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Endothelial injury and thrombotic microangiopathy in COVID-19: Treatment with the lectin-pathway inhibitor narsoplimab

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ABSTRACT

In COVID-19, acute respiratory distress syndrome (ARDS) and thrombotic events are frequent, life-threatening complications. Autopsies commonly show arterial thrombosis and severe endothelial damage. Endothelial damage, which can play an early and central pathogenic role in ARDS and thrombosis, activates the lectin pathway of complement. Mannan-binding lectin-associated serine protease-2 (MASP-2), the lectin pathway's effector enzyme, binds the nucleocapsid protein of severe acute respiratory syndrome-associated coronavirus-2 (SARS-CoV-2), resulting in complement activation and lung injury. Narsoplimab, a fully human immunoglobulin gamma 4 (IgG4) monoclonal antibody against MASP-2, inhibits lectin pathway activation and has anticoagulant effects. In this study, the first time a lectin-pathway inhibitor was used to treat COVID-19, six COVID-19 patients with ARDS requiring continuous positive airway pressure (CPAP) or intubation received narsoplimab under compassionate use. At baseline and during treatment, circulating endothelial cell (CEC) counts and serum levels of interleukin-6 (IL-6), interleukin-8 (IL-8), C-reactive protein (CRP) and lactate dehydrogenase (LDH) were assessed. Narsoplimab treatment was associated with rapid and sustained reduction of CEC and concurrent reduction of serum IL-6, IL-8, CRP and LDH. Narsoplimab was well tolerated; no adverse drug reactions were reported. Two control groups were used for retrospective comparison, both showing significantly higher mortality than the narsoplimab-treated group. All narsoplimab-treated patients recovered and survived. Narsoplimab may be an effective treatment for COVID-19 by reducing COVID-19-related endothelial cell damage and the resultant inflammation and thrombotic risk.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, causing COVID-19) was identified as a clinical syndrome in Hubei province China in December 2019 and spread rapidly (Zhou et al., 2020). By late February 2020, a fast-growing number of COVID-19 cases were diagnosed in the northern Italian region of Lombardy (Remuzzi and Remuzzi, 2020). A primary cause of death in COVID-19 is severe respiratory failure. Lung tissue in patients who died of COVID-19 shows high concentrations of SARS–CoV-2 RNA (Wichmann et al., 2020) and

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the same intense inflammatory changes seen in previously reported coronaviruses SARS-CoV (SARS) and MERS-CoV (MERS), and anti-inflammatory strategies are being evaluated for COVID-19 treatment (Xu et al., 2020; Horby et al., 2020; Gritti et al., 2020). Thrombosis has also been reported in SARS and COVID-19 infection (Magro et al., 2020; Ding et al., 2003; Wichmann et al., 2020). Similar to SARS and MERS, COVID-19 can cause life-threatening acute respiratory distress syndrome (ARDS) (Guan et al., 2020). A central pathological component of COVID-19 and of the exudative phase of ARDS is endothelial injury and activation (Varga et al., 2020; Ackermann et al., 2020; Green, 2020; Teuwen et al., 2020; Goshua et al., 2020; Thompson et al., 2017). Endothelial injury can also cause microvascular angiopathy and thrombosis. Endothelial activation further enhances the local inflammatory environment. Importantly, as demonstrated in human in vitro and animal studies, endothelial injury specifically activates the lectin pathway of complement on the endothelial cell surface (Collard et al., 2000).

The lectin pathway and its effector enzyme mannan-binding lectin-associated serine protease-2 (MASP-2) have been directly linked to the lung injury in coronavirus infection. Specifically, SARS-CoV-2 nucleocapsid protein, as well as those of SARS and MERS, have been shown to activate MASP-2, and MASP-2 deposits are seen in the vasculature of lung tissue of COVID-19 patients (Gao et al., 2020; Magro et al., 2020). Complement activation has been demonstrated to contribute to pulmonary injury in SARS and MERS infections in murine models, with increased complement factors seen in pulmonary tissue and complement blockade mitigating lung injury (Gralinski et al., 2018; Jiang et al., 2018). The rapid time-course to protection of respiratory function in these models suggests that the lectin pathway drives complement activation in both SARS and MERS (Gralinski et al., 2018; Jiang et al., 2018). Additionally, lung tissue from deceased COVID-19 patients shows components of the lectin and terminal complement pathways, specifically MASP-2, complement factor 4d (C4d) and C5b-9 (i.e., the membrane attack complex) (Gao et al., 2020; Magro et al., 2020).

Narsoplimab (Omeros Corporation) is a high-affinity fully human immunoglobulin gamma 4 (IgG4) monoclonal antibody that binds MASP-2 and blocks lectin pathway activation (Fig. 1). Narsoplimab is the subject of a rolling Biologics License Application with the United States Food and Drug Administration (FDA) for the treatment of hematopoietic stem cell transplant-associated thrombotic microangiopathy (HSCT-TMA) and has been granted FDA's Breakthrough Therapy designation for this indication. As with COVID-19, endothelial injury is a central component of the pathophysiology of HSCT-TMA (Jodele et al., 2014) and activates the lectin pathway (Collard et al.,

