




# Evaluation of the potential of Rejuveinix plus dexamethasone against sepsis

Fatih M Uckun<sup>\*1,2</sup> , Muhammad Saeed<sup>3</sup>, Mustafa Awili<sup>3</sup>, Ibrahim H Ozercan<sup>4</sup>, Sanjive Qazi<sup>2</sup>, Cynthia Lee<sup>1</sup>, Adeel Shibli<sup>3</sup>, Alan W Skolnick<sup>5</sup>, Alonso Prusmack<sup>6</sup>, Joseph Varon<sup>6</sup>, Cesar IP Barrera<sup>6</sup>, Cemal Orhan<sup>7</sup> , Michael Volk<sup>1</sup> & Kazim Sahin<sup>7</sup> 

<sup>1</sup>Drug Discovery Program, Reven Pharmaceuticals, Westminster, CO 80234, USA

<sup>2</sup>Department of Developmental Therapeutics, Immunology & Integrative Medicine, Ares Pharmaceuticals, St Paul, MN 55110, USA

<sup>3</sup>PRX Research & Dallas Regional Medical Center, Dallas, TX 75149, USA

<sup>4</sup>Department of Pathology Faculty of Medicine, Firat University, Elazig, 23119, Turkey

<sup>5</sup>Memorial Hermann Memorial City Medical Center, Houston, TX 77024, USA; HD Research, Bellaire, TX 77401, USA

<sup>6</sup>United Memorial Medical Center, Houston, TX 77091, USA

<sup>7</sup>Department of Animal Nutrition, Faculty of Veterinary, Firat University, Elazig, 23119, Turkey

\*Author for correspondence: Tel.: +1 612 443 4607; [fatih.uckun@aresmit.com](mailto:fatih.uckun@aresmit.com)

**Aim:** Our main objectives were to compare the effects of Rejuveinix (RJX), dexamethasone (DEX) and their combination on the severity of sepsis and survival outcome in an animal model of fatal sepsis. **Methods:** We used the LPS plus D-galactosamine mouse model of sepsis to compare the anti-inflammatory activities of RJX, dexamethasone and a combination of RJX plus DEX. Additionally, we examined the clinical feasibility and tolerability of combining RJX with DEX in COVID-19 patients in a clinical phase I study. Data were analyzed using standard methods. **Results & conclusion:** RJX exhibited potent anti-inflammatory activity in the murine sepsis model. The combination of RJX plus DEX was more effective than either agent alone, decreased the inflammatory cytokine responses and associated organ damage, and improved the survival outcome in mice. In the phase I clinical study, RJX plus DEX was well tolerated by COVID-19 patients.

First draft submitted: 17 February 2022; Accepted for publication: 1 August 2022; Published online: 2 September 2022

**Keywords:** COVID-19 • inflammation • LPS • RJX • sepsis • vitamins

Sepsis represents a strong systemic inflammatory response to an infection with a potentially fatal outcome due to its complications [1–9]. Severe viral sepsis caused by SARS-CoV-2 shows a rapid progression associated with cytokine release syndrome and a high case fatality rate in high-risk COVID-19 patients [10–16]. Our antisepsis drug candidate, Rejuveinix (RJX), comprises several anti-inflammatory and antioxidant vitamins [17–28]. It has exhibited promising single-agent anti-inflammatory activity in mice challenged with LPS plus D-galactosamine (LPS-GalN) [17]. Specifically, it decreased the inflammatory cytokine responses and improved survival when administered prophylactically in mice challenged with LPS-GalN. Furthermore, preliminary experiments have suggested that it may also be effective in reversing sepsis if administered after the onset of sepsis [17,19]. In addition, RJX showed a favorable clinical safety and pharmacokinetics profile in a recently completed randomized, double-blind, placebo-controlled phase I ascending dose-escalation study in healthy volunteers (Clinical Trial Registration: NCT03680105 [ClinicalTrials.gov]) [17].

The primary objective of the present study was to compare the effects of RJX, the standard anti-inflammatory drug dexamethasone (DEX) and their combination on the severity of sepsis and survival outcome in an animal model that employed LPS-GalN to induce fatal sepsis. We hypothesized that RJX, especially when combined with DEX, would improve the survival outcome of mice developing systemic inflammation after injection with a lethal dose of LPS-GalN [23]. Here, we first confirm and extend our previous study of the single-agent activity of RJX in mice challenged with LPS-GalN [17,23]. We demonstrate that RJX – at a dose level >ten-times lower than its clinical maximum tolerated dose (MTD) – exhibits potent single-agent anti-inflammatory activity and is as effective as DEX at standard dose levels in this model of fatal sepsis [23]. The combination of RJX plus DEX immediately and profoundly decreased the inflammatory cytokine (IL-6, TNF- $\alpha$ ) responses to LPS-GalN,

mitigated the inflammatory tissue damage in the lungs and liver, and prevented a fatal outcome [23]. Although the treatments were started after the onset of fulminant cytokine storm characterized by markedly elevated serum IL-6 and TNF- $\alpha$  levels and systemic inflammation, as well as severe lung damage, a near-complete recovery of the inflammatory lung injury was achieved within 24 h and the survival outcome was improved [23]. We also report data from an early-stage clinical evaluation of RJX in COVID-19 patients with systemic inflammation.

## Materials & methods

### Rejuveinix

RJX is a formulation that contains multiple antioxidant vitamins with anti-inflammatory properties [17,21].

