Pregnancy test[13]	X															
Sample for viral load[14]			X		X		X		Days 8, 10, 12, 14							
Clinical response assessment[15]	X	X	X	X	X	X	X	X	X	X						
Mandatory hospitalization[16]		X	X	X	X	X	X	X								
Leflunomide/placebo[17]		Loading dose			X	X	X	X	X							
Cholestyramine[18]											X[18]					
CLIA teriflunomide levels												X[19]		X[19]		
PT/INR[20]											X[19]	X[20]		X[20]		

Research blood for Synold lab PK/PD	X	X	X	X	X	X	X	X	X[7]	Days 21,28		X		X		
Research blood for Pichiorri lab	X				X				Day 8	Days 21,28						

1. All screening procedures to be performed within 4 days prior to start of treatment.
2. Informed consent process to be fully documented: e.g. prospective participant had sufficient time for deliberation, all questions were answered, treatment options provided by the physician, full study reviewed including risks, and a copy of signed consent given to participant.
3. Registration -See **Section 4.3** for slot reservation and registration process. Treatment must begin within 7 days of registration. Documentation providing Investigator's confirmation that all eligibility criteria are met must be available prior to registration.
4. Medical history - will be obtained by the investigator or qualified designee. Includes information on demographics, all active conditions, and any prior conditions that are considered to be clinically significant by the PI. A disease history, including the date of initial diagnosis and list of all prior anti-COVID-19 treatments, and responses and duration of responses to these treatments, will also be recorded.
5. Concomitant medications - All concomitant medications and treatments must be recorded in the CRF. Any medication received up to 30 days prior to screening visit, including any prescription, over the counter, or natural/herbal preparations taken will be reviewed during screening to determine subject eligibility. New medications started during the trial through up to 30 days from last dose of study therapy will be recorded. Concomitant treatments that are required to manage a subject's medical condition during the study will also be recorded in the CRF. The medication record will be maintained following enrollment including any changes to the dose or regimen.
6. Vital signs - include BP, HR, and temperature. As part of screening visit, vitals should be obtained within 4 days prior to first dose of study agent. Vitals will also be obtained during treatment. Significant findings that were present prior to the signature of the informed consent must be included in the Medical History eCRF page. Significant new findings that begin or worsen after time of participant enrollment must be recorded on the Adverse Event eCRF page.
7. Daily if inpatient; twice a week, at least 3 days apart, if outpatient
8. Adverse event monitoring - Toxicities and adverse events (AEs) will be assessed at each timepoint using the CTCAE v5.0. AEs will be monitored from the time the participant is enrolled to the study. Participants will be instructed to report all AEs during the study and will be assessed for the occurrence of AEs throughout the study. All AEs (serious and non-serious) must be recorded on the source documents and routine AE collection via CRFs (per **Section 14.6**) regardless of the assumption of a causal relationship with the study drugs. Toxicities which occur prior to the start of treatment will not be subject to analysis.
9. A tele-health visit can be performed in lieu of in-person as appropriate.
10. Electrocardigram to be performed at baseline and as clinically indicated thereafter.
11. AST, ALT, and bilirubin values will be obtained and recorded in the electronic case report form.
12. Liver function tests must be performed weekly during cholestyramine administration
13. Pregnancy test – serum or urine test for women of childbearing potential only.
14. Measure viral load every 2 days by PCR of nasopharyngeal swab and tracheal aspirates if on ventilator (if safely obtainable) to assess the kinetics of viral replication from day 1 to day 14.
15. Clinical response assessment – See **Section 11**
16. Mandatory hospitalization – The first 7 days of treatment/placebo will be inpatient, with hospital discharge contingent on improvement of COVID symptoms to moderate severity or better and resolution of treatment-related adverse events to ≤ grade 1 or a return to baseline level(s).

17. Leflunomide - The first 3 loading doses will be given at a dose of 100 mg, after which treatment will continue at a dose of 20, 40, or 60 mg daily, depending on the dose level (phase 1) or RP2D (phase 2). In phase 1, leflunomide will be given in 20 mg or 100 mg pills. In phase 2, 20 mg pills will be used, either leflunomide or placebo.
18. Cholestyramine (for participants given leflunomide only) - Participants who have completed the 28-daytreatment/monitoring period will undergo oral administration of cholestyramine 8 grams oral suspension three times daily for 11 days, starting 29 days post-initiation of protocol therapy. *Phase 1*: If the participant discontinues leflunomide because of study withdrawal or toxicities that are at least possibly related to leflunomide, cholestyramine should be given immediately after leflunomide discontinuation. *Phase 2*: If the participant discontinues leflunomide or placebo because of study withdrawal or toxicities that are at least possibly related to leflunomide, the participant should become unblinded to the treatment assignment (see **Section 4.4**). Only participants who were given leflunomide should be given cholestyramine.
19. After the treatment with cholestyramine, CLIA laboratory testing will be performed to verify plasma levels less than 0.02 mg/L or 0.02µg/mL by two separate tests at least 14 days apart. If plasma levels are higher than 0.02 mg/L or 0.02µg/mL, additional cholestyramine treatment will be considered. The amount of additional cholestyramine is at the discretion of the treating physician and should take into account the remaining plasma level of teriflunomide, with consultation with Timothy Synold.
20. PT/INR, vitamin K—Because cholestyramine administration may lead to bleeding abnormalities due to vitamin K deficiency, PT/INR should be measured before cholestyramine is given, at the end of its 11-day course, and ≥14 days after its termination. Intravenous vitamin K may be given.

11.0 ENDPOINT DEFINITIONS/MEASUREMENT OF EFFECT

11.1 Primary Endpoints

11.1.1 Phase 1 Segment

The primary endpoint is toxicity. Toxicity will be graded according to the NCI-Common Terminology Criteria for Adverse Events version 5.0. Dose limiting toxicity (DLT) is defined below. The maximum tolerated dose (MTD) will be based on the assessment of DLT during the 28-day treatment period.

DLT is defined as any of the following:

- **Hematologic DLT events (any AEs considered at least possibly attributable to study treatment):**
  - For patients with *solid tumor malignancies*, all grade 3 or 4 hematologic AEs
  - For patients with *hematologic malignancies*, the following AEs will be considered DLTs if they are determined as not attributable to underlying hematological malignancy
    - Grade 4 neutropenia (ANC <500/mm^3);
    - Grade 3 or 4 febrile neutropenia;
    - Grade 4 thrombocytopenia (<25,000/mm^3);
    - Grade 3 thrombocytopenia (<50,000/mm^3) with bleeding;
- **Non-Hematologic AEs (any treatment emergent AEs):**
  - ≥ Grade 3 non-hematologic toxicity excepting the following:
    - Alopecia;
    - Grade 3 nausea/vomiting/diarrhea for less than 72 hours treated with adequate antiemetic and other supportive care;
    - Grade 3 fatigue for < 1 week
    - ≥ Grade 3 electrolyte abnormalities that are not clinically complicated and resolve spontaneously or to conventional medical interventions within 72 hours
    - ≥ Grade 3 amylase or lipase elevation not associated with symptoms or clinical manifestations of pancreatitis
  - Treatment-emergent increase in serum ALT or AST to > 3x ULN associated with an increase in serum total bilirubin to > 2x ULN (consistent with Hy's Law).

Any treatment-emergent Grade 5 AE that occurs during the 28-day treatment period that is not due to underlying malignancy will be considered a DLT.

11.1.2 Phase 2 Segment

- The primary endpoint is best response/clinical improvement attained by day 28 that is sustained for at least 2 days. Response/clinical improvement is defined as a ≥2-point improvement in clinical status from day 1, on a 7-point ordinal scale.

