The Use of Etoposide for Covid-19 Treatment

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Content

1. Abstract

SARS-CoV-2, or Covid-19, has been classified as a Public Health Emergency of International Concern by the World Health Organisation (WHO (1)). Although vaccines are currently being administered, there is still no effective treatment for Covid-19. Because of this, there is a lot of interest for potentially effective Covid-19 therapeutics. One of these therapeutic agents is etoposide. Etoposide gained interest as a potential Covid-19 therapeutic after it had been established that the severe phase of Covid-19 resembles the disease course of hemophagocytic lymphohistiocytosis (HLH). Etoposide is a chemotherapeutic agent that is also used as the standard therapy against HLH. Both in HLH and in severe Covid-19, there is an excessive response of the immune system, leading to activation of T-cells and macrophages, eventually leading to excessive release of cytokines. This cytokine storm causes multi-organ damage, which can be fatal (2) (3). In this essay, the background of Covid-19 and etoposide is discussed and the available clinical data on etoposide as a treatment against Covid-19 is analysed. As clinical data on the use of etoposide against Covid-19 is limited, prospects of etoposide as a Covid-19 therapeutic are difficult to determine. However, based on case reports and available literature on the use of etoposide against Covid-19, etoposide has potential as a therapeutic agent against Covid-19.

2. Introduction

2.1. Covid-19 and therapy

In December 2019, the first report was made of severe acute respiratory syndrome coronavirus 2019 (SARS-CoV-2) in Wuhan, China (1). This disease, which has now taken 3.803.592 lives and caused 175.686.814 cases of infection worldwide, still continues to hold its grip on society today (4). The unforeseen economic and social impact, next to the immense impact on human health, has led the World Health Organisation (WHO) to declare coronavirus disease 2019 (Covid-19) as a Public Health Emergency of International Concern. Covid-19 causes respiratory distress and it has a mortality rate of about 3% (1). Although various vaccines have been produced and many doses have been administered, there is still a need for an effective therapeutic against Covid-19 infection. This is especially relevant for the severe cases of hospitalized Covid-19 patients.

2.1.1. Origin of Covid-19

Covid-19 is not the first coronavirus to spread and cause outburst of severe acute respiratory syndrome. In 2002, 8089 people were infected with severe acute respiratory syndrome coronavirus (SARS-CoV), eventually leading to 774 deaths. The disease was first reported in China but eventually people from five different continents became infected. In 2012, Middle East respiratory syndrome coronavirus (MERS-CoV) led to the infection and death of 2500 and 858 people respectively. MERS originated in Saudi Arabia and eventually spread to 27 different countries. Of all three coronaviruses, SARS-CoV, MERS-CoV and SARS-CoV-2, bats are considered to be the primary reservoir of infection. SARS-CoV-2 is similar to a virus that resides in bats; SARS-like bat CoV. However, humans cannot be directly infected with SARS-like bat CoV's. Thus, it is probable that SARS-like bat CoV underwent mutational changes in an intermediate host. It has been reported that there are structural similarities between Pangolin-CoV and SARS-CoV-2, therefore is has been suggested that pangolins functioned as intermediate host for SARS-CoV-2. However, to confirm if the virus was transferred to humans via an intermediate host, this still needs to be proved (1).

2.1.2. SARS-CoV-2 mechanism

SARS-CoV-2 consists out of a 29,9 kb sized positive sense RNA genome. For viral replication, the virus contains 15 non-structural proteins. The virus also contains 4 structural proteins: matrix protein, spike glycoprotein, nucleocapsid protein and small envelope protein. The spike glycoprotein has an important function in the infiltration of the virus into human cells. The spike glycoprotein is embedded on the surface of the virus and is responsible for receptor recognition: the spike glycoprotein binds to the cellular receptor human angiotensin-converting enzyme 2 (ACE2), which is expressed on the epithelial cells in organs such as the heart, lung, intestine, kidney and testis. The spike protein consists out of the S1 and the S2 functional domains. The S1 domain binds to the ACE2 receptor via the receptor binding domain (RBD). The binding of the RBD to the ACE2 receptor initiates a conformational change in the S2 domain, that causes uncovering of the fusion peptide that mediates the virus-cell membrane fusion. This leads to entry of the virus into the cytoplasm of the host cell through endocytosis, where the viral RNA is exposed and translated into polyproteins. In the cell, the viral RNA genome is replicated and translated into structural proteins and two glycoproteins. Eventually, the genomic RNA, formed glycoproteins and nucleocapsid proteins are assembled in the endoplasmic reticulum-Golgi compartment to form the mature virus, which is then released from the cell through exocytosis (1) (3) (5).