### LPS-GalN model of fatal cytokine storm & sepsis

The RJX doses were 0.7 ml/kg (~14  $\mu$ l/20 g mouse) and 1.4 ml/kg (~28  $\mu$ l/20 g/mouse), and they were administered as intraperitoneal (ip.) bolus injections. The doses of the active ingredients at the 0.7 ml/kg dose were 31.5 mg/kg vitamin C, 2.2 mg/kg vitamin B1, 0.09 mg/kg vitamin B2, 4.2 mg/kg vitamin B3, 0.1 mg/kg vitamin B5, 4.2 mg/kg vitamin B6, 0.07 mg/kg vitamin B12 and 28.3 mg/kg magnesium sulfate. The doses for the active ingredients at the 1.4 ml/kg dose were 63 mg/kg vitamin C, 4.4 mg/kg vitamin B1, 0.18 mg/kg vitamin B2, 8.4 mg/kg vitamin B3, 0.2 mg/kg vitamin B5, 8.4 mg/kg vitamin B6, 0.14 mg/kg vitamin B12 and 56.6 mg/kg magnesium sulfate [17]. These sub-MTD dose levels were based on the observation of single-agent activity in the LPS-GalN mouse model of sepsis [17]. The 0.7 ml/kg mouse dose corresponds to a human equivalent dose (HED) of 0.057 ml/kg of RJX, which is 7.5% of its MTD of 0.759 ml/kg determined in the previously published randomized phase I clinical trial in healthy volunteers [17]. The 1.4 ml per kg mouse dose corresponds to HED of 0.114 ml/kg of RJX, which is 15% of its MTD [23].

In the reported experiments, the Guide for the Care and Use of Laboratory Animals was followed. The study received approval from the Animal Care and Use Committee of Firat University (no. 420629). Male BALB/c mice (6–8 weeks old and weighing on average 20 g), which were purchased from Laboratory Animal Center of Firat University, were randomly divided into different treatment groups, as previously reported [17]. In order to induce fatal cytokine release syndrome as well as acute lung and liver injury, mice were challenged with an ip. injection of LPS plus D-galactosamine (Sigma, MO, USA). Each mouse received a 500  $\mu$ l ip. injection of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) [23]. Treatments were delayed until 2 h post-LPS-GalN injection when mice had a fulminant systemic inflammation with very severe lung and liver damage, as well as markedly elevated inflammatory cytokine levels [23]. Vehicle control mice were treated with 0.5 ml normal saline (NS) instead of RJX. NS was administered ip. 2 h after LPS-GalN. Test mice received either RJX (0.7 ml/kg or 1.4 ml/kg) or DEX (0.1 mg/kg, 0.6 mg/kg or 6.0 mg/kg) as a monotherapy in a side-by-side comparison. We also examined a combination of 0.7 ml/kg RJX with either 0.6 mg/kg or 6 mg/kg DEX at 2 h post-LPS-GalN injection [23]. Drugs were administered ip. in a total volume of 0.5 ml. Tissue levels of oxidative stress biomarkers superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px), ascorbic acid levels and malondialdehyde were determined to examine the effects of RJX, DEX and RJX + DEX on the sepsis-associated oxidative stress. Levels of IL-6 and TNF- $\alpha$  in serum samples were measured by quantitative ELISA using the commercially available Quantikine ELISA kits, as previously reported [17]. At the time of death, blood samples were obtained for biomarker studies. In addition, lungs and liver specimens were subjected to histopathologic examination, as described previously [17].

### Statistical analysis

Standard statistical methods were used in analyzing the experimental data. The Kaplan–Meier method was applied to the analysis of the survival outcome data within the limitations of a small sample size, as reported [17].

### Clinical phase I study

The study examined the safety of RJX plus institutional standard of care (SOC) in hospitalized patients with COVID-19. Patients received daily 40 min ( $\pm$ 10 min) infusions of 20 ml RJX mixed with 100 ml normal saline (total volume = 120 ml) intravenously plus SOC for 7 consecutive days. The details, including the statistical analysis of the clinical data, are provided in the Supplementary Data. The study was performed under IND149585 at the following four centers: 1) Memorial Hermann Memorial City Medical Center, TX, USA; 2) Christus Health Santa Rosa Hospital, TX, USA; 3) LFROS Research and United Memorial Medical Center, TX, USA; and 4) PRX Research and Dallas Regional Medical Center, TX, USA.

## Results

### Effectiveness of treatment with RJX in a side-by-side comparison to DEX in reversing acute lung & liver injury & improving survival in mice injected with LPS-GalN

