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A Multicenter, Randomized, Double Blind, Placebo Controlled Phase II Trial of Intravenous Inflammasome Inhibitor (NuSepin) for the Treatment of COVID-19 Patients

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Review Article

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ABSTRACT

Background: More effective and safer anti-inflammatory drugs are necessary to improve clinical outcomes and prevent long term sequelae of patients with COVID-19 pneumoniae.

Objectives: Here we evaluated whether a novel inflammasome inhibitor (NuSepin) provides greater benefit than placebo in patients with COVID-19 pneumonia.

Methods: We conducted a double-blind, randomized, placebo controlled phase ii trial of intravenous NuSepin, which is an NLRP3 inflammasome inhibitor targeting GPCR19, in adults who were hospitalized with COVID-19 pneumoniae in Romania from September 2020 to March 2021. Patients were randomly assigned to receive 0.1 mg/kg NuSepin, 0.2 mg/kg NuSepin, or placebo (b.i.d., i.v. infusion) for up to 14 d or until complete remission, together with standard of care, and were monitored for another 14 d. The primary outcome was the Time to Clinical Improvement (TTCI), defined as a decline of two Ordinal Scales (OS) from randomization on a six category OS that ranges from 1 (discharged with normal activity) to 6 (death) (primary TTCI,=TTCI_P). In addition, clinical improvement was also assessed by aggregated National Early Warning Score 2 (NEWS2), and secondary TTCI (TTCI_S) was defined by time to NEWS2=0 from randomization, which is maintained for 24 h.

Results: By pairwise comparison of TTCI_P, NuSepin 0.2 mg/kg showed a 1.34-fold (95%CI:0.70~2.59) and 1.93-fold (95% CI: 0.96~3.85) higher recovery Rate Ratio (RR) than placebo in modified Intention to Treatment (mITT) set and in Per-Protocol (PP) set, respectively. The median difference in TTCI_S was 3.5 days between the NuSepin 0.2 mg/kg group and the placebo group (p=0.016, PP set with baseline NEWS2 ≥ 5), which favored improved recovery in the NuSepin group. The overall RR was 3.4, which favors the NuSepin 0.2 mg/kg group when the effects of covariates (use of anti-viral drugs and baseline NEWS2 ≥ 5) were adjusted (p=0.0026). At day 4, CRP levels in 52% of patients in the NuSepin 0.2 mg/kg group returned within the normal range, which is comparable to 20% of patients in the placebo group. At the end of the study day, blood pro-inflammatory cytokines levels were significantly lower in the NuSepin group and the percentage of patients with pro-inflammatory cytokines within normal ranges were higher in the NuSepin group (35%~60%) than the placebo group (11%~33%). Serious adverse events were reported in a patient who received NuSepin 0.2 mg/kg (4.5%), but this was found to be unrelated to

NuSepin treatment.

Conclusions: Considering the facts that (1) NuSepin has a favorable and tolerable safety profile and (2) a significant increase in RR and a reduction in the time to NEW2=0 was observed, and (3) a significant decrease in blood pro-inflammatory cytokines, clinical improvement of hospitalized moderate-to-severe COVID-19 patients might be achieved with NuSepin 0.2 mg/kg significantly faster than placebo.

Keywords: COVID-19; Pneumonia; Inflammasome; NEWS2; NuSepin treatment

INTRODUCTION

COVID-19 is caused by infection with Severe Acute Respiratory Syndrome–Coronavirus-2 (SARS-CoV-2). More than 670 million cases and 6.7 million deaths have been confirmed globally in the last 3 years. In March 2020, the World Health Organization (WHO) officially designated COVID-19 as a pandemic disease. The genome, positive sense single stranded RNA, of the SARS-CoV-2 virus has mutated, and several types of mutated viruses have now spread worldwide, with increased infectivity and increased chance for escaping from neutralizing antibodies induced by vaccines.

Fortunately, most COVID-19 patients develop only mild disease, with approximately 15% developing moderate to severe disease that necessitates hospitalization and support with supplementary oxygen. Less than 5% of COVID-19 patients require admission to an Intensive Care Unit (ICU). In severe cases of COVID-19 pneumonia, patient's situation is complicated by Acute Respiratory Distress Syndrome (ARDS), sepsis, septic shock, and multiorgan failures [1]. Patients in old age, and with co-morbid disease, such as hypertension, diabetes and obesity are reported to be at substantially higher risk of mortality [2].

Early in the clinical course of COVID-19, the disease is by tissue damages due to replication of SARS-CoV-2. In the later stage of clinical course, the disease is exacerbated by local and systemic inflammatory responses to damaged tissues as well as to SARS-CoV-2. In many COVID-19 patients, Cytokine Release Syndrome (CRS) is frequently found. The inflammatory cytokines potentiate recruitment of inflammatory monocytes, neutrophils, and T-cells in tissues of patients suffering from COVID-19. The systemic inflammation resultant from CRS leads to hyper coagulation, increased endothelial permeability, multi-organ dysfunction, and eventually death [3].