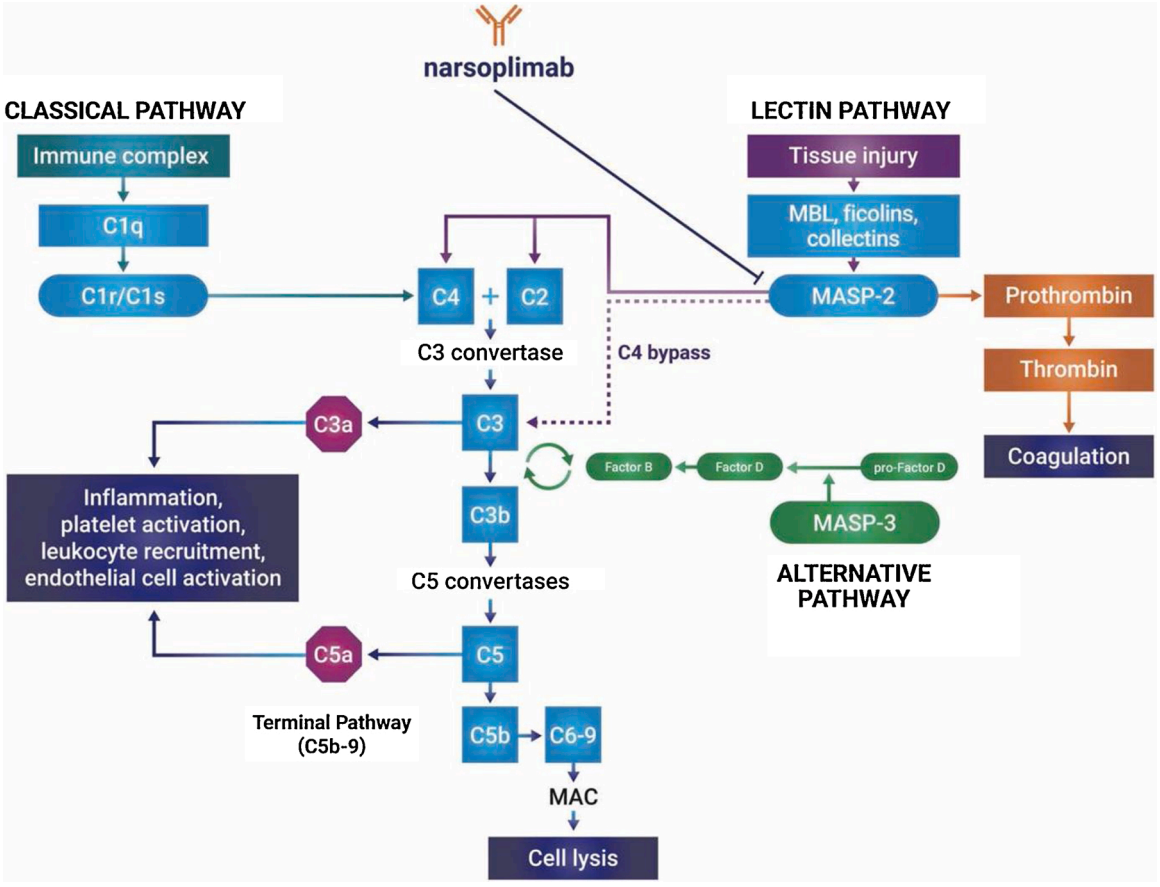

Fig. 1. The complement activation pathways and narsoplimab mechanism of action. Narsoplimab, by blocking MASP-2, inhibits activation of the lectin pathway (LP). Part of the innate immune system, the LP is activated by microorganisms or injured cells. Microorganisms display carbohydrate-based pathogen-associated molecular patterns (PAMPs) and injured host cells display damage-associated molecular patterns (DAMPs) on their surfaces. DAMPs are not displayed on healthy cells but become exposed with cell injury. Lectins, carrying MASP-2, bind to the PAMPs or DAMPs, localizing lectin pathway activation to the vicinity of the cell surface. Activated MASP-2 cleaves complement factor 2 (C2) and C4, initiating a series of enzymatic steps that result in the production of the anaphylatoxins C3a and C5a and formation of C5b-9 (the membrane attack complex), and can also directly cleave C3 through the C4-bypass mechanism. The alternative pathway acts as an amplification loop, further enhancing lectin pathway-mediated complement activation. Unlike C3 or C5 inhibition, MASP-2 inhibition does not interfere with the classical pathway, preserving the adaptive immune response and the antigen-antibody complex-mediated lytic response needed to fight infection.
MASP-2 also acts directly on the coagulation cascade and the contact system, cleaving prothrombin to thrombin and forming fibrin clots. Narsoplimab not only inhibits lectin pathway activation but also blocks microvascular injury-associated thrombus formation as well as MASP-2-mediated activation of kallikrein and factor XII.

2000). In its pivotal, single-arm clinical trial for HSCT-TMA, narsoplimab demonstrated marked clinical and laboratory improvement. In a high-risk population and using a rigorous response-based composite measure consisting of organ function, transfusion burden, and laboratory values (i.e., platelet and lactate dehydrogenase (LDH)), 54 % of all narsoplimab-treated patients and 65 % of those receiving at least 4 weeks of protocol-specified treatment achieved a complete response compared to the FDA-agreed efficacy threshold of 15 % (Rambaldi et al., 2020). Narsoplimab also is in Phase 3 clinical trials for immunoglobulin A (IgA) nephropathy and atypical hemolytic uremic syndrome for which the drug has received FDA's Breakthrough Therapy and Fast Track designations, respectively.

Complement inhibition has been proposed as a treatment for severe COVID-19, but clinical data supporting this therapeutic approach are scant (Campbell and Kahwash, 2020; Mastaglio et al., 2020; Gao et al., 2020; Diurno et al., 2020). Given the heavy disease burden in Italy, evidence linking lectin pathway activation to coronavirus-related pathophysiology, and the efficacy and safety of narsoplimab in the endothelial-injury syndrome HSCT-TMA, we treated patients with severe COVID-19 infection and ARDS with narsoplimab under a compassionate-use program at Papa Giovanni XXIII Hospital in Bergamo. This represents the first time that a lectin pathway inhibitor has been used to treat patients with COVID-19, and here we report our initial clinical experience.

2. Methods

2.1. Study oversight

This investigation and all assessments in all patients, including those in the control groups, were conducted at Azienda Socio-Sanitaria Territoriale Papa Giovanni XXIII in Bergamo, Italy and approved by the Institutional Ethics Committee and the Agenzia Italiana del Farmaco. All study patients provided informed consent. All data were collected and analyzed by the authors.

2.2. Histopathology

Standard hematoxylin and eosin staining (H&E) and immunohistochemistry were performed on formalin-fixed, paraffin-embedded samples obtained from pathological autopsies of COVID-19 patients. H&E-stained sections were reviewed by two pathologists (A.G. and A.S.). Immunohistochemical analysis of the human endothelial cell marker (CD34) was performed with Bond Ready-to-Use Antibody CD34 (Clone QBEnd/10, Leica Biosystems, Germany), an antibody optimized for use with Bond Polymer Refine Detection. The assay was performed on an automated stainer platform (Leica Bond-3, Leica Biosystems, Germany) using a heat-based antigen retrieval technique (Bond Epitope Retrieval) as recommended by the manufacturer. Cytoplasmatic staining of endothelium in the capillaries of pulmonary alveoli indicated positive results.

2.3. Circulating endothelial cells (CEC) identification and count

CEC were measured by flow cytometry using peripheral blood collected with EDTA. After an erythrocyte bulky-lysis step, samples were labeled for 20 min at room temperature with the following: anti-CD45 V500-C (clone 2D1), anti-CD34 PerCP-CY5.5 (clone 8G12) (BD Biosciences, USA), and anti-CD146 PE (clone P1H12) (BD Biosciences-Pharmingen, USA). At least 1×10^6 events/sample with total leukocyte morphology were acquired by flow cytometry (FACSLyric, BD Biosciences, USA). To reduce operator-induced variability, all samples were analyzed by the same laboratory technician. CEC values were calculated by a dual-platform counting method using the lymphocyte subset as reference population as previously reported (Almici et al., 2017).