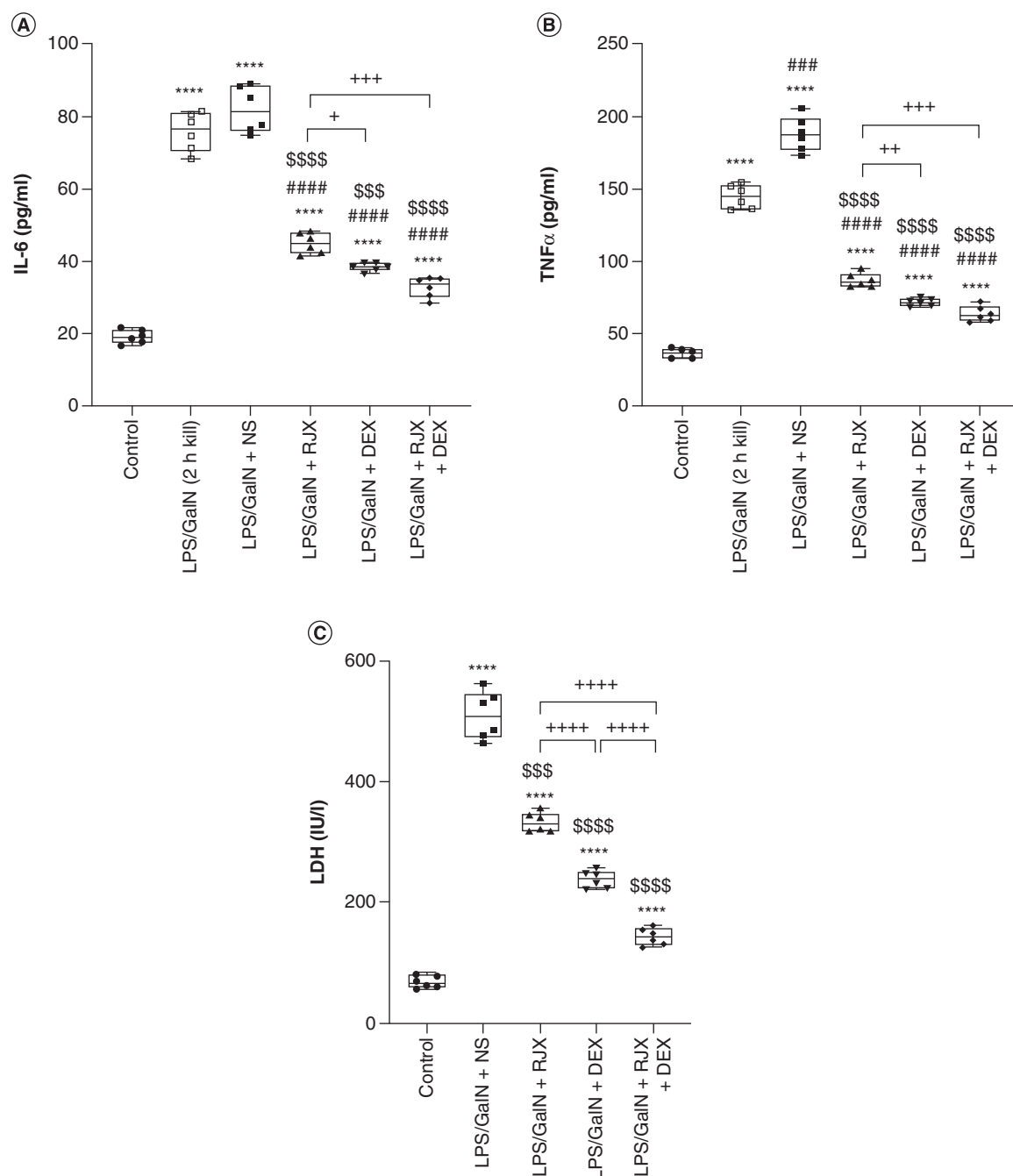
LPS-GalN-challenged mice experience a rapid onset systemic inflammation with markedly elevated inflammatory cytokine levels as well as severe lung injury, as early as 2 h after the administration of LPS-GalN [17]. We first set out to determine the effects of RJX versus DEX on the LPS-GalN-induced inflammatory cytokine response in BALB/c mice [23] (Supplementary Figure 1). At 2 h post-injection of LPS-GalN, the serum IL-6 and TNF- $\alpha$  levels were markedly elevated in electively terminated control mice consistent with an inflammatory cytokine response. A single low-dose RJX at the assigned low dose levels of 0.7 ml/kg (HED: 0.057 ml/kg; 7.5% of human MTD) and 1.4 ml/kg (HED: 0.114 ml/kg; 15% of human MTD), was administered at 2 h after LPS-GalN injection, and effectively reversed the LPS-GalN-induced increased serum levels of the pro-inflammatory cytokines IL-6 and TNF- $\alpha$  within 24 h [23] (Supplementary Figure 1). A 1.4 ml/kg low-dose (15% of MTD) RJX was significantly more effective than a single DEX injection at the standard 0.6 mg/kg dose level (HED: 0.05 mg/kg; 4 mg standard dose for an 80 kg person) and comparable to the 6.0 mg/kg high-dose DEX (HED: 0.49 mg/kg; 39 mg dose for an 80 kg person, which is higher than the standard 4–8 mg dose levels for DEX) (Supplementary Figure 1). The observed reversal of the inflammatory cytokine response by RJX (0.7 or 1.4 ml/kg) or DEX (0.6 or 6.0 mg/kg) was associated with a partial reversal of the lung and liver injury that was documented at 2 h post-LPS-GalN injection when treatments were initiated (Supplementary Figure 2–4). Specifically, as evidenced in Supplementary Figures 2A & 3, RJX at 0.7 ml/kg (mean  $\pm$  standard error [SE] acute lung injury [ALI] score:  $2.7 \pm 0.2$ ) or 1.4 ml/kg (mean  $\pm$  SE ALI score:  $2.3 \pm 0.2$ ) low dose levels as well as DEX at the 0.6 mg/kg (HED: 0.05 mg/kg; 4 mg standard dose for an 80 kg person) (mean  $\pm$  SE ALI score:  $2.8 \pm 0.3$ ) dose level (but not DEX at 0.1 mg/kg dose level; mean  $\pm$  SE ALI score:  $3.5 \pm 0.2$ ) were capable of partially reversing the lung injury that was documented at 2 h post-LPS-GalN injection when treatments were initiated (mean  $\pm$  SE ALI score:  $3.0 \pm 0.3$ ), as measured by the lung histopathological scores (i.e., ALI scores) [23]. The best results were obtained with a 6.0 mg/kg suprathreshold high-dose DEX (HED: 0.49 mg/kg; 39 mg dose for an 80 kg person) (mean  $\pm$  SE ALI score:  $1.8 \pm 0.3$ ) (Supplementary Figure 3A). By comparison, the lung damage further progressed in control mice treated with NS (vehicle) (mean  $\pm$  SE ALI score:  $3.7 \pm 0.1$ ) [23]. Similar to its effects on the LPS-GalN-induced ALI, 0.7 ml/kg or 1.4 ml/kg low-dose RJX, as well as standard 0.6 mg/kg and very high 6.0 mg/kg dose levels of DEX (but not DEX at 0.1 mg/kg dose level), significantly reduced the liver injury (Supplementary Figures 2 & 4). The histopathological liver damage scores (mean  $\pm$  SE) were  $0 \pm 0$  for control mice not challenged with LPS-GalN,  $3.5 \pm 0.3$  for mice electively terminated 2 h post LPS-GalN,  $3.5 \pm 0.2$  for mice treated with NS post LPS-GalN,  $3.3 \pm 0.2$  for 0.1 mg/kg DEX,  $2.7 \pm 0.2$  for 0.6 mg/kg DEX,  $2.3 \pm 0.2$  for 6.0 mg/kg DEX,  $2.5 \pm 0.2$  for 0.7 ml/kg RJX and  $2.3 \pm 0.2$  for 1.4 ml/kg RJX [23].