  The ordinal scale is an assessment of the clinical status at a given study day. Each day, the worst (i.e., lowest ordinal) score from the previous day will be recorded. For example, on Day 3, the lowest ordinal score from Day 2 is obtained and recorded for Day 2. The scale is as follows:

  1. Death
  2. Hospitalized, on invasive mechanical ventilation or ECMO
  3. Hospitalized, on non-invasive ventilation or high flow oxygen devices
  4. Hospitalized, requiring low flow supplemental oxygen
  5. Hospitalized, not requiring supplemental oxygen - requiring ongoing medical care (COVID-19 related or otherwise)

6. Hospitalized, not requiring supplemental oxygen - no longer requires ongoing medical care (other than per protocol RDV administration)
7. Not hospitalized

- The co-primary endpoint is toxicity graded by NCI CTCAE v5. Unacceptable Toxicity (UT) follows the same definition as DLT for the phase 1 segment (**Section 11.1.1**).

11.2 Secondary Endpoints

11.2.1 Phase 1:

- Best response/clinical improvement attained by day 28 that is sustained for at least 2 days. Response/clinical improvement is defined as a ≥2-point improvement in clinical status from day 1, on a 7-point ordinal scale, (as described in **Section 11.1.2**).

11.2.2 Phase 1 and Phase 2:

- Clinical improvement, time to;
- SpO2 > 93% on room air, time to;
- First negative SARS-CoV-2 result, by PCR, time to;
- Oxygen therapy, type and duration (days);
- Hospitalization (days);
- Mechanical ventilation required (yes, no) and duration (days);
- Vital status (alive/dead), cause of death;
- Plasma trough teriflunomide concetrations by LC-MS/MS.

11.3 Exploratory Endpoints:

Phase 1 and Phase 2:

- Cytokine profile by Human Cytokine 30-Plex Antibody Bead Kit;
- Immune effector cell profile of peripheral blood mononuclear cells (PBMC) by CyTOF using custom antibody panel of 37 cell surface markers.
- Viral load measured by polymerase chain reaction (PCR) assay of viral RNA from nasopharygeal swab

12.0 STATISTICAL CONSIDERATIONS

12.1 Study Design

12.1.1 Phase 1 Design (Single-arm, Single-agent, Multi-center):

Prior to formally initiating a multi-center, randomized, placebo-controlled, double-blind, phase 2 pilot trial, a multi-center single-arm phase 1 trial will be conducted to assess the safety and tolerability of leflunomide when combined with COVID-19 SOC. Because the objective is to keep the DLT probability to low levels, a modified rolling-six design will govern the dose escalation (de-escalation), as opposed to methods that calibrate to a DLT rate. Up to three doses of leflunomide will be considered: 20 mg/day (DL-1), 40 mg/day (DL1-starting dose), and 60 mg/day (DL2) as shown in **Table 12.1**. The treatment cycle length is 28 days; patients will receive only one cycle of treatment. There will be no dose escalation within a patient.

This multi-center phase 1 dose-escalation trial is designed to determine the MTD and RP2D. The RP2D of leflunomide will generally be the MTD, but it may be less than the MTD based on a review of available data. Extensive pharmacokinetic and pharmacodynamic studies are also incorporated into this protocol and will be helpful in determining the RP2D.

Table 12.1. Dose Levels

Dose Level	Leflunomide Dose*
-1	20 mg PO daily
(starting dose) **1**	40 mg PO daily
2	60 mg PO daily

*NOTE: Leflunomide is administered with a loading dose of 100 mg for the 1st three doses.

Dose Escalation Rules: This phase 1 trial will employ a modified rolling six design, the IQ 3+3, [7], a more conservative version of the rolling 6 design of Skolnik, et al [46]. In this design, at most 3 patients will be under observation for DLT (during the 28-day evaluation period) on the current test dose level at any time. Patients who are not evaluable for dose escalation will be replaced. Once each patient completes the 28-day DLT evaluation period and passes without a DLT, an additional patient *may* be accrued on that dose level for up to 6 patients. Once 3 patients have completed the 28-day DLT evaluation period with no patient at that dose level experiencing a DLT, the dose can be escalated. Up to 3 additional patients may be treated at the current dose level. Although this design does not require that 6 patients be treated, no more than 6 evaluable patients will be accrued to any dose level during the dose finding segment of this study. If at any time, the dose level has 1 documented DLT with fewer than 6 evaluable patients, accrual will continue until 6 patients are evaluable. Escalation will terminate as soon as two or more patients experience a DLT attributable to the study treatment. MTD will be the highest dose in which ≤ 1/6 patients experience a DLT. These rules are detailed in **Table 12.2**. The MTD will be the highest dose in which ≤ 1/6 patients experience a DLT. The RP2D of leflunomide will generally be the MTD, but it may be less than the MTD based on a review of available data, including pharmacokinetic and pharmacodynamic studies, and clinical activity.

Table 12.2: Dose Escalation Rules

# Patients on Current Level			Action
With DLT^	**Evaluable**	**Evaluable + At Risk^**	
0	0	1-2	Accrue next patient at this level*
0	0	3	Hold accrual
0	1	1-3	Accrue next patient at this level
0	1	4	Hold accrual
0	2	2-4	Accrue next patient at this level
0	2	5	Hold accrual
0	3-6	3-6	Accrue next patient at the next higher level*,+
1	1	1-2	Accrue next patient at this level
1	1	3	Hold Accrual
1	2	2	Accrue next patient at this level
1	2	3-4	Hold accrual
1	3-5	3-5	Accrue next patient at this level
1	3-5	6	Hold accrual
1	6	6	Accrue next patient at the next higher level*
2**	any	any	Accrue next patient at next lower level (max 6)

^: DLT: a patient with a documented DLT

Evaluable: a patient who is either fully evaluable for toxicity for the purpose of dose escalations or has a DLT

At Risk: a patient who is on treatment and has not yet passed the evaluation period nor had a DLT

*: During the dose-escalation portion, if higher dose level is already closed, the next lower dose will accrue to a total of 6 patients, with 2 or higher DLTs requiring further dose de-escalation.

+: Although under this scenario escalating to the next higher dose level is suggested, additional patients can be accrued to the current level -up to n=6 patients.

**: Patients treated on a higher dose will have their treatment modified to the dose below the dose level with 2 DLTs, if pending patients have DLT.

12.1.2 Phase 2 Pilot Design (Two-arm, Randomized, Double-blind, Multi-center):

The phase 2 segment will be a multi-center, randomized, placebo-controlled, double blind, phase 2 pilot trial of two treatments; Arm 1: leflunomide plus standard of care, Arm 2: placebo plus standard of care. The study design consists of randomization in a 1:1 ratio. The randomization will be stratified by COVID-19 risk (Lower: 1 additional risk factor; High: ≥2 additional risk factors) and concomitant SOC anti-viral treatment (SOC1: FDA approved/EUA COVID-19 anti-viral agent; SOC2: other/none) to limit imbalance between the two treatment arms. Risk factors considered -beyond the risk associated with cancer diagnosis: age (≥65), COPD, diabetes, hypertension, cardiovascular disease, obesity, and time from most recent cancer related treatment to trial enrollment (≤ 3 months) [8-11]. We allow two additional risk factors in the high-risk group since the majority of cancer patients are over 65 and we did not want this risk to obscure all of the others.