For these reasons, treatment with anti-viral drugs or neutralizing antibodies are necessary to inhibit viral replication in the early phase of infection, while anti-inflammatory therapeutics are necessary in the early and late stages of COVID-19 to ameliorate inflammation caused by tissue destruction by viruses. Anti-inflammatory drugs could be beneficial to maintain SpO_2, which constitutes the most crucial clinical parameter for the patients to thrive, by resolving lung inflammation, and by preventing long term sequalae incurred by lung fibrosis [4]. Therefore, anti-inflammatory drugs combined with anti-viral drugs could decrease mortality and expedite clinical recovery without any sequalae that are frequently found in moderate to severe patients [5].

Fortunately, dexamethasone, one of the anti-inflammatory drugs, improves survival of hospitalized patients with COVID-19 who receive supplemental oxygen. WHO panels have recommended the use of systemic corticosteroids in patients with severe COVID-19. However, the WHO and the U.S. Food and Drug Administration (FDA) also have warned that treatment of systemic corticosteroids could increase the risk of infection in COVID-19 patients [6-9]. Meta-analyses showed that steroids delayed viral clearance and increased long term mortality by prothrombotic side effects in COVID-19 patients. Not only in COVID-19 cases, corticosteroid also delayed virus clearance in MERS and SARS patients, and increased mortality in severe pneumonia caused by influenza viruses [10].

LITERATURE REVIEW

In addition to corticosteroids, several anti-inflammatory drugs have been tested in COVID-19 patients such as tocilizumab (anti-Interleukin (IL)-6 receptor monoclonal antibody) that decreases levels of IFN-γ, IL-6 and TNF-α. JAK inhibitors decreased risk of death or respiratory failure among patients hospitalized with COVID-19 pneumonia. Considering medical unmet needs with current anti-inflammatory drugs, the better anti-inflammatory drugs, and better combinations of several anti-inflammatory drugs with different mode of actions are necessary not only for COVID-19 but also for future viral pandemics to combine with anti-viral drugs [11-16].

We demonstrated that Taurodeoxycholate (TDCA), a G-protein Coupled Receptor (GPCR) 19 agonist, could attenuate systemic inflammation. The GPCR19 pathways regulate inflammation by inhibiting activation of macrophages, attenuate tissue damage by inhibiting inflammation, and regulate inflammasomal activation. GPCR19-cAMP-PKA axis is crucial in regulating activation of NLRP3 inflammasome. TDCA is an active pharmaceutical ingredient of NuSepin that suppresses NLRP3 inflammasome activation. We found that TDCA could alleviate systemic inflammation in a cecal ligation and puncture induced or LPS induced mice sepsis model. The production of inflammatory cytokines, such as TNF-α, MCP-1, IL-6 and IL-1β, was significantly inhibited by TDCA in these models. In addition, TDCA increases

the number of Myeloid-Derived Suppressor Cells (MDSC) of which phenotype is different from conventional MDSCs. TDCA controls alternative splicing, chromatin silencing and translation of the immune proteome of MDSCs, which increases the anti-inflammatory potency of MDSCs. For example, Cathelicidin Antimicrobial Peptide (CAMP) was up-regulated by TDCA treatment, which inhibits expression of TNF-α, CXCL8, IL6, CCL2, IL-1β, leukotriene B4 and nitric oxide [17].

NuSepin induced no systemic toxicity at a dose of 10 mg/kg/d in rats, which is >30 times higher than the effective dose observed in a mouse sepsis model. In a phase I clinical trial, healthy volunteers received 0.1 mg/kg, 0.2 mg/kg, 0.4 mg/kg, 0.8 mg/kg, 1.6 mg/kg NuSepin, or placebo. Among the 39 subjects who received NuSepin, there were no serious adverse events and all adverse events by NuSepin treatment resolved spontaneously without any treatment in phase I trial. A single intravenous administration of NuSepin in a dose range of 0.1 mg/kg to 1.6 mg/kg in healthy volunteers exhibited dose dependent pharmacokinetic properties.

In this study, we report the safety and efficacy of NuSepin for the treatment of COVID-19 patients in randomized, double blind, placebo controlled phase II trials.