RJX treatment significantly improved the survival of mice (Supplementary Figure 5). By comparison, treatment with NS included as vehicle control did not reverse the fulminant cytokine response or prevent its progression. Notably, 0.7 ml/kg low-dose RJX was moderately more effective than DEX at a 0.1 mg/kg low dose level (median survival: 15.1 h vs 5.1 h; 24-h mortality: 50% vs 83.3%), and it was as effective as DEX at the standard dose 0.6 mg/kg (Supplementary Figure 5). Notably, at a dose level of 1.4 ml/kg, which corresponds to 15% of its clinical MTD, RJX reduced the mortality to 40% (median survival >24 h). These results were very similar to and statistically not different from the 33.3% mortality (median survival >24 h;  $p = 0.99$ ) achieved with DEX at the suprathreshold 6.0 mg/kg dose level that is 4.9–9.8-fold higher than the standard 4–8 mg clinically applied dose levels for DEX [23] (Supplementary Figure 5).

### Effectiveness of treatment with RJX in combination with DEX in reversing fatal cytokine storm, acute lung & liver injury & improving survival in mice injected with LPS-GalN

Low-dose RJX (0.7 ml/kg) plus high-dose DEX (6.0 mg/kg) effectively reversed the increased serum levels of the systemic inflammation markers (IL-6, TNF- $\alpha$  and lactate dehydrogenase) within 24 h, and it appeared to be overall more effective than DEX alone or RJX alone (Figure 1) [23]. The serum levels of lactate dehydrogenase, a biomarker of systemic inflammation and tissue damage, were significantly lower in mice treated with the RJX + DEX combination than mice treated with RJX alone ( $p < 0.0001$ ) or DEX alone ( $p < 0.0001$ ) (Figure 1).

As there was significant residual tissue damage in the lungs and liver of surviving LPS-GalN-challenged mice treated with RJX or DEX (even at the 6.0 mg/kg high dose level), we next sought to determine if a combination of low-dose RJX (0.7 ml/kg) and high-dose DEX (6.0 mg/kg) could improve the tissue healing and the survival



**Figure 1. Therapeutic use of low-dose RJX plus suprathreshold high-dose DEX combination after onset of systemic inflammation and lung injury reverses inflammatory cytokine response and systemic inflammation in the LPS-GalN mouse model of fatal cytokine storm and sepsis.** Depicted are the effects of RJX, DEX and RJX + DEX combination treatments on serum levels of IL-6 (A), TNF-α (B) and LDH (C) [23]. Groups of six BALB/c mice were treated with intraperitoneal injections of RJX (sixfold diluted, 4.2 ml/kg, 0.5 ml/mouse), DEX (6 mg/kg, 0.5 ml/mouse), RJX + DEX (0.5 ml/mouse) or vehicle (NS, 0.5 ml/mouse) 2 h post-injection of LPS-GalN. Except for untreated control mice (control), each mouse received 0.5 ml of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) intraperitoneally. The depicted whisker plots represent the median and values. Welch's analysis of variance and Tamhane's T2 *post hoc* test were used for comparing the results among different treatment groups. Statistical significance between groups is shown by \*\*\*\**p* < 0.0001 as compared with control group; ###*p* < 0.001; #####*p* < 0.0001 as compared with LPS/GalN (2 h kill) group; \$\$\$*p* < 0.001; \$\$\$\$*p* < 0.0001 as compared with LPS/GalN + NS and +*p* < 0.05; ++*p* < 0.01; +++*p* < 0.001; ++++*p* < 0.0001 pairwise comparisons between the groups. DEX: Dexamethasone; GalN: Galactosamine; LDH: Lactate dehydrogenase; LPS: Lipopolysaccharide; NS: Normal saline; RJX: Rejuveinix.