Instead of a randomized study design that would utilize a larger number of subjects -to assure sufficient power to detect differences with statistical significance, this small randomized study screens for the potential of leflunomide based simply on observing an improved response. Within each arm, clinical activity will be assessed and an estimate of clinical improvement (response rate) will be generated. Given guidance provided by the FDA and uncertainty regarding 1) the safety of leflunomide in cancer patients with COVID-19 disease, and 2) the natural course of SARS-CoV2 infection, a placebo-control arm will allow for better interpretation of both safety and activity.

Our target difference in response rate is 20%. As designed, with 30 patients (15 patients per arm), this trial is powered to observe a superior response rate in the leflunomide arm if leflunomide is associated with a true increase in the response rate of 20%. If leflunomide is associated with a greater number of responses this will be considered evidence in support of a larger definitive study. However, given the rapidly changing landscape of COVID-19 treatment, additional factors may be considered when determining future investigation of leflunomide in addition to considerations for the variability in patient's status and SOC delivered not considered in the risk factors. Toxicity, feasibility, and overall survival will also guide further investigation of leflunomide.

12.1.3 Evaluable Subjects, Subject Replacement

Evaluable for Toxicity: All patients who receive at least one dose of study agent(s) will be evaluable for toxicity.

Evaluable for DLT: **Evaluable for Dose-Escalation/De-Escalation Criteria:** Participants will be considered evaluable for dose-escalation criteria if they receive ≥79% of leflunomide, which is 11 of 14 days of leflunomide treatment, and are followed for 28 days, or experience a DLT. All participants who are not evaluable for dose-escalation criteria will be replaced. Note: While patients who receive <79% of the intended dose of leflunomide will be considered inevaluable for determination of MTD, if a patient

discontinues leflunomide due to toxicities prior to receiving 79% of the dose, the patient will be considered evaluable and will not be replaced.

Evaluable for Response Criteria: Participants will be considered evaluable for response if they are confirmed eligible, receive at least one dose of study agent (leflunomide or placebo) and have had their clinical status, on a 7-point ordinal scale, re-evaluated (Phase 2 primary endpoint).

12.1.4 Sample Size and Accrual Rate:

In general, the total sample size for the phase 1 segment will depend on the number of dose levels evaluated to determine the RP2D. Assuming the highest dose tested is well tolerated, we expect to enroll and treat approximately 9-12 patients, with a maximum of approximately 18 patients (24 absolute maximum due to the rules of the IQ 3+3 design employed). The phase 2 segment is expected to treat 15 patients per arm, for an overall total of n=30 patients. Assuming 2-3 patients are enrolled each month, across the two segments, accrual is expected to be completed in 12-15 months.

12.2 Phase 2: Stopping Rule for Excessive Toxicity

Study accrual will be temporarily suspended to allow for full review of data, if in either arm, after the first 6 patients are enrolled, ≥33% of patients experience unacceptable toxicity (UT) in the first 28 days. UT follows the same definition as DLT for the phase 1 segment (**Section 11.1.1**). Note: Patients with ongoing toxicity (cycle 1 toxicity persisting beyond day +28) will be followed until resolution or stability. After consideration by the study team [study PI(s), statistician, etc.] and DSMC, a decision will be made as to whether accrual/treatment can be resumed safely or whether protocol modifications are necessary. Patient accrual will not resume until approved by the DSMC and IRB.

12.3 Statistical Analysis Plan

Patient demographic and baseline characteristics, including age, gender, medical history, and prior therapy, will be summarized using descriptive statistics. For continuous variables, descriptive statistics [number (n), mean, standard deviation, standard error, median (range)] will be provided. For categorical variables, patient counts and percentages will be provided.

Primary Safety Analysis: Observed toxicities will be summarized, for all dose levels of the phase 1 segment and for the phase 2 segment, in terms of type (organ affected or laboratory determination), severity, time of onset, duration, serum concentration of the active leflunomide metabolite, probable association with the study treatment and reversibility or outcome.

Primary Activity Analysis: The primary analysis set for the clinical activity analysis in the phase 2 segment is defined as the full analysis set, which will include all participants who (1) were randomized and (2) have received at least 1 dose of leflunomide/placebo. Participants will be grouped according to the treatment to which they were randomized. The response rate will be calculated as the percent of treated patients; the exact 95% confidence interval will be calculated for this estimate. Time to response and survival will be estimated using the product-limit method of Kaplan and Meier.

Pharmacokinetics: Because serum concentration of the metabolite, teriflunomide (A77 1726), can be variable, and because some studies have found an association between serum concentration of this metabolite and RA response, samples will be collected throughout the study for analysis by the Analytical Pharmacology Core Facility (APCF) for an exploratory analysis relating metabolite levels to safety and response outcomes. At the conclusion of the study, total and free teriflunomide levels will be summarized at sampling time points. Descriptive statistics will be used to characterize possible inter-patient variability and relationship to dose, toxicity and response for future studies, including guidance with respect to RP2D

selection. Additional testing and analysis will be done on collected samples to assess, in an exploratory manner.

13.0 DATA HANDLING, DATA MANAGEMENT, RECORD KEEPING

13.1 Source Documents

Source documents are original documents, data, and records (e.g., medical records, pharmacy dispensing records, recorded data from automated instruments, laboratory data) that are relevant to the clinical trial. The investigator or their designee will prepare and maintain adequate and accurate source documents. These documents are designed to record all observations and other pertinent data for each patient enrolled in this clinical trial. Source documents must be adequate to reconstruct all data transcribed onto the case report forms.

13.2 Data Capture Methods and Management

Data for this trial will be collected using City of Hope's electronic capture system that is compliant with 21 CFR Part 11.

Study personnel will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF).

13.3 Case Report Forms/Data Submission Schedule

Study personnel will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF) when the information corresponding to that visit is available.

The investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the investigator. All case report forms must be completed by designated study personnel. The completed case report forms must be reviewed, signed and dated by the Investigator or designee in a timely fashion.

All data will be collected using electronic data collection, stored as indicated in Section 13.2, and will be submitted according to the timelines indicated in Table 13.3.

Table 13.3 Data Submission Schedule

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration
On Study Forms	Within 14 calendar days of registration
Baseline Assessment Forms	Within 14 calendar days of registration
Treatment Forms	Within 10 calendar days of treatment administration
Adverse Event Report Forms	Within 7 calendar days of AE assessment/notification
Response Assessment Forms	Within 10 calendar days of the response assessment
Other Assessment Forms (concomitant medications)	Within 10 calendar days of the assessment
Off Treatment/Off Study Forms	Within 10 calendar days of end of treatment/study
Follow up/Survival Forms	Within 14 calendar days of the follow up activity

13.4 Regulatory Records

The investigator will maintain regulatory records, including updating records in accordance with Good Clinical Practice guidelines and FDA regulations.

14.0 REPORTING OF ADVERSE EVENTS, UNANTICIPATED PROBLEMS & OTHER EVENTS OF INTEREST

14.1 COH Data and Safety Monitoring Plan

Definition of Risk Level

This is a Risk Level 4 study as defined in the City of Hope Institutional Data and Safety Monitoring Plan. This determination was made because this study involves a COH IND.

14.2 Dose Escalation

This study will utilize the Phase I Tracking Log to monitor data and safety for dose escalation. The Tracking Log will contain dose levels administered, DLT, DLT-defining adverse events, and any details regarding dose level escalation. The record of doses administered and resultant adverse events will be included in the PMT Report.

14.3 Adverse Event Definitions

The PI or designee will be responsible for determining the event name, assessing the severity (i.e., grade), expectedness, and attribution of all adverse events.

Adverse Event (AE) - An adverse event is any untoward medical experience or change of an existing condition that occurs during or after treatment, whether or not it is considered to be related to the protocol intervention.