2.1.3. Symptoms of Covid-19

Covid-19 presents as a disease in which the clinical features are varied and nonspecific. It has been reported that symptoms arise after an incubation period between 2 to 14 days and that in fatal situations the period from onset of symptoms to death ranged from 6 to 41 days. It has been reported that of the confirmed Covid-19 cases, approximately 80% suffered from mild to moderate disease conditions. Of the diagnosed Covid-19 patients, around 12% was from the elderly population (5). Classification of Covid-19 patients can range from asymptomatic to critical. Patients with a mild disease presentation can have symptoms of acute and upper respiratory tract infection, such as myalgia, fever, cough, fatigue or digestive symptoms, such as abdominal pain, nausea, diarrhoea or vomiting. In more severe cases, Covid-19 presents as pneumonia with hypoxemia. The situation becomes critical when the patient develops acute respiratory distress syndrome (ARDS) potentially attended with shock, heart failure, myocardial injury, encephalopathy, coagulation dysfunction or kidney injury. It has been reported that this critical disease phase of Covid-19 resembles the disease course of hemophagocytic lymphohistiocytosis (HLH) (2). This finding has let to new insights and possibilities in the research for a therapeutic against Covid-19.

2.1.4. HLH and Covid-19

In critically ill COVID-19 patients suffering from ARDS there has been a presentation of systemic hyperinflammation and immune dysregulation, wherein there is upregulation of various factors such as pro-inflammatory cytokines and chemokines (6) (2). In Covid-19, there has been an upregulation of IL-2, IL-7, IL-10, granulocyte-colony stimulating factor (G-CSF), interferon-inducible protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP1), macrophage inflammatory 1A (MIP1A) and tumour-necrose-factor-α (TNFα) (7). Other reports have also mentioned increased levels of IL-1B and interferon-γ (3). These developments often lead to multiorgan damage in COVID-19 patients (6). Another interesting observation was the presence of patchy inflammatory cellular infiltration and multinucleated giant cells in a Covid-19 patient's lung. It has been suggested that the multinucleated giant cells might have been hemophagocytes. This overall disease presentation resembles the course of disease in HLH, which is a type of cytokine storm syndrome (2).

Cytokine Storm Syndromes (CSS) are a group of disorders in which there is usually a display of inflammation that often leads to hemodynamic instability, followed by multiple organ dysfunction and potentially death (8). Although the pathogenesis of CSS can vary greatly, both as a result of different etiologies as well as differences within specific etiologies, the same outcome is usually observed in CSS. In case of a virally stimulated CSS, the pathogenesis is initiated by the microorganism causing excessive proliferation and activation of macrophages and T-cells. This leads to a range of effects: excessive Th1-cell activation, excessive secretion of cytokines that activate macrophages and cytotoxic T-lymphocytes, increased phagocytosis in macrophages and proliferation of cytotoxic T-lymphocytes. The microorganism also activates dendritic cells and NK cells. The overall effect of this process is a cytokine storm. Under these pathological conditions, cytokines do not only exert their effects locally, but they can also act in an endocrine manner leading to a systemic response. This eventually leads to damage in target cells in various organs, with often a fatal outcome (7).

The cytokine storm syndrome HLH is characterised by an excessive response of the immune system, leading to activation of histiocytes such as macrophages and dendritic cells, T- and natural killer (NK) cells with prominent hemophagocytosis in primarily the bone marrow (9). In Covid-19 a similar cytokine profile has been observed as in HLH. In HLH, the cytokine profile leads to a hyperinflammatory syndrome which is often the cause of cytopenia's, persistent fevers, elevated ferritin, and ARDS (3). This eventually leads to multiorgan failure and severe hypercytokinaemia (2). There are two forms of HLH: primary or familial HLH and secondary HLH. Primary HLH is an autosomal recessive hereditary

condition, that usually presents itself within the first two years of life. Secondary HLH is usually the result of an underlying cause, such as an auto-immune disease or viral infection (9).

