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GM-CSF Neutralization With Lenzilumab in Severe COVID-19 Pneumonia: A Case-Cohort Study

Zelalem Temesgen, MD; Mariam Assi, MD; F.N.U. Shweta, MBBS; Paschalis Vergidis, MD; Stacey A. Rizza, MD; Philippe R. Bauer, MD, PhD; Brian W. Pickering, MD; Raymund R. Razonable, MD; Claudia R. Libertin, MD; Charles D. Burger, MD; Robert Orenstein, DO; Hugo E. Vargas, MD; Raj Palraj, MBBS; Ala S. Dababneh, MD; Gabrielle Chappell; Dale Chappell, MD, MBA; Omar Ahmed, PharmD; Reona Sakemura, MD, PhD; Cameron Durrant, MD, MBA; Saad S. Kenderian, MD; and Andrew D. Badley, MD

Abstract

Objective: To assess the efficacy and safety of lenzilumab in patients with severe coronavirus disease 2019 (COVID-19) pneumonia.

Methods: Hospitalized patients with COVID-19 pneumonia and risk factors for poor outcomes were treated with lenzilumab 600 mg intravenously for three doses through an emergency single-use investigational new drug application. Patient characteristics, clinical and laboratory outcomes, and adverse events were recorded. We also identified a cohort of patients matched to the lenzilumab patients for age, sex, and disease severity. Study dates were March 13, 2020, to June 18, 2020. All patients were followed through hospital discharge or death.

Results: Twelve patients were treated with lenzilumab; 27 patients comprised the matched control cohort (untreated). Clinical improvement, defined as improvement of at least 2 points on the 8-point ordinal clinical endpoints scale, was observed in 11 of 12 (91.7%) patients treated with lenzilumab and 22 of 27 (81.5%) untreated patients. The time to clinical improvement was significantly shorter for the lenzilumab-treated group compared with the untreated cohort with a median of 5 days versus 11 days (P=.006). Similarly, the proportion of patients with acute respiratory distress syndrome (oxygen saturation/fraction of inspired oxygen<315 mm Hg) was significantly reduced over time when treated with lenzilumab compared with untreated (P<.001). Significant improvement in inflammatory markers (C-reactive protein and interleukin 6) and markers of disease severity (absolute lymphocyte count) were observed in patients who received lenzilumab, but not in untreated patients. Cytokine analysis showed a reduction in inflammatory myeloid cells 2 days after lenzilumab treatment. There were no treatment-emergent adverse events attributable to lenzilumab.

Conclusion: In high-risk COVID-19 patients with severe pneumonia, granulocyte-macrophage colony-stimulating factor neutralization with lenzilumab was safe and associated with faster improvement in clinical outcomes, including oxygenation, and greater reductions in inflammatory markers compared with a matched control cohort of patients hospitalized with severe COVID-19 pneumonia. A randomized, placebo-controlled clinical trial to validate these findings is ongoing (NCT04351152).

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Affiliations are at the end of this article.

The clinical manifestations of coronavirus disease 2019 (COVID-19), the disease caused by severe acute respiratory coronavirus 2 (SARS-CoV-2) infection, range from asymptomatic disease to severe pneumonia.[1,2] Although viral evasion of host immune response and virus-induced cytopathic effects are believed

to be critical for disease progression, most deaths associated with COVID-19 are attributed to the development of an immune hyper-response and resultant acute respiratory distress syndrome (ARDS) and multi-organ failure.[3]

The immune hyper-response is characterized by an elevation of inflammatory cytokines resulting in fever, hypotension, capillary leak syndrome, pulmonary edema, disseminated intravascular coagulation, respiratory failure, and ARDS.[4,5] A similar immune hyper-stimulation has been previously described in patients with autoimmune and lymphoproliferative diseases,[6] as well as in patients with B-cell malignancies receiving chimeric antigen receptor T-cell (CART) therapy, and has been named cytokine release syndrome (CRS).[7,8] Over the last 5 years, preclinical studies and correlative science from clinical trials in CART therapy have shed light on the pathophysiology, development, characterization, and management of CRS.[5,9]

Cytokine release syndrome during CART therapy is characterized by activation of myeloid cells and release of inflammatory cytokines and chemokines, including interleukin-6 (IL-6), granulocyte-monocyte colony-stimulating factor (GM-CSF), monocyte chemoattractant protein -1 (MCP-1), macrophage inflammatory protein 1α (MIP-1α), Interferon gamma-induced protein 10 (IP-10), and IL-1.[4,7,10] The cascade, once initiated, can quickly evolve into a cytokine storm, resulting in further activation, expansion, and trafficking of myeloid cells, leading to abnormal endothelial activation, increased vascular permeability, and disseminated intravascular coagulation.[11,12]

Similar to CRS in patients receiving CART therapy, the immune hyper-response in patients with COVID-19 has been associated with elevation of C-reactive protein (CRP), ferritin, and IL-6, as well as correlating with respiratory failure, ARDS, and adverse clinical outcomes.[13-17] Most significantly, high levels of GM-CSF–secreting T cells (Th^{GM} cells) have been associated with disease severity, myeloid cell trafficking to the lungs, and intensive care unit (ICU) admission.[18] The elevation in inflammatory cytokine levels indicates that post–COVID-19 immune hyper-stimulation is caused by a similar mechanism, induced by activation of myeloid cells and their trafficking to the lung, resulting in lung injury and ARDS.[18] Tissue CD14+ myeloid cells produce GM-CSF and IL-6, further triggering a cytokine storm cascade.[18] Single-cell RNA sequencing of bronchoalveolar lavage samples from COVID-19 patients with severe ARDS demonstrated an overwhelming infiltration of newly arrived inflammatory myeloid cells compared with mild COVID-19 disease and healthy controls, consistent with a hyper-inflammatory immune-mediated pathology.[19]

With this understanding of the pathophysiology of COVID-19, modalities to target inflammatory cytokines and suppress or prevent immune hyper-stimulation after COVID-19 have been investigated in pilot clinical trials. Controlled clinical trials using IL-6 blockade as well as other immunomodulatory molecules targeting receptor tyrosine kinase are ongoing.