METHODOLOGY

Study design

The efficacy and safety of two doses (0.1 mg/kg and 0.2 mg/kg) of NuSepin for the treatment of adult patients diagnosed with SARS-CoV-2 were evaluated by a phase ii, multi-center, randomized, double blinded, placebo controlled study. Enrollment began in September 2020 and ended in March 2021. Individuals with laboratory confirmed SARS-CoV-2 infection by PCR test were recruited at five study centers across Romania. Eligible patients were randomly assigned in a 1:1:1 ratio to receive either NuSepin 0.1 mg/kg, NuSepin 0.2 mg/kg, or placebo along with standard-of-care. The test doses were selected based on dose efficacy relationship shown in preclinical test, PK profiles and No Observed Adverse Effect Level (NOAEL) published earlier. Briefly, NOAEL was 10 mg/kg in rats and dogs and Human Equivalent Dose (HED) was lower when calculated with NOAEL in rats (1.38 mg/kg=10 mg/kg x (0.15/60)0.33) than dogs (5.54 mg/kg), respectively. The body weights of rats, dogs and humans were considered as 0.15, 10 and 60 kg, respectively. After dividing NOAEL with safety factor (=10), the lowest dose (0.1 mg/kg) was selected for phase I trial. Mice showed dose dependent survival after i.v. infusion of NuSepin (0.5~4 mg/kg). C_{max} of TDCA in mice after infusion of 4 mg/kg (HED=0.28 mg/kg) NuSepin was like to C_{max} of 0.2 mg/kg NuSepin in phase I clinical trial. For these reasons, we tested 0.1 and 0.2 mg/kg of NuSepin in phase II trial.

Patients are considered to have moderate to severe disease if they scored a baseline National Early Warning Score 2 (NEWS2) ≥ 5 at randomization day. NuSepin was infused intravenously B.I.D. from day 1 through day 14 or until hospital discharge. All patients received standard of care of the trial site. The protocol was approved by the Central Ethics Committee (CEC), Local Ethics Committee (LEC) at each site where applicable and the national agency for medicines and medical devices of Romania. Administration of anti-viral drugs was not specified in the protocol of the phase II trial of NuSepin. The patients received anti-viral drugs solely according to primary investigators discretion, included in the standards of care. Written informed consent was obtained from each patient or from the patient's legally authorized representative if the patient was unable to provide consent [18].

For the study protocol, the IXRS (IRT system) was used to randomize and assign subjects to one of the three respective treatment groups *via* a central randomization methodology. Under this randomization design, a central randomization list was generated, in which the three treatment groups (and corresponding randomization numbers) were statistically randomized through a permutation and sorted based on sequence number. When a subject is randomized into the study, the system identifies the lowest available sequence number in the randomization list and assigns the corresponding randomization number and associated treatment group to the subject. Once a randomization number is assigned to a subject, it is marked so it cannot be assigned to another subject. The IXRS follows the same process as each subject is randomized into the study in sequential order [19].

Participants

Participants who met the following inclusion criteria were included in the study: age between 18-80 years, SARS-CoV-2 infection confirmed by PCR for the first time within 144 h prior to randomization, pneumonia based on chest X-ray/CT scan, blood leukocyte count >4.0 x 10^9/L and lymphocyte count >0.7 x 10^9/L, $SpO_2 \leq 94\%$ on room air or PaO_2/FIO_2 ratio<300 mgHg, and for females with a negative pregnancy test prior to treatment. Because NuSepin inhibits NLRP3 inflammasome of innate immune cells such as macrophages and neutrophils, we could not estimate serious immuno-suppression in COVID-19 patients with lymphopenia when both innate (by NuSepin) and adaptive immunity (by lymphopenia) are suppressed. For the ethical reason, we did not enroll patients with lymphopenia because efficacy data of NuSepin in patients have not been available. Exclusion criteria were as follows: Alanine amino Transferase (ALT)/ Aspartate amino Transferase (AST)>5 x Upper Limits of Normal (ULN), reduced renal function with estimated glomerular filtration rate <30 mL/min, pregnant or breast feeding women, evidence of multiorgan failure, and steroid treatment for any reason within 72 h prior to enrolment. As we excluded lymphopenic patients because of uncertain serious immunosuppression by NuSepin, we could not use steroid as one of the standard of care combined with NuSepin ethically. Once the efficacy of NuSepin being tested first, we planned to test efficacy of NuSepin combined

with steroids, while monitoring immunological parameters more carefully. In addition, WHO published guidance on the use of corticosteroids in patients with COVID-19 as one of the standard of care on September 2020 after the NuSepin protocol was approved on Aug 2020. Although the preprint of RECOVERY's results on dexamethasone treatment was available in June 2020, we could not include use of corticosteroid as standard of care while the NuSepin protocol was under review by the CEC and LEC in Romania [20].

Study protocol and measurements

From day -5 to 0, the patients were screened to assess eligibility for inclusion and exclusion and enrolled into any of the three treatment arms at day 1. During the treatment period, the patient received treatment with NuSepin 0.1 mg/kg, NuSepin 0.2 mg/kg, or placebo. The test drugs were given until complete remission up to 14 d. After the treatment was discontinued, the patients were followed-up for another 15 d until the End of the Study visit (EoS). The safety of test drugs was monitored by laboratory tests according to the discretion of the primary investigators. The routine laboratory tests were performed at a central laboratory of each trial site.