2.4. Serum levels of cytokines

Levels of interleukin-1β, interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10, tumor necrosis factor, and interleukin-12p70 were analyzed in a single serum sample by flow cytometry (BD CBA Human Inflammatory Cytokines Kit, BD Biosciences, USA).

2.5. Patients

All narsoplimab-treated patients were admitted to the hospital between March 11 and March 23, 2020. Over this 13-day span, the total daily number of COVID-19 patients hospitalized on the wards ranged from 405 to 542. During this same time period, an average of 140 Helmet-continuous passive airway pressure (CPAP) devices were utilized on a daily basis, and a median of 82 patients (range 66–91) were managed each day in the ICU. Of these ICU patients, 61 met the Berlin criteria for ARDS (PaO2/FiO2 ratio <100 is severe ARDS; 100–200 is moderate; >200 and ≤ 300 is mild) (Ferguson et al., 2012) on March 11, 2020 and 80 on March 23, 2020 (Fagiuoli et al., 2020).

All study patients had laboratory-confirmed COVID-19 infection. SARS-CoV-2 genome from nasal and respiratory samples was detected by different molecular methods including GeneFinder™ COVID-19 Plus RealAmp Kit (ELIThech Group, France) and Allplex™ 2019-nCoV Assay (Seegene Inc, Arrow Diagnostics S.r.l., Italy). After purification of viral RNA from clinical samples, RdRp, E and N viral genes were detected by real-time polymerase chain reaction according to World Health Organization protocol (Corman et al., 2020). To be eligible for narsoplimab treatment, COVID-19-confirmed patients were required to be adults (>18 years of age), have ARDS according to the Berlin criteria and require CPAP. While all enrolled patients empirically received azithromycin 500 mg once daily, patients with active systemic bacterial or fungal infections requiring antimicrobial therapy were not eligible for narsoplimab treatment.

2.6. Narsoplimab treatment, supportive therapy and outcome assessment

Narsoplimab 4 mg/kg was administered intravenously twice weekly for 2–4 weeks. At study initiation, dosing duration was set at 2 weeks but was increased empirically when the first patient treated with narsoplimab experienced a clinical and laboratory-marker recurrence after cessation of treatment at 2 weeks, subsequently resolving with an additional week of dosing. All patients received routine supportive care per our hospital's guidelines at the time of the study, including prophylactic enoxaparin (Clexane, Sanofi Aventis) 4,000 IU/0.4 mL, azithromycin (Zitromax,Pfizer SpA, Italy) 500 mg once daily, hydroxychloroquine (Plaquenil, Sanofi Aventis) 200 mg twice daily, darunavir and cobicistat (Rezolsta, Janssen-Cilag S.p.A., Italy) 800/150 mg once daily. Beginning March 27, 2020 per updated institutional guidelines, all COVID-19 patients in our hospital received methylprednisolone 1 mg/kg, which was administered to five of the six narsoplimab-treated patients. All respiratory support was provided according to institutional treatment algorithms.

In addition to CEC counts and cytokine levels, clinical and laboratory measures, including blood counts, LDH and C-reactive protein (CRP) levels, were collected on all narsoplimab-treated patients per standard clinical practice. Routine blood examinations were collected prior to each narsoplimab dose and then twice weekly. Respiratory function was evaluated daily. All patients received chest computed tomography (CT) at hospital admission to document interstitial pneumonia and, if clinically indicated, during hospitalization to document pulmonary embolism.

2.7. Statistical analysis

Demographic and clinical patient data are presented as frequency with percentage for categorical variables and median with range for

continuous ones. Difference in CEC value between normal and COVID-19 patients was assessed with Mann-Whitney *U* test Repeated measures analysis was performed to test differences in CEC and cytokine levels during narsoplimab treatment at appropriate timepoints; non-parametric Friedman test was used, and pairwise-comparisons were performed using paired Wilcoxon signed-rank test. Decreasing trend of LDH and CRP levels during treatment were evaluated with non-parametric Spearman test between the observations and time. Significance at 5% was fixed. Analysis was performed using R software (version 3.6.2).

3. Results

3.1. Treatment with narsoplimab

Table 1 summarizes the clinical characteristics of the six narsoplimab-treated patients. Median age was 56.5 years, and 83 % were male. All patients were overweight or obese based on a body mass index (BMI) ≥25 and ≥30, respectively. At enrollment, all had pneumonia/ARDS requiring CPAP with two patients rapidly deteriorating and requiring intubation soon after enrollment. Narsoplimab treatment was started within 48 h of CPAP initiation.

Fig. 2 summarizes the clinical outcomes observed in these narsoplimab-treated patients.

In four patients, enoxaparin was given at therapeutic doses (100 IU/kg twice daily) due to CT scan-documented pulmonary thromboses (patients #4 and #6), medical decision (patient #3) or rapidly deteriorating respiratory function requiring intubation (patient #5). Median follow-up was 27 days (range 16–90), and patients were administered narsoplimab twice weekly with a median of 8 total narsoplimab doses (range 5–8). Following treatment, all patients improved clinically. Four patients (67 %) reduced ventilatory support from CPAP to non-rebreather or Venturi oxygen mask after a median of 3 narsoplimab doses (range 2–3). In three of these patients, oxygen support was weaned and then discontinued, and discharge followed a median of 6 (range 5–8) total narsoplimab doses. In patient #4, a contrast-enhanced CT scan documented massive bilateral pulmonary thromboses 4 days following enrollment. Enoxaparin was added to ongoing narsoplimab dosing, and rapid clinical and radiographic (repeat CT scan) improvement was documented 11 days later (Fig. 3), subsequently allowing discharge from the hospital. In the two remaining patients (#5 and #6), rapidly worsening severe ARDS was documented soon after enrollment. In patient #5, severe ARDS (PaO_2/FiO_2 of 55) required intubation at day 4. Nonetheless, subsequent clinical outcome was rapidly favorable, and the patient was discharged from the intensive care unit after 3 days.

Following 2 days of CPAP, he stabilized with low-flow oxygen support and was subsequently discharged from the hospital. Patient #6 had PaO_2/FiO_2 of 60 and severe ARDS at enrollment, requiring intubation 2 days later. Her course was complicated by massive bilateral pulmonary thromboses and nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) infection. Her condition improved and, after 18 days, she was extubated, tracheostomized and supported with oxygen. Her MRSA progressively improved, oxygen support was removed and, at day 85, she was discharged. No treatment-related adverse events were reported in this study.