Reporting Non-serious Adverse Events – Adverse events will be collected after the patient is given the study treatment or any study related procedures. Adverse events will be monitored by the PMT. Adverse events that do not meet the criteria of serious OR are not unanticipated problems will be reported only in the PMT Report.

Serious Adverse Event (SAE) [Modified from the definition of unexpected adverse drug experience in 21 CFR 312.32] - defined as *any expected* or *unexpected adverse event*s that result in any of the following outcomes:

- Death
- Is life-threatening experience (places the subject at immediate risk of death from the event as it occurred)
- Unplanned hospitalization (equal to or greater than 24 hours) or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Secondary malignancy*
- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring

intensive treatment in the emergency room or at home, blood dyscrasias of convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

*Modified from 21 CFR 312.32.

Reporting Serious Adverse Events - begins after study treatment or any study related procedures. All SAEs occurring during this study, whether observed by the physician, nurse, or reported by the patient, will be reported according to the approved City of Hope's Institutional policy. Serious Adverse Events that require expedited reporting will be submitted electronically using iRIS.

Assessment of Adverse Event Name and Grade

The authorized investigator will be responsible for determining the event name, assessing the severity (i.e., grade), expectedness, and attribution of all adverse events.

Adverse events will be characterized using the descriptions and grading scales found in the most recent version of the NCI CTCAE v5.0. A copy of the scale can be found at https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm. The determination of severity for all other events not listed in the CTCAE should be made by the investigator based on medical judgment and the severity categories of Grade 1 to 5 as defined below:

- Grade 1 (mild) – An event that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- Grade 2 (moderate) – An event that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the subject.
- Grade 3 (severe) – An event that requires intensive therapeutic intervention. The event interrupts usual activities of daily living, or significantly affects the clinical status of the subject.
- Grade 4 (life threatening) – An event, and/or its immediate sequelae, that is associated with an imminent risk of death or with physical or mental disabilities that affect or limit the ability of the subject to perform activities of daily living (eating, ambulation, toileting, etc).
- Grade 5 (fatal) – Death (loss of life) as a result of an event.

Expected Adverse Event - Any event that does not meet the criteria for an unexpected event, OR is an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

Unexpected Adverse Event [21 CFR 312.32 (a)] – An adverse event is unexpected if it is not listed in the investigator's brochure and/or package insert; is not listed at the specificity or severity that has been observed; is not consistent with the risk information described in the protocol and/or consent; is not an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

14.4 Adverse Event Attribution

The following definitions will be used to determine the causality (attribution) of the event to the study agent or study procedure.

- **Unrelated –** The event is clearly related to other factors such as the participant's clinical state, other therapeutic interventions, or concomitant medications administered to the participant.

- **Unlikely –** The event is doubtfully related to the investigational agent(s). The event was most likely related to other factors such as the participant's clinical state, other therapeutic interventions, or concomitant drugs.
- **Possible –** The event follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the participant's clinical state, other therapeutic interventions, or concomitant drugs.
- **Probable –** The event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug. The event cannot be reasonably explained by other factors such as the participant's clinical state, therapeutic interventions, or concomitant drugs.
- **Definite –** The event follows a reasonable temporal sequence from the time of drug administration, follows a known response pattern to the study drug, cannot be reasonably explained by other factors such as the participant's condition, therapeutic interventions, or concomitant drugs, AND occurs immediately following study drug administration, improves upon stopping the drug, or reappears on re-exposure.

14.5 Pregnancies

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female participant occurring between the time participant receives the first dose of protocol therapy until after the 11-day washout with cholestyramine, are considered immediately reportable events. Protocol therapy is to be discontinued immediately, and cholestyramine washout commenced. **The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to the Study PI immediately and the DCC within 24 hours of awareness**. The female subject may be referred to an obstetrician-gynecologist (preferably one with reproductive toxicity experience) or another appropriate healthcare professional for further evaluation.

The Investigator should make every effort to follow the female participant until completion of the pregnancy per institutional policies, and should notify the Study PI.

Abnormal pregnancy outcomes and neonatal deaths that occur within 28 days of birth should be reported as an SAE per expedited reporting guidelines.

Any infant death after 28 days that the Investigator suspects is related to the in utero exposure to protocol therapy should also be reported as an SAE per expedited reporting guidelines (see **Section 14.7**).

14.5.1.1 Male participants

If a female partner of a male participant becomes pregnant, the male participant should notify the Investigator, and the pregnant female partner should be advised to call their healthcare provider immediately.

The Investigator should make every effort to follow the outcome of the pregnancy per institutional policies, and should notify the Study PI.

14.6 Routine AE Collection and Reporting Guidelines

AEs will be collected from the signing of informed consent until ending study participation. Routine AE reporting will occur via data entry into the study eCRF. AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must also be reported in routine study data submissions.

14.6.1 Routine AE Collection

AEs recorded in the CRF include:

Highest grade of Grade 1-5 AEs, excepting the following for which all grade changes and start and stop dates must be captured:

- Diarrhea
- Vomiting
- Allergic reaction/hypersensitivity
- Electrolyte/metabolic toxicity
- Fever
- Hypoxia
- Any new infection that occurs on study, regardless of infecting agent (i.e., viral or non-viral) should be captured. Additionally, the site of infection and source of culture (BAL, tracheal aspirate, sputum, blood, urine, etc.) should also be recorded.

All SAEs, and whether hospitalization occurred (or prolongation of hospitalization). Hospitalization for expedited reporting purposes is defined as an inpatient hospital stay ≥ 24 hours.

14.6.2 Other Mandated Data Collection

The following protocol-specific data must be recorded in the CRF:

- Number of days between onset of symptoms and initiation of treatment.
- Identity of all investigational antivirals (if any) that were used and their time of administration.

14.7 Expedited Reporting

The table below indicates what events to report to expeditiously.

Table 14.7 Expedited Reporting Guidelines

Time point	What to report
From signing of the consent to study completion	• All UPs
For the time period beginning at treatment through 28 days following cessation of treatment **All reportable events will require follow up until stabilization or resolution per the agreement of the Study PI.**	• All SAEs regardless of relationship to protocol therapy unless they meet exceptions to expedited reporting • All AEs that meet the definition of a UP • pregnancies and lactation
From Day 1 of protocol therapy up to day 11 of cholestyramine washout during follow-up	• Pregnancies and lactation
Post Safety follow-up to removal from study	• All SAEs that are considered probably or definitely related to leflunomide.

NOTE: All events reported expeditiously require follow-up reporting until the event is resolved, stabilized, or determined to be irreversible by the investigator.

The DCC should be consulted prior to ending the follow-up of events that have stabilized.

14.7.1 Expedited reporting guidelines **(COH only)**

14.7.1.1 To the DCC/Study PI

All events that meet the criteria specified in Table 14.7 will be reported to the DCC and Study PI within 24 hours of notification that the event met the expedited reporting criteria.

Email the following information to DCC@coh.org and the Study PI: Participant ID, date the event met criteria for reporting, whether the event meets the definition of serious, whether the event is an unanticipated problem, grade of event, attribution of event, whether the event is a known expected toxicity to study agent.

14.7.1.2 To the COH DSMC/IRB

Serious Adverse Events that require expedited reporting and unanticipated problems will be reported according to the approved City of Hope's Institutional policy via electronic submission in iRIS. This includes all SAEs and UPs that meet COH DSMC/IRB expedited reporting criteria that occurred at COH and non-COH sites.