Our group has developed GM-CSF depletion as a strategy to mitigate CRS following CART therapy. We have shown that GM-CSF neutralization results in a reduction in IL-6, MCP-1, macrophage inflammatory protein 1 alpha (MIP-1α), IP-10, vascular endothelial growth factor (VEGF), and tumor necrosis factor-α (TNFα) levels, showing that GM-CSF is an upstream regulator of many inflammatory cytokines that are important in the pathophysiology of CRS.[20] GM-CSF depletion results in modulation of myeloid cell behavior, a specific decrease in their inflammatory cytokines, and a reduction in tissue trafficking,[20] while enhancing T-cell apoptosis machinery.[21] These biological effects prevented both CRS and neuroinflammation after CART therapy in preclinical models and are being tested in a phase Ib/II clinical trial (NCT 04314843).

Lenzilumab is a first-in-class recombinant monoclonal antibody targeting human GM-CSF, with potential immunomodulatory activity, high binding affinity in the

picomolar range, 94% homology to human germline, and low immunogenicity. Following intravenous administration, lenzilumab binds to and neutralizes GM-CSF, preventing GM-CSF binding to its receptor, thereby preventing GM-CSF—mediated signaling to myeloid progenitor cells.[22] Lenzilumab has been studied across four completed clinical trials in healthy volunteers and patients with asthma, rheumatoid arthritis, and chronic myelomonocytic leukemia.[23,24] A total of 113 individuals received lenzilumab in these trials. Lenzilumab was well tolerated with a low frequency and severity of adverse events.[23,24]

Given the hypothesized role of GM-CSF in the pathogenesis of COVID-19—related immune hyper-response, along with our studies showing that GM-CSF depletion prevents CRS and modulates myeloid cell behavior in preclinical models,[20] lenzilumab therapy was offered to patients hospitalized with severe COVID-19 pneumonia who had clinical and/or biomarker evidence for increased risk of progression to respiratory failure.

METHODS

Patients

Hospitalized patients with COVID-19 confirmed by reverse transcriptase-polymerase chain reaction for the SARS-CoV-2 and radiographic findings consistent with COVID-19 pneumonia were considered for treatment with lenzilumab through an emergency investigational new drug (IND) program. Active systemic infection with bacteria, fungi, or other viruses was an exclusion criterion. Informed consent and Institutional Review Board approval was obtained for each patient. A request for lenzilumab under a US Food and Drug Administration (FDA) emergency use IND program was submitted to the FDA in accordance with agency guidelines.[25] A control cohort of patients who did not receive lenzilumab (untreated) was identified from an electronic registry of more than 1900 COVID-19 patients in the same health care centers as the lenzilumab-treated patients, and were matched to cases on sex and age within a tolerance of 5 years. Patients in the untreated group were further matched to patients in the lenzilumab group for disease severity (hospitalized with COVID-19 pneumonia, at least one risk factor for poor outcome from COVID-19, and required oxygen supplementation without mechanical ventilation). At the time of their selection for the untreated group, the clinical outcomes of these patients were not known. Lenzilumab-treated patients (treated) received lenzilumab 600 mg administered via a 1-hour intravenous infusion every 8 hours for a total of three doses (1800 mg). Study dates were March 13, 2020, to June 18, 2020. All patients (lenzilumab-treated and untreated) were followed through hospital discharge or death.

Study Assessments

All laboratory tests and radiologic assessments were performed at the discretion of the treating physician and per standard clinical management processes. Vital signs were monitored before and upon completion of each lenzilumab infusion. Demographics, co-existing conditions, laboratory and radiographic data, as well as clinical data, adverse events, and outcomes were captured from the electronic health record until discharge or death. Similarly, for lenzilumab-treated patients, data were collected up to the date of discharge or death. For untreated patients, baseline was considered their first day of hospitalization. Baseline values for the lenzilumab-treated group were defined as those values obtained before lenzilumab administration, either on the day of administration for patients who receive lenzilumab on the first day of hospital admission or the day before the administration for patients that received lenzilumab after the first day of admission. Cytokine analysis was performed on serum isolated from one patient who had samples available pre- and post-lenzilumab treatment. Serum was diluted 1:2 with human serum matrix before following the manufacturer's protocol for Milliplex Human Cytokine/Chemokine MAGNETIC BEAD Premixed 38 Plex Kit

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TABLE 1. Demographics and Baseline Characteristics[a,b]

Characteristics	Lenzilumab group (n=12)	Control group (n=27)	*P*
Age (range), years	65 (52-70)	68 (61-76)	.25
Male	8 (66.7)	19 (70.4)	>.99
Female	4 (33.3)	8 (29.6)	>.99
Race			
White	9 (75.0)	17 (63.0)	.79
Asian	2 (16.7)	5 (18.5)	>.99
American Indian/Native American	1 (8.3)	0 (0.0)	.36
Comorbidities			
Diabetes mellitus	7 (58.3)	14 (51.9)	>.99
Hypertension	7 (58.3)	na	na
Obesity (BMI > 30 kg/m^2)	6 (50.0)	9 (33.3)	.54
Coronary artery disease	2 (16.7)	4 (15)	>.99
Kidney transplantation	1 (8.3)	na	na
Obstructive lung disease	4 (33.3)	na	na
Chronic obstructive pulmonary disease	2 (16.7)	11 (40.7)	.47
Reactive airway disease	1 (8.3)	na	na
Temperature, °C	38 (37.25-38.5)	37.5 (37.1-38.4)	.76
Inflammatory markers before treatment			
CRP (≤8.0 mg/mL)	103.2 (52.7-159.9)	74.4 (42.2-131.5)	.25
Ferritin (24-336 μg/L)	596.0 (358.3-709.0)	673.0 (406.8-1012.8)	.75
IL-6 (≤1.8 pg/mL)	30.95 (24.18-34.05)	29.20 (13.55-40.70)	.87
D-dimer (≤500 ng/mL)	829 (513.5-1298.5)	916.0 (585.0-1299.0)	.84
Lymphocyte count before treatment (0.95-3.07×10^9/L)	0.75 (0.55-1.04)	0.76 (0.59-1.01)	.91
Oxygen therapy before treatment			
Nasal cannula (=4 clinical ordinal endpoint scale)	8 (66.7)	20 (74.0)	>.99
High-flow oxygen/NIPPV (=3 clinical ordinal endpoint scale)	4 (33.3)	7 (25.9)	.73
Invasive ventilation (=2 clinical ordinal endpoint scale)	0 (0.0)	0 (0.0)	>.99
SpO_2/FiO_2 before treatment	280.9 (252.5-317.9)	289.1 (254.9-342.0)	.98