3.2. Thrombosis, endothelial cell damage, and inflammatory markers

From March 13 through March 16, 2020 soon after the COVID-19 outbreak began in the Bergamo area, our hospital's pathology department performed autopsies on an initial group of 20 deceased patients. Prior to their deaths, all of these patients, as did the patients treated with narsoplimab in the current study, required advanced respiratory support with CPAP or invasive mechanical ventilation. Consistent with the clinical picture of frequently lethal pulmonary thromboembolism, the lungs and liver of most patients showed extensive thromboses.

Table 1
Features of the six study patients at enrollment.

Clinical characteristics	All patients (N = 6)
Age – years, median (range)	56.5 (47–63)
Sex – no. (%)	
Female	1 (17)
Male	5 (83)
Weight – kilograms, median (range)	86 (82–100)
BMI – kilograms/m^2; median (range)	28 (26.8–32)
Time from symptom onset to hospital admission – days, median (range)	8.5 (3–12)
Fever[#] **on admission to the hospital** – no. (%)	6 (100)
Other Symptoms – no. (%)	
Cough	1 (17)
Anorexia	2 (33)
Fatigue	4 (67)
Shortness of breath	5 (83)
Nausea or vomiting	1 (17)
Diarrhea	2 (33)
Headache	1 (17)
Coexisting disorder – no. (%)	
Diabetes	1 (17)
Hypertension	1 (17)
Dyslipidemia	2 (33)
Obesity (BMI ≥ 30 kg/m^2)	2 (33)
Overweight (BMI ≥ 25 kg/m^2)	4 (66)
ARDS severity at enrollment – no. (%)	
Mild	3 (50)
Moderate	2 (33)
Severe	1 (17)
Time from hospitalisation to start of treatment – days, median (range)	2 (1–4)
Time from CPAP initiation to start of treatment – no. (%)	
0–24 h	4 (67)
24–48 h	2 (33)
Radiologic findings	
Abnormality on chest radiography – no. (%)	
Bilateral interstitial abnormalities	6 (100)
Laboratory findings	
PaO_2:FiO_2 ratio – median (range)	175 (57.5–288)
Circulating endothelial cell count – median (range)	334 (0–9315)
White cell count – per mm^3, median (range)	8335 (6420–10,120)
>10,000 per mm^3 – no. (%)	2 (33)
<4000 per mm^3 – no. (%)	0 (0)
Lymphocyte count – per mm^3, median (range)	875 (410–1290)
Platelet count – x 10^3 per mm^3, median (range)	282 (199–390)
Distribution of other findings (laboratory reference ranges)	
C-reactive protein (0.0–1.0 mg/dL)	14 (9.5–31.3)
Lactate dehydrogenase (120/246 U/L)	518.5 (238–841)
Aspartate aminotransferase (13–40 U/L)	78.5 (51–141)
Alanine aminotransferase (7–40 U/L)	73 (37–183)
Creatinine (0.3–1.3 mg/dL)	0.85 (0.38–1.33)
D-dimer[*] (<500 ng/mL)	1250.5 (943–1454)
Haptoglobin (36–195 mg/dL)	368.5 (270–561)
Complement C3[**] (79–152 mg/dL)	101 (60–126)
Complement C4[**] (16–38 mg/dL)	21 (2–37)
Concomitant treatments	
Antiretroviral therapy – no. (%)	
Darunavir + Cobicistat	6 (100)
Systemic steroid therapy – no. (%)	5 (83)
After the 1st dose of narsoplimab	2 (33)
After the 2nd dose of narsoplimab	1 (17)
After the 3rd dose of narsoplimab	1 (17)
After the 4th dose of narsoplimab	1 (17)

ARDS: Acute Respiratory Distress Syndrome; ICU: Intensive Care Unit; CPAP: Continuous Positive Airway Pressure.

[#] Defined as body temperature >37.5 °C.

[*] Data available only for 4 patients.

[**] Data available only for 5 patients.

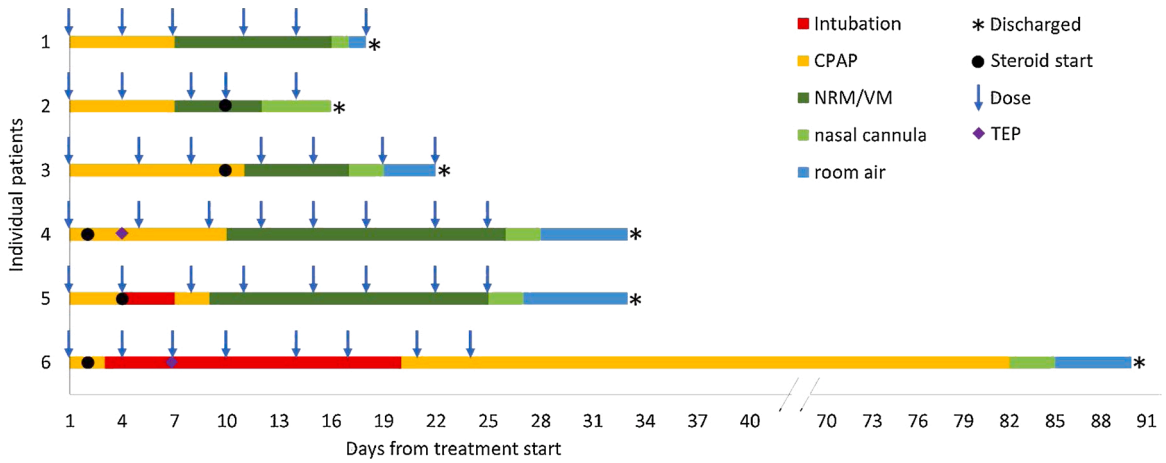

Fig. 2. Clinical outcome of patients treated with narsoplimab. The bar colors indicate the different oxygen support (CPAP, yellow; mechanical ventilation with intubation, red; non-rebreather oxygen mask, green; low flow oxygen by nasal cannula, light green; room air, blue). Narsoplimab doses are marked by blue arrows. Black circles indicate the beginning of steroid treatment (day +2 in patients #4 and #6, day +4 in patient #5 and day +10 in patient #2 and #3). Black asterisk denotes discharge from hospital. CPAP = continuous positive airway pressure; NRM = non-rebreather oxygen mask; VM = Venturi mask; TEP = pulmonary thromboembolism.