14.7.1.3 To Participating Investigators

- Report all expedited reportable AEs to participating investigators as an IND Safety Report occurring within 30 calendar days of receipt of sponsor (lead site) notification, and indicate whether or not a protocol and/or consent form change is required. A cover letter will indicate the protocol title, the IND#, whether the FDA was informed, and, for non-COH sites, a statement that the report should be submitted to their local IRB for review as an IND safety report if applicable per local IRB policy.

- Circulate to all participating sites for submission to their IRBs the COH PMT report and DSMC recommendation, in accordance with NIH guidance.

14.7.2 Expedited reporting guidelines **(non-COH sites only)**

14.7.2.1 To the DCC/Study PI

All events that meet the criteria specified in Table 14.7 will be reported to the DCC and Study PI within 24 hours of notification that the event met the expedited reporting criteria.

1. Document/describe the AE/UP on each of the following:
    a. MedWatch 3500A or local IRB submission document*
    MedWatch 3500A is downloadable form at http://www.fda.gov/medwatch/getforms.htm
    *The local IRB submission document may be used if the document template is approved by the DCC
    b. Expedited Reporting Coversheet. The Expedited Reporting Coversheet is found in Appendix D. A modifiable Microsoft Word document is also available from the DCC. An electronic signature on the document will be accepted.
2. Scan and email above documents to DCC@coh.org with the subject title as "Leflunomide COVID SAE COH IRB #20291".
    a. All expedited reports received at this account are immediately forwarded to the Study PI, and to DCC personnel.

b. While not required, if available and applicable, please also include the local IRB submission for this event in the submission.

3. If an email receipt from DCC personnel is not received within one working day, please call (626)-218-7904 and/or email DCC@coh.org.

14.7.2.2 To the local IRB

- Non-COH participating sites will report to their local IRB per their site's specific institutional and IRB guidelines.
- As soon as possible, non-COH sites will provide to the DCC copies of the IRB submission and corresponding IRB response.

14.8 Reporting to the FDA

The study PI (or designee) will be responsible for contacting the Office of IND Development and Regulatory Affairs (OIDRA) at COH to ensure prompt reporting of safety reports to the FDA. OIDRA will assist the PI with the preparation of the report and submit the report to the FDA in accordance with the approved City of Hope's Institutional policy.

Serious Adverse Events meeting the requirements for expedited reporting to the Food and Drug Administration (FDA), as defined in 21 CFR 312.32, regardless of the site of occurrence, will be reported as an IND safety report using the MedWatch Form FDA 3500A for Mandatory Reporting.

The criteria that require reporting using the MedWatch 3500A are:

- Any unexpected fatal or life threatening adverse experience associated with use of the drug must be reported to the FDA **no later than 7 calendar days** after initial receipt of the information [21 CFR 312.32(c)(2)]
- Any adverse experience associated with use of the drug that is both serious and unexpected must be submitted **no later than 15 calendar days** after initial receipt of the information [21 CFR 312.32(c)(1)]
- Any follow-up information to a study report shall be reported **as soon as** the relevant information becomes available. [21 CFR 312.32(d)(3)]

In addition, on behalf of the study PI, OIDRA will submit annually within 60 days (via COH OIDRA) of the anniversary of the date the IND went into effect, an annual report to the FDA which is to include a narrative summary and analysis of the information of all FDA reports within the reporting interval, a summary report adverse drug experiences, and history of actions taken since the last report because of adverse drug experiences.

15.0 ADHERENCE TO THE PROTOCOL & REPORTING OF PROTOCOL DEVIATIONS

A deviation is a divergence from a specific element of a protocol. It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. Protocol deviations may be on the part of the subject, the investigator, or study staff.

15.1 Definitions

15.1.1 Unplanned Deviations:

- **Emergency modifications -** Investigators may implement a deviation from the protocol to eliminate an immediate hazard for the protection, safety, and well-being of the study patient to trial participants without prior COH IRB or Sponsor approval.
- **Deviations Discovered After They Have Occurred.**

Unplanned deviations from the protocol must be documented in study subject source documents.

15.1.2 Planned Non-Emergency Deviations (Single Subject Exception)

A **planned deviation** involves circumstances in which the specific procedures called for in a protocol are not in the best interests of a specific patient. It is a deviation that is anticipated and receives prior approval by the Study PI and the COH IRB.

15.2 Reporting of Deviations

15.2.1 Reporting Unplanned Deviations

For any such deviation, the Study PI will notify the COH DSMC and IRB within 5 calendar days of its occurrence via iRIS in accordance with the Clinical Research Protocol Deviation policy.

A list of these deviations, regardless of the site of occurrence, will be submitted along with the Protocol Management Team (PMT) reports to the COH DSMC.

For non-COH sites:

- The local IRB and/or DSMC must be notified according to local institutional policies.
- The study Principal Investigator and the DCC (dcc@coh.org) must be notified by email as soon as practical (within 24 hours of notification of the event). This email should provide input on the following:
  - Description of the event
  - Impact on participant safety or the safety to others
  - Impact on the study design
  - A corrective and preventative action plan

15.2.2 Reporting Planned Non-Emergency Deviations/ Single Subject Exceptions

Any planned deviation must be submitted as a “planned protocol deviation” via iRIS in accordance with IRB guidelines and the Clinical Research Protocol Deviation policy. An IRB approved planned deviation does not need to be submitted as a deviation to the DSMC.

All planned non-emergency deviations from the protocol must have **prior** approval by the Study Principal Investigator, the Site Principal Investigator, COH IRB, and when applicable, the local IRB.

16.0 STUDY OVERSIGHT, QUALITY ASSURANCE, & DATA AND SAFETY MONITORING

16.1 Study PI Responsibilities

The Study PI is responsible for the conduct of the clinical trial, including overseeing that sponsor responsibilities are fulfilled as defined in § 21 CFR 312.

16.2 All Investigator Responsibilities

All investigators agree to:

- Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when necessary to protect the safety, rights or welfare of subjects.
- Personally conduct or supervise the study (or investigation).
- Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
- Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
- Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
- Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
- Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
- Promptly report to the IRB and the Sponsor all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
- Seek IRB and Sponsor approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
- Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

16.3 Protocol Management Team (PMT)

The PMT minimally consisting of the Study Principal Investigator, Site Lead Investigators, collaborating investigators, the research nurse, the clinical research associate/coordinator, and the study biostatistician is responsible for ongoing monitoring of the data and safety of this study.

The PMT will meet (in person or via teleconference) at least monthly, and will meet at least quarterly with the study biostatistician, to review study status. This review will include, but not be limited to, reportable AEs and UPs, and an update of the ongoing study summary that describes study progress in terms of the study schema. The meeting will be a forum to discuss study related issues including accrual, SAE/AEs experienced, study response, deviations/violations and study management issues. The appropriateness of further subject enrollment and the specific intervention for subsequent subject enrollment are addressed, including the implementation of stopping rules.

Minutes of these discussions will be taken to document the date of these meetings, attendees and the issues that were discussed. Copies of these minutes will be maintained in the Regulatory Binder.

16.4 Monitoring/ Auditing

Clinical site monitoring/auditing is conducted to ensure that the rights of human subjects are protected, that the study is implemented in accordance with the protocol and regulatory requirements, and that the quality and integrity of study data and data collection methods are maintained. Monitoring/auditing for this study will be performed by the City of Hope Office of Clinical Trials Auditing and Monitoring (OCTAM).

The Investigator will permit the study monitors and appropriate regulatory authorities direct access to the study data and to the corresponding source data and documents to verify the accuracy of this data. The Investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure

that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

Details of clinical site monitoring are documented in the OCTAM SOP. This document specifies the frequency of monitoring, monitoring procedures, the level of clinical site monitoring activities (e.g., the percentage of subject data to be reviewed), and the distribution of monitoring reports. Staff from OCTAM will conduct monitoring activities and provide reports of the findings and associated action items in accordance with the details described in the SOP. Documentation of monitoring activities and findings will be provided to the study team, and the COH DSMC.

