

A Prospective, Single-Center, Randomized Phase 2 Trial of Etoposide in Severe COVID-19

Meredith Halpin¹, Adam Lerner¹, Manish Sagar², Praveen Govender³, Bhavesh Shah¹, Janice Weinberg⁴, Shayna Sarosiek⁵ and Mark Sloan^{1*}

¹Department of Medicine Section of Hematology and Oncology, Boston University Chobanian & Avedisian School of Medicine, Boston, United States of America

²Department of Medicine Section of Infectious Disease, Boston University Chobanian & Avedisian School of Medicine, Boston, United States of America

³Department of Medicine Section of Pulmonology, Boston University Chobanian & Avedisian School of Medicine, Boston, United States of America

⁴Department of Public Health, Boston University School of Public Health, Boston, United States of America

⁵Department of Oncology, Dana Farber Cancer Institute, Boston, United States of America

Abstract

The systemic inflammatory response seen in patients with severe COVID-19 shares many similarities with the changes observed in Hemophagocytic Lymphohistiocytosis (HLH); a disease characterized by excessive immune activation. Many patients with severe COVID qualify for a diagnosis of HLH. Etoposide, an inhibitor of topoisomerase II is used to control inflammation in HLH. This randomized, open-label, single center phase II trial attempted to determine whether etoposide can be used to blunt the inflammatory response in severe COVID. This trial was closed early after eight patients were randomized. This underpowered trial did not meet its primary endpoint of improvement in pulmonary status by two categories on an 8 point ordinal scale of respiratory function. There were not significant differences in secondary outcomes including overall survival at 30 days, cumulative incidence of grade 2 through 4 adverse events during hospitalization, duration of hospitalization, duration of ventilation and improvement in oxygenation or $\text{paO}_2/\text{FIO}_2$ ratio or improvement in inflammatory markers associated with cytokine storm. A high rate of grade 3 myelosuppression was noted in this critically ill population despite dose reduction, a toxicity which will limit future attempts to explore the utility of etoposide for virally-driven cytokine storm or HLH.

Keywords: COVID-19; Etoposide; Clinical trial

Abbreviations: BMC: Boston Medical Center; HLH: Hemophagocytic Lymphohistiocytosis

Introduction

The initial wave of COVID-19 swept through the Northeast United States in the spring of 2020. At that time Boston Medical Center (BMC), a safety net hospital, had the largest proportional load of patients with COVID-19 infection in the city of Boston. In April 2020 seven out of 10 patients admitted to BMC patients had COVID-19 [1]. An alarming report from New York in April 2020 described the mortality for mechanically ventilated COVID-19 patients to be between 25 and 97% [2]. Reliable information on the risks and benefits of dexamethasone, remdesivir, therapeutic anticoagulation, tocilizumab and other interventions were not available during the first year of the pandemic as multi-center clinical trials were initiated, accrued and analyzed [3-6]. In the interim, these medications and others were used outside the context of clinical trials either as hospital policy or on a case-by-case basis.

It was noted early in the course of the pandemic that patients with severe disease often had evidence of a profound systemic inflammatory response [7]. Elevations in markers of systemic inflammation such as ferritin, C-Reactive Protein (CRP), D-dimer, and interleukin-6 were seen, a constellation of findings described as 'cytokine storm' [8]. The cytokine storm observed in COVID-19 infection has similarities to the patterns of immune dysregulation observed in Hemophagocytic Lymphohistiocytosis (HLH); a disease characterized by excessive immune activation with laboratory and clinical evidence of inflammation [9]. An early autopsy series of COVID-19 patients from Boston Medical Center showed that three of 4 cases had histologic evidence of profound hemophagocytosis; several of these patients qualified for a formal diagnosis of secondary HLH based upon H-score [10].