Endpoints

The primary outcome is Time to Clinical Improvement (TTCI), defined as the time (in days) from randomization until a decline of two categories on a six-category Ordinal Scale (OS) of clinical status (TTCI_P (primary)). The six category OS is as follows: (6) death; (5) ICU, requiring ECMO and/or Invasive Mechanical Ventilation (IMV); (4) ICU/hospitalization, requiring Non-Invasive mechanical Ventilation (NIV)/ High Flow Nasal Cannula (HFNC) therapy; (3) hospitalization, requiring supplemental oxygen (such as Low Flow Nasal Cannula (LFNC) or facial mask); (2) hospitalization, not requiring supplemental oxygen; and (1) complete clinical remission, *i.e.,* no fever, normal respiratory rate, oxygen saturation return to normal, and cessation of cough.

The secondary outcomes were time to complete clinical remission evaluated by NEWS2, all cause mortality, duration (days) of mechanical ventilation, duration (days) of extracorporeal membrane oxygenation, duration (days) of supplemental oxygenation, length of hospital stay (days), length of ICU stay (days), and percentage of patients with CRP<10 mg/L or <30% decreases from screening. In this report, we compared TTCI_S (secondary), days to normalization of SpO_2, and supplementary oxygen free days after hospitalization between the groups. TTCI_S is defined as the time (in days) from randomization until a day when the NEWS2 score reaches zero in which clinical stage patients are under normal consciousness, normal vital signs (respiratory rate, heart pulse rates, systolic blood pressure, and body temperature) and normal SpO_2 without supplementary oxygen which is maintained for 24 h. All serious adverse events were recorded from day 1 to EoS. Safety assessments were based on the incidence of treatment emergent adverse events (TEAEs) in all three treatment groups.

Statistical analysis

The Safety Analysis Set (SAS) consists of all randomized patients (N=64). The Intention-To-Treatment (ITT) set consists of all SAS patients receiving at least one dose of study medication and having at least one post-baseline efficacy measurement (N=64). Because the baseline OS was two in three cases (106-09, 106-12, and 106-13), the TTCI_P, defined as “decrease in 2 OS”, could not be determined. For this reason, these patients were not included in the modified ITT (mITT) population (N=61). The Per-protocol Population (PP) included patients from mITT set without any major protocol deviations described in the approved synopsis. In the NuSepin 0.1 mg/kg group, three patients (106-26, 106-31, and 106-34) did not complete the study due to treatment with steroids. In the NuSepin 0.2 mg/kg group, one patient (104-46) did not complete the study due to steroid treatment. The 102-1 patient was not included in the PP set since the primary endpoints could not be assessed. In the placebo group, two patients discontinued due to steroid treatment (106-18 and 106-20). A patient (106-13) in the NuSepin 0.1 mg/kg group who withdrew consent was not included in the PP set.

TTCIs was also characterized with descriptive statistical methods, including calculation of the mean, median, and standard error. Mean and standard error were analyzed without the censored data. Kaplan–Meier survival curves were constructed to show cumulative recovery over the EoS and median TTCIs, and the log-rank test was used to test statistical significance of differences. For the analysis of TTCI_P and TTCI_S, the Hazard Ratio (HR) and statistical significance from the Cox proportional hazard regression model were used to estimate the recovery Rate Ratio (RR, =HR) between groups. CRPs were summarized by descriptive statistics, including the mean and standard error, and statistical significance was assessed using a Wilcoxon Rank sum test. The primary or secondary outcomes were also analyzed in subgroups of patients stratified with baseline NEWS2 ≥ 5 (moderate-to-severe patients) on admission or use of anti-viral drugs during the study period.

Because we completed the phase II clinical trial to test the null hypothesis proposing the relationship between NuSepin and clinical improvement of COVID-19 patients, treatment with anti-viral drugs was not specified in the approved protocol. Administration of the anti-viral drugs was solely determined according to primary investigators’ discretion based on the clinical situation of each patient. Data from patients who received Remdesivir were not included in the analysis because the number of patients who received Remdesivir was limited (one or two in each group). Moreover, two patients who received more than two types of anti-viral drugs were not included in the analysis to clarify the effects of a specific type of anti-viral drugs.

RESULTS

Study population

Of the 95 patients who were assessed for eligibility, 64 underwent randomization, of which 22 were assigned to the NuSepin 0.1 mg/kg group, 22 were assigned to the NuSepin 0.2 mg/kg group, and 20 were assigned to the placebo group. The median age of the patients was 62 (NuSepin 0.1 mg/kg), 59 (NuSepin 0.2 mg/kg), and 63 (placebo) years-old. 45% (NuSepin 0.1 mg/kg), 55% (NuSepin 0.2 mg/kg), and 50% (placebo) were male. 100% of patients were enrolled in Romania and were Caucasian. The patients had one (n=20 (31%)), two (n=19 (30%)) or more (n=13 (20%)) comorbidities at enrollment, most commonly hypertension (n=35 (55%)), obesity (n=19 (30%)), and diabetes mellitus (n=16 (25%)).