2.1.5. Covid-19 current treatment options

The development of vaccines against Covid-19 has progressed at accelerating speed, leading to the production of various vaccines that are being administered to a growing part of the population. Nevertheless, people suffering from an active Covid-19 infection cannot be treated with a vaccine. Especially in situations where the disease progresses to a critical point, there is a demand for a therapeutic agent that effectively diminishes the effects of the disease and enhances the prospects for critical Covid-19 patients. In the search for an effective therapeutic, there have been various approaches. In one such an approach, it has been the aim to develop a new agent. However, this process is time consuming and expensive. Therefore, another approach has been to repurpose established drugs that also appear be effective against Covid-19. Some of the medicines that have been analysed are remdesivir, dexamethasone, tocilizumab and hydroxychloroquine (5) (10).

The principle that analysing these specific medicines is based on, is that the pathogenesis of Covid-19 is driven by two main processes. Initially after infection, the disease is driven primarily by replication of the virus. Subsequently, when the infection has advanced, the disease becomes more driven by a dysregulated immune and inflammatory response. For this reason, it is probable that antiviral therapies are more effective in the initial stage of the disease and that immunosuppressive or anti-inflammatory therapies are more advantageous in the progressed stages of the disease (10). Focussing on this latter, more critical stage of disease, there has been a lot of attention on the chemotherapeutic etoposide. This therapeutic is registered for the application against various tumours and haemato-oncological diseases, but recent reports have demonstrated its possible efficacy in the treatment of critically ill Covid-19 patients.

2.2. Etoposide: a podophyllotoxin-derived drug

2.2.1. Origin and structure of Etoposide

Etoposide is an alkaloid and a synthetic derivative of podophyllotoxin, which is an aryltetralin-type lignan (11) (12). Podophyllotoxin is present in numerous plant species that belong to over 60 vascular families, but its most common sources are the two perennial herbs *Sinopodaphyllum hexandrum Royle*, or Himalayan mayapple, and *Podophyllum peltatum*, or American mayapple (13) (12). Both these herbs are spread across Western China and the Himalayan region (12). Since a long time, these herbs have been used in traditional medicine, such as Traditional Chinese medicines. In 1983, etoposide was approved by the FDA as cancer therapy against various cancer types (14).

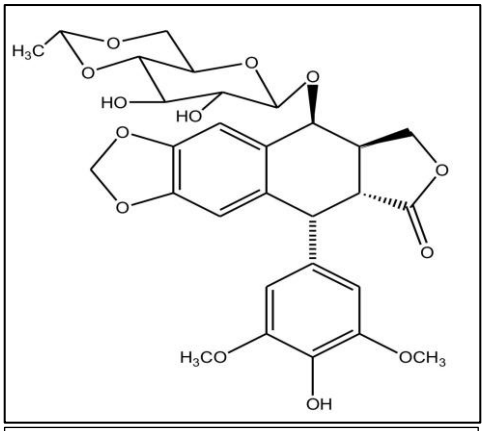

Figure 1. Molecular structure of etoposide (12).

2.2.2. Applications of Etoposide

Etoposide is a chemotherapeutic agent and is administered against testicular carcinoma, ovarian carcinoma, high-risk gestational trophoblastic neoplasia, small cell lung cancer (SCLC) and against haemato-oncological diseases such as Hodgkin-lymphoma, non-Hodgkin-lymphoma and acute myeloid

leukaemia. Podophyllotoxin-derivatives cause the inhibition of tumour-growth and regression of tumours. This effect is based on the binding of the derivatives to the topoisomerase II-DNA complex, which impedes reversible single-stranded DNA breaks to be reversed. This leads to the accumulation of single-strand DNA breaks and eventually to the occurrence of double-stranded DNA breaks, leading to cell damage and cell death (15). Moreover, the use of etoposide is not limited to application as a chemotherapeutic agent.