Treatments for HLH are given with the intent of decreasing the inflammatory response and include etoposide, an inhibitor of Topoisomerase II. Etoposide is used to selectively ablate activated T

cells to control the immunoregulatory disorder in HLH [11]. Etoposide impacts T cells directly and has a broader spectrum of cytokine modulation than tocilizumab or anakinra, which target a single cytokine [11]. Support for the use of etoposide in the treatment of HLH in adults is based upon the results of HLH-94, a large prospective pediatric study. In that study investigators used a regimen that included an 8 week induction with dexamethasone and etoposide [12]. This regimen allowed the majority of patients to survive until bone marrow transplantation. In a retrospective multicenter study of 162 patients with HLH looking at predictors of mortality, use of etoposide was associated with improved survival [13]. Etoposide has been previously used as salvage therapy after anti-cytokine therapy for patients with severe avian Influenza A and COVID-19 [14,15]. This clinical trial explored the utility of etoposide to alleviate cytokine storm and improve lung and multi-organ dysfunction in patients with life threatening COVID-19.

Materials and Methods

Trial design and randomization

This was designed as a randomized, open label, single center phase II clinical trial to evaluate the safety and efficacy of etoposide in patients with COVID-19 infection and evidence of cytokine storm. From May 15, 2020 until January 22, 2021, we enrolled hospitalized adults (>18 years of age) with COVID-19 infection as confirmed by PCR that required intubation due to COVID-19 related respiratory

***Corresponding author:** Mark Sloan, Department of Medicine, Section of Hematology and Oncology, Boston University Chobanian & Avedisian School of Medicine, Boston, United States of America; E-mail: Mark.Sloan@bmc.org

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illness. Eligibility criteria required evidence of cytokine storm as defined by a peak ferritin >10,000 ng/mL or, peak ferritin >500 ng/mL with one or more of the following: LDH >500 U/L, D-dimer >1000 ng/mL, CRP>100 mg/L or White Blood Cells (WBC) >15 k/uL. Patients were excluded if they were pregnant or breastfeeding, had a creatinine clearance <15 mL/min, bilirubin >3 mg/dL, AST or ALT>5 times the upper limit of normal, platelets <50,000/mm³ or required ECMO. An amendment to the protocol allowed a single vasopressor. Enrollment targeted patients who had either failed or were considered ineligible for anti-cytokine therapy. Previous biologic therapy was allowed after a three half-life wash out period. The study protocol was approved by the Food and Drug Administration as an Investigational New Drug (IND) and by the Institutional Review Board at Boston Medical Center. It was supervised for safety by an independent medical monitor and registered with clinicaltrials.gov. Informed consent was obtained from the patient or the legally authorized representative.

Eligible patients were randomized in a 3:1 allocation ratio to receive Etoposide administered intravenously at a dose of 150 mg/m² on Days 1 and 4 or standard of care treatment. Dose reductions of 50% were used for total bilirubin 1.5-3/0 mg/dL, AST >2.5 times the upper limit of normal. The dose was reduced 30% if albumin was <3.0 g/dL and 25% if creatinine clearance was 15-50 mL/min. Standard of care treatment evolved considerably while the study was open. In addition to the etoposide, therapies such as dexamethasone and remdesivir, and interventions such as prone ventilation were allowed at the discretion of the treating clinician.

Evaluations

The patient's clinical status was assessed at screening, day 1 prior to treatment, days 4 and 7, and at discharge or death. For patients randomized to standard of care, the day of randomization was considered day 0 of treatment. No placebo or blinding was used in this study.

Outcome measures

The primary outcome was improvement in pulmonary status by two categories on an 8 point ordinal scale of respiratory function. Secondary outcomes included overall survival at 30 days, cumulative incidence of grade 2 through 4 adverse events during hospitalization, duration of hospitalization, duration of ventilation and improvement in oxygenation or paO₂/FIO₂ ratio. Improvement in inflammatory markers associated with cytokine storm (ferritin, CRP, d-dimer) was assessed at day 30 as were WBC and platelet count nadir and at day 30. In cases where patients were discharged or died before day 30, or if the day 30 value was missing the last lab value checked was used for analysis.

