A Phase II Single-Center, Randomized, Open-Label, Safety and Efficacy Study of Etoposide in Patients with COVID-19 Infection

Simple Title: Etoposide in COVID-19 patients

IRB #: H-40102

NCT #: NCT04356690

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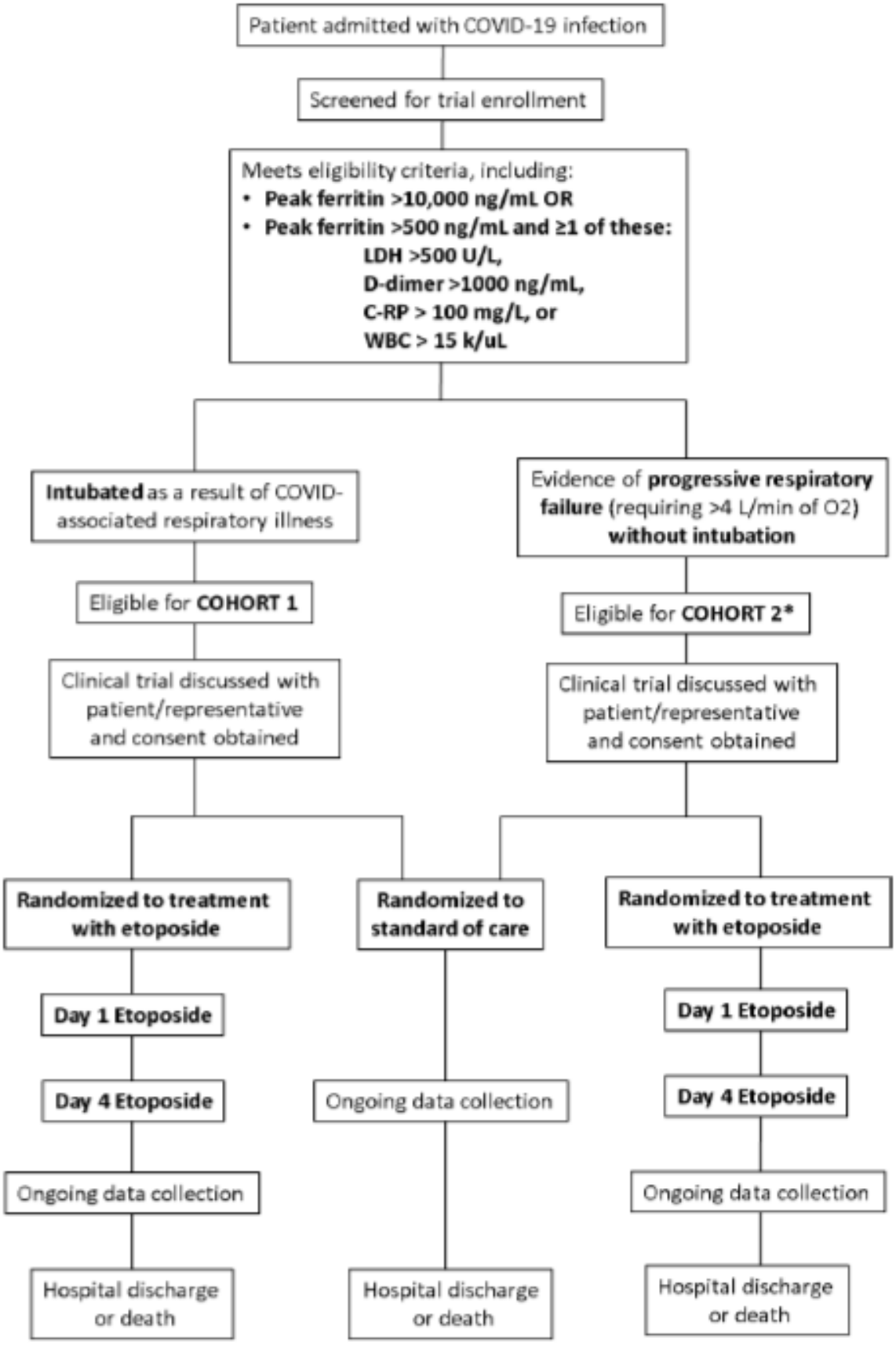
IND #: 149640

Protocol Version # 2
Amendment 1
Original version: 18 May 2020

CONFIDENTIAL



*Cohort 2 to be opened for enrollment after evaluation of data from initial enrollment of Cohort 2 (see Section 4.2)

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1. BACKGROUND

1.1 Cytokine Storm in COVID-19 and Hemophagocytic Lymphohistiocytosis (HLH)

Evidence is accumulating that patients with severe COVID-19 infection have a cytokine storm syndrome. Cytokine changes similar to secondary hemophagocytic lymphohistiocytosis (HLH) have been seen in COVID-19 and are associated with disease severity. The cytokines known to be elevated in HLH and COVID-19 are interleukin (IL)-2, IL-7, granulocyte colony stimulating factor, interferon-γ inducible protein 10, monocyte chemoattractant protein 1, macrophage inflammatory protein 1-α, and tumor necrosis factor-α.[1] A recent retrospective, multicenter study of 150 confirmed COVID-19 cases in Wuhan, China, demonstrated predictors of mortality which included elevated ferritin (mean 1297.6 ng/mL in non-survivors v. 614.0 ng/mL in survivors; $p<0.001$) and IL-6 (mean 11.4 ng/mL in non-survivors v. 6.8 in survivors; $p<0.0001$).[2] This suggests that mortality in COVID-19 may be partially driven by virally associated hyperinflammation with resulting increase in markers of inflammation. Cytokine modulation with monoclonal antibodies such as tocilizumab (anti-IL-6) and anakinra (anti-IL-1) are being studied elsewhere in phase III trials and are being given on an ad hoc basis at Boston Medical Center. The safety and efficacy of these medications in COVID is unproven. The Infectious Disease Society of America (IDSA) recommends against treatment with biologic outside the context of a clinical trial. Treatment with etoposide will impact the cells elaborating multiple cytokines and may provide more robust reduction in inflammation. Etoposide has an established and predictable safety profile; its potential efficacy is supported by previous studies in HLH[3,4].