[a]BMI = body mass index; CRP = C-reactive protein; FiO_2 = fraction of inspired oxygen; IL = interleukin; na = not available; NIPPV = noninvasive positive pressure ventilation; SpO_2 = oxygen saturation.
[b]Values shown are n (%) unless otherwise specified.

(Millipore Sigma, Ontario, Canada). Data were collected using Luminex (Millipore Sigma, Ontario, Canada).

Statistical Methods

Continuous variables at baseline are represented using the median and interquartile range and compared using a Wilcoxon rank-sum test. Proportions between groups at baseline were compared using the Fisher exact test. We used an 8-point ordinal outcome scale to define clinical status: (1) death; (2) hospitalized, on invasive mechanical ventilation or extracorporeal membrane oxygenation; (3) hospitalized, on noninvasive ventilation or high-flow oxygen devices; (4) hospitalized, requiring supplemental oxygen; (5) hospitalized, not requiring supplemental oxygen — requiring ongoing medical care (COVID-19–related or otherwise); (6) hospitalized, not requiring supplemental oxygen — no longer requires ongoing medical care; (7) not hospitalized, limitation of activities; and (8) not hospitalized, no limitations of activities (as recommended by the WHO R&D Blueprint Group).[26] We defined clinical improvement as improvement of at least 2 points on the 8-point ordinal scale, with

the main outcome for our observation designated the time to clinical improvement. Statistical significance for differences in temperature, serum CRP concentration, serum IL-6 concentration, absolute lymphocyte counts (ALC), and platelet counts from baseline versus 4 days post-treatment was determined using a paired t-test. Day 4 was determined as the last value for statistical analysis because data post−day 4 were not available for more than 50% of this cohort. For the untreated cohort, the first day of hospitalization was used as baseline and day 4 of hospitalization as the relevant time to measure change from baseline. Differences in mean change between lenzilumab-treated and -untreated groups were assessed for statistical significance with an independent two-sample Student *t* test comparing baseline and last values as defined above. Differences in mean oxygen saturation (SpO_2)/fraction of inspired oxygen (FIO_2) ratio over time between the treated and untreated groups were assessed using a repeated-measures analysis of variance (ANOVA) test. The proportion of patients with ARDS ($SpO_2/FIO_2<315$) over time between lenzilumab-treated and -untreated groups was assessed using a repeated-measures ANOVA test. The significance of proportional changes between groups was assessed by calculating the odds ratio. The time to event analyses was portrayed by Kaplan-Meier plots, and curves were compared with a log-rank test. GraphPad Prism version 8.0.0 for Windows was used to perform analysis (GraphPad Software, San Diego, CA).

RESULTS

Patients and Baseline Characteristics

Twelve patients received full treatment with three doses of lenzilumab administered 8 hours apart. Twenty-seven patients comprised the matched control cohort. The baseline demographic and clinical characteristics of lenzilumab-treated and -untreated patients are summarized in Table 1.

In the lenzilumab group, 5 of 12 (41.7%) patients received other pharmacotherapies targeting COVID-19 besides lenzilumab. Three patients received hydroxychloroquine, one of these also received tocilizumab, an IL-6 inhibitor; one patient each received remdesivir or systemic steroids. Among the untreated cohort, 20 of 27 (74.1%) received COVID-19− directed therapies; 5 of these patients received more than one modality of treatment. Three patients received hydroxychloroquine with azithromycin, 7 patients received systemic corticosteroids, 4 patients each received tocilizumab or remdesivir, and 1 patient each received ritonavir-boosted lopinavir or ribavirin.

At baseline, all patients, lenzilumab-treated and -untreated, required oxygen supplementation, but not mechanical ventilation. In the lenzilumab group, one patient was on noninvasive positive-pressure ventilation (NIPPV), 8 (66.7%) were on low flow oxygen, 3 (25.0%) were on high-flow oxygen. Among untreated patients, 2 (7.4%) were on NIPPV, 20 (74.1) were on low-flow oxygen, and 5 (18.5%) were on high-flow oxygen at baseline. In the lenzilumab group, the median SpO_2/FIO_2 ratio was 281 mm Hg, with SpO_2/FIO_2 ratios below 315 mm Hg in 8 (66.7%) patients, and below 235 mm Hg in 3 (25.0%) patients. In the untreated group, baseline median SpO_2/FIO_2 was 289.1 mm Hg, with SpO_2/FIO_2 ratios below 315 mm Hg in 15 (55.6%) patients and below 235 mm Hg in 6 (22.2%) patients. Additionally, 6 (50.0%) patients were febrile within 24 to 48 hours before lenzilumab administration, with a median temperature of 38.3°C. Nine (33.3%) untreated patients were febrile at baseline with a median temperature of 38.8°C.