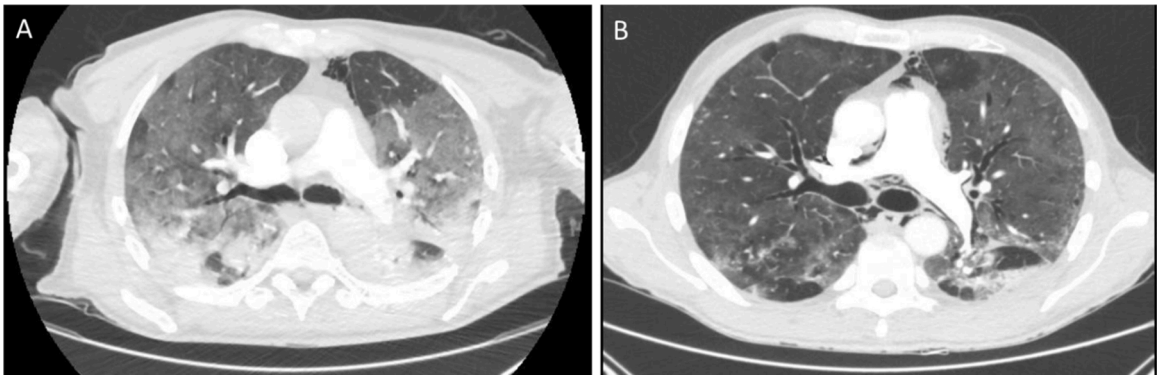

Fig. 3. Serial CT-scan images obtained on a 59-year-old man (patient #4) with COVID-19 pneumonia treated with narsoplimab. (Panel A) *Day 5 after enrollment*: Severe interstitial pneumonia with diffuse ground-glass opacity involving both the peripheral and central regions. Consolidation in lower lobes, especially in the left lung. Massive bilateral pulmonary emboli with filling defects in interlobar and segmental arteries (not shown). (Panel B) *Day 16 after enrollment.* Ground-glass opacity significantly reduced with almost complete resolution of parenchymal consolidation. "Crazy paving" pattern with peripheral distribution, especially in the lower lobes. Evident pneumomediastinum. Minimal filling defects in subsegmental arteries of right lung (not shown).

Histologically, arterial thromboses were evident in septal vessels of the lung, including areas unaffected by the destructive inflammatory process. Immunohistochemical staining for CD34 (an endothelial cell marker) demonstrated severe endothelial damage with cell shrinkage, degenerated hydropic cytoplasm and adhesion of lymphocytes to endothelial surfaces (Fig. 4).

Based on these initial observations and published findings in acute graft-versus-host disease (GVHD) in which immune-mediated attack of vascular endothelial cells leads to their detachment from the vessel wall and release into circulation (Almici et al., 2017), prior to initiation of the study with narsoplimab we began measuring CEC counts in a non-study cohort of molecularly confirmed COVID-19 patients randomly selected in our hospital. In this non-study cohort of 33 COVID-19 patients, we found that CEC/mL of peripheral blood (median 110, range 38–877) were significantly increased compared to healthy controls (median 7, range 0–37) (P = 0.0004), (Fig. 5).

Because our hospital established guidelines implementing standard steroid use for COVID-19 patients 16 days after the initiation of this study, five of the six narsoplimab study patients received steroid treatment as supportive therapy, beginning 2–10 days following initiation of narsoplimab dosing (Fig. 2). For this reason, CEC counts were also evaluated in a separate group of four patients (all female, median age 83 years with a range of 62–90 years, three requiring oxygen by mask and one on CPAP) receiving only steroids. In these four patients, the CEC counts evaluated after 48 h were found to be unaffected by steroid administration (p = 0.38). In two additional patients receiving only steroids, CEC counts were evaluated at baseline and after 4 weeks of steroid-inclusive supportive treatment. In the first patient, whose clinical course progressively worsened, CEC counts remained unaffected (271/mL vs. 247/mL) while, in the second, clinical improvement was accompanied by a simultaneous decrease of CEC (165 vs. 65/mL).

In the six narsoplimab-treated patients, CEC/mL were markedly increased at baseline (median 334, range 0−9315). With narsoplimab, CEC counts rapidly decreased after the second (median 92 CEC/mL, range 18–460, 6 patients), fourth (median 72.5, range 0–593, 6 patients) and sixth (median 59, range 15–276, 4 patients) doses of treatment (p = 0.01) (Fig. 5). Serum concentrations of IL-6, IL-8, CRP and LDH also markedly decreased with narsoplimab treatment (Fig. 6).

4. Discussion

The findings in this study further indicate that endothelial injury is central to the pathophysiology of COVID-19-related lung injury (Varga et al., 2020; Green, 2020; Ackermann et al., 2020; Teuwen et al., 2020;