8-Point ordinal scale of respiratory function

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

Statistical analysis

The study originally planned to enroll 32 patients with a 3:1 allocation ratio. If fully accrued and 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 would 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. This study as originally designed was not powered to detect the minimal clinically meaningful difference in outcome, rather it hoped to provide initial signal of efficacy if improvement in response was observed.

The study was terminated early. Patients who did not receive etoposide (including the patient randomized to intervention who did not receive the etoposide) were included in the standard of care group in this modified intention to treat analysis. Only the patients who received etoposide were evaluable for toxicity. A Fisher's exact test was used to determine if there was a significant association between receiving etoposide and dichotomous variables (2 point improvement in the ordinal scale and overall survival at 30 days). A Wilcoxon rank sum test was used to determine significance of the secondary outcomes. NCSS Statistical Software was used for analysis and (α=0.05) was used for the level of statistical significance.

Results

Patients

A total of 13 patients underwent screening. Of these patients, eight gave informed consent and underwent randomization. All patients were on mechanical ventilation and had received prior therapies as detailed in Table 1. Several patients enrolled had already been on a ventilator for many days (median 6.6, range 1-17 days). Only one patient was randomized to standard of care. One patient was assigned to treatment but did not receive etoposide because they progressed to hemodialysis after randomization but prior to treatment. The six patients randomized to treatment received both doses of etoposide. Five of the six patients (83%) who received etoposide required dose reductions for abnormal liver function or decrease albumin (Table 1 and Table 3).

Subject Number	Approximate Age (yrs)	Gender	Prior Treatment	Peak Ferritin	D-Dimer, add time (D1 or screen)	CRP	Days from intubation to consent or treatment	Dose Reduction based on enrollment labs
1	51-55	Male	Anakinra	14038	852	443.4	11	None
2	61-65	Female	Sarilumab, Dexamethasone	4553	1201	309.1	17	50%: LFT
3	61-65	Male	Remdesivir, Dexamethasone	5086	989	242.7	4	50%: LFT & albumin
4	71-75	Male	Dexamethasone	5441	1162	223.7	NA-get the number	
5	51-55	Male	Dexamethasone	3486	2445	138.1	4	30%: Albumin
6	75-80	Female	Dexamethasone	513	279	68.6	NA-get the number	

7	85-90	Male	Dexamethasone, Ivermectin, Remdesivir	1053	281	56.9	3	30%: albumin
8	75-70	Male	Dexamethasone, Remdesivir	1753	671	74.3	6	30%: albumin

Table 1: Baseline Characteristics of COVID-19 Patients

Subject Number	Assigned Treatment	Score at enrollment	Score at Discharge	Outcome at Discharge	Days from treatment/enrollment extubation	Days from treatment/enrollment to discharge or death
1	Etoposide	6	1	Home on Room Air	12	22
2	Etoposide	6	6	Rehabilitation with a tracheostomy & weaning ventilator support	More than 30	38
3	Etoposide	6	8	Deceased		8
4	Etoposide, never received (analyzed as SOC)	6	8	Deceased		20
5	Etoposide	6	1	Home on Room Air	5	11
6	Standard of Care	6	1	Home on Room Air	15	28
7	Etoposide	6	8	Deceased		11
8	Etoposide	6	8	Deceased		12

Table 2: Results of patients with Etoposide and standard of care.

Subject number	Dose Reduction	Adverse Events
1	None	Grade 1: Anemia, Leukopenia
2	50%: Baseline LFT Day 4-30% for Albumin <3	Grade 2 Anemia Worsening renal function
3	50%: Baseline LFT & albumin	Grade 1 Transaminitis Grade 3 Thrombocytopenia Grade 2 Anemia Grade 4 Neutropenia Neutropenic Fever
4	NA (No Albumin)	None
5	30%: baseline albumin	None
6	NA (No Albumin)	None
7	30%: Baseline albumin	Ventilator associated pneumonia (not neutropenic)
8	30%: Baseline albumin	Grade 4 Anemia Grade 4 Neutropenia Grade 4 Thrombocytopenia

Table 3: Dose reduction and adverse events for treatment.