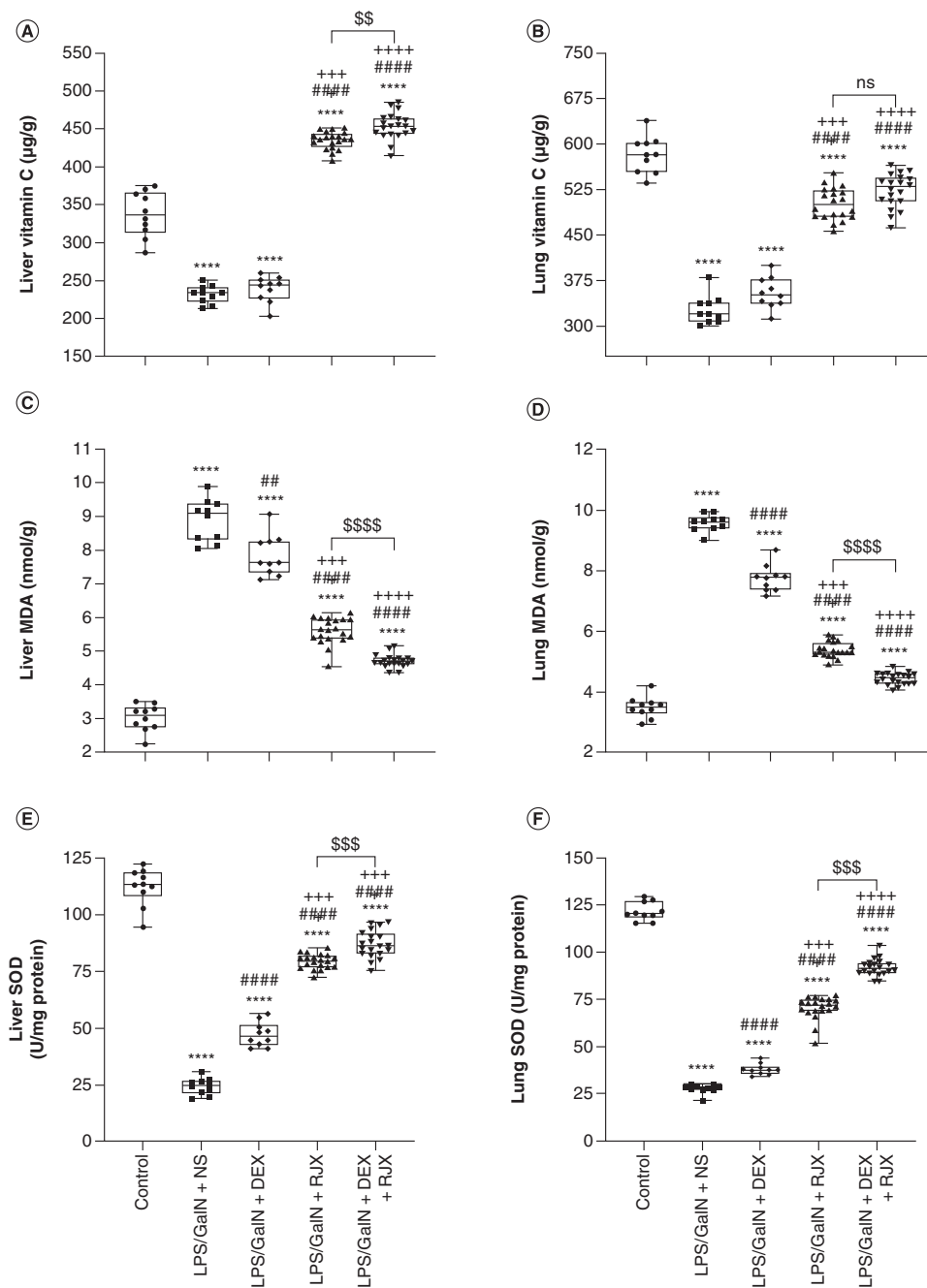
outcome after LPS-GalN exposure [23]. Treatments were initiated at 2 h after LPS-GalN injection at a time of documented active systemic inflammation. LPS-GalN caused severe inflammation and oxidative stress in the lungs and liver with enhanced lipid peroxidation, as measured by a marked elevation of malondialdehyde levels, and decreased tissue levels of vitamin C as well as the antioxidant enzymes GSH-Px that inhibit lipid peroxidation and SOD that converts superoxide anion radicals that contribute to lipid peroxidation into hydrogen peroxide and oxygen. Low-dose RJX alone or in combination with DEX significantly suppressed the oxidative stress, as documented by increases in vitamin C, GSH-Px and SOD levels that were reduced by LPS-GalN (Figure 2A, C & E depict the liver tissue levels; B, D & F depict the lung tissue levels). Notably, delayed treatments with RJX, DEX or RJX + DEX starting at 2 h after the LPS-GalN injection partially reversed the inflammatory lung damage within 24 h [23], as evidenced by significantly reduced histopathological lung/ALI scores (Figures 3 & Supplementary Figure 6). The tissue healing activity of the combination was more pronounced than the tissue healing activity of RJX alone or DEX alone and resulted in a near-complete recovery of the severe lung damage (Figures 3 & Supplementary Figure 6).

RJX + DEX combination also decreased the liver tissue damage (Figure 3) [23]. While 12 of 12 mice (100%) treated with either RJX alone (n = 6) or DEX alone (n = 6) had moderate-to-severe residual damage in either their lungs or liver, two of the six mice (33.3%) treated with the combination regimen had no or minimal damage (i.e., histopathological damage scores: 0–1) in both organs (p = 0.098, Fisher's exact test). RXX + DEX was more effective than RJX alone or DEX alone in improving the survival outcome (Supplementary Figure 7). In contrast to the rapid death of all control mice treated with NS (median survival: 4.3 h post-LPS-GalN or 2.3 h after administration of NS), 100% of mice treated with RJX + DEX survived the LPS-GalN challenge (median survival: >24 h post LPS-GalN or >22 h after initial administration of RJX + DEX) (Supplementary Figure 7). By comparison, the combined group of mice treated with monotherapy (i.e., RJX alone or DEX alone) (n = 12) had a 24-h survival rate of 41.7% (monotherapy with RJX or DEX vs combination therapy with RJX + DEX: log-rank  $X^2 = 3.053$ ; p = 0.081).