17 patients (27%) were mild (baseline NEWS2<5), 11 patients (17%) were moderate (baseline NEWS2=5~6), and 36 patients (56%) were severe (baseline NEWS2 ≥ 7) at randomization day. Patients with baseline OS=4 were 27.3% (N=6) and 10.0% (N=2) in the NuSepin 0.2 mg/kg group and placebo group, respectively. No patients were with baseline OS=5. 38 patients (59.4%) received anti-viral drugs, such as lopinavir-ritonavir, remdesivir or favipiravir, etc. No significant differences in baseline demographic factors were observed between groups.

Primary endpoint

In mITT population, the median TTCI_P was 11 days (95% CI:8~13), 9.5 days (95% CI:7~11), and 10 days (95% CI: 9~13) in the NuSepin 0.1 mg/kg, NuSepin 0.2 mg/kg and placebo groups, respectively (p=0.54) by Log-Rank test. By pairwise comparison of TTCI_P, NuSepin 0.2 mg/kg showed a 1.34 fold (95%CI:0.70~2.59) and 1.93-fold (95% CI: 0.96~3.85) higher recovery Rate Ratio (RR) than placebo in modified Intention to Treatment (mITT) set and in Per-Protocol (PP) set, respectively. In the PP set with baseline NEWS2 >=5 at randomization day, 37.5% and 8.3% of patients recovered at day 9 in the NuSepin 0.2 mg/kg group and placebo group, respectively (p=0.028). None of the patients recovered until day 4. At day 15, 100% and 94.1% of patients recovered in the NuSepin 0.2 mg/kg group and the placebo group, respectively.

Additional endpoints

The median time to NEWS2 score=0 maintained for 24 hours (TTCI_S) was further compared between NuSepin group and placebo group. Because NuSepin 0.1 mg/kg group did not show statistically significant differences with placebo, the data are not listed in this report. When the effects of baseline covariates were analyzed, use of anti-viral drugs (RR=3.7 (95% CI: 1.4~10.2), p=0.01) and baseline NEWS2 ≥ 5 at randomization day (RR=2.7 (95% CI: 1.2, 6.4), p=0.02) significantly affected on the TTCI_S. However, age, gender and underlying comorbidities did not effect on the recovery rate ratio determined by TTCI_S. When these two covariates of PP set were adjusted, overall RR was 3.4 (95% CI: 1.5~7.4, p=0.0026) between NuSepin 0.2 mg/kg group and placebo group that favors the NuSepin group in recovery rate.

In moderate to-severe patients (baseline NEWS2 ≥ 5 at randomization day), the difference in TTCI_S between NuSepin 0.2 mg/kg group and placebo was 3.5 days in both ITT set (p=0.086) and PP set (p=0.016). RR between NuSepin 0.2 mg/kg group and placebo was 1.9 (ITT set, p=0.1) and 2.7 (PP set, p=0.02), respectively, favoring improved recovery in NuSepin 0.2 mg/kg group significantly better than placebo group.

When NuSepin 0.2 mg/kg was combined with anti-viral drugs, median TTCI_S was 9.0 days (7.0~12.0), which is comparable with the group of placebo+anti-viral drugs of 13.0 days (4.0~ND) (p=0.013, ITT set). The RR in these patients was 3.5 (1.2~10.0), which favors the group of NuSepin 0.2 mg/kg+anti-viral drugs compared with the group of placebo+anti-viral drugs (p=0.02).

Because supplementary oxygen free days are crucial parameter to define clinical remission of ARDS patients, we compared supplementary oxygen free days between the groups. After adjusting the effects of baseline NEWS2 at randomization day, the RR was 2.2 (95% C.I., 1.0~4.6, p=0.04), which favors longer supplementary oxygen free days in the NuSepin 0.2 mg/kg group than in the placebo group.

C-Reactive Protein (CRP) levels decreased after randomization both in the NuSepin 0.2 mg/kg group and the placebo group. However, the CRP level in the NuSepin 0.2 mg/kg group was normalized faster than that in the placebo group. At day 4, CRP levels in 52% of patients in the NuSepin 0.2 mg/kg group returned within normal range, which is comparable to 20% of patients in the placebo group.