Seven (58.3%) lenzilumab-treated and 19 (70.4%) -untreated patients had lymphopenia at baseline, with an absolute lymphocyte count less than 0.95 × 109/L. Median lymphocyte count before treatment was 0.75 and 0.76 in the treated and untreated groups, respectively ($P=.91$). All lenzilumab patients and 26 (96.3%) untreated patients had an elevation in at least one inflammatory marker at baseline. Eleven (91.7%) treated patients had elevated CRP values above the upper limit of normal (>8.0 mg/L), with a

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TABLE 2. Clinical Outcomes[a,b]

	Lenzilumab group (n=12)	Control group (n=27)	*P*
Incidence of clinical improvement	11 (91.7)	22 (81.5)	.43
Days to clinical improvement	5 (1-14)	11 (4-42)	.006
Days to discharge from hospital	5 (3-19)	11 (4-42)	.008
Mean temperature reduction, °C	1.075	0.459	.02
Days to resolution of fever	2 (1-6)	1 (1-3)	.22
Incidence of IMV	1 (8.3)	10 (37.0)	.10
Incidence of death	1 (8.3)	5 (18.5)	.43
Incidence of IMV and/or death	1 (8.3)	11 (40.7)	.07

[a]IMV = invasive mechanical ventilation.
[b]Values are n (%) unless otherwise noted.

median of 103.2 mg/L. Baseline CRP values were available for 17 (63.0%) of patients among the untreated group, all of which were above the upper limit of normal, with a median of 74.4 mg/L. All 11 patients in the lenzilumab group with IL-6 levels available at baseline had elevated values above the upper limit of normal (>1.8 pg/mL), with a median of 30.95 pg/mL. Similarly, all seven patients in the untreated cohort with IL-6 levels available at baseline had elevation of IL-6, with a median of 29.2 pg/mL. Ten (83.3%) patients in the lenzilumab group had elevated ferritin values above the upper limit of normal (>336 μg/L), with a median of 596 μg/L, compared with 12 of 14 (85.7%) untreated patients with available ferritin levels, with a median of 673 μg/L. Of the 11 patients in the lenzilumab group with D-dimer levels available at baseline, 9 of 11 (81.8%) had values above the upper limit of normal (>500 ng/mL), with a median of 829 ng/mL. Of the 13 untreated patients with D-dimer levels available at baseline, eleven (84.6%) had elevated levels, with a median of 916 ng/mL (*P*=.84).

Clinical Outcomes

The proportion of patients who achieved clinical improvement, defined as improvement of at least 2 points on the 8-point ordinal clinical endpoints scale, was comparable in both groups: 11 of 12 (91.7%) patients in the lenzilumab group and 22 of 27 (81.5%) patients in the untreated group (*P*=.43) (Table 2). However, the time to clinical improvement was significantly shorter for patients who received lenzilumab compared with the untreated group (median 5 days [range, 1 to 14] vs 11 days [range, 4 to 42], x^2=7.43, *P*=.006) (Figure 1A). The median length of hospital stay following lenzilumab administration was significantly shorter than the median length of hospital stay for patients in the untreated group (5 days [range, 3 to 19] vs 11 days [range, 4 to 38], *P*=.008) (Table 2).

Ventilator-free survival was better in the lenzilumab cohort compared with the untreated group, but did not reach statistical significance (x^2=3.67, *P*=.06) (Figure 1B). Only one (8.3%) patient in the lenzilumab group progressed to mechanical ventilation and death. In comparison, 10 (37.0%) patients in the untreated group progressed to mechanical ventilation, and 5 (18.5%) patients died (*P*=.10 and *P*=0.43, respectively) (Table 2).

Mean baseline SpO_2/FIO_2 were comparable between the lenzilumab group and the untreated group (285.0 mm Hg vs 285.7 mm Hg, *P*=.98). However, there was a statistically significant difference in mean SpO_2/FIO_2 between the lenzilumab and the untreated groups over time post-treatment (*P*<.001) (Figure 2A). The proportion of patients free of ARDS (who achieved a SpO_2/FIO_2 of 315 mm Hg or higher) by the end of observation was comparable between the 2 groups: 11 (91.7%) patients in the lenzilumab group had achieved a SpO_2/FIO_2 of 315 mm Hg or higher, compared with 22

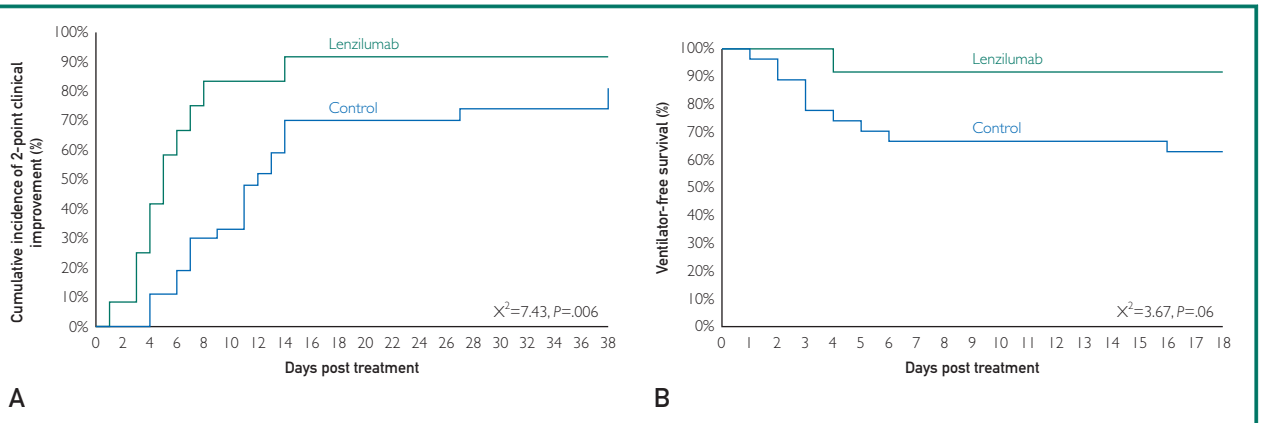

FIGURE 1. Clinical outcome measures of patients with severe coronavirus disease 2019 pneumonia (lenzilumab-treated patients versus controls). A, Cumulative percentage of patients with at least a 2-point improvement in the 8-point ordinal clinical endpoint scale estimated by Kaplan-Meier curve and compared by log-rank test. B, Mechanical ventilator-free survival estimated by Kaplan-Meier curve and compared by log-rank test.