As mentioned before, it has been reported that severe COVID-19 can cause HLH. In 1994, the discovery was made that etoposide could also be applied as a therapeutic against HLH. Etoposide appeared to improved the prospects for HLH patients, by increasing the 5 year survival rate of patients with 21-54% (14). Nowadays, the standard of care for secondary HLH is an etoposide-based protocol and it has been shown to be effective in HLH secondary to Epstein-Barr-virus (EBV) and hemagglutinine-1-neuraminidase virus (H1N1-virus). For this reason, etoposide also gained interest as a therapy against HLH secondary to COVID-19 (2).

2.2.3. Etoposide and safety considerations

Despite the possible beneficial applications of etoposide, there are some negative aspects accompanied with the use of the therapeutic. First of all, the bioavailability of etoposide is variable and decreases with a higher dose; the bioavailability is approximately 76% in a dose of 100 mg and 48% in a dose of 400 mg (15). Another safety issue in the use of etoposide, is that the compound has a low water solubility. Because of this, formulation in aquatic solution is not possible. Instead, etoposide has to be formulated containing excipients, such as ethanol, Tween 80, benzyl alcohol or polyethylene glycol, that may increase the toxicity of intravenous administration. However, recently the solubility of etoposide was enhanced by introduction of etoposide phosphate. This is a prodrug that can be absorbed rapidly and that is converted in vivo to etoposide, which has made the administration of etoposide less harmful. Furthermore, due to the clearing effect after metabolization in the liver by cytochrome P450, it is necessary to administer large doses of etoposide. Because of this, the administered therapeutic circulates to non-targeted organs, enhancing the occurrence of side effects (14).

There are serious side effects that are associated with the use of etoposide, such as neurotoxicity, nephrotoxicity, bone marrow suppression, hair loss, mucositis, cardiotoxicity and immunosuppression. Some of these adverse effects are the result of the targeting mechanism of etoposide. For example, podophyllotoxin-derivates target fast-dividing cells. This also results in the targeting of various fast-dividing normal cells, such as hair follicle cells and bone marrow cells, causing bone marrow suppression and hair loss. Nephrotoxicity is another side effect of etoposide and it can be induced by the generation of reactive oxygen species (ROS) and activation of extracellular regulated protein kinases (ERK) in the human-kidney-2 (HK-2) cells, leading to necrosis. Other side effects can be caused by 0-Quinones, which are reactive metabolites originating from natural catechol products, such as podophyllotoxins. o-Quinones are formed by oxidation of the catechol and have a variety of targets, such as physiological metabolizing enzymes. One of these targeted metabolizing enzymes is glutathione (GSH). Through alkylation or through oxidation of GSH, oxidative stress in normal cells and depletion of GSH is caused, contributing to a variety of side effects (14).

2.2.4. Etoposide against HLH in Covid-19

Because of the evident similarities in the disease course of HLH and critical phase Covid-19, there have been many reports on the possible efficacy of etoposide as a therapy against critical phase Covid-19. In a performed study, a murine model was used to elucidate the mechanism of etoposide as a therapy against HLH. In this study it was observed that etoposide caused prolonged survival by considerably

alleviating all symptoms of the murine HLH. The mechanism of etoposide was found to be involved with potent deletion of activated T cells and effective suppression of inflammatory cytokine production. What made this effect remarkable, was that etoposide had no direct anti-inflammatory effect on dendritic cells or macrophages, neither did it cause elimination of quiescent naïve or memory T cells (16) (2). By eliminating activated T cells and suppressing inflammatory cytokine production, etoposide may control splenomegaly, fevers, cytopenia's, hypofibrinogenemia, hypertriglyceridemia and hyperserotonaemia resulting from HLH (3). Moreover, it has been shown in murine models that low-dose etoposide can renew cytotoxic T lymphocytes, which allows the elimination of SARS-CoV-2 infected cells, activated macrophages and associated immunomodulatory abnormalities of SARS-CoV-2 infection (17).

However, as mentioned previously, etoposide has severe side effects and it has a low bioavailability (14). Therefore, before etoposide can be registered as a therapy against critical phase Covid-19 infection, more research is needed on the efficacy and the safety of etoposide in these circumstances. Furthermore, as mentioned before there are other medicines under investigation for possible repurposed application against Covid-19 (5) (10). These issues give rise to the question: What are the prospects of etoposide as a therapy against Covid-19?