Documentation of monitoring/auditing activities and findings by OCTAM will be provided to the study team, the Site Lead Investigator, Study PI, and the COH DSMC.

16.5 Quality Assurance

The City of Hope Clinical Research Information Support will provide quality assurance.

16.6 City of Hope Data and Safety Monitoring Committee (DSMC)

The DSMC is a multidisciplinary committee charged with overseeing the monitoring of safety of participants in clinical trials, and the conduct, progress, validity, and integrity of the data for all clinical trials that are sponsored by City of Hope. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. The committee reviews the progress and safety of all active research protocols that are not monitored by another safety and data monitoring committee or board.

The COH DSMC will review and monitor toxicity and accrual data from this trial. Information that raises any questions about participant safety will be addressed with the Principal Investigator, statistician and study team.

The DSMC will review the study's status every 3 months and/or more frequently if necessary. The DSMC will review up-to-date participant accrual; summary of all adverse events captured via routine and expedited reporting; a summary of deviations; any response information; monitoring reports, and summary comments provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request. A review of outcome results (response, toxicity and adverse events) and factors external to the study (such as scientific or therapeutic developments) is discussed, and the Committee votes on the status of each study.

Data and safety will be reported to the COH DSMC using the PMT report and submitted 3 months from the date of activation.

17.0 ETHICAL AND REGULATORY CONSIDERATIONS

17.1 Ethical Standards

This study will be conducted in conformance with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research (US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, April 18, 1979) and the Declaration of Helsinki.

17.2 Regulatory Compliance

This study is to be conducted in compliance with the IRB approved protocol and according to the following considerations:

- US Code of Federal Regulations (CFR) governing clinical study conduct
  - Title 21 Part 11 – Electronic Records; Electronic Signatures
  - Title 21 Part 50 – Protection of Human Subjects
  - Title 21 Part 54 – Financial Disclosure by Clinical Investigators
  - Title 21 Part 56 – Institutional Review Boards
  - Title 21 Part 58 – Good Laboratory Practice for Nonclinical Laboratory Studies
  - Title 21 Part 312 – Investigational New Drug Application
  - Title 45 Part 46 – Protection of Human Subjects
- US Federal legislation, including but not limited to
  - Health Insurance Portability and Accountability Act of 1996
  - Section 801 of the Food and Drug Administration Amendments Act
- Applicable state and local laws. For research occurring in California, this includes but is not limited to State of California Health and Safety Code, Title 17
- Applicable NIH policies and procedures
- Applicable institutional research policies and procedures

17.3 Institutional Review Board

An Institutional Review Board (IRB) that complies with the federal regulations at 45 CFR 46 and 21 CFR 50, 56 and State of California Health and Safety code, Title 17, must review and approve this protocol, informed consent form and any additional documents that the IRB may need to fulfill its responsibilities (Investigator's Brochure, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) prior to initiation of the study. Revisions to approved documents will require review and approval by the IRB before the changes are implemented in the study. All institutional, NCI, Federal, and State of California regulations must be fulfilled.

Each participating non-COH institution must provide for the review and approval of this protocol and the associated informed consent documents by an appropriate IRB holding a current US Federal wide Assurance issued by and registered with the Office for Human Research Protections (OHRP). The protocol and consent will be reviewed and approved by the COH IRB before submission to a participating site IRB.

The IRB's written unconditional approval of the study protocol and the informed consent document must be in the possession of the investigator, and, for external sites, the possession of the DCC, before the study is initiated.

The IRB will be informed of serious unexpected, unanticipated adverse experiences, and unanticipated problems occurring during the study, and any additional adverse experiences in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

17.4 Informed Consent

Each participating non-COH institution will be provided with a model informed consent form. Each institution may revise or add information to comply with local and/or institutional requirements, but may not remove procedural or risk content from the model consent form. Furthermore, prior to submission to

the site's IRB (initial submission and amendments), the consent and accompanying HIPAA form, if separate to the consent, must be reviewed and approved by the DCC.

The Principal Investigator or IRB approved named designee will explain the nature, duration, purpose of the study, potential risks, alternatives and potential benefits, and all other information contained in the informed consent document. In addition, they will review the experimental subject's bill of rights and the HIPAA research authorization form. Prospective participants will be informed that they may withdraw from the study at any time and for any reason without prejudice, including as applicable, their current or future care or employment at City of Hope or participating institution or any relationship they have with City of Hope. Prospective participants will be afforded sufficient time to consider whether or not to participate in the research.

After the study has been fully explained, written informed consent will be obtained from either the prospective participant or his/her guardian or legal representative before study participation. The method of obtaining and documenting the informed consent and the contents of the consent must comply with the ICH-GCP and all applicable regulatory requirements.

A copy of the signed informed consent will be given to the participant or his/her legally authorized representative. The original signed consent must be maintained by the investigator and available for inspection by sponsor designated representatives, or regulatory authority at any time.

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation.

17.5 Participant Withdrawal

Participants may withdraw from the study at any time and for any reason without prejudice. The withdrawal must be documented per institutional policies. The COH DCC should be promptly notified of the change in participant status.

Participant withdrawal may consist of any of the following with regard to study procedures and data collection:

- Withdrawal from study treatment, but agreement to continue with active study procedures and chart review and survival follow-up.
- Withdrawal from study treatment and all active procedures, but agreement for chart review and survival follow-up.
- Withdrawal from study treatment, all active procedures, and any future data collection.

Participants who agreed to the collection of research blood samples may withdraw consent to use their specimens, if they are not yet processed as detailed in the consent form. Once the PI and site PI is notified of this withdrawal of informed consent, the research specimens will not be used in any research. At that time, any of the existing specimens will be destroyed.

17.6 Special and Vulnerable Populations

17.6.1 Women and Minorities

The study is open to anyone regardless of gender, race or ethnicity. Efforts will be made to extend the accrual to a representative population. If differences in outcome that correlate to gender, racial, or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

It is important to enroll subjects with diverse backgrounds, to help ensure that the treatment is safe and effective for everyone, especially given the disproportionate impact of COVID-19 on racial and ethnic minorities. We will work with our existing Minority Recruitment Task Force and Minority Outreach Coordinator to ensure that these expected accruals are met. We are also recruiting patients from USC Medical Center, which has a high minority population.

Pregnant women are excluded because the effects of the regimens on fetuses are unknown.

17.6.2 Pediatric Population

Pediatric participants (< 18 years of age) are excluded from this study since safety and effectiveness of protocol therapy has not yet been defined for the study population. Additional studies may be performed in the pediatric population once safety and effectiveness of protocol therapy is defined in the adult study population.

17.6.3 HIV Positive Individuals

Participants with HIV are not excluded unless their CD4+ T cell count is <200. An infectious disease consultation is indicated for patients with HIV, as there is a minor drug interaction with a commonly used HIV medication (tenofovir alafenamide) and leflunomide. A simple substitution can be made, which does not preclude patients on this medication from being included in the study.

17.6.4 Vulnerable Populations

Per 45 CFR §46.111 (a)(3) and 45 CFR §46, Subparts B-D identifies children, prisoners, pregnant women, mentally incapacitated persons, and economically or educationally disadvantaged persons as vulnerable populations.