(81.5%) patients in the untreated group (P=.43). However, the proportion of patients free of ARDS (with SpO_2/FIO_2 of 315 mm Hg or higher) was significantly increased in the lenzilumab group over time compared with untreated (P<.001) (Figure 2B).

Laboratory Markers

Baseline and follow-up values that would allow comparative analysis were available for the following laboratory markers for both lenzilumab-treated and -untreated groups, including CRP, ALC, and platelet counts. Baseline and follow-up values of IL-6 were available only for patients who received lenzilumab.

The lenzilumab group showed significant reductions in mean CRP values compared with baseline (172.2 mg/L vs 36.4 mg/L, P=.04). A reduction of at least 50% was observed in mean CRP levels in six patients (50.0%) by day 2. In contrast, the untreated group did not have a significant reduction in mean CRP (120.6 mg/L vs 121.7 mg/L, P=.98). The reduction in mean CRP after 4

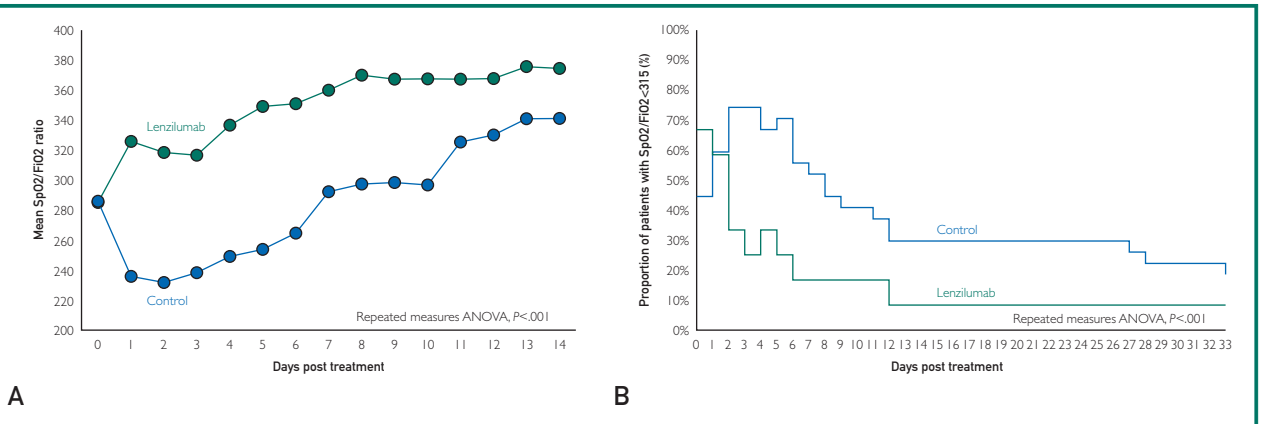

FIGURE 2. Measurement of oxygenation status of patients treated with lenzilumab versus controls. A, Change in mean oxygen saturation (SpO_2)/fraction of inspired oxygen (FIO_2) ratio displayed at baseline (day 0) through day 14 post-therapy and compared by repeated measures analysis of variance (ANOVA). B, Percentage of patients with acute respiratory distress syndrome (defined as SpO_2/FIO_2 <315 mm Hg) and compared by repeated measures ANOVA.

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TABLE 3. Laboratory Markers[a]

	Lenzilumab group (n=12)	Control group (n=27)	*P*
CRP reduction	135.8	-0.95	.01
IL-6 reduction	20.1	na	na
ALC increase	0.46×10^9/L	0.03×10^9/L	.04
PLT increase	52.5	63.2	.61

[a]ALC = absolute lymphocyte count; CRP = C-reactive protein; IL-6 = interleukin 6; na = not available; PLT = platelet count.

days of treatment was significantly greater in the lenzilumab group than in the untreated group (mean CRP reduction, 135.8 vs. -0.95; *P*=.01) (Table 3).

Increase in mean ALC was significantly greater among the lenzilumab-treated cohort compared with the -untreated group: 0.46 × 109/L versus 0.03 × 109/L, *P*=.04) (Table 3). Significant increases in mean platelet count from baseline were noted among both treated and untreated groups (52.5 10_9/L, *P*=.002 and 63.2, *P*<.001, respectively). However, the difference between the two groups was not statistically significant (*P*=.61) (Table 3).

Compared with baseline, there was a decrease in IL-6 concentration on day 4 following lenzilumab administration (28.6 pg/mL vs 8.52 pg/mL, *P*=.02). A decrease of at least 50% was observed in IL-6 values in four lenzilumab-treated patients (33.3%) by day 4.

Analysis of human cytokines comparing pretreatment 48 hours post-lenzilumab treatment in one patient revealed significant reduction in multiple cytokines and chemokines involved in the cytokine storm (granulocyte colony-stimulating factor [G-CSF], macrophage-derived chemokine [MDC], GM-CSF, IL-1α, interferon gamma [IFN-γ], IL-7, fms-related tyrosine kinase 3 ligand [FLT-3L], IL-1rα, IL-6, and IL-12p70) (Table 4 and Figure 3).

Safety of Lenzilumab Treatment

Lenzilumab was well-tolerated in all patients. One patient with a history of restless leg syndrome reported a "pins and needles" sensation during the first dose of lenzilumab; those symptoms resolved and did not recur with subsequent infusions of lenzilumab. There was no significant difference in mean absolute neutrophil count or hemoglobin values between baseline and day 4 post lenzilumab (5.1×109/L vs 4.8 x109/L, *P*=.27; 12.9 g/dL vs 11.4 g/dL, *P*=.89; respectively). In one patient, hemoglobin values dropped from 10.3 g/dL on day 0 to 7.9 g/dL on day 6. This patient had undergone a renal biopsy on day 2; imaging revealed a subcapsular hematoma. At the last study observation, the patient remained anemic at 9.3 g/dL. No treatment-emergent adverse events attributable to lenzilumab were noted.