3. Trials and tests on Etoposide as COVID-19 treatment

Since etoposide gained interest as a possible therapeutic for critically ill Covid-19 patients, it has been analysed in various settings. There are two case reports on the use of etoposide in Covid-19 patients (6) (3). Besides that, etoposide is currently being investigated in a phase 2 clinical trial (18).

3.1. Case report: Etoposide treatment adjunctive to immunosuppressants in Covid-19

During a study period of March 2 to April 10, 2020, 13 Covid-19 patients received 50-150 mg/m^2 etoposide. These patients came out of a patient population of 709 Covid-19 patients. Two of these 13 patients had to be excluded from the study because they were already intubated. Of the 11 included patients, 2 were female and 9 were male and the range of the age was 41 to 79, with a median age of 58. In appendix I, table 1 the clinical characteristics of the patients are shown. The PaO_2/FiO_2 ratio at admission ranged from 52 to 174, with a median of 98. It was observed that after treatment with etoposide, the PaO_2/FiO_2 ratio had improved to an average of 195%. Of the 11 included patients, 9 patients fully recovered and were eventually discharged, 3 patients needed mechanical ventilation and 2 patients died following thrombotic complications. Patient 4 had a notable improved respiratory function, which led to extubation. However, 2 days after extubation the patient developed massive cerebral ischemic stroke and died 16 days after admission. Furthermore, patient 5 improved and recovered from severe ARDS with profound leukopenia. However, the patient died after discharge from the ICU at day 24. The cause of death was probably from massive pulmonary thromboembolism. In patient 5, there had been no adverse effects which could be attributed to etoposide (6).

It was mentioned that in treating the Covid-19 patients with etoposide, 1-2 doses were enough to induce clinical improvement in critically ill patients. Clinical improvement was determined based on inflammatory serum markers, respiratory support and requirement of vasopressor therapy. From these results it was concluded that etoposide is an effective and safe adjunctive salvage treatment for Covid-19 patients whom are critically ill. However, the limitations of the study were also pointed out: the number of participants in the study was low and there was no comparison group, due to the severe state of the patients included. Moreover, all patients had been treated previously with tocilizumab and methylprednisolone and thus both drugs were possible confounders. Nonetheless, none of the patients who had received etoposide had responded well to previously administered drugs. Etoposide was administered to these patients because of worsening of their conditions, implicated that etoposide was the drug that eventually positively affected these patients (6).

3.2. Case report: Etoposide as salvage therapy in Covid-19

A female 66-year-old patient was admitted with complaints of shortness of breath, insomnia and malaise for 5 days. The patient had a medical history of type 2 diabetes, hyperlipidaemia and hypertension. After laboratory research it became evident that the patient was suffering from an urinary tract infection and from acute kidney injury. After the patient had developed acute hypoxic respiratory injury and was not responsive to the supplemental oxygen, she was admitted to the ICU were the patient eventually received intubation. The patient was tested positive for SARS-CoV-2 and was thereafter enrolled in a randomized placebo-controlled double-blind clinical trial for the anti-IL-6 drug sarilumab. However, the patient did not respond to this treatment and even developed a worsening cytokine storm. At this point, the patient was started on treatment with the anti-IL-1 receptor antagonist anakinra and a 3-day course of immunoglobulin IV (IVIG). Although the patient initially responded well to this treatment, she had a complicated and prolonged ICU course. The patient developed ventilator-associated pneumonia, and on day 18 of hospitalization, the patient also