In the phase 1 portion, cognitively impaired adults are excluded from enrollment, because the safety and feasibility of the treatment should be established before cognitively impaired patients participate in a trial that requires self-administration of leflunomide and cholestyramine and some self-monitoring of toxicities during the outpatient period. After the feasibility and safety of the treatment is established, cognitively impaired adults may enroll in the phase 2 portion, provided that they have adequate psychosocial support as assessed by a psychologist or social worker. In such instances, informed consent will be sought and documented from the prospective participant's legally authorized representative in agreement with institutional policies and local IRB approval.

Economically/educationally disadvantaged persons are not actively targeted for participation, nor are they excluded from participation. This study does not pose additional risks for economically/educationally disadvantaged persons than for the general population.

17.7 Participant Confidentiality

Participant confidentiality is strictly held in trust by the investigators, study staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples in addition to any study information relating to participants.

This research will be conducted in compliance with federal and state requirements relating to protected health information (PHI), including the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). HIPAA regulations require a signed subject authorization informing the subject of the nature of the PHI to be collected, who will have access to that information and why, who will use or disclose that information, and the rights of a research participant to revoke their authorization for use of their PHI. In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation

of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

Release of research results should preserve the privacy of medical information and must be carried out in accordance with Department of Health and Human Services Standards for Privacy of Individually Identifiable Health Information, 45 CFR 164.508. When results of this study are reported in medical journals or at meetings, identification of those taking part will not be disclosed and no identifiers will be used.

Medical records of subjects will be securely maintained in the strictest confidence, according to current legal requirements. Data will be entered, analyzed and stored in encrypted, password protected, secure computers that meet all HIPAA requirements. All data capture records, drug accountability records, study reports and communications will identify the patient by initials and the assigned patient number.

Source documents provided to the DCC for the purpose of auditing or monitoring will be de-identified and labeled with the study number, subject ID, and if applicable patient initials.

The investigator/institution will permit direct access to source data and documents by sponsor representatives, the FDA, and other applicable regulatory authorities. The access may consist of trial-related monitoring, including remote monitoring, audits, IRB/IEC reviews, and FDA/regulatory authority inspections. The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

Participant specimens with a limited data set will be provided to research laboratories. The specimens will be labeled with the study number, subject (accession) ID, date and time point of collection. The key to the code will be maintained in the COH clinical trials management system which is a secure environment.

17.8 Use of Unused (Leftover) Specimens Collected for this Trial

Unused samples in existence at study completion (i.e. completion of all research activities under this study) will either be: (a) placed in a COH IRB approved CTCL biorepository (COH#15185) with some clinical information and potentially PHI attached or (b) discarded.

With regard to which option will apply, each site IRB may choose to either: (a) leave the determination to the participant via a question in the informed consent document, which would be communicated to the study registrar (DCC) at the time of participant registration, OR b) may choose to make a single determination on behalf of their respective participants, and communicate that determination to their respective participants via the informed consent.

17.9 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study Sponsor (City of Hope) prior to participation in this study. All City of Hope investigators will follow the City of Hope conflict of interest policy.

17.10 Financial Obligations, Compensation, and Reimbursement of Participants

Leflunomide will be provided free of charge to participants.

Neither the research participant nor the insurance carrier will be responsible for the research procedures related to this study.

Standard of care drugs or procedures provided during the course of study participation will be the responsibility of the research participant and/or the insurance carrier. The participant will be responsible for all copayments, deductibles, and other costs of treatment and diagnostic procedures as set forth by the insurance carrier. The participant and/or the insurance carrier will be billed for the costs of treatment and diagnostic procedures in the same way as if the participant were not in a research study.

In the event of physical injury to a participant resulting from research procedures, appropriate medical treatment will be available at City of Hope or at the non-COH site to the injured participant. There are no plans for City of Hope to provide financial compensation in the event of physical injury to a participant.

The research participant will not receive reimbursement or payment for taking part in this study.

17.11 Publication/ Data Sharing

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by City of Hope for the purposes of performing the study, will be published or passed on to any third party without the written approval of the Study PI. Any investigator involved with this study is obligated to provide City of Hope with complete test results and all data derived from the study.

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement between City of Hope and participating non-COH institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

In accordance with the U.S. Public Law 110-85 (Food and Drug Administration Amendments Act of 2007 or FDAAA), Title VIII, Section 801, this trial will be registered onto ClinicalTrials.gov. Results will be reported on ClinicalTrials.gov generally within 12 months after the completion date unless criteria to delay submission are met per the final rule.

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APPENDIX A. REGISTRATION COVER SHEET

COH IRB# 20291: ***A Phase 1/2 Trial of Leflunomide for the Treatment of COVID-19 in Patients with a Concurrent Malignancy***

Data Coordinating Center:
City of Hope
1500 Duarte Road
Duarte, CA 91010
Tel: (626)-218-7904
Email: DCC@coh.org (use #secure# in subject line)

Site Principal Investigator
Name:

CRA/Study Coordinator:	Contact Number:
Patient's Initials: (F M L):	Institution: PI/ Sub-Investigator:
Patient's DOB:	IRB approval valid until (date):
Sex: _____Male _____Female	Date Informed Consent Signed: Projected start date of treatment:

Race		Ethnicity		Method of Payment:_____	
				Codes:	
☐	Black	☐	Hispanic		
☐	Caucasian	☐	Non-Hispanic	**01** Private	**06** Military or Veterans Adm. sponsored
☐	Asian	☐	Other_____	**02** Medicare	**07** Self-pay (no insurance)
☐	American Indian			**03** Medicare & private ins.	**08** No means of payment (no insurance)
☐	Native Hawaiian/Pacific Islander			**04** Medicaid	**09** Unknown
☐	Other_____ _____			**05** Medicaid & Medicare	

Reason for Screen Failure:

Reason for Failing to Initiate Protocol Therapy:

APPENDIX B. NON-RECOMMENDED COVID THERAPIES

Hydroxychloroquine/chloroquine ± azithromycin

Recommendation: Not recommended, based on insufficient clinical trial and observational data.

Dosing and Pharmacology:

Adults	≥50 kg: Hydroxychloroquine 800 mg on day 1, 400 mg PO daily for 4-7 days of total treatment based on clinical evaluation Azithromycin 500 mg on day 1, then 250 mg daily on days 2-5

Adverse Effects:

- HCQ: QT interval prolongation, hypoglycemia, neuropsychiatric effects (agitation, confusion, delirium, hallucination), agranulocytosis, bone marrow suppression, G6PD-related hemolysis), retinopathy, hepatic impairment.
- Azithromycin: Cardiac arrhythmias, QT prolongation, GI adverse reactions (diarrhea, nausea, vomiting, abdominal pain)

Lopinavir/ritonavir ± ribavirin, interferon-beta-1b

Recommendation: LPV/r is not recommended based on negative clinical trial data for COVID-19. Insufficient data regarding the efficacy of ribavirin and interferon beta-1b to recommend its use.

Dosing:

- Lopinavir 400 mg and ritonavir 100 mg q12h
- Ribavirin 400 mg q12h
- Interferon beta-1b 8 million IU on alternate days (max 3 doses).

Adverse Effects:

- LPV/r: moderate diarrhea, nausea, abnormal LFTs; potential drug interactions (CNI, mTOR inhibitors, azoles)
- Ribavirin: anemia, flu-like symptoms;
- IFN beta-1b: abnormal LFTs, injection site reactions, lymphopenia

Ivermectin

Recommendation: Not recommended. No known published data to support efficacy or safety in treatment of COVID-19.

Nitrazoxanide

Recommendation: Not recommended; No known published data to support efficacy or safety in treatment of COVID-19.