Hemophagocytic lymphohistiocytosis is a rare disorder of excessive immune activation with both laboratory and clinical evidence of severe inflammation. The etiologies of this syndrome include genetic mutations of cytotoxic function (familial HLH), as well as secondary causes such as infections, malignancy, or metabolic conditions (acquired HLH). HLH is well described in the pediatric population, but has also been described and studied in adult populations. Hypercytokinemia mediates the clinical and laboratory presentation. Familial HLH typically presents in early childhood and is caused by mutations in genes important for NK and T-cell cytotoxic function.[5] Secondary HLH is the phenotype that occurs in both children and adults in the absence of a known genetic cause. A retrospective study from the Mayo Clinic of 62 patients with secondary HLH revealed the cause to be a malignancy (52%), infection (34%), autoimmune disorder (8%), or unknown cause (6%). The most common malignancy observed was T cell lymphoma (59%) and the most common infection was Epstein Barr Virus (EBV) (26%).[6]

HLH is diagnosed by a constellation of signs, symptoms, and laboratory abnormalities. Multiorgan dysfunction and critical illness are common. In 2004, The Histiocyte Society proposed an updated set of criteria to aid in identification of patients with HLH for clinical trials, which include molecular testing consistent with HLH or 5 of 8 of the following criteria: fever, splenomegaly, cytopenias affecting ≥2 lineages, hypertriglyceridemia and/or hypofibrinogenemia, hemophagocytosis (in bone marrow, spleen, or lymph node), hyperferritinemia, impaired NK cell function, and elevated soluble CD25 (sCD25) (ie, sIL2R).[7] Other common findings include transaminitis, coagulopathy, hyponatremia, edema, rash, hypoalbuminemia, elevated lactate dehydrogenase (LDH), C-reactive protein, and d-dimer, and neurologic symptoms ranging from focal deficits to altered mental status.[8] Elevated ferritin

occurs in the vast majority of patients with HLH but is nonspecific. The published criteria use a ferritin cutoff value of ≥500 μg/L when diagnosing HLH. This is based on the HLH-94 study that showed a sensitivity of 84% at this level. Subsequent studies have demonstrated that a higher threshold of ≥10,000 μg/L has a 90% sensitivity and 96% specificity for HLH.[9] The diagnosis of secondary HLH can be challenging to make because the symptoms and laboratory features may overlap with other severe illnesses such as sepsis and hematologic malignancy, including disorders which are known to trigger HLH. Fardet *et al* created and validated the HScore, which includes 9 weighted variables based on a retrospective cohort of 312 patients.[10] Some patients with cytokine storm from COVID-19 seen at Boston Medical Center meet formal HLH criteria, although bone marrow biopsies to confirm hemophagocytosis have not been routinely performed to make this assessment. Autopsies done on COVID patients at Boston Medical Center show evidence of hemophagocytosis in the lymph nodes and spleen, thereby suggesting that the true incidence of HLH in COVID patients is higher than pre-mortem estimates.

1.2 Etoposide Background and Proposed Mechanism of Action

Etoposide has been used to successfully treat HLH. It inhibits topoisomerase II, which leads to double stranded DNA breaks. In a murine model of HLH, etoposide selectively depleted activated T cells leading to suppression of inflammatory cytokines and improved survival.[11] Of note in this same study, dexamethasone had minimal benefit, despite the fact that dexamethasone is included in many clinical HLH regimens with the goal of further suppression of hypercytokinemia and inflammation. Etoposide may be more effective than cytokine-specific therapies because it eliminates the cells that are elaborating the cytokines, rather than targeting a specific cytokine in the evolving cytokine storm.

There are no prospective trials for treatment of HLH in adults, and treatment is largely based on the HLH-94 study, a large prospective pediatric study in patients less than 16 years old without history of immunosuppression or malignancy. In that study, the investigators used a regimen that included an 8 week induction with dexamethasone and etoposide.[3] Response to treatment was determined by clinical and laboratory evidence of resolution, including fever curve, trend of sIL2R, LFTs, fibrinogen and ferritin level. Timely diagnosis and early administration of therapy has been demonstrated to improve survival. In a retrospective multicenter study of 162 patients with HLH looking at predictors of mortality, use of etoposide was associated with better survival.[4]

Etoposide is an already FDA approved drug that is comparably inexpensive and currently available. Etoposide has an established safety profile and its use is supported by previous studies in HLH[3,4]. We propose a Phase II study of etoposide for the treatment of cytokine storm in severe COVID-19 infection.

1.3 Rationale for Etoposide Clinical Study

The cytokine storm related to COVID-19 appears to be similar to the hyperinflammation seen in other virally triggered secondary HLH. Others have postulated that the combination of topoisomerase I and topoisomerase II inhibitors could potentially be an effective treatment to

protect COVID 19 patients from cytokine storms.[12] We posit that the cytokine storm seen in COVID-19 infection resembles secondary HLH seen as a result of other viral infections, resulting in an aberrant and overly exuberant cytokine response which is deleterious and leads to multiorgan dysfunction.

The rationale for the use of etoposide to treat the cytokine storm in COVID-19 is the high mortality associated with the hyperinflammatory response to the virus, which is similar to that seen in secondary types of HLH. Early use of etoposide has been shown to improve survival in secondary HLH.[13] Autopsy studies of ARDS in COVID patients show a high number of cytolytic T cells in the lungs of such patients.[14] Early autopsy results of COVID patients at BMC demonstrate significant hemophagocytosis in lymph nodes and spleen. Comparable studies in the related coronavirus infection SARS have demonstrated hemophagocytosis, a hallmark of HLH.[15] By targeting the T cells and monocytes driving the cytokine storm in patients with the more severe forms of COVID infection, we hope to alleviate the progression of lung and multi-organ dysfunction characteristic of patients who die from this illness.