developed worsening hypoxia and significant thrombocytopenia (3). Treatment with bivalirudin (direct thrombine inhibitor (19)) was started and it was confirmed that the patient had a pulmonary embolism. Moreover, the patient was treated for heparin-induced thrombocytopenia (HIT). On day 20 of hospitalization, the patient developed persistent fevers, worsening hypoxia and an increase in inflammatory markers (e.g. CRP and ferritin). A bronchial washing was performed, but the results were negative for any acute infection. A broncho-alveolar lavage (BAL) was performed and the polymerase chain reaction (PCR) for SARS-CoV-2 performed on the fluid was positive. Eventually it was decided to treat the patient with etoposide. Treatment with trimethoprim-sulfamethoxazole was initiated as a therapy against pneumocystis pneumonia and acyclovir was initiated as a prophylaxis against herpes simplex. Thereafter, treatment with dexamethasone combined with 50 mg/m^2 IV etoposide was initiated. Treatment with etoposide and dexamethasone evoked a fierce clinical response and the oxygenation and inflammatory markers improved significantly. A second dose of etoposide was given after 7 days. The patient was eventually discharged from the ICU, was liberated from the ventilator and was transferred to an acute rehab unit. The patient is undergoing physical therapy and has not had any readmissions since discharge (3).

Furthermore, it was mentioned that the patient had not developed cytopenia's as a side effect of etoposide. However, the patient did develop a mild transaminitis after receiving the second dose of etoposide. This demonstrated the hepatoxic side effects of etoposide. It was also addressed that in administering etoposide, development of other hematologic malignancies is possible and should be considered (3).

3.3. Phase II clinical trial: Etoposide in patients with Covid-19 infection

On May 8, 2020, the phase II clinical trial evaluating the efficacy and safety of etoposide as a therapy for Covid-19 was initiated. This study is a randomized and open-labelled study. In this study, the patients are treated with intravenously administered etoposide, in a dose of 150 mg/m^2, at days 1 and 4. The participants will receive a subsequent dose of etoposide in the scenario that both the investigator and the treating physician are under the impression that the patient will benefit clinically from the etoposide therapy but subsequently recurrent clinical deterioration is evident. Randomization was performed with a 3:1 allocation ratio and no placebo is used. Subjects randomized to control will receive standard of care treatment. In this clinical trial, a total of 8 participants are enrolled. These participants had to meet the established criteria. Participants had to have an age of 18 years of higher, all sexes were allowed and no healthy volunteers were used. Inclusion criteria and exclusion criteria are displayed in appendix II, table 2. In the study there are four arms: 2 experimental arms and 2 no intervention arms. The experimental arm of cohort 1 includes participants that are on ventilation and receive 150 mg/m^2 daily on days 1 and 4. The no intervention arm of cohort 1 is the control group of cohort 1. In this group the participants are on ventilation and are given standard of care therapy. The experimental arm of cohort 2 includes participants that are not on ventilation and are given etoposide 150 mg/m^2 daily on days 1 and 4. The no intervention arm of cohort 2 is the control group of cohort 2. These participants are not on ventilation and are given standard of care therapy (18).

The motivation for this study is the high mortality rate that accompanies the hyperinflammatory response in Covid-19 infection. In the Boston Medical Centre after autopsy of Covid-19 patients, it was observed that there was significant hemophagocytosis present in the spleen and lymph nodes, denoting the occurrence of an HLH disease process in critically ill Covid-19 patients. By using etoposide to target the monocytes and T cells that cause the cytokine storm in severely ill Covid-19 patients, the aim is to reduce the progression of multi-organ dysfunction, which is characteristic for fatal Covid-19 infection. It is expected that the study completion date is in June 2022 (18).

4. Discussion

The aim of this essay was to determine what the prospects of etoposide are as a therapy against Covid-19. In the introduction it became apparent that Covid-19 is a serious issue that has affected many aspects of society worldwide. Although the exact origin of SARS-CoV-2 is still to be elucidated, part of the mechanism and the clinical effects of the virus are already clarified. This information aids in finding new drugs and repurposing registered drugs as possible therapies against Covid-19. The main weapon of SARS-CoV-2 is the spike protein, with which the virus can infiltrate host cells and replicate (5). When the disease progresses after the initial infection, the hosts immune and inflammatory response can cause a range of effects that eventually lead to critical disease presentation of Covid-19 in patients (6). In critically ill Covid-19 patients, the disease progresses in such a way that it resembles the disease course of the cytokine storm syndrome HLH. The array of cytokines and chemokines that are released in this process, can cause multi-organ damage and can eventually lead to a fatal outcome (2). The standard treatment for HLH is etoposide. Because of the resemblance in the disease course of HLH and severe Covid-19, etoposide has also gained interest as a possible therapy against Covid-19 (2). It has been reported that etoposide alleviates all symptoms of HLH by deletion of activated T cells and suppression of inflammatory cytokine production. Moreover, in murine models has been shown that etoposide can renew cytotoxic T lymphocytes, which allows the elimination of SARS-CoV-2 infected cells, associated immunomodulatory abnormalities of SARS-CoV-2 and activated macrophages (17).