At End of Study (EoS), blood pro-inflammatory cytokines levels were significantly lower in the NuSepin 0.2 mg/kg group, and the percentage of patients with pro-inflammatory cytokines within normal ranges was higher in patients in the NuSepin 0.2 mg/kg group compared with those in the placebo group. For example, IL-8 levels (pg/ml) in patients of the NuSepin 0.2 mg/kg group (12.8 ± 2.2) at EoS were significantly lower than those of the randomization day (22.1 ± 4.0, p <0.05). In comparison, IL-8 levels in patients of the placebo group were not significantly decreased at EoS day (259.4 ± 167.8) compared with those of randomization day (22.0 ± 3.2). IL-8 levels returned within normal ranges in 55% of patients in the NuSepin 0.2 mg/kg group and in 33% of patients in the placebo group. At EoS day, the levels of TNF-α (pg/ml) in patients of the NuSepin 0.2 mg/kg group were (7.4 ± 0.7) significantly lower than those of patients in the placebo group (24.8 ± 12.3, p=0.007), and TNF-α levels returned within normal ranges in 35% of

patients in the NuSepin 0.2 mg/kg group and in 11% of patients in the placebo group. Furthermore, IL-6 levels (pg/ml) returned within normal ranges in 60% of patients in the NuSepin 0.2 mg/kg group and in 33% of patients in the placebo group. IL-6 levels in patients in the NuSepin 0.2 mg/kg group at EoS (9.7 ± 5.1) were significantly lower than those of randomization day (19.0 ± 3.9, $p<0.05$). Because no deceased cases were observed in either the NuSepin or placebo groups, we were unable to compare 28-d mortality.

Safety outcomes

In the SAS population, which includes all 64 patients, 25% of patients experienced more than one Adverse Event (AE). The total number of events was 31. 27.3% (6 patients, 12 events) of the 0.1 mg/kg NuSepin group, 27.3% (6 patients, 12 events) of the 0.2 mg/kg NuSepin group, and 20.0% (4 patients, 7 events) of the placebo group reported at least one treatment emergent AE. AEs were mild and recovered by EoS. The relationship between AEs and NuSepin was determined to be 'possible' when graded as "probable, possible, or definite". There was one serious AE of dyspnea in the NuSepin 0.2 mg/kg arm which was "not related" to NuSepin. The most common AE was increases in ALT, *i.e.,* 1 case (5%), 2 cases (9%), and 2 cases (10%) in the NuSepin 0.1 mg/kg, NuSepin 0.2 mg/kg, and placebo arm, respectively.

DISCUSSION

In this small multicenter phase II trial involving 64 hospitalized patients, the differences in primary endpoint (TTCI_P) between groups did not reach statistical significance at a threshold of 5%. However, after adjusting significant baseline imbalances of covariates (use of anti-viral drugs and baseline NEWS2 at randomization day), the overall RR was 3.4-fold higher in the NuSepin 0.2 mg/kg group compared with the placebo group, suggesting a higher probability of clinical improvement by NuSepin 0.2 mg/kg, when TTCI_S was analyzed. Without adjusting baseline imbalances, RR of the moderate-to-severe patients was 2.7 ($p=0.02$), which favored improved recovery (TTCI_S) in the NuSepin 0.2 mg/kg group. These findings suggests that NuSepin stabilize respiratory rate, pulse rates, systolic blood pressure, body temperature and blood SpO_2 without supplementary oxygen, significantly faster than placebo.

In addition to faster normalization of unstable vital signs, NuSepin 0.2 mg/kg also normalized level of blood pro-inflammatory cytokines that are frequently elevated in COVID-19 patients, faster than placebo. Interleukin (IL)-8, neutrophil chemotactic factor, induces chemotaxis of granulocytes including neutrophils, crucial for migration toward the site of inflammation. Considering macrophages that express GPCR19, a target of NuSepin, produce IL-8, NuSepin might play role as a GPCR19 agonist in patients as like as preclinical settings. Because IL-6 level of a patient in placebo group was outlier (632 pg/ml), the mean baseline IL-6 level in placebo group (51.0 ± 138.2 pg/ml, 20.5 ± 4.8 without an outlier) was much higher than 0.1 mg/kg (22.5 ± 22.7 pg/ml) or 0.2 mg/kg (19.0 ± 18.4 pg/ml) NuSepin group. The differences between groups did not reach statistical significance at a threshold of 5% ($p=0.3$ by t-test).

Mortality of COVID-19 has decreased recently. This might be due to immunity established by vaccination and well-organized guidelines to manage COVID-19 patients. Nonetheless, more efficacious anti-viral drugs combined with better anti-inflammatory drugs are still needed to decrease hospital stays, ICU stays, and mortality as low as clinical parameters of infection by seasonal Influenza viruses to co-exist with SARS-CoV-2 in an endemic era.

The clinical spectrum of COVID-19 includes asymptomatic infection, mild, moderate, severe, and critical illness. In this study, the patients with baseline NEWS2 ≥ 5 at randomization day was stratified as moderate to severe illness. In comparison, the moderate illness corresponds to patients who have SpO_2 ≥ 94% on room air according to COVID-19 Treatment Guidelines from NIH, USA. Considering that the lowest NEWS2 score of patients in the severe stratum of the NIH Guidelines is 5, moderate to severe strata in our study (NEWS2 ≥ 5 at randomization day) corresponds to severe to critical strata suggested by the NIH Guidelines.