Potential concerns about the use of etoposide and dexamethasone for COVID-19 infected patients include decreased immune response and pathogen clearance. Studies in humans and animals indicate that corticosteroid immunosuppression (both inhaled and systemic) impairs induction of anti-viral type-I interferon responses to a range of respiratory viruses.[16] Opinions on the use of steroids in COVID-19 are mixed, and based on limited data. A retrospective cohort study of 201 patients with confirmed COVID-19 pneumonia in Wuhan China, suggested that risk factors associated with the development of ARDS and progression from ARDS to death included older age, and neutrophilia, as well as organ and coagulation dysfunction (eg, higher lactate dehydrogenase [HR, 1.61; 95% CI, 1.44-1.79; and HR, 1.30; 95% CI, 1.11-1.52, respectively] and D-dimer [HR, 1.03; 95% CI, 1.01-1.04; and HR, 1.02; 95% CI, 1.01-1.04, respectively]). Among patients with ARDS, treatment with methylprednisolone decreased the risk of death (HR, 0.38; 95% CI, 0.20-0.72).[17] The CDC cautions interpretation of this data given its observational by nature, and patients with MERS-CoV or influenza who were given corticosteroids were more likely to have prolonged viral replication, receive mechanical ventilation, and have higher mortality.[18] Given the data in MERS and influenza, and the lack of randomized data demonstrating benefit of corticosteroids, others caution against the use of steroids in COVID 19 patients.[19] For these reasons in the study proposed here, we do not plan to use corticosteroids as part of the treatment protocol.

Another potential concern is that decrease in cytotoxic T cell and humoral immune response with the administration of cytotoxic chemotherapy. Prolonged etoposide therapy has been shown to reduce cytotoxic T cell activity, macrophage/monocyte number and function, and humoral responses in animal models, some of which initiate low dose daily etoposide **prior to** a viral challenge.[20,21] In HLH, etoposide has been argued to be effective as a result of its ability to ablate activated T cells.[11] In the study proposed here, etoposide will be used well after patients have developed an established COVID infection and we plan to use a very limited dose and schedule of this drug so as to minimize the likelihood of arresting the production of neutralizing antibodies to COVID-19, but still allow ablation of cytolytic T cells that are leading to cytokine activation. To our knowledge, no studies have examined the immunologic effect of brief etoposide therapy relatively late in a human viral infection. Etoposide is used in the treatment of

HLH associated with Epstein Barr Virus (EBV) infection without known worsening of viral infection. In studies of hematologic malignancy patients who were inoculated with a varicella zoster vaccine, then underwent high dose chemotherapy followed by autologous stem cell transplant, neutralizing antibody production was detected despite this myeloablative regimen.[22]

1.4 Rationale for Dose Selection

In this trial, patients will be treated with a markedly abbreviated course of the dosing regimen used in the current standard adult HLH protocol. In the standard HLH regimen, etoposide is given at 150 mg/m2 twice a week for two weeks, followed by weekly therapy, in combination with dexamethasone. In this trial, patients will receive the standard initial two doses of etoposide in the first week (150 mg/m2 IV x 2) on day 1 and 4 of treatment. Further etoposide therapy will not be given unless the assessment of the patient's subsequent course is that they improved after etoposide treatment but then had recurrent clinical deterioration, at which point further etoposide therapy would be allowed (according to the HLH-94 protocol)[3] after discussion with the investigator.

The rationale for such an abbreviated course of etoposide is three fold. First, the immune hyperactivation in COVID infections may differ from that of patients with classic secondary HLH and may require only a relatively brief period of pharmacologic down-regulation. Second, the development of neutralizing antibodies to COVID are likely to be an important aspect of recovery from this illness and prolonged treatment with etoposide could theoretically blunt such a response. Third, prolonged etoposide therapy will induce more significant myelosuppression in a group of patients who will be susceptible to bacterial super-infection. The proposed two doses of IV etoposide is not expected to cause deep or prolonged myelosuppression.

2. STUDY PLAN

2.1 Study Design

This is a randomized, open-label phase II study designed to evaluate the safety and efficacy of etoposide in patients with COVID-19 infection. Treatment will be comprised of etoposide administered intravenously at a dose of 150 mg/m^2 on Days 1 and 4 in patients with COVID-19 infection meeting eligibility criteria. Subsequent doses of etoposide will be allowed if the investigator and treating physician believe the patient had clinical benefit from etoposide therapy but subsequently has evidence of recurrent clinical deterioration.

Two cohorts of patients will be treated with etoposide. Cohort 1 will be patients who require intubation due to their severe COVID-19 related respiratory illness. Cohort 2 will be COVID-19 patients who have progressive pulmonary dysfunction and evidence of severe inflammation but who are not yet intubated. Although prior treatment for COVID with anti-cytokine therapy will not exclude a patient from enrollment on this trial, a "washout" period of at least 3 half-lives will be required prior to initiation of etoposide therapy. Enrollment will begin with cohort 1, following which a decision will be made to open cohort 2 or not, as outlined further in this document.