In order to proof the efficacy and safety of etoposide as a therapeutic in critically ill Covid-19 patients, extensive research and clinical trials are essential. Covid-19 is a relatively new disease, which is why research on the application of etoposide against Covid-19 is still limited. Since the 8th of May, 2020, the efficacy and safety of etoposide as a Covid-19 therapeutic agent are being analysed in a phase II clinical trial. The expected completion date of this trial is June 2022 (18). Therefore, based on the phase II clinical trial, nothing yet can be said on the safety or efficacy of etoposide. However, based on two case reports, the efficacy of etoposide against Covid-19 in critically ill Covid-19 patients seems to be potentially potent.

In the case report concerning the treatment of 13 critically ill Covid-19 patients with etoposide, it was concluded that etoposide is an effective salvage treatment for Covid-19 patients who are critically ill. Of the 11 included patients, 9 fully recovered after treatment with etoposide (6). In the case report concerning the 66-year-old female patient, this same conclusion was drawn; etoposide is a potent therapeutic against severe Covid-19 (3). In both case reports, the patients had previously been treated with other drugs aiming to oppose the progression of Covid-19. However, these therapies had been unsuccessful where etoposide had succeeded, implicating a relative improved efficacy of etoposide compared to the other drugs. Of course, the reliability of the information these case reports provide is limited and prone to bias. Moreover, the previous administration of other drugs against Covid-19 prior to the etoposide treatment, abates the reliability of the proposed potency of etoposide. Thus, in order to determine the efficacy of etoposide against severe Covid-19, more data is necessary. Clinical studies are required in which control groups are used, inclusion and exclusion criteria are clearly determined and confounders are avoided.

The same reasoning applies for the safety of etoposide as a therapeutic agent against Covid-19. Based on the phase II clinical trial, nothing can be said concerning the safety and the information given by the two case reports has limited reliability. In the case report concerning the treatment of 13 critically ill Covid-19 patients with etoposide, it was mentioned that in none of the participants side effects had been observed that were associated with etoposide (6). In the case report concerning the 66-year-old female patient, etoposide had caused hepatoxic side effects, although these were relatively mild (3). Again, the reliability of the information these case reports provide is limited and prone to bias and clinical studies are required to draw valid conclusions on the safety of etoposide as a therapy against

Covid-19. Furthermore, both case reports emphasised that etoposide has severe side effects. As was also mentioned in the introduction, some side effects of etoposide are neurotoxicity, bone marrow suppression and cardiotoxicity. These are side effects that need to be taken into careful consideration in the use of etoposide. The safety of the use of etoposide is only adequate, when the benefits of the treatment outweigh the possible negative consequences.

As the prospects of etoposide as a therapy against Covid-19 are in large measure dependent on the assessment of the safety and efficacy, it is difficult to make assumptions on this topic. However, when the efficacy and safety of etoposide appear to be appropriate, the potential role of etoposide in Covid-19 will be significant yet very specific, as it will only be applied in critical cases of Covid-19 infection. Other medicines such as tocilizumab, methylprednisolone, sarilumab and anakinra appeared insufficiently potent in the treatment of severe Covid-19. However, as this information is based on just 2 case reports, this gives no conclusive answer on the efficacy of these drugs. In the case reports, etoposide was given as a salvage therapy, implicating the importance of etoposide in saving lives of critically ill Covid-19 patients. Therefore, prospects of etoposide as a therapeutic agent against severe Covid-19 might be great, provided the efficacy and safety of the drug are adequate.