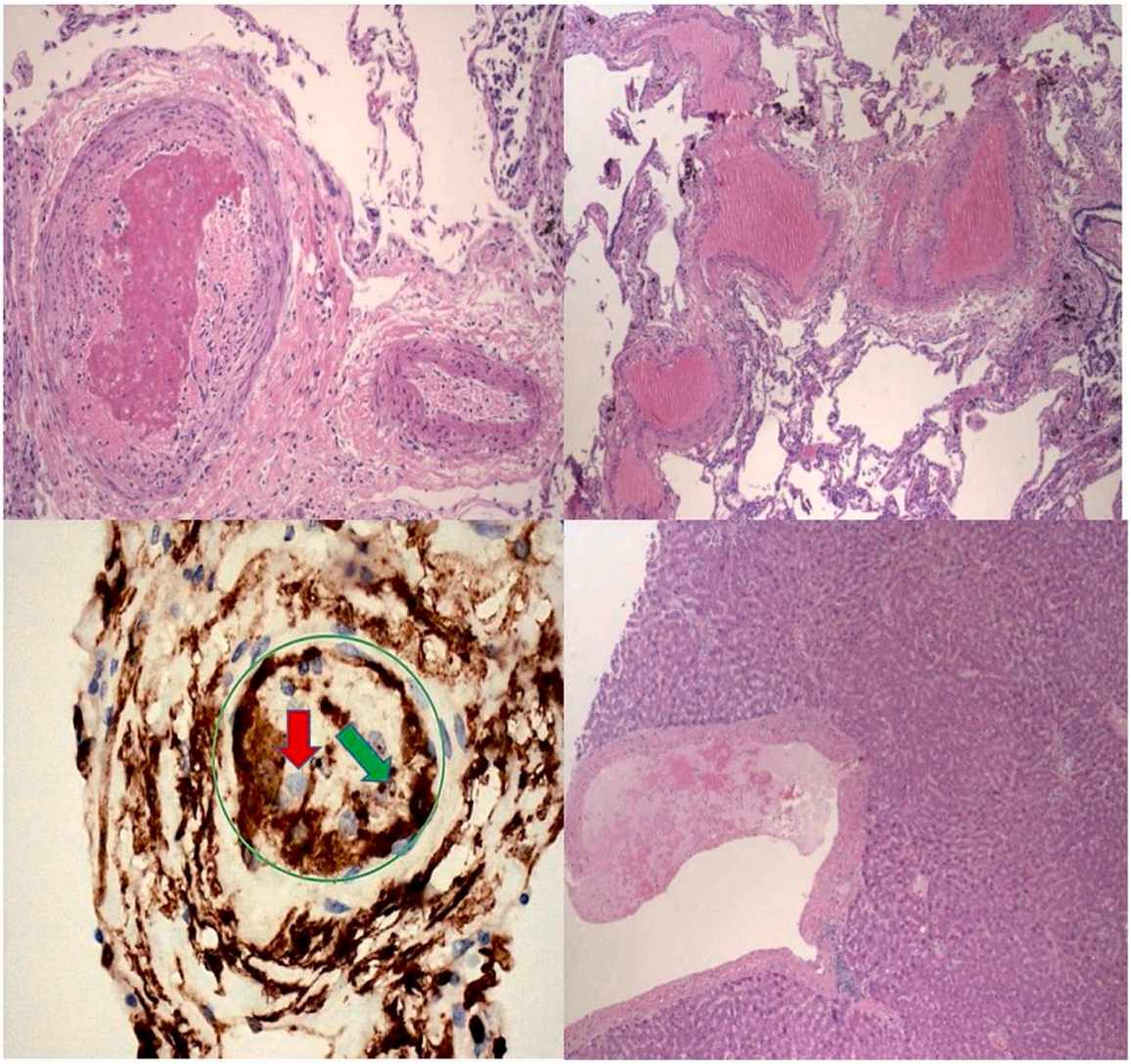

Fig. 4. Vascular damage in COVID-19 patients. Arterial involvement by thrombotic process in septal blood vessels of the lung; note initial organization of thrombus in the arterial lumen (upper left) (H&E, 400×). Similar pathologic features are extensively notable in most septal vessels in lung areas unaffected by the destructive inflammatory process (upper right) (H&E, 400×). Medium-diameter lung septal blood vessel (green circle) with complete lumen thrombosis; immunohistochemical brown staining for CD34 (endothelial cell marker) demonstrates severe endothelial damage with cell shrinkage, degenerated hydropic cytoplasm (green arrow) and adhesion of lymphocytes on endothelial cell surfaces (red arrow, bottom left). Vascular alteration also observed in liver parenchyma with large-vessel lumens partially obstructed by thrombosis (bottom right) (H&E, 400×).

Goshua et al., 2020) and provide previously unreported evidence that MASP-2 and the lectin pathway may be important in the pathophysiology of COVID-19. Patients with severe respiratory failure demonstrated not only markedly elevated levels of CRP and LDH (Guan et al., 2020) but also IL-6 (Gritti et al., 2020), IL-8 and CEC. The novel observation of elevated CEC is consistent with the histopathological findings in the lung and liver showing marked endothelial injury and thrombosis in COVID-19 patients. The multi-organ microvascular histopathological changes, specifically the formation of microvascular thrombi, resemble those of HSCT-TMA, further supporting the role of endothelial injury in COVID-19-related pulmonary injury. In HSCT-TMA, endothelial injury can result from conditioning regimens, immunosuppressants, GVHD and infections (Ackermann et al., 2020; Varga et al., 2020). In COVID-19, endothelial injury appears to be caused by direct viral infection. Given the known relationship between endothelial injury and multi-organ thrombotic microangiopathy in HSCT-TMA and the mounting evidence for a similar relationship in COVID-19, it is reasonable that the pathophysiologic events that follow endothelial injury – and lead to diffuse TMA – are also similar. Endothelial injury, regardless of cause, activates the lectin pathway of complement on the endothelial cell surface (Collard et al., 2000). In its pivotal HSCT-TMA trial, the MASP-2 inhibitor narsoplimab demonstrated marked improvement in laboratory and clinical endpoints, including survival (Rambaldi et al., 2020). In the current study, inhibition of MASP-2 and the lectin pathway by narsoplimab was associated with clinical improvement and survival in all COVID-19 patients treated with the drug.

The lectin pathway of complement is part of the innate immune response. Activation of the lectin pathway is initiated by members of the MASP enzyme family (MASP-1, MASP-2 and MASP-3) (Schwaeble et al., 2002), which complex in blood with lectins, specifically mannan-binding lectin, the ficolins and collectins. These lectins recognize and bind to carbohydrate patterns found on surfaces of pathogenic microorganisms or injured host cells, including damaged endothelial cells (Collard et al., 2000), targeting MASPs to their site(s) of action and leading to their activation.

MASP-2, the key enzyme responsible for lectin pathway activation, binds and undergoes activation by the COVID-19 N protein (Gao et al.,

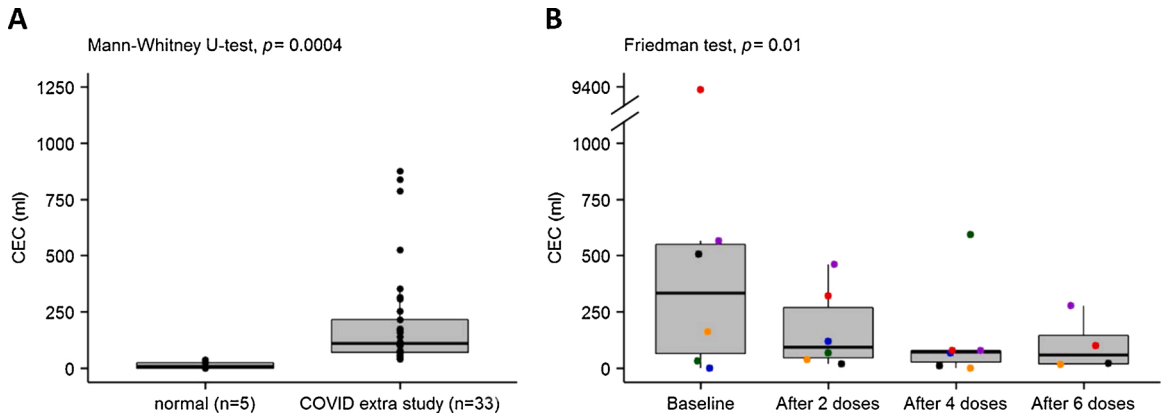

Fig. 5. Circulating endothelial cells in normal controls and COVID-19 patients. Boxes represent values from the first to the third quartile, horizontal line shows the median value, and dots show all patient values. (Panel A) CEC counts evaluated in healthy controls (n = 5) and in COVID-19 patients not selected for this study (n = 33). (Panel B) CEC counts evaluated at baseline and after the second (6 patients), fourth (6 patients) and sixth dose (4 patients) of treatment with narsoplimab. Each patient is identified by a specific color: #1, green, #2 blue, #3 yellow, #4 purple, #5 red, #6 black. In patient #1, CEC count evaluated after the 5th dose of narsoplimab was 63/mL (not shown).