2.2 Number of sites

One (Boston Medical Center, 820 Harrison Ave, FGH-2, Boston, MA 02118)

2.3 Number of patients

- 32 patients in each cohort with 3:1 allocation ratio (24 etoposide and 8 control per cohort)
- 64 patients total

2.4 Primary Objective

- Improvement in pulmonary status by two-categories on 8 point ordinal scale of respiratory function (See Section 8.2)

2.5 Secondary Objectives

- Overall Survival at 30 days is a key secondary endpoint.
- Improvement in inflammatory markers associated with cytokine storm (Ferritin, C-Reactive Protein (CRP), d-dimer, white blood cell count)
- Cumulative incidence of grade 3 and 4 adverse events during hospitalization
- Cumulative incidence of serious adverse events during hospitalization
- Duration of hospitalization
- Duration of ventilation
- Ventilator free days (reintubations or death within 28 days will result in zero ascribed time off ventilator prior to reintubation)
- Improvement in oxygenation or paO2/FIO2 ratio

3. PARTICIPANT SELECTION

3.1 Screening, Recruitment, and Consenting

The investigators will recruit participants from within Boston Medical Center on the inpatient setting. It will be standard for COVID-19 positive patients to be screened to determine if they may be eligible for any available clinical trials. A screening consent is not obtained, as the pre-screening review is conducted prior to contacting the patient to discuss the trial. At that time no data will be collected by the study team. The medical records are reviewed to determine if a patient may or may not be potentially eligible for a clinical trial.

If the patient is potentially eligible, the inpatient nurse, treating physician or trial representative will inform the patient or the patient's legally authorized representative (LAR) of the availability of a clinical trial. No research procedures are performed until after written consent is obtained.

If the patient agrees to discuss and receive information about the study, a copy of the IRB-approved Informed Consent Form will be provided to the patient by a member of the patient's clinical care team to avoid using additional personal protective equipment (PPE). In the event the

patient is too ill to make medical decisions, the patient's LAR will be asked to review the informed consent form, which may be sent electronically.

The inpatient provider will contact the research nurse and provide the phone number of the patient or the LAR. The research nurse and/or investigator will call the patient or the LAR and will remotely conduct the consent discussion, reviewing all elements of consent and emphasizing the voluntary nature of research participation. Additional family members or friends may join the call at the subject's request. Once all questions have been asked and answered to the patient's/LAR's satisfaction, the research nurse will offer to end the call so the patient/LAR can be given the opportunity to review the consent form and discuss with family members (remotely) and/or other physicians. The patient/LAR will have as long to decide as possible within the confines of the clinical urgency. Once the patient/LAR is satisfied that all questions have been answered, if he/she wishes to participate, he/she will contact the research nurse or the research nurse will reach out to the patient/LAR again if requested by the patient/LAR. The study team will have arranged for a witness to join the telephone call at this point, and consent will be obtained remotely, with the patient or LAR directing the witness to sign on their behalf. The informed consent will also be documented in the medical record.

The study team member conducting the consent discussion and the witness will both sign and date the consent form external to the patient's room. The signed consent form will be filed in the research records. The patient will keep the unsigned copy.

Potential participants who are non-English speaking will be consented using the Short Form (if IRB approved). A hospital interpreter, or an interpreter on the phone line, will be used throughout the consent process and throughout their participation in the study. The interpreter will sign as an impartial witness.

Documentation that informed consent occurred prior to the subject's entry into the study and the informed consent process will be recorded in the subject's source documents.

3.2 Inclusion Criteria (applicable for patients enrolled for treatment or data collection)

- Age 18 years or older
- Confirmed COVID-19 infection
- Evidence of cytokine storm defined as:
    - Peak ferritin > 10,000 ng/mL OR
    - Peak ferritin > 500 ng/mL and one or more of the following at any time during hospital admission:
        - Lactate dehydrogenase > 500 U/L, d-dimer >1000 ng/mL, C-reactive protein > 100 mg/L, or WBC > 15 k/uL
- Cohort 1: Intubated status as a result of COVID infection-associated respiratory illness

- Cohort 2 (if activated): Evidence of progressive respiratory failure (requiring >4 L/min of supplemental oxygen to maintain SaO_2 greater than 92%) without intubation

3.3 Exclusion Criteria

- Pregnancy or breastfeeding
- History of severe hypersensitivity to etoposide products
- Absolute neutrophil count (ANC) < 1000 cells/mm^3
- Platelet count <50,000/mm^3
- Bilirubin > 3.0 mg/dL
- Aspartate OR alanine aminotransferase > 5.0 x ULN
- Creatinine Clearance < 15 mL/min (calculated by Cockcroft and Gault formula)
- Requiring continuous renal replacement therapy
- Requiring > 1 vasopressor medications
- Requiring extrcorporeal membrane oxygenation (ECMO)
- Other active, life-threatening infections
- Anti-cytokine treatment (including anakinra or IL6 antibodies eg tocilizumab, sarilumab) administration within three half-lives of the medication used
- Hydroxychloroquine, colchicine, azithromycin, doxycycline—if administered for COVID infection—must be discontinued for at least 24 hrs prior to randomization.
- Has a history or current evidence of any condition, therapy or laboratory abnormality that might confound the results of the study, interfere with subject participation, or is not in the best interest of the patient to participate, in the opinion of the investigator.
- Inability to consent and no legally authorized representative
- Poorly controlled HIV infection (CD4 count <100 cells/mm^3)

4. INVESTIGATIONAL PLAN

4.1 Overall Design

Open-label, randomized Phase II study of etoposide administered intravenously at a dose of 150 mg/m^2 once daily on Days 1 and 4 for a total of two doses to inpatient patients with moderate and severe COVID-19 who have consented to treatment on this clinical trial. Dose reductions will be performed as outlined in Section 5.

This study includes a screening period, a treatment period, and a follow-up period for adverse events that will include the remainder of hospitalization, with additional assessments at days 30 and 60 as outlined in the study calendar (Section 6). After obtaining informed consent, the patients randomized to etoposide will begin treatment with the study drug. Subjects randomized to control will receive standard of care treatment. No placebo will be used. Subjects will be monitored for adverse events and to evaluate study outcomes. Patients removed from the study due to any unacceptable adverse events will also be followed until time of death or hospital discharge.

For patients randomized to standard of care, day 1 will be considered day of randomization. These patients will be followed to record the outcome of all primary and secondary objectives until time of death or hospital discharge, whichever occurs earlier.