DISCUSSION

There is no therapy with proven efficacy against COVID-19 at present. Based on the pathophysiology of immune hyper-response following SARS-CoV-2 infection,[1,27] along with our preclinical work,[20] we hypothesized that lenzilumab-induced GM-CSF depletion prevents immune hyper-stimulation in COVID-19 and progression to severe disease or death. We report our observations from the first-ever use of lenzilumab to neutralize GM-CSF in the treatment of COVID-19. Lenzilumab was offered through a compassionate single-use IND to patients with severe and critical COVID-19 pneumonia. To provide further context for our observations, we compared outcomes noted in the patients who received lenzilumab with that of a cohort of patients hospitalized with COVID-19 pneumonia and who matched the lenzilumab patients in sex and age as well as being comparable in requiring oxygen supplementation but not mechanical ventilation and having at least one risk factor associated with poor COVID-19 outcomes.[28,29]

TABLE 4. Serum Inflammatory Cytokine Levels Pre- And Post-Lenzilumab Treatment (Day -1 and Day 2) in a Patient With Severe Coronavirus Disease 2019 Pneumonia[a]

Cytokines/chemokines	Pre-lenzilumab (mean)	Post-lenzilumab (mean)
EGF, pg/mL	35	30.15
FGF-2, pg/mL	238.53	156.085
G-CSF, pg/mL	179.275	88.175
FLT-3L, pg/mL	22.555	3.67
GM-CSF, pg/mL	54.315	36.755
IFN-α2, pg/mL	125.2	84.755
IFN-γ, pg/mL	11.755	5.42
IL-12p40, pg/mL	35.85	18.435
MDC, pg/mL	144.625	103.935
IL-12p70, pg/mL	12.23	4.97
IL-13, pg/mL	23.48	18.93
IL-15, pg/mL	10.715	10.385
IL-1ra, pg/mL	264.25	99.165
IL-1a, pg/mL	637.55	271.9
IL-5, pg/mL	1.68	1.49
IL-6, pg/mL	123.445	64.515
IL-7, pg/mL	11.37	3.26
IL-8, pg/mL	33.35	25.68
VEGF, pg/mL	79.915	53.125
MIP-1a, pg/mL	11.715	8.515
GRO, pg/mL	98.015	158.7
IL-10, pg/mL	18.8	21.92
MCP-3, pg/mL	79.335	84.2
sCD40L, pg/mL	557.875	920.99
IL-4, pg/mL	263.88	288.995
MCP-1, pg/mL	494.59	666.1
MIP-1ß, pg/mL	43.2	44.205
TNF-ß, pg/mL	60.345	84.255

[a]EGF = epidermal growth factor; FGF-2 = fibroblast growth factor 2; FLT-3L = fms-related tyrosine kinase 3 ligand; G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; GRO = growth-regulated oncogene; IFN = interferon; IL = interleukin; MCP-1 = monocyte chemoattractant protein; MDC = macrophage-derived chemokine; MIP-1a = macrophage inflammatory protein; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

Our primary clinical outcome was time to clinical improvement, with clinical improvement defined as at least a 2-point improvement in the 8-point ordinal scale. In this group of high-risk patients with severe COVID-19 pneumonia, treatment with lenzilumab was associated with a significantly shorter time to clinical improvement compared with the matched cohort. Improvement in oxygen requirement was noted among lenzilumab-treated as well as -untreated patients. However, the proportion of patients free of ARDS (SpO_2/FIO_2 of 315 mm Hg or higher) was significantly greater in the lenzilumab group over multiple times. Ventilator-free survival favored the lenzilumab cohort. Among patients in the lenzilumab group, improvement in clinical parameters was accompanied by significant improvement in inflammatory markers and markers of disease severity. This was not observed for patients in the untreated group. The reduction in mean CRP in the lenzilumab group was significantly greater than in the untreated group; increases in mean ALC were statistically significant in patients who received lenzilumab, but not in the untreated control group. We have recently shown that GM-CSF depletion results in modulation of apoptosis pathways in T cells.[22] It is unclear at this time if the increase in lymphocyte count is secondary to clearance of SARS-CoV-2 virus, overall improvement of inflammation, or a direct effect of GM-CSF on T cells. A significant improvement in platelet count was noted both among lenzilumab-treated and -untreated patients. This may reflect an overall improved coagulopathy associated with COVID-19.[30] Significant improvement in mean IL-6 was also noted following lenzilumab administration. These results are consistent with our original hypothesis, and corroborate our laboratory findings following GM-CSF depletion in preclinical models of CRS after CART cell therapy. Figure 4 depicts a proposed mechanism for the role of GM-CSF in CRS post−COVID-19.

Targeting individual cytokines downstream in the inflammatory cascade of CRS, such as IL-6, have not shown improved clinical outcomes in COVID-19. However, the clinical benefit observed with broad immunosuppression with dexamethasone suggests that a hyper-inflammatory immune response is pathologic in latter stages of COVID-19. Neutralization of GM-CSF, which is upstream in the CRS cascade, may provide better suppression of the hyper-inflammatory immune response than IL-6 receptor antagonists alone while sparing the lympholytic