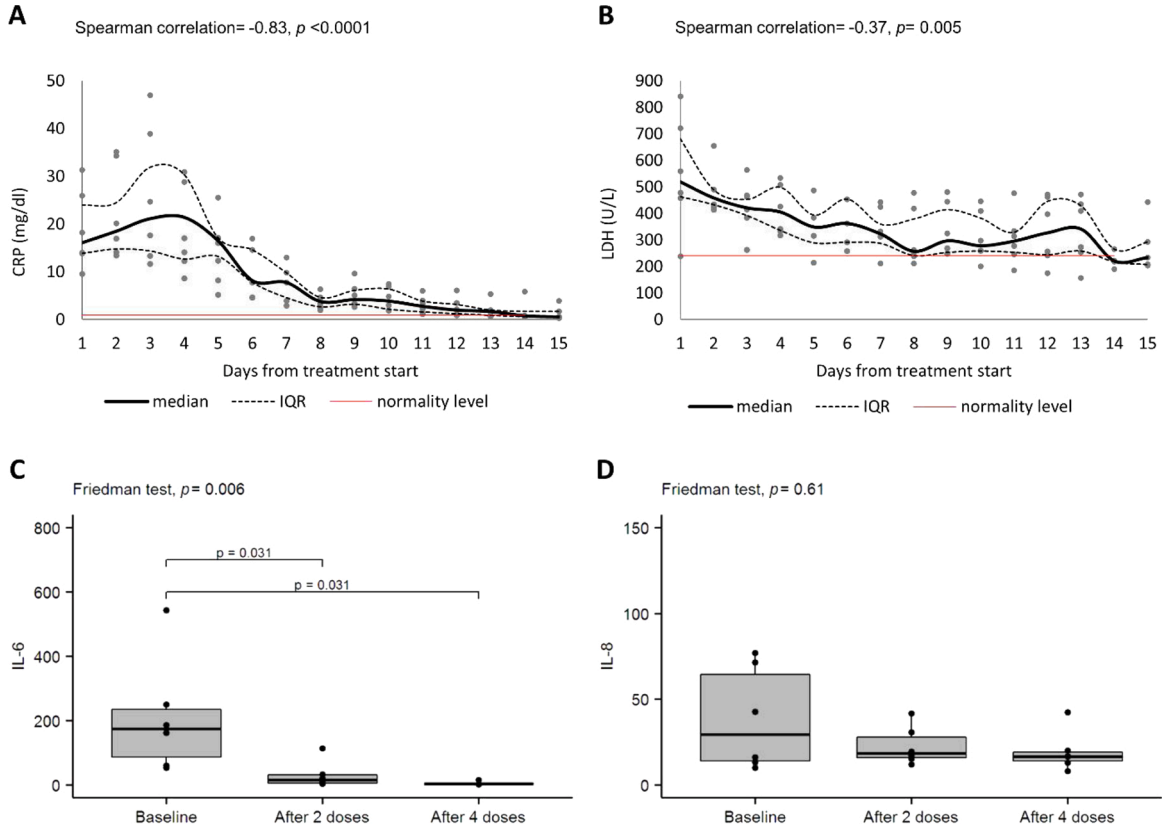

Fig. 6. Serum levels of lactate dehydrogenase (LDH), C-reactive protein (CRP), IL-6 and IL-8 at baseline and at different time points after narsoplimab treatment. (Panel A and B) CRP and LDH levels at each day following treatment start. Black lines represent median and interquartile range (IQR), red line represents normality level and dots show all patient values. (Panel C and D) IL-6 and IL-8 at baseline and after treatment (second and fourth or fifth doses) with narsoplimab. Boxes represent values from the first to the third quartile, horizontal line shows the median value, and dots show all patient values. Only significant pairwise-comparisons are shown.

2020) and has been found in the microvasculature of lung tissue in patients with severe COVID-19 (Magro et al., 2020). Activated MASP-2 initiates a series of enzymatic steps that results in production of the anaphylatoxins C3a and C5a and in formation of the membrane attack complex C5b-9 (Dobo et al., 2018), which can induce proinflammatory responses and cause cell lysis and death. MASP-2 can also cleave C3 directly through the C4 bypass (Yaseen et al., 2017). Importantly, MASP-2 is located upstream in the lectin pathway, so inhibition of MASP-2 does not interfere with the lytic arm of the classical pathway (i.e., C1r/C1s-driven formation of the C3 and C5 convertases), preserving the adaptive immune response needed to fight infection (Schwaeble et al., 2011) (Fig. 1).

In addition to its role in complement, MASP-2 acts directly on the coagulation cascade and the contact system, cleaving prothrombin to thrombin and forming fibrin clots (Gulla et al., 2010; Krarup et al., 2007). Narsoplimab not only inhibits lectin pathway activation but also

blocks microvascular injury-associated thrombus formation as well as MASP-2-mediated activation of kallikrein and factor XII (Demopulos et al., 2020; Omeros Corporation, 2013a). These activities could contribute to beneficial effects by inhibiting microvascular thrombosis, which may have played an important therapeutic role in the narsoplimab-treated patients, particularly those who suffered massive pulmonary thromboses. Narsoplimab does not prolong bleeding time nor does it affect prothrombin or activated partial thromboplastin times (Omeros Corporation, 2019; 2013b), and no bleeding was observed in the patients we treated. It appears that narsoplimab may block coagulation resulting from endothelial damage (associated with factor XII activation) but not extracellular matrix-related (factor VII-driven) coagulation. Additional studies are underway to determine in more detail the mechanism(s) by which narsoplimab affects coagulation.