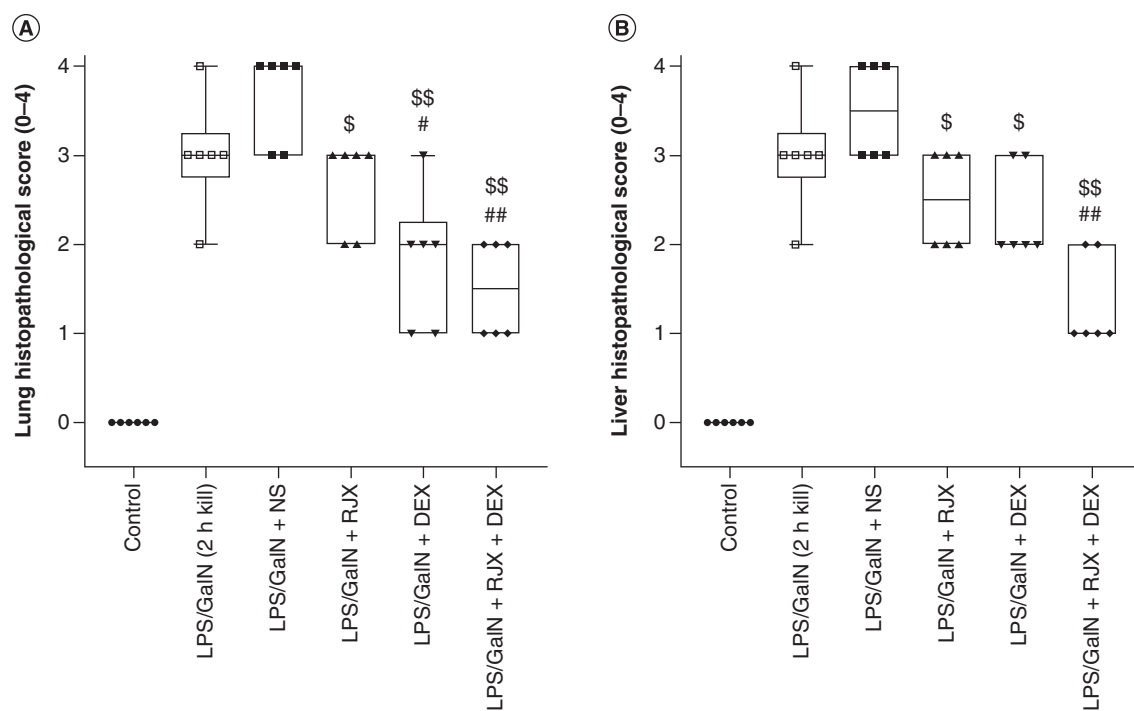
We next evaluated the efficacy of a combination regimen that employs low-dose RJX (0.7 ml/kg) plus standard-dose DEX (0.6 mg/kg) [23]. Notably, delayed treatments with low-dose RJX plus standard-dose DEX starting at 2 h after the LPS-GalN injection reversed the lung damage, as evidenced by significantly reduced histopathological lung scores (Figure 4). The tissue-healing activity of the combination was more pronounced than the tissue-healing activity of RJX alone or DEX alone (Figure 4A). Hence, although treatments were delayed until the onset of fulminant cytokine storm and systemic inflammation as well as very severe lung damage, a near-complete recovery of the inflammatory lung injury was achieved in the vast majority of mice within 24 h. Similar to its effects on the LPS-GalN induced ALI, RJX + DEX combination significantly reduced the liver injury (Figure 4B). While five of ten mice (50%) treated with standard-dose DEX alone and 14 of 20 mice (70%) treated with low-dose RJX alone had severe residual lung injury (histopathological lung score  $\geq 3$ ), only two of 20 (10%) of mice treated with the DEX + RJX combination had severe residual lung damage. The observed superiority of the combination regimen was statistically significant (Figure 4C). Similarly, while five of ten DEX-treated mice (50%) and 12 of 20 RJX-treated mice (60%) had severe residual liver damage, only two of 20 mice (10%) treated with the DEX + RJX combination had severe residual liver damage, and this difference was statistically significant (Figure 4C). In contrast to the rapid death of all control mice treated with NS (median survival: 5.1 h post LPS-GalN or 3.1 h after the administration of NS), 15 of 20 (75%) of mice treated with RJX + DEX survived the LPS-GalN challenge (median survival: >24 h post LPS-GalN) (Figure 5). By comparison, the combined group of mice treated with monotherapy (i.e., RJX alone or DEX alone) (n = 30) had a 24-h survival rate of 53% (i.e., 16 of 30 mice) (monotherapy with RJX or DEX vs combination therapy with RJX + DEX: log-rank  $X^2 = 3.977$ ; p = 0.046).

### Clinical data from the phase I study of RJX in high-risk COVID-19 patients

Thirteen hospitalized adult patients with high-risk COVID-19 received daily intravenous infusions of RJX (maximum one cycle = 7 consecutive days) in combination with institutional SOC. Preliminary data from this phase I study indicated that RJX plus DEX and other components of the SOC were well tolerated. The results are detailed in Supplementary Figures 8–16 & Supplementary Tables 1–6. Improvements have been observed in serum inflammatory markers as well as clinical parameters. The outcome data for 12 evaluable patients are shown in Supplementary Table 6. This early data must be interpreted with due caution within the confines of a nonrandomized phase I study.



**Figure 2. Tissue-level *in vivo* antioxidant activity of RJX, DEX and RJX + DEX in a mouse model of fatal cytokine storm, sepsis, systemic inflammation, ARDS and multiorgan failure.** BALB/c mice were treated with intraperitoneal injections of RJX (n = 20, sixfold diluted, 4.2 ml/kg, 0.5 ml/mouse), DEX (n = 10, 6 mg/kg, 0.5 ml/mouse), RJX + DEX (n = 20, 0.5 ml/mouse) or vehicle (NS, 0.5 ml/mouse) 2 h post-injection of LPS-GalN. We used previously published methods and statistical analysis tools [17,21]. Except for untreated control mice (control, n = 10), each mouse received 0.5 ml of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) intraperitoneally. The depicted whisker plots represent the median and values. **(A, C, E & F)**, Welch's analysis of variance and Games-Howell *post hoc* test were used for comparing the results among different treatment groups. **(B & D)** Analysis of variance and Tukey's *post hoc* test were used for comparing the results among different treatment groups. Statistical significance between groups is shown by \*\*\*\*p < 0.0001 as compared with control group; ##p < 0.01; #####p < 0.0001 as compared with LPS/GalN + NS group; +++++p < 0.0001 as compared with LPS/GalN + DEX group and \$\$\$p < 0.01; \$\$\$\$p < 0.001; \$\$\$\$\$p < 0.0001 pairwise comparisons between the groups. ARDS: Acute respiratory distress syndrome; DEX: Dexamethasone; GalN: Galactosamine; LPS: Lipopolysaccharide; NS: Normal saline; RJX: Rejuveinix; SOD: Superoxide dismutase.