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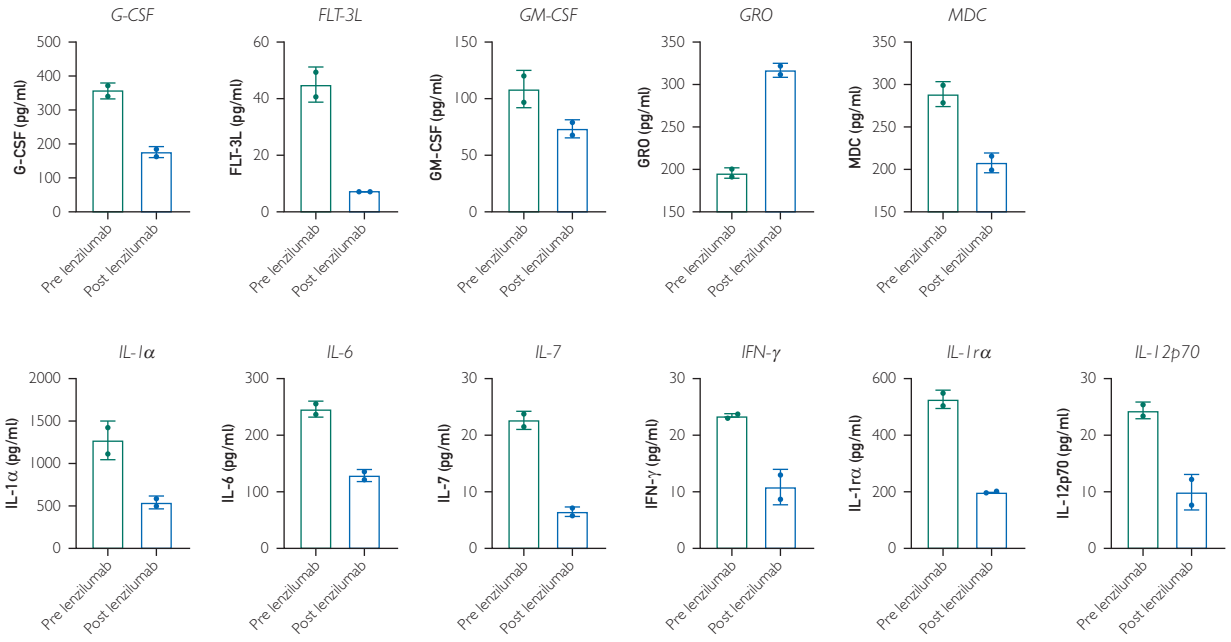

FIGURE 3. Lenzilumab treatment results in improved inflammatory cytokines in a patient with severe coronavirus disease 2019 pneumonia. Inflammatory cytokine levels on day 1 and day 2 post-lenzilumab treatment (*=$P<.05$, **=$P<.01$). FLT-3L = fms-related tyrosine kinase 3 ligand; G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; GRO = growth-regulated oncogene; IFNγ = interferon gamma; IL = interleukin; MDC = macrophage-derived chemokine.

effects of broad immunosuppression with steroids.

Several patients (5 in the lenzilumab group and 20 in the untreated group) received other pharmacotherapies targeting COVID-19. These treatment decisions were not done systematically and the number of patients who received each individual therapy is so small that any meaningful analysis of their potential contribution to patients' outcomes cannot be made.

The use of lenzilumab was safe, without any adverse events attributable to lenzilumab. Numerically, more patients in the matched cohort required mechanical ventilation or died compared with patients receiving lenzilumab. However, this was not statistically significant. Although there is a theoretical concern for bone marrow toxicity when GM-CSF is depleted, lenzilumab treatment was not associated with any hematologic toxicity in this cohort. There were no infusion reactions following lenzilumab treatment.

Study Limitations

Our report has several limitations. First, the sample size is small. Second, as lenzilumab was offered under emergency single-use IND conditions; all management decisions, including prescribing medications and laboratory/radiologic monitoring, were at the discretion of the treating clinicians. There was heterogeneity in the treatment specifics of individual patients as well as the laboratory and other diagnostic data that were collected. Although we have attempted to provide context to our observations by including a matched cohort, this is not a randomized controlled clinical trial. Therefore, we cannot, with full confidence, declare that all of the clinical improvement that we observed in our patients was clearly and solely attributable to lenzilumab. However, the better outcomes in patients who received lenzilumab compared with patients in the matched cohort are very encouraging and will be further addressed in the upcoming randomized National Institutes of Allergy