4.2 Patient cohorts

This protocol has two patient cohorts, including cohort 1) severe COVID-19 infection and cohort 2) moderate COVID-19 infection (as defined in the inclusion criteria).

Upon protocol activation, cohort 1 will open to accrual. We will randomize and enroll 10 patients and then perform interim safety analysis prior to further enrollment or opening of cohort 2. See Section 7.2 for statistical design and section 9.5 for stopping rules.

Following treatment with two doses of etoposide (Day 1 and Day 4) in the first 10 participants in cohort 1, safety data including adverse events and patient outcomes will be submitted to the COVID Research Committee for review. At that time, the committee will determine whether they feel that enrollment may be expanded to include cohort 2. If necessary, the COVID Research Committee may ask for safety data to be presented after treating the full 32 patients on cohort 1 prior to opening cohort 2. Only after permitted by the COVID Research Committee will 32 patients on cohort 2 be enrolled and treated. The COVID Research committee is not tasked with medical monitoring, pharmacovigilance or applying stopping rules for this study.

4.3 Randomization

Randomization will be performed using a 3:1 allocation ratio using a block size of 4 separately in each cohort.

4.4 Schema

Etoposide 150 mg/m2 administered intravenously once daily on Days 1 and 4.
If the treating clinicians feel that the patient initially benefited from etoposide but then has evidence of relapse of cytokine storm, the patient may continue on the standard HLH-94 etoposide schedule of day 8, 11, 18, 25 after discussion with one of the study investigators (prior to day 8 dosing).

4.5 Criteria for removal from protocol treatment

- Completion of study (either death or completion of day 60 survival assessment)
- Unacceptable toxicity
- Participant withdrawal for any reason

5. DRUG FORMULATION AND ADMINISTRATION

5.1 Etoposide Chemistry, Mechanism of Action, and Formulation

Chemistry: Etoposide is a semi-synthetic podophyllotoxin derivative from the plant podophyllum peltatum, and has antineoplastic properties in experimental animals and in man.

Mechanism of action: The epipodophyllotoxins exert specific spindle poison activity with metaphase arrest, but in contrast to the vinca-alkaloids, have an additional activity of inhibiting

cells from entering mitosis. Suppression of tritiated thymidine, uridine, and leucine incorporation in human cells in tissue culture suggests effects against DNA, RNA and protein synthesis.

Formulation: 100mg of VP-16 is supplied as 5ml or 10ml solution in sterile multiple dose vials for injection. The pH of the yellow clear solution is 3-4. Each ml contains 20mg VP-16, 2mg citric acid, 30mg of benzyl alcohol, 80mg polysorbate 80/tween 80, 650mg polyethylene glycol 300, and 30.5% (v/v) alcohol. VP-16 must be diluted prior to use with either 5% Dextrose Injection, USP, or 0.9% sodium chloride injection, USP. The time before precipitation occurs depends on concentration, however when at a concentration of 0.2mg/ml it is stable for 96 hours at room temperature and at 0.4mg/ml it is stable for 48 hours. Concentrations over 0.5mg/ml is stable for at least 24 hours at room temperature.

5.2 Etoposide Availability

Etoposide is commercially available.

5.3 Etoposide Administration

Etoposide 150 mg/m^2 will be administered over 60 minutes intravenously on Days 1 and 4. Admixed in NS-500ml.

If the treating clinicians feel that the patient initially benefited from etoposide but then has evidence of relapse of cytokine storm, the patient may continue on the standard HLH etoposide schedule of day 8, 11, 18, 25 after discussion with one of the study investigators.

5.4 Etoposide Expected Toxicities and Management

Reversible myelotoxicity has been uniformly observed to be the major toxicity for VP-16 and to represent the only clinically significant side effect. Following a single IV injection, peak myelotoxicity occurs at seven to nine days. Following daily injections for five to seven days, myelotoxicity is maximal between 12-16 days from the initiation of therapy. Bone marrow suppression is mainly manifested as granulocytopenia, with thrombocytopenia and anemia occurring to a lesser extent. Gastrointestinal toxicities including transient modest nausea, vomiting, and diarrhea, are common. Other reactions could include aftertaste, rash, pigmentation, pruritis, abdominal pain, constipation, and dysphagia. Occasional alopecia is reported. VP-16 does not produce phlebitis or nephrotoxicity. Rarely anaphylactic-like reactions have been reported, as well as, hypotension. The drug is to be infused over 60 minutes in this study to decrease the potential for hypotension, and the blood pressure and pulse will be monitored during the infusion. Occasionally, chills, fever, peripheral neurotoxicity, stomatitis, hepatotoxicity, transient cortical blindness and radiation recall dermatitis may be a result of VP-16 administration. Stomatitis is usually associated with high dose regimens containing etoposide. The occurrence of acute leukemia has been reported rarely in patients treated with VP-16 in association with other antineoplastic agents. Etoposide can cause fetal harm when administered to a pregnant women. It has been shown to be teratogenic in mice and rats. In females of reproductive potential, etoposide may cause infertility and result in amenorrhea. Premature menopause can occur with etoposide. Recovery of menses and ovulation is related to

age at treatment. Males In male patients, etoposide may result in oligospermia, azoospermia, and permanent loss of fertility. Sperm counts have been reported to return to normal levels in some men, and in some cases, have occurred several years after the end of therapy Secondary leukemias associated with etoposide have been reported. Women of childbearing potential will be advised to avoid becoming pregnant for at least 6 months after the final dose of etoposide. Males with female partners of reproductive potential will be advised to use effective contraception for 4 months after the final etoposide dose.

Safety of etoposide for use by nursing women has not been established.