Lectin pathway inhibition has not previously been investigated as a treatment for COVID-19. All patients in this study had COVID-19-related respiratory failure. Following treatment with the MASP-2 inhibitor narsoplimab, all patients recovered and were able to be discharged from the hospital, further supporting the importance of the lectin pathway in COVID-19 pathophysiology. In each case, COVID-19 lung injury had progressed to ARDS requiring CPAP prior to narsoplimab treatment. Two patients continued to deteriorate following the first dose and required invasive mechanical ventilation. Both patients were subsequently able to discontinue invasive mechanical ventilation with continued narsoplimab treatment. Two patients (one intubated and the other on CPAP) experienced massive bilateral pulmonary thromboses, and both patients completely recovered with narsoplimab, possibly benefitting from the drug's anticoagulant effects. The temporal patterns of laboratory markers (CEC, IL-6, IL-8, CRP and LDH) were consistent with the observed clinical improvement and with the hypothesized mechanism of action. In particular, CEC counts appear to be a reliable tool to evaluate endothelial damage and treatment response in this disease. The temporal improvement of IL-6 and IL-8 with narsoplimab treatment suggests that lectin pathway activation may precede cytokine elevation in COVID-19 and that lectin pathway inhibition has a beneficial effect on the cytokine storm described in patients with COVID-19 infection (Xiong et al., 2020). Two weeks of narsoplimab dosing was planned initially but was increased to 3–4 weeks following the rise in CEC in patient #1 when dosing was first discontinued. With the third week of dosing, the patient's CEC counts again improved (63/mL after the fifth dose). Rebound pulmonary signs and symptoms have not been observed following 4 weeks of narsoplimab treatment. No narsoplimab-related adverse events were observed.

Use of other complement inhibitors in COVID-19 have been reported. AMY-101, a compstatin-based C3 inhibitor (Mastaglio et al., 2020), was used in one patient and eculizumab was administered together with antiviral and anticoagulant therapy to four patients (Diurno et al., 2020). These five patients were on CPAP and survived. Two COVID-19 patients on high-flow nasal oxygen received a C5a antibody in conjunction with supportive therapy, including antiviral therapy, following steroid treatment. These two patients also survived (Gao et al., 2020). Collectively, these reports support our findings with narsoplimab. Notably, unlike C3 and C5 inhibitors, the MASP-2 antibody narsoplimab fully maintains classical complement pathway function and does not interfere with the adaptive immune response or the antigen-antibody complex-mediated lytic response (Schwaeble et al., 2011). No evidence of narsoplimab-related infection risk has been observed in narsoplimab clinical trials.

While this was a compassionate use, single-arm study, two different control groups provide a retrospective comparison. The first was described in a recently published article by Gritti et al. (Gritti et al., 2020) evaluating the use of siltuximab, an IL-6 inhibitor, in COVID-19 patients. The siltuximab study and our narsoplimab study share the same lead investigators (G.G. and A.R.), entry criteria and patient characteristics (i.e., demographics, symptoms, comorbidities, ARDS severity, laboratory values and respiratory support at enrollment). In that study, mortality rates in the siltuximab-treated and the control groups were 33 % and 53 %, respectively. The second retrospective comparator is represented by the 33 patients who were randomly selected within our hospital to assess the viability of CEC measurements in COVID-19 patients. Of these 33 patients, 22 met the same entry criteria and had similar baseline characteristics as the narsoplimab-treated patients. Median baseline CEC count, however, in the control group compared to that in the narsoplimab-treated group was 101/mL versus 334/mL, respectively. Interestingly, 20 of these 22 patients (91 %) were treated with IL-6 inhibitors (tocilizumab or siltuximab) and/or steroids, and the group had an overall 30-day mortality of 32 %. The mortality rate was still 31 % when the outcome analysis was restricted to 16 patients matched for age to narsoplimab-treated patients (median 58 years, range 51–65 years). In this latter group, 94 % received IL-6 and/or steroid therapy and the median baseline CEC count at 55/mL was six-fold lower than in the narsoplimab-treated patients.

The use of steroids in COVID-19 has resulted in reports of mixed outcomes (Veronese et al., 2020). Most recently, the Randomised Evaluation of COVID-19 therapy (RECOVERY) trial, demonstrated that dexamethasone reduced 28-day mortality in patients on invasive mechanical ventilation by 28.7 % (29.0 % versus 40.7 % with usual care), by 14 % (21.5 % versus 25.0 % with usual care) in those receiving oxygen support without invasive mechanical ventilation and had no effect on mortality in patients not receiving respiratory support at randomization (17.0 % versus 13.2 % with usual care) (Horby et al., 2020). Based on these data and the experience at our hospital, we believe that steroids have a role to play in treating COVID-19 patients with respiratory dysfunction, acting to tamp down the inflammatory response. In the narsoplimab-treated group, one (patient #1) of the six patients did not receive steroids. Subsequently, in late March 2020, institutional guidelines were updated, requiring that all patients in our hospital receive steroids. Of the five narsoplimab-treated patients who received steroids, two (patients #2 and #3) initiated them after already improving such that CPAP was no longer required or was discontinued the following day (Fig. 2). As described previously, we evaluated CEC counts in a separate group of four patients receiving only steroids for a short duration, and the counts were found to be unaffected by steroid administration. This suggests that any beneficial effect of steroids on COVID-19-associated endothelial damage may be delayed and had little effect on the recovery course of patients #2 and #3.

Our findings have several limitations. First, this is a small, uncontrolled case series and patients were heterogeneous in clinical presentation. Second, although COVID-19 treatment was standardized at our institution, data collection in this compassionate-use program was not prospectively defined. Third, the treatment regimen was empirical, and it is not known if a longer or more frequent treatment regimen would affect our findings. Finally, the narsoplimab-treated patients received other therapies as part of supportive care.

Despite these limitations, our findings strongly suggest that endothelial injury-induced activation of MASP-2 and the lectin pathway play a central role in the pathophysiology of COVID-19-related lung injury. The improvements in clinical status and laboratory findings following narsoplimab treatment are notable. While not definitive, these findings strongly suggest meaningful clinical efficacy and provide supportive evidence related to the drug's mechanism of action and the pathophysiology of the disease. Lectin pathway inhibition by narsoplimab appears to be a promising treatment of COVID-19-related lung injury and endothelial damage-associated thromboses, and further investigation is warranted.

Authorship contributions

GG, MCM, MF, GB, ASa, FL, CP, FB, SF, FDM, LL, GR: Patient data collection and acquisition; analysis & interpretation of data

ASo, AG: Histopathology analysis

AR, SW, GD: Conception and design of the study; wrote the manuscript.

Transparency document

The Transparency document associated with this article can be found in the online version.

Declaration of Competing Interest

Since the Editor-in-Chief of Immunobiology, Hans-Wilhelm Schwaeble, has been collaborating with and consulting for Omeros Corporation, this manuscript was edited by Associate Editor Teizo Fujita, who has no conflict of interest to declare.

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SW, GD: employed by and holds stock in Omeros Corporation.

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