Janus Kinase (JAK) inhibitors (e.g., baricitinib, ruxolitinib)

Recommendation: Not recommended. Broad immunosuppressive effect of JAK inhibitors outweighs the potential for benefit.[12]

Dosing:

- Baricitinib: Dosage information not yet available.
- Ruxolitinib: 5 mg twice daily for 14 days with possible extension to 28 days (NCT04362137).

This list is not intended to be all-inclusive. The treating physician should consider the strength of the evidence in terms of risks and benefits when considering concomitant medications.

APPENDIX C: RT-PCR PROTOCOL

I. PURPOSE

Define proper techniques for preparing RT-PCR plates. Establish protocols for creating and loading reagent mixes, controls, and clinical samples into RT-PCR plate.

II. SCOPE

This SOP is applicable to all staff in TNCL who will be loading and starting RT-PCR plates.

III. QUALITY CONTROL

- Each new LOT number of reagents will be validated before use with patient specimens
- All patient specimens will be associated with LOT numbers used to test it
- Reagents will be revalidated when necessary (deviation from storage temperature, etc.)
- Reagents will not be used past expiration
- Control samples will be included with each run of each assay. These controls will monitor performance of the reagents and potential contamination. Ct values for all controls will be recorded and monitored for variation over time
- Patient samples determined to yield an “inconclusive” result will be retested as defined by

IV. DEFINITIONS

PCR - polymerase chain reaction

RT-PCR - reverse transcription PCR

V. PROCEDURES

<u>Applicable theory</u>

- PCR involves cyclical stages of heating and cooling defined as denaturation, annealing, and extension. These stages allow for exponential amplification of very specific target regions of a genome. The target region is determined by the oligonucleotide “primers” used in the PCR. Due to the exponential nature of PCR, even small

levels of nucleic acid contamination can produce substantial contamination in the final product. Aseptic technique is *critical*.

- Real-time PCR is a polymerase chain reaction with an additional oligonucleotide fluorescent probe. The probe is designed to anneal to the target region, then release a fluorescent molecule with each stage of amplification. The release of fluorescent molecules is captured by a very sensitive camera, allowing the PCR product generated to be quantified.
    - Reference TNCL-0802 Primer preparation for primer/probe handling information
- RT-PCR utilizes a reverse transcription reaction prior to performing PCR on the reverse transcription product. Reverse transcription is the process by which a reverse transcriptase enzyme synthesizes cDNA (complementary DNA) from total RNA or mRNA (messenger RNA)
- Enzymes are very specifically folded proteins that are sensitive to temperature and prone to physical and chemical inactivation. Enzymes should not be vortexed vigorously, and should be kept cold for as long as possible until PCR run begins.

Reagents, equipment, supplies

- Reliance One-Step Multiplex Supermix, Bio-Rad, **Master Mix** [-20C]
- Assay primer mix, 20X [-20C]
- Water, nuclease free [ambient]
- Positive template controls (SARS-CoV-2, human genomic RNA) [-20C]

- Micropipettes: p20, p200, p1000
- Cold block for 1.5-2mL tubes [4C]
- Cold block for 96-well microplate [4C]
- Racks for 1.5-2mL tubes
- Rack for 96-well microplate

- 1.5 or 2mL nuclease free tubes
- 96-well PCR plate
- Microseal 'B' PCR Plate Sealing Film
- Micropipette tips: p20, p200, p1000
- RNAse Away
- Powder-free disposable gloves
- Laboratory coat

Assay set up

- Thaw one SARS-CoV-2 genomic RNA working stock on a cold block (benchtop)
- Thaw one Human genomic RNA working stock on a cold block (benchtop)
- Create aliquot of molecular grade water for the day (a new aliquot will be made every day) in a 1.5 or 2mL tube. Make sure to make aliquots from same backstock of water every day so the LOT# does not change

These steps should be performed in the PCR workstation, wearing a washable lab coat and new powder-free gloves.

- Thaw and maintain RT-PCR reagents on a cold block
    - Reliance One-Step Multiplex Supermix, Bio-Rad, **Master Mix** (4X)
    - Primer mixes (20X)
- Pre-label 1.5 or 2mL nuclease free tube with the following information
    - Assay name (TG-N2, TG-S4, RP), date, initials
- Prepare adequate quantity (use reagent calculator on Google Drive) of the reagent mix below for the number of samples being processed in the tubes labeled from the previous step

REAGENT AND VOLUME

Nuclease free water

Reliance One-Step Multiplex Supermix, Bio-Rad, 12 µL

Master Mix (2X), 5 µL

Primer mix (20X), 1 µL

- In the PCR hood workstation, add 18µL reagent mix to appropriate wells of a 96-well plate.
- Seal plate (temporary seal) for transport to benchtop nucleic acid addition area
- Move reagent plate to benchtop template addition area
- Add 2µL RNA from each patient specimen into appropriate wells of the 96-well plate (sample is added to one well per assay for three assays, TG-N2/TG-SPIKE4/CDCRP, therefore the sample will be loaded into a total of 3 wells on the plate)
- Add 2µL no template control (water, nuclease free) to the NTC wells for all 3 assays

- Add 2µL SARS-CoV-2 genomic DNA to the positive control well of assays TG-N2 and TG-SPIKE4.
- Add 2µL human genomic RNA to the positive control well of the CDC-RNAse P assay.
- Seal the plate using Microseal 'B' PCR Plate Sealing Film, secure the plate seal by using a plate sealing device to prevent evaporation during RT-PCR
- Centrifuge plate to bring all liquid to the bottom of the wells. Make sure there are no bubbles on the bottom of the wells
- Load plate onto Bio-Rad CFX96 Connect real-time PCR detection system instrument according to CFX instrument loading protocol

SOP Revision Number	Date issued	Author (initials)	Reviewer (Initials)	Summary of revisions
1				Draft
2				
3				

APPENDIX D: EXPEDITED REPORTING COVERSHEET

NOTIFICATION OF UNANTICIPATED PROBLEM/SERIOUS ADVERSE EVENT

For Use by Participating Institutions Only

THIS FORM ALONG WITH A COPY OF THE MEDWATCH 3500 OR IRB REPORTING FORM MUST BE **EMAILED** TO DCC@COH.ORG WITHIN 24 HOURS OF KNOWLEDGE OF ONSET OF SERIOUS ADVERSE EVENT OR UNANCTICIPATED PROBLEM

COH IRB #_____- Participating Site IRB # _____

From:	Date:
Phone No.:	Email:
Reporting Investigator:	
Event:	
Participant ID:	Institution:
Date Event Met Reporting Criteria (as defined in protocol):	

Type of Report:	☐ Initial ☐ Follow-up
CTCAE Grade:	☐ G1/mild ☐ G2/moderate ☐ G3/severe ☐ G4/life threatening ☐ G5
Attribution to **Agent xx:**	☐ Not Applicable* ☐ Unrelated ☐ Unlikely ☐ Possible ☐ Probable ☐ Definite
Attribution to **Agent xxy**:	☐ Not Applicable* ☐ Unrelated ☐ Unlikely ☐ Possible ☐ Probable ☐ Definite
Historical/Known Correlation to **Agent xx**:	☐ Expected ☐ Unexpected
Historical/Known Correlation to **Agent xxy**:	☐ Expected ☐ Unexpected
Meets Definition of Serious AE:	☐ Serious ☐ Non-serious
Meets Definition of Unanticipated Problem:	☐ UP ☐ Not a UP
Has the event been reported to the following institution's IRB?	☐ No ☐ Yes; Date:_____/____/_____

*** Not Applicable should only be used if subject has not received this agent.**

Authorized Investigator Signature:	Date:____/____/____