5.5 Etoposide Dose Modifications

Etoposide dosing in Hepatic and Renal Insufficiency

T. Bili 1.5 – 3.0 mg/dL	50% dose reduction
T. Bili > 3.0 mg/dL	Omit dose
AST > 2.5 x ULN	50% dose reduction
Albumin < 3.0 g/dL	30% dose reduction
Creatinine Clearance 15-50 mL/min*	25% dose reduction
Creatinine Clearance <15 mL/min	Omit dose
Abs Neutrophil Count <500/uL	Hold until ANC > 500/uL

*calculated by Cockcroft Gault equation

5.6 Etoposide Drug Interactions

Patients receiving concomitant medications with known interactions with etoposide, as mentioned below, will be monitored closely for adverse events.

- Cisplatin: Co-administration of cisplatin may increase exposure to etoposide.
- Highly protein-bound drugs: Phenylbutazone, sodium salicylate, and aspirin displaced protein-bound etoposide in vitro.
- Select antiepileptic medications: Co-administration with antiepileptic medications including phenytoin, phenobarbital, carbamazepine, and valproic acid may increase etoposide clearance.
- Etoposide may be a substrate of the P-glycoprotein (P-gp) transporter system based upon in vitro studies

Co-administration of etoposide with warfarin may result in elevated international normalized ratio (INR). If patients taking warfarin the INR will be monitored at least twice weekly.

6. STUDY PROCEDURES & CALENDAR

REQUIRED STUDIES	Screening	Day 1, Day 4 and Day 7$^{\Sigma\#}$	Prior to discharge	Day 30*
PHYSICAL				
History & Physical Exam	X	X	X	X
Height, Weight, Body Mass Index, and Body Surface Area	X			
Vital Signs	X	X	X	X
Toxicity Notation	X	X	X	X
Respiratory Status on 8 point scale^	X	X	X	X
HISTORY				
Concomitant medicines (including COVID therapies, ie hydroxychloroquine, tocilizumab, anakinra) with date of initiation	X			
Smoking status	X			
Comorbidities	X			
Symptoms, including date of symptom onset	X			
LABORATORY				
Beta HCG (for women of childbearing potential)	X			
CBC with Differential^{+}	X	X	X	X
Comprehensive metabolic panel^{+}	X	X	X	X
Lactate Dehydrogenase	X	X	X	X
D-dimer	X	X	X	X
Ferritin	X	X	X	X
C-reactive protein	X	X	X	X
PaO2/FIO2 (P/F) ratio (cohort 2 only)	X	X	X	X
INR (if on warfarin)$^{\Omega}$	X			
ADDITIONAL TESTING				
Electrocardiogram (ECG)	X			

$^{\#}$ For subjects randomized to treatment, the data for Day 1 and Day 4 will be collected prior to the etoposide dose (within 24 hours).
*****Information to be obtained as feasible, if patient has been discharged attempts will be made to obtain the information from the patient's primary care provider. If such results are not available, participant will be contacted by the research nurse to assess toxicities.
^ Mortality assessment (included on the 8 point scale) will also be recorded at Day 14 and 60, in addition to Day 30 mentioned above
$^{+}$ CBC and comprehensive metabolic panel will be measured at least twice weekly during hospitalization. These tests will also be obtained pre-dose 8, 11, 18, and 25 for those patients who require additional etoposide dosing as described above (Section 4.3 and 5.3)
$^{\Omega}$ INR to be repeated at least twice weekly for patients on warfarin
$^{\Sigma}$ Day 1 of treatment for patients randomized to standard of care (SOC) will be considered the day of randomization

7. STATISTICAL CONSIDERATIONS

7.1 Sample Size Considerations

This study will be performed with two cohorts 1) severe COVID-19 infection and 2) moderate COVID-19 infection (as defined in inclusion criteria).
The effect of the intervention will be evaluated in each cohort separately. A total of 32 patients will be enrolled in each cohort with a 3:1 allocation ratio (24 Etoposide and 8 control per cohort). Assuming a 30% response rate in the control group for the primary outcome of a 2 point improvement in respiratory status on an 8 point scale at Day 14, we can detect a 50% increase in response in the Etoposide group (80% response rate in the Etoposide group) with a 2-sided α of 0.05 and 80% power. While this study is not powered to detect a statistically significant difference in outcome, it will provide initial evidence of efficacy if improvement in response is observed.

7.2 Statistical Analysis

Tabulations overall, and by treatment group, will be produced for appropriate demographic, baseline, efficacy, and safety parameters, with number and percentages reported for categorical variables and the mean, median, standard deviation, and range for continuous variables. All analyses will be performed at an α=0.05 level using SAS v.4.

Efficacy analyses will be performed in an intention-to-treat (ITT) population consisting of all patients randomized. The difference in ordinal scale improvement rates between treatment arms will be calculated with a 95% confidence interval and formally compared using Fisher's Exact Test . For secondary analysis of the primary endpoint, logistic models individually adjusted for age, diabetes, chronic lung disease/moderate or severe asthma, immunocompromise, liver disease, heart disease/hypertension, smoking and obesity (BMI > 40), residence in long-term care facility, will also be examined with odds ratios and 95% confidence intervals reported.

The analysis of secondary time to event endpoints including survival at 30 days, duration of hospitalization, duration of ventilation and ventilator free days, will be compared between treatment arms based on a log-rank test. The median will be estimated with a 95% CI for each treatment arm using the Kaplan-Meier method. Cox proportional Hazards models adjusted individually for age, diabetes, chronic lung disease/moderate or severe asthma, immunocompromise, liver disease, heart disease/hypertension, smoking and obesity (BMI > 40), residence in long-term care facility, will also be examined with odds ratios and 95% confidence intervals reported.. Changes in inflammatory markers and in oxygenation or paO2/FIO2 ratio, based on available recorded measurements, will be compared between treatment arms using a two sample t-test, or a Wilcoxon Rank Sum test, as appropriate. Incidence of grade 3 or 4 AEs and serious AEs will be compared using Fisher's Exact test.