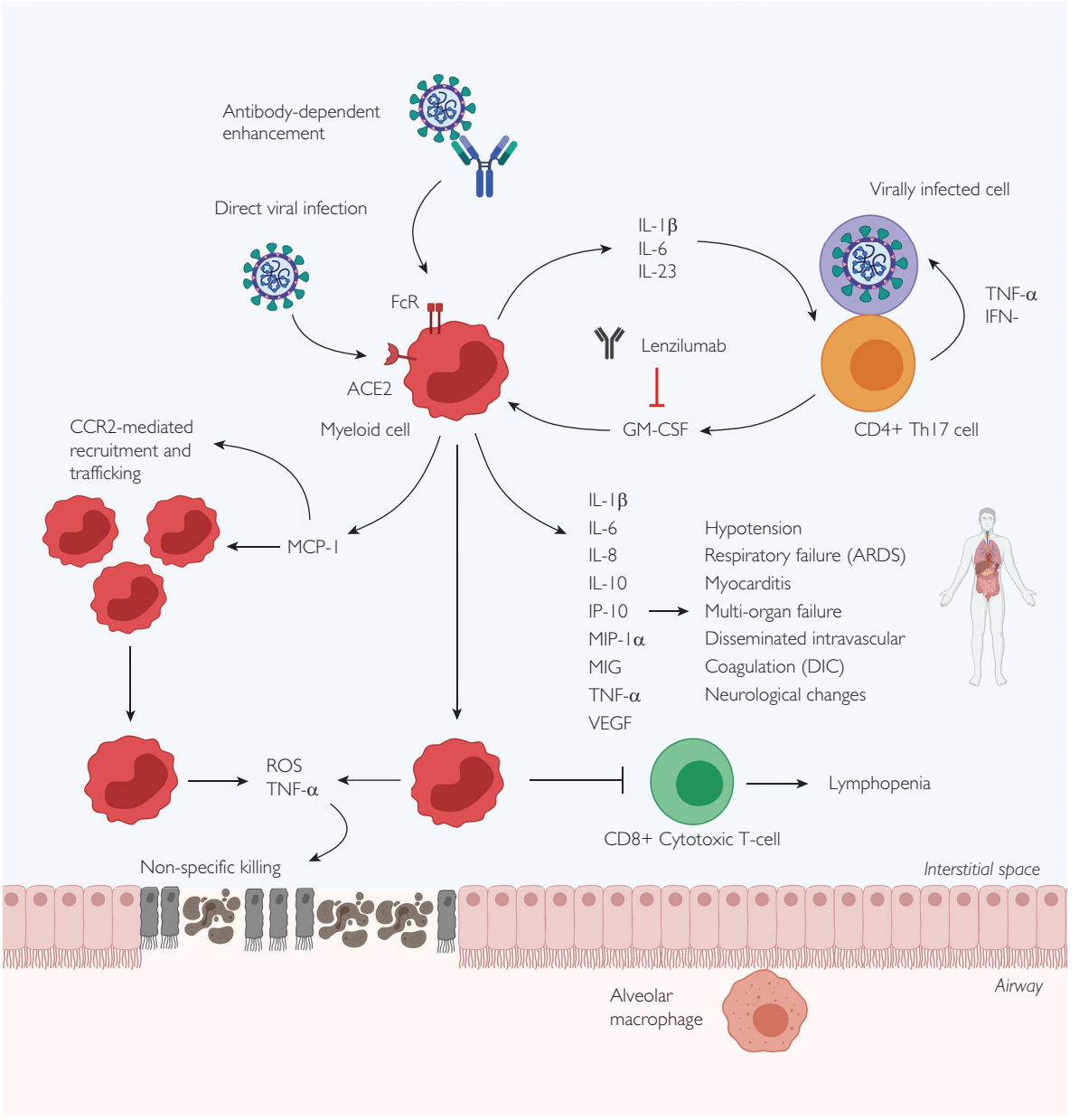

FIGURE 4. Proposed mechanim for granulocyte-macrophage colony-stimulating factor (GM-CSF) neutralization in coronavirus disease 2019 — associated cytokine storm. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infects monocytes/macrophages direcly via the angiotensin-converting enzyme 2 (ACE-2) receptors and through antibody-dependent enhancement. Infection with SARS-CoV-2—induced a T-cell response through the activation of ThGM and Th17 cells. Granulocyte-macrophage colony-stimulating factor production by ThGM cells further stimulated monocytes and initiates an immune hyper-inflammatory response. Activated monocytes result in production of myeloid derived cytokines, propagation of cytokine storm, trafficking of blood derived monocytes to the lungs, acute respiratory distress syndrome (ARDS), and respiratory failure. Granulocyte-macrophage colony-stimulating factor — activated monocytes induce T-cell death and result in lymphopenia and worse clinical outcomes. CCR2 = C-C chemokine receptor type 2; FcR = interferon; IFN = interferon; IL = interleukin; MCP-1 = monocyte chemoattractant protein-1; MIG = monokine induced by gamma; MIP-1α = macrophage inflammatory protein-1α; ROS = reactive oxygen species; TNFα = tumor necrosis factor α; VEGF = vascular endothelial growth factor.

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and Infectious Diseases—sponsored Big Effect Trial in addition to the phase III clinical trial (NCT04314843) that has recently been initiated.

CONCLUSION

We administered lenzilumab under a single-use emergency IND compassionate program to 12 patients with severe COVID-19 pneumonia with risk factors for disease progression. Lenzilumab use was associated with faster improvement in clinical status and oxygenation, as well as greater reductions in inflammatory markers and markers of severity compared with the matched cohort. Lenzilumab was well tolerated; no treatment-emergent adverse events attributable to lenzilumab were observed.

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Drs Temesgen, Kenderian, and Badley contributed equally.

Affiliations: From the Division of Infectious Diseases (Z.T., M.A., F.N.U.S., P.V., S.A.R., R.R.R., R.P., A.S.D., A.D.B.), Division of Pulmonary and Critical Care Medicine (P.R.B.), Department of Anesthesia and Critical Care Medicine (B.W.P.), T Cell Engineering (R.S., S.S.K.), Division of Hematology (R.S., S.S.K.), Department of Immunology (S.S.K.), and Department of Molecular Medicine (S.S.K., A.D.B.), Mayo Clinic, Rochester, MN; the Division of Infectious Diseases (C.R.L.) and the Division of Pulmonary Medicine (C.D.B.), Mayo Clinic, Jacksonville, FL; the Division of Infectious Diseases (R.O.), and the Division of Gastroenterology and Hepatology (H.E.V.), Mayo Clinic, Scottsdale AZ; and the Humanigen, Inc, Burlingame, CA (G.C., D.C., O.A., C.D.).

Potential Competing Interests: Dr Kenderian is an inventor on patents in the field of CAR immunotherapy that are licensed to Novartis (through an agreement between Mayo Clinic, University of Pennsylvania, and Novartis) and Mettaforge (through Mayo Clinic). Drs Sakemura and Kenderian are inventors on patents in the field of CAR immunotherapy that are licensed to Humanigen (through Mayo Clinic). Dr Kenderian receives research funding from Kite, Gilead, Juno, Celgene, Novartis, Humanigen, MorphoSys, Tolero, Sunesis, and Lentigen; and has participated in advisory boards with Kite, Juno, Novartis, and Humanigen. Dr Badley is a consultant for AbbVie; is on scientific advisory boards for Nference and Zentalis; and is founder and President of Splissen Therapeutics. The remaining authors report no potential competing interests.

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Correspondence: Address to Zelalem Temesgen, MD, Department of Infectious Diseases, Mayo Clinic, 200 First St SW, Rochester, MN 55905 (Temesgen.zelalem@mayo.edu).

ORCID

Zelalem Temesgen: https://orcid.org/0000-0001-9179-6697; Mariam Assi: https://orcid.org/0000-0003-2045-4715; F.N.U. Shweta: https://orcid.org/0000-0001-6634-6272; Philippe R. Bauer: https://orcid.org/0000-0001-8429-3581; Raymund R. Razonable: https://orcid.org/0000-0001-5248-0227; Ala S. Dababneh: https://orcid.org/0000-0003-3831-995X; Saad S. Kenderian: https://orcid.org/0000-0003-2767-3830; Andrew D. Badley: https://orcid.org/0000-0001-7796-7680

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