5. Conclusion

Covid-19 is a disease that has caused much misery throughout the world. Although not all the aspects of the virus and the disease it causes are clarified, much has been discovered in a relatively short time span. Severe Covid-19 causes a disease pattern that shares great similarities with the cytokine storm syndrome HLH. Because of this finding, the HLH therapeutic etoposide is under investigation as a possible therapeutic against Covid-19. However, clinical data on the safety and efficacy of etoposide as a therapy against Covid-19 is very limited. At the moment a phase II clinical trial is performed analysing the efficacy and safety of etoposide against severe Covid-19. Until this trial, and potentially other clinical trials, has been completed, prospects of etoposide as a therapeutic against Covid-19 are difficult to determine. However, based on case reports and available literature on etoposide, potent efficacy of etoposide against severe Covid-19 is conceivable.

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Appendix I – Case Report: Etoposide treatment adjunctive to immunosuppressants in Covid-19

Table 1. Clinical characteristics of the 13 Covid-19 patients treated with etoposide. Study period from March 2 to April 10, 2020 (6).

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13
Age	56	41	63	70	60	70	55	57	64	58	79	73	42
Sex	Male	Male	Male	Male	Female	Male	Male	Female	Male	Male	Male	Male	Male
Hypertension/ Obesitiy	No	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes	Yes	Yes
pO2/FIO2 ratio	150	94	150	73	98	96	112	63	52	174	63	118	154
pCO2 (mmHg)	29	32	44	36	36	39	38	46	45	48	28	34	33
Ferritin (mg/ml)	2020	1492	4014	4543	1316	1835	3232	3054	3359	2029	1697	3770	2665
CRP (mg/ml)	345	181	94	204	93	185	243	89	123	103	244	126	93
D-dimers (µg/ml)	3,4	5,5	1,8	7,8	16,5	1,1	1,2	1,9	2,3	0,3	2,8	10,3	0,6
Lymphocytes abs ($*10^3$/µl)	0,3	0,4	0,4	0,1	0,3	0,6	0,6	1,8	0,5	0,5	0,2	0,4	0,1
Dose of etoposide (mg/m^2)	80	80	100	50	100	100	150	150	150	150	50	50	174
Total number of doses	1	1	2	1	2	1	2	2	2	2	2	1	1
Post etoposide PO2/FIO2	430	452	435	-	200	445	287	120	160	321	180	120	340
Etoposide administrated in ICU	Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	No	Yes	No	No

Mechanical ventilation	Noninvasive	Noninvasive	Noninvasive	Invasive	Invasive	Noninvasive	Spontaneous	Nonintvasive	Invasive	Spontaneous	Invasive	Noninvasive	Spontaneous
Outcome	Discharged	Discharged	DNR	Death	Discharged	Discharged	Discharged	Discharged	Discharged	Discharged	Hospitalized	Death	Discharged
Hospital stay (days)	15	15	13	11	13	14	7	20	5	5	15	14	32
Cytopenia > 2 lines	No	No	No	No	Yes	No	No	No	Yes	No	No	Yes	No
Infections	No	No	No	No	Enterococcus	No	No	No	HSV-1	No	No	No	No

Appendix II – Phase II clinical trial etoposide as Covid-19 treatment

Table 2. Inclusion and exclusion criteria of the phase II clinical trial on the safety and efficacy of etoposide as a treatment against Covid-19 (18).

Inclusion criteria	**Exclusion criteria**
Confirmed Covid-19 infection	Pregnancy or breastfeeding
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 white blood count > 15 k/microlitre	History of severe hypersensitivity to etoposide products
Cohort 1: intubated status as a result of Covid infection-associated respiratory illness	Absolute neutrophil count (ANC) < 1000 cells/mm^3
Cohort 2 (if activated): evidence of progressive respiratory failure (requiring >4 L/min of supplemental oxygen to maintain oxygen saturation greater than 92%) without intubation	Platelet count < 50,000/mm^3
-	Bilirubin > 3,0 mg/dL
-	Aspartate OR alanine aminotransferase > 5.0 x upper limit of normal
-	Creatinine Clearance < 15 mL/min (calculated by Cockcroft Fault formula)
-	Requiring continuous renal replacement therapy
-	Requiring vasopressors
-	Requiring extracorporeal membrane oxygenation (ECMO)
-	Other active, life-threatening infections
-	Anti-cytokine treatment (including anakinra or Interleukin 6 antibodies e.g. 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 hours 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)