Additional safety analyses will be based on the reported adverse events and other clinical information. Safety analyses will be performed in the all-treated (AT) population, consisting of all patients who took at least one dose of study treatment. The safety and tolerability of Etoposide will be evaluated by means of drug-related AE reports, physical examinations, and laboratory safety evaluations and compared between groups using Fisher's exact test. The first 10 patients allocated to the Etoposide group in the ventilated cohort will be evaluated for safety endpoints prior to initiating enrollment in the non-ventilated group.

8. MEASUREMENT OF EFFECT

8.1 Definitions

Evaluable for toxicity: All participants who receive at least one dose of study treatment will be evaluable for toxicity from the time of their first treatment.

Evaluable for objective response: Only those participants who have received at least one dose of etoposide will be eligible for objective response.

For purposes of data collection, day 1 of patients on the standard of care arm will be the day of randomization.

8.2 Response Definitions

8-Point Ordinal Scale:

- 8 - Death;
- 7 - Ventilation in addition to extracorporeal membrane oxygen (ECMO), continuous renal replacement therapy (CRRT), or need for vasopressors (dopamine ≥5 µg/kg/min OR epinephrine ≥0.1 µg/kg/min OR norepinephrine ≥0.1 µg/kg/min);
- 6 - Intubation and mechanical ventilation;
- 5 - Non-invasive mechanical ventilation (NIV) or high-flow oxygen;
- 4 – Hospitalized, requiring oxygen by mask or nasal prongs;
- 3 - Hospitalization without oxygen supplementation;
- 2 – Discharged from hospital either to home with supplemental oxygen OR to inpatient rehabilitation/skilled nursing facility (+/- supplemental oxygen);
- 1 – Discharged to home without supplemental oxygen

When calculating Ventilator Free days, reintubations or re-hospitalizations or death occurring in the first 28 days should result in zero ascribed to time off the ventilator prior to reintubation

When calculating days of hospitalization, re-hospitalization or death occurring in the first 28 days should result in zero ascribed to time out of the hospital prior to readmission.

When determining days of supplemental oxygen use and need for supplemental oxygen, the patient will only be considered to no longer need supplemental oxygen if a room air saturation is ≥ 90% after five minutes of rest.

9. ADVERSE EVENTS AND UNANTICIPATED EVENTS

9.1 Adverse Event Definition

An adverse event is any untoward or unfavorable physical or psychological occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

Toxicity will be scored using CTCAE Version 5.0 for toxicity and adverse event reporting. A copy of the CTCAE Version 5.0 can be downloaded from the CTEP homepage (HTTP://CTEP.INFO.NIH.GOV). All appropriate treatment areas will have access to a copy of the CTCAE Version 5.0. All adverse clinical experiences, whether observed by the investigator

or reported by the subject, must be recorded, with details about the duration and intensity of each episode, the action taken with respect to the test drug, and the subject's outcome. The investigator must evaluate each adverse experience for its relationship to the test drug, its expectedness, and for its seriousness.

The investigator must appraise all abnormal laboratory results for their clinical significance. If any abnormal laboratory result is considered clinically significant, the investigator must provide details about the action taken with respect to the test drug and about the subject's outcome.

9.2 Serious Adverse Event (SAE) Definition

Serious Adverse Event: According to the FDA's Code of Federal Regulations Title 21 Part 314.80, a SAE is any untoward medical occurrence that results in any of the following outcomes:

- Death
- A life-threatening event (an event that places the subject at immediate risk of death from the event as it occurred)
- Inpatient hospitalization or prolongation of an existing hospitalization
- A persistent or significant disability / incapacity
- A congenital anomaly / birth defect
- Important medical events based upon appropriate medical judgment that may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition of serious.

Expectedness: Unexpected events include any adverse drug experience that is not listed in the current package insert for the drug product (e.g., all previously unobserved or undocumented events).

Relatedness: Indicates that a determination has been made that an event had a reasonable possibility of being related to exposure to the product. Examples of causality include not related, unlikely, possibly, probable, definitely related and unable to be determined.

9.3 Unanticipated Problem (UP) Definition

Unanticipated Problem is defined as an event, experience or outcome that meets **all three** of the following criteria:

- is unexpected; AND
- is related or possibly related to participation in the research; AND
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Death due to progressive COVID infection will not be considered an unexpected event.

Reporting of AEs, SAEs, and UPs to the IRB and FDA will take place according to institutional IRB and FDA policy (see Section 10). AEs and SAEs will be reported from the first dose of study drug through hospital discharge. All SAEs should continue to be monitored until they are

resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

9.4 Interim Analysis

Interim analysis will occur after 10 patients are enrolled into cohort 1. Safety data including adverse events and patient outcomes will be presented to the medical monitor (and COVID research committee as detailed in section 4.2). If cohort 2 is activated, an interim analysis will also be performed after 10 patients have enrolled in that cohort.

9.5 Stopping Rules

Accrual in a cohort will continue after the interim analysis if ≥ 1 of the treated patients in that cohort meets the primary endpoint of 2 point improvement in respiratory status on the 8 point scale at day 14. If cohort 1 is stopped, enrollment in cohort 2 (if open) may still continue.

10. DATA MANGEMENT

10.1 Analyses and Reporting

Data generated by the methods described in the protocol will be recorded in the subjects' medical records and/or study progress notes. The investigator will be the assessor of the primary endpoint. Data will be directly inputted into an electronic system. Data will be analyzed and reported after study is completed or meaningful endpoints are reached. All subsequent data collected will be analyzed and reported in a follow-up clinical report.

The index data for patients on treatment is the day 1 dose of etoposide. For patients enrolled into the standard of care arm, day 1 will be the day of randomization.