In endemic era of SARS-CoV2, the management of severe to critical patients might be more crucial than mild to moderate cases socioeconomically. Severe to critical cases of COVID-19 are frequently associated with cytokine release syndrome and thromboembolic complications that leads to respiratory failure, cardiac dysfunction, and septic shock. It has been suggested that hemodynamic and pulmonary management of severe to critical adult COVID-19 patients should follow the protocols for adult patients with septic shock as published in the surviving sepsis campaign: International guidelines for management of sepsis and septic shock.

For the stratification of clinical status of sepsis patients and COVID-19 patients, the quick Sequential Organ Failure Assessment (qSOFA), NEWS2, CURB-65, and Systemic Inflammatory Response Syndrome (SIRS) criteria have been used. Among these tools, NEWS2 showed better negative predictive value of 98.0% for early mortality and superior accuracy to predict severity of COVID-19 than CURB-65, qSOFA, and COVID-GRAM, although it is still controversial. Six vital signs (respiratory rate, blood oxygen saturation, systolic blood pressure, pulse rate, and body temperature) in addition to level of consciousness (or new confusion) and dependency on supplementary oxygen, are assessed by NEWS2 aggregate scoring. The sensitivity and specificity of a baseline NEWS2 score ≥ 6 in predicting severe disease is 80.0% and 84.3%, respectively. Consequently, NEWS2 score ≥ 5 has been used as a key threshold indicating poor prognosis that needs to be managed as sepsis patients. In comparison, a recent multicenter study showed that baseline NEWS2 had poor-to-moderate discrimination to predict clinical outcomes of severe COVID-19 patients at 14 day.

In two ways, NEWS2 could be used in clinics, 1) to predict short term or long term prognosis of patients based on baseline NEWS2 in order to detect early warning signal and 2) to stratify clinical status of patients based on stability of vital signs and dependency on supplementary oxygen in order to assess clinical improvement daily. In this study, we measured NEWS2 score of patients to assess clinical improvement daily in terms of stability of vital signs, which is the most crucial parameter in managing severe to critical patients in intensive care unit.

An advantage of NEWS2 compared to the other tools used for scoring clinical status is that both hypoxemia status and dependency on supportive oxygen treatment are included as scoring parameters, which is crucial immediate outcomes of pulmonary management of COVID-19 pneumonia. Considering that stabilization of abnormal respiratory rate, blood oxygen saturation, systolic blood pressure, pulse rate, and body temperature is immediate goal for pulmonary and hemodynamic management of severe to critical COVID-19 patients, NEWS2 might be used as a surrogate composite outcome measure to assess the clinical efficacy of test therapeutics for COVID-19 patients daily.

In underdeveloped countries without enough supplies of vaccines or anti-viral drugs, number of severe-to-critical COVID-19 patients may not decrease for a while. In these countries where hospital resources such as supplementary oxygen devices are limited, de-escalation of supplementary oxygen supply need to be evaluated as a primary endpoint (TTCI_P) in clinical trials carefully. Because supplementary oxygen might not be de-escalated appropriately according to clinical status of patients due to limited supply with devices and overload of patients in the clinics. In this study, de-escalation of supplemental oxygen support (TTCI_P, assessed by OS) was not correlated with TTCI_S. For these reasons, time to normalization of pulmonary and hemodynamic parameters (measured by NEWS2 daily) might be better composite outcome measure for clinical trials in the underdeveloped countries without enough hospital resources.

CONCLUSION

Considering that NuSepin was not associated with an increased risk of adverse events, NuSepin could be considered as relevant in the context of a pandemic, in which NuSepin modestly expedites stabilization of vital signs after admission and normalization of blood levels of pro-inflammatory cytokines, especially in moderate to severe patients. These findings suggest that inflammasome inhibitor might be one of the candidate that can be combined with various anti-inflammatory drugs and anti-viral drugs for COVID-19 patients.

Limitations

The present study has several limitations because of limited sample size. Large scale validation study would increase the generalizability of our results. Only patients who were hospitalized were enrolled; none of the patients was admitted to ICU; none of the patients was in ordinal scale 5 (on 6 categories) on admission. It is also possible that this population may not be as severely ill as populations enrolled in other RCTs. For example, about 15% of patients in RECOVERY were receiving invasive mechanical ventilation. The NuSepin trial recruited none of the participants who were invasively ventilated. For this reason, the results about severe to critical COVID-19 are not conclusive. The patient with leukocyte count >4.0 x 10^9 and lymphocyte count >0.7 x 10^9 were chosen as inclusion criteria because of ethical reasons due to uncertainty of NuSepin in terms of immunosuppression. Because lymphopenia is common in COVID-19 and is correlated to severity of illness, future validation study would clarify the efficacy of NuSepin in lymphopenia patients. While there is some precedent for the use of time to clinical improvement on the ordinal scale (TTCI_P) in previously published studies for COVID-19 patients, use of "time to NEWS2 of 0" (TTCI_S) as the outcome measure might be a matter for debate. Nonetheless, this is a clinical trial of NLRP3 inflammasome inhibitor targeting GPCR19 showing promising results in terms of time to normalizing unstable pulmonary and hemodynamic vital signs of COVID-19 patients, for the first time on the literature.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The protocol was approved by the central ethics committee, Local Ethics Committee (LEC) at each site where applicable, and the national agency for medicines and medical devices of romania. Written informed consent was obtained from each patient or from the patient's legally authorized representative if the patient was unable to provide consent.