**Figure 3.** *In vivo* treatment activity of low-dose RJX, suprathreshold high-dose DEX and their combination on lung and liver histopathological scores in the LPS-GalN mouse model of fatal cytokine storm and sepsis. We used previously published methods and statistical analysis tools [17,21]. Groups of six BALB/c mice were treated with intraperitoneal injections of RJX (sixfold diluted, 4.2 ml/kg, 0.5 ml/mouse), DEX (6.0 mg/kg, 0.5 ml/mouse) or vehicle (NS, 0.5 ml/mouse) 2 h post-injection of LPS-GalN. Except for untreated control mice (control), each mouse received 0.5 ml of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) intraperitoneally. The tissue damage in the (A) lungs and (B) liver were scored using previously published grading scales [17,21]. Mann-Whitney *U* test was used for comparing the results among different treatment groups.

Statistical significance between groups is shown by #*p* < 0.05; ##*p* < 0.01 as compared with LPS/GalN (2 h kill) group, \$*p* < 0.05; \$\$*p* < 0.01 as compared with LPS/GalN + NS group.

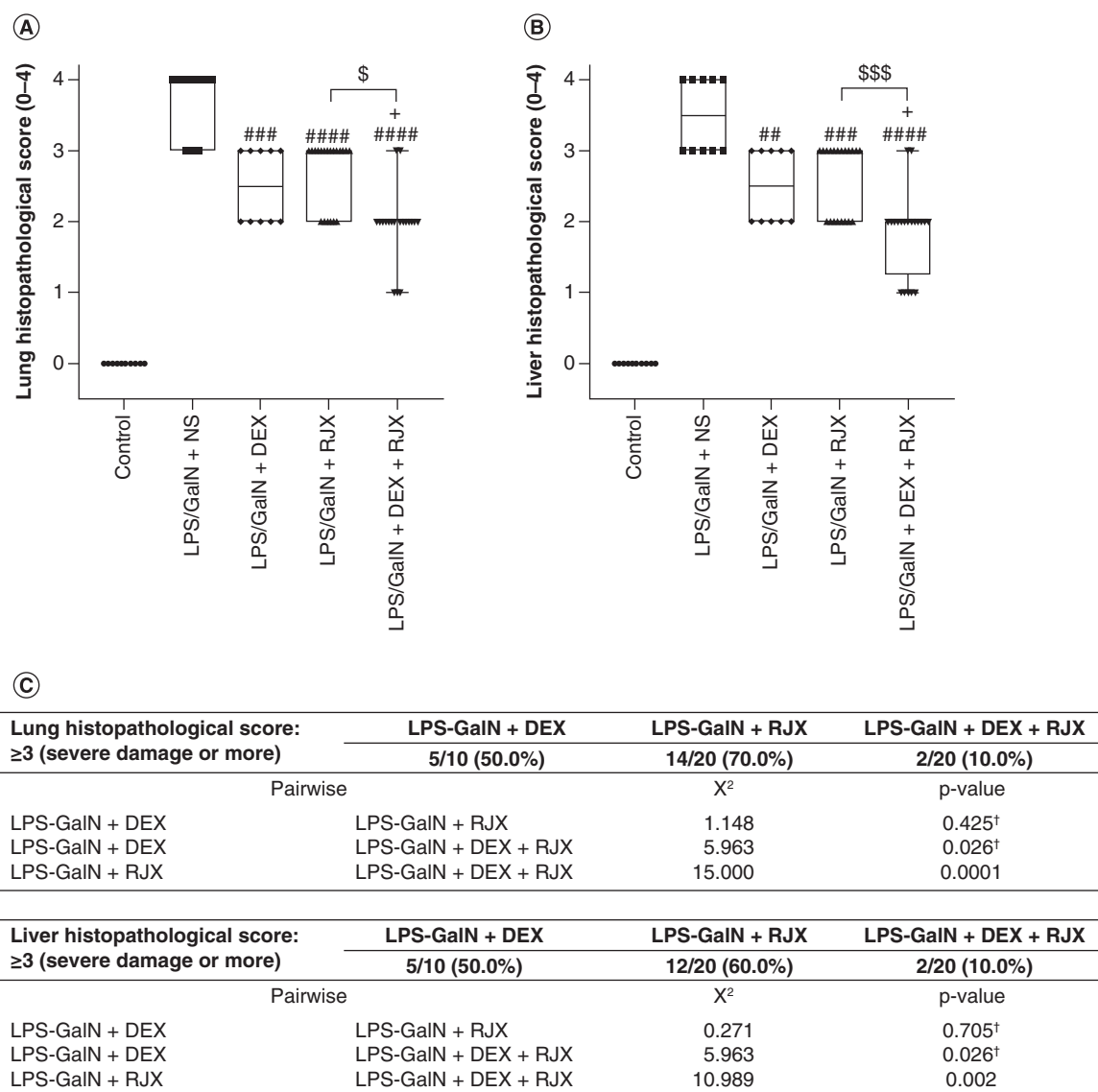
DEX: Dexamethasone; GalN: Galactosamine; LPS: Lipopolysaccharide; NS: Normal saline; RJX: Rejuveinix.

## Discussion

Sepsis is a major cause of mortality [1,3] and new treatment regimens are urgently needed for sepsis patients. COVID-19 has become a leading cause of death globally [29–31]. Patients with high-risk COVID-19 with viral sepsis are in urgent need of effective strategies that can prevent and/or reverse the systemic inflammatory process and its often fatal complications, including acute respiratory distress syndrome (ARDS) and multiorgan failure [11–13,15–17,29–31]. DEX has been shown to improve the survival outcome of patients with ARDS [32]. Similar findings have been reported from other studies [33,34].

RJX is an emerging anti-inflammatory drug candidate to prevent and treat sepsis, including bacterial sepsis in neutropenic cancer patients as well as viral sepsis in COVID-19 patients [17]. The active ingredients of RJX have demonstrated potential as antisepsis therapeutics [17]. However, while several studies appeared to demonstrate therapeutic benefit, no meaningful clinical activity was observed in other studies [20,22,24–27,35–37]. Additional studies are needed to clarify the reported discrepancies, which may in part relate to differences in eligibility criteria and timing of interventions.