The Principal Investigator at BMC/BU Medical Campus will report Unanticipated Problems, safety monitors' reports, and Adverse Events to the BMC/BU Medical Center IRB and FDA in accordance with established policies:

- Unanticipated Problems occurring at BMC/BU Medical Campus will be reported to the IRB within 7 days of the investigator learning of the event.
- Unanticipated Problems occurring at BMC/BU Medical Campus will be reported to the FDA within 15 days of the investigator learning of the event.
- Unexpected fatal or life-threatening suspected adverse events occurring at BMC/BU Medical Campus will be reported to the IRB within 7 days of the investigator learning of the event.
- Reports from the designated medical monitor with recommended changes will be reported to the IRB within 7 days of the investigator receiving the report.
- Adverse Events (including Serious Adverse Events) will be reported in summary at the time of continuing review, along with a statement that the pattern of adverse events, in total, does not suggest that the research places subjects or others at a greater risk of harm than was previously known.

- Reports from designated medical monitor with no recommended changes will be reported to the IRB at the time of continuing review.

10.2 Data Safety Monitoring

Toxicity and accrual monitoring will be performed on a routine basis by the study investigators. Subjects will undergo toxicity assessment and laboratory tests according to the study calendar in Section 7. The clinical status and laboratory reports of the study participants will be assessed routinely by the co-investigators throughout the study. The investigators, Medical Monitor and data analyst will remain as broadly informed as possible regarding emerging evidence from related studies as well as from the conduct of this protocol.

In addition, a Medical Monitor has been appointed to provide additional data safety monitoring. The Medical Monitor is a qualified clinician with relevant expertise, but no direct connection with the research, whose primary responsibility is to provide independent safety monitoring in a timely fashion. The PI will submit a report to the Medical Monitor which will include a list of all adverse events and serious adverse events (regardless of relatedness). In addition, any unanticipated problems involving risks to participants or others and participant deaths will be reported to the monitor as soon as possible and within 24 hours. Additional data may be requested by the Medical Monitor, and interim statistical reports may be generated as deemed necessary and appropriate.

The Medical Monitor is expected to provide a formal, unbiased written report evaluating individual and cumulative participant safety data. Although the PI is responsible for assigning causality and expectedness, the medical monitor will comment on whether or not he is in agreement. As an outcome of each review, the Medical Monitor will make a recommendation as to the advisability of proceeding with study intervention, and to continue, modify, or terminate this trial.

Medical Monitor reports will be submitted to the PI following each review which the PI will, in turn, submit to the IRB. The study will not stop enrollment awaiting these reviews, though the Medical Monitor may recommend temporary or permanent cessation of enrollment based on their safety reviews.

Additional monitoring will also be performed by the COVID Research Committee after 10 patients have been randomized in cohort 1. At that time, the committee will determine whether they feel that enrollment may be expanded to include cohort 2, or may request that data be presented again when all patients on cohort 1 have been treated.

10.3 Study Monitoring and Auditing

Investigator responsibilities are set out in the ICH guideline for Good Clinical Practice (GCP) and in the US Code of Federal Regulations (CFR).

Investigators must enter study data onto CRFs or other data collection system. The Investigator will permit study-related monitoring visits and audits by IRB/EC review, and regulatory

inspection(s) (e.g., FDA, EMEA, TPP), providing direct access to the facilities where the study took place, to source documents, to CRFs, and to all other study documents.

11. REGULATORY CONSIDERATIONS

11.1 Protocol Amendments

Any amendment to this protocol must be agreed to by the Principal Investigator. Written verification of IRB approval will be obtained before any amendment is implemented.

11.2 Protocol Deviations

When an emergency occurs that requires a deviation from the protocol for a subject, a deviation will be made only for that subject. A decision will be made as soon as possible to determine whether or not the subject (for whom the deviation from protocol was effected) is to continue in the study. The subject's medical records will completely describe the deviation from the protocol and state the reasons for such deviation. In addition, the Investigator will notify the IRB in writing of such deviation from protocol. Non-emergency minor deviations from the protocol will be permitted with approval of the Principal Investigator.

11.3 Institutional Review Board/Ethics Committee Approval

The protocol for this study has been designed in accordance with the general ethical principles outlined in the Declaration of Helsinki. The review of this protocol by the IRB/EC and the performance of all aspects of the study, including the methods used for obtaining informed consent, must also be in accordance with principles enunciated in the declaration, as well as ICH Good Clinical Practice Guidelines, Title 21 of the Code of Federal Regulations (CFR), Part 50 Protection of Human Subjects and Part 56 Institutional Review Boards.

The Investigator will be responsible for preparing documents for submission to the relevant IRB/EC and obtaining written approval for this study. The approval will be obtained prior to the initiation of the study.

The approval for both the protocol and informed consent must specify the date of approval, protocol number and version, or amendment number.

Any amendments to the protocol after receipt of IRB/EC approval must be submitted by the Investigator to the IRB/EC for approval. The Investigator is also responsible for notifying the IRB/EC of any serious deviations from the protocol, or anything else that may involve added risk to subjects.

Any advertisements used to recruit subjects for the study must be reviewed and approved by the IRB/EC prior to use.

11.4 Study Records Requirements

The Investigator must ensure that the records and documents pertaining to the conduct of the study and the distribution of the study drug, that is copies of CRFs and source documents (original documents, data, and records [e.g., hospital records; clinical and office charts; laboratory notes; memoranda; subject's diaries or evaluation checklists; SAE reports, pharmacy dispensing records; recorded data from automated instruments; copies or transcriptions certified after verification as being accurate copies; microfiches; photographic negatives, microfilm, or magnetic media; x-rays; subject files; and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical study; documents regarding subject treatment and study drug accountability; original signed informed consents, etc.]) be retained by the Investigator for as long as needed to comply with institutional policies, which generally requires records to be kept for 7 years after closure of the clinical trial. The Investigator agrees to adhere to the document/records retention procedures by signing the protocol.

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