Conflicts of interest statement

This study received funding from Shaperon Inc; CL, CM, OACS and AC: Grant from Shaperon Inc. for the submitted work. The funder had the following involvement with the study; SYS, SHL and HSA are employees of Shaperon Inc. CL, CM, OACS and AC was involved in the data collection. SYS, SHL and HAS are involved in the study design, analysis, interpretation of data and writing of this article. All authors are involved in the decision to submit it for publication. The patent "Composition for treating COVID-19 comprising taurodeoxycholic acid or pharmaceutically acceptable salts thereof as an active ingredient: PCT/KR2021/011236" was invented by SYS, et al., and applied by Seoul National University R&DB Foundation. The exclusive license for the patent was transferred from SNU R&DB Foundation to Shaperon Inc. SYS is a founder of Shaperon Inc. and its current CEO.

REFERENCES

1. Li C, et al. The impact of receptor binding domain natural mutations on antibody recognition of SARS-CoV-2. Signal Transduct Target Ther. 2021;6:132.
2. Gandhi RT, et al. Mild or moderate Covid-19. N Engl J Med. 2020;383:1757-1766.
3. Rosas IO, et al. Tocilizumab in Hospitalized Patients with Severe Covid-19 Pneumonia. N Engl J Med. 2021;384:1503-1516.
4. Zhang K, et al. The prognostic accuracy of national early warning score 2 on predicting clinical deterioration for patients with covid-19: A systematic review and meta-analysis. Front Med (Lausanne). 2021;8.
5. Mokhtari T, et al. COVID-19 and multiorgan failure: A narrative review on potential mechanisms. J Mol Histol. 2020;51:613-628.
6. Hosseinzadeh R, et al. Should all patients with hypertension be worried about developing severe coronavirus disease 2019 (COVID-19)? Clin Hypertens. 2021;27:3.
7. Zhou Y, et al. Obesity and diabetes as high risk factors for severe coronavirus disease 2019 (Covid-19). Diabetes Metab Res Rev. 2021;37:3377.
8. Que Y, et al. Cytokine release syndrome in COVID-19: A major mechanism of morbidity and mortality. Int Rev Immunol. 2021:1-14.
9. Moore JB, et al. Cytokine release syndrome in severe COVID-19. Science. 2020;368:473-474.
10. Borges L, et al. COVID-19 and Neutrophils: The relationship between hyperinflammation and neutrophil extracellular traps. Mediators Inflamm. 2020:8829674.
11. Monari C, et al. A focus on the nowadays potential antiviral strategies in early phase of coronavirus disease 2019 (Covid-19): A narrative review. Life. 2020;10.
12. Taylor PC, et al. Neutralizing monoclonal antibodies for treatment of COVID-19. Nat Rev Immunol. 2021;21:382-393.
13. Horby P, et al. Dexamethasone in hospitalized patients with Covid-19. N Engl J Med. 2021;384:693-704.
14. Tortajada C, et al. Corticosteroids for COVID-19 patients requiring oxygen support? Yes, but not for everyone: Effect of corticosteroids on mortality and intensive care unit admission in patients with COVID-19 according to patients oxygen requirements. J Med Virol. 2021;93:1817-1823.
15. Stebbing J, et al. COVID-19: combining antiviral and anti-inflammatory treatments. Lancet Infect Dis. 2020;20:400-402.
16. van Paassen J, et al. Corticosteroid use in COVID-19 patients: A systematic review and meta-analysis on clinical outcomes. Crit Care. 2020;24:696.
17. Ni YN, et al. The effect of corticosteroids on mortality of patients with influenza pneumonia: A systematic review and meta-analysis. Crit Care. 2019;23:99.
18. Hui DS, et al. Systemic corticosteroid therapy may delay viral clearance in patients with middle east respiratory syndrome coronavirus infection. Am J Respir Crit Care Med. 2018;197:700-701.
19. Babaei F, et al. Review of registered clinical trials for the treatment of COVID-19. Drug Dev Res. 2021;82:474-493.
20. Guimaraes PO, et al. Tofacitinib in patients hospitalized with Covid-19 pneumonia. N Engl J Med. 2021;385:406-415.