Primary outcome

Two of 6 (33%) patients randomized to receive etoposide met the primary endpoint of improvement of two points on the respiratory ordinal scale. One patient was discharged to rehab with a tracheostomy requiring ongoing ventilator support so did not meet the primary outcome measure but ultimately recovered. The patient randomized to treatment who did not receive etoposide due to rapid decline in renal function died 20 days after enrollment. The one patient randomized to standard of care was discharged on room air at day 28, thereby meeting the primary endpoint (50%). Three of 6 patients (50%) treated with etoposide died on the ventilator. The cause of death was hypoxemic respiratory failure from COVID lung injury in all cases. There was no significant association between receiving etoposide and a 2 point improvement on the ordinal respiratory scale (2 of 6 etoposide patients (33%) vs 1 of 2 (50%) SOC patients, $p>0.999$, Fisher's exact test) (Table 2).

Secondary Outcomes

There was no significant association between receiving etoposide

and survival at day 30 in a modified intention to treat analysis, (3 of 6 (50%)) for etoposide, 1 of 2 (50%) for SOC $p>0.999$; Fisher's exact test). Likewise, there were no significant associations between change in ferritin (1235 ng/L etoposide vs -1771 ng/L $p=0.14$), CRP (-49 mg/L etoposide vs -74 mg/L SOC, $p=0.52$) or d-dimer (480 ng/mL etoposide vs -2243 ng/mL SOC, $p=0.64$) from pretreatment levels until day 30 (or discharge or death) levels.

Safety and adverse events

Despite dose reductions, four patients of 6 (66%) experienced bone marrow suppression during the study period related to etoposide, with two patients (33%) experiencing grade 4 neutropenia and grade 3 or 4 thrombocytopenia. One patient experienced febrile neutropenia requiring broad spectrum antibiotics. Three patients (50%) died or had care withdrawn while still in their hematologic nadir although none of these deaths were from a complication of myelosuppression (new infection or bleeding). One patient developed grade 1 AST/ALT elevation and one patient had grade 4 acute kidney injury requiring dialysis.

Discussion and Conclusion

Etoposide is useful to control the dire immune dysregulation seen in Haemophagocytic Lymphohistiocytosis. The similarities between HLH and the pathologic inflammation seen in severe COVID suggested that it may also be helpful to blunt the impact of cytokine storm from COVID. This trial attempted to evaluate the utility of etoposide in critically-ill patients with severe COVID requiring mechanical ventilation.

Accrual to this study was halted early due to the rapidly changing demographics of the infection, emergence of other available treatment options and eventually the development of effective vaccines. The limited sample size hampers interpretation. From the available data, inconsistent myelosuppression is the major adverse effect of etoposide for COVID, despite the use of dose reductions. This toxicity will limit etoposide's applicability in critically ill patients with COVID. Further limitations in this study include the rapid changes in standard of care for COVID that occurred while this study was open. It is also acknowledged that the intervention for many of these patients occurred after the initial period of cytokine storm had resolved. Acknowledging these significant limitations, there was no definite trend in improvement in respiratory function or secondary outcome measure that supports further exploration in this population.

Declaration

This study was planned, conducted and reported in accordance with the Helsinki Declaration as revised in 2013. Authorization to conduct this research was approved by the Institutional Review Board at Boston Medical Center and the protocol was approved by the Food and Drug Administration under an Investigational New Drug (IND) application. It was supervised for safety by an independent medical monitor and registered with clinicaltrials.gov. Informed consent was obtained from the patient or the legally authorized representative. Nonessential identifying details including specific age has been omitted to protect anonymity.

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Author declarations

I confirm all relevant ethical guidelines have been followed, and any necessary IRB and/or ethics committee approvals have been obtained.

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