Inflammatory cytokines play an important and causal role in the development of potentially life-threatening complications of COVID-19-associated viral sepsis in adults as well as multisystem inflammatory syndrome in children [9,38–46]. Recently, we demonstrated that RJX prevents in the LPS-GalN mouse model of sepsis the marked increase of each of these cytokines in the serum as well as lungs and liver [17]. Furthermore, preliminary evidence has suggested that it may also be effective in reversing sepsis if administered after the onset of sepsis [19]. Here we report on the activity of a combination of RJX plus DEX in therapeutic settings in a preclinical sepsis model. The combination of RJX plus DEX immediately and profoundly decreased the inflammatory cytokine responses



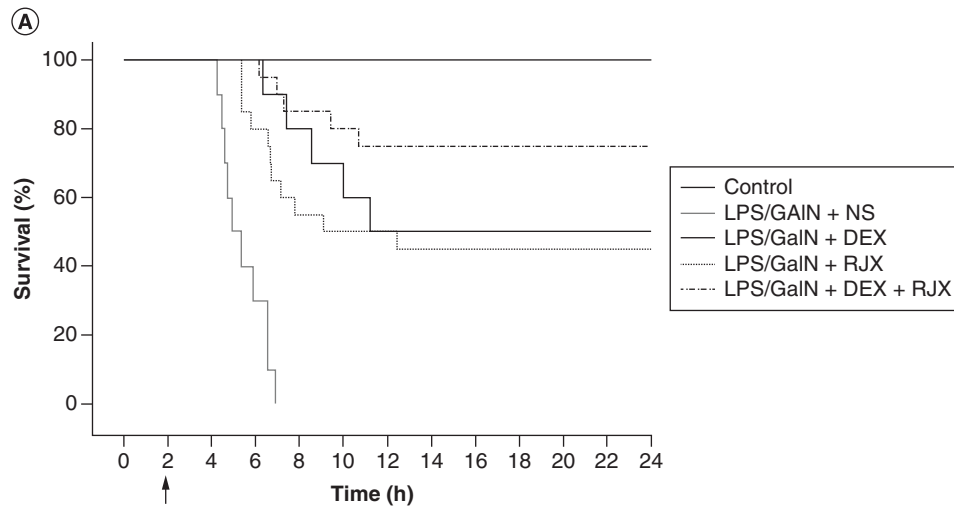
**Figure 4.** *In vivo* treatment activity of RJX, DEX and RJX + DEX on lung and liver histopathological scores in the LPS-GalN mouse model of fatal cytokine storm, sepsis, systemic inflammation, ARDS and multiorgan failure. BALB/c mice were treated with intraperitoneal injections of RJX (n = 20, sixfold diluted, 4.2 ml/kg, 0.5 ml/mouse), DEX (n = 10, 0.6 mg/kg, 0.5 ml/mouse), RJX + DEX (n = 20, 0.5 ml/mouse) or vehicle (NS, 0.5 ml/mouse) 2 h post-injection of LPS-GalN. Except for untreated control mice (control, n = 10), each mouse received 0.5 ml of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) intraperitoneally. The depicted whisker plots represent the median and values. The tissue damage in the (A) lungs and (B) liver were scored using previously published grading scales [17,21]. Kruskal–Wallis test and Mann–Whitney U test were used for comparing the results among different treatment groups. (C) For severe damage, lung and liver histological scores were compared by Pearson’s Chi-Square or Fisher’s Exact test. <sup>†</sup>Fisher’s exact Chi-Square test used.

Statistical significance between groups is shown by ## p < 0.01; ### p < 0.001; #### p < 0.0001 as compared with LPS/GalN + NS group; + p < 0.05 as compared with LPS/GalN + DEX group and \$ p < 0.05; \$\$\$ p < 0.001 pairwise comparisons between the groups.

ARDS: Acute respiratory distress syndrome; DEX: Dexamethasone; RJX: Rejuvenix.

to LPS-GalN, mitigated the inflammatory tissue damage in the lungs and liver, and prevented a fatal outcome. Although the treatments were started after the onset of fulminant cytokine storm characterized by markedly elevated serum cytokine levels and systemic inflammation as well as severe lung damage, a near-complete recovery of the inflammatory lung injury was achieved within 24 h, and the survival outcome was improved. The superiority of the combination regimen was particularly striking when we compared the histopathological data on sepsis-associated





**(B)**

Survival analysis	Censored	Events	n	Proportion (%)	Median
Control	10	0	10	0.0	≥24 h
LPS/GaIN + NS	0	10	10	100.0	5.1 h
LPS/GaIN + DEX	5	5	10	50.0	17.6 h
LPS/GaIN + RJX	11	9	20	45.0	10.8 h
LPS/GaIN + DEX + RJX	15	5	20	25.0	≥24 h

**(C) Pairwise comparisons**

		Log-rank		p-value summary
		Chi-square	p-value	
Control	LPS/GaIN + NS	21.837	0.0001	****
Control	LPS/GaIN + DEX	6.389	0.0115	*
Control	LPS/GaIN + RJX	7.624	0.0947	**
Control	LPS/GaIN + DEX + RJX	2.793	0.0040	**
LPS/GaIN + NS	LPS/GaIN + DEX	18.258	0.0001	****
LPS/GaIN + NS	LPS/GaIN + RJX	20.151	0.0001	****
LPS/GaIN + NS	LPS/GaIN + DEX + RJX	36.369	0.0001	****
LPS/GaIN + DEX	LPS/GaIN + RJX	0.313	0.5759	ns
LPS/GaIN + DEX	LPS/GaIN + DEX + RJX	1.583	0.2083	ns
LPS/GaIN + RJX	LPS/GaIN + DEX + RJX	4.365	0.0367	*
Monotherapy DEX or RJX	LPS/GaIN + DEX + RJX	3.977	0.0461	*

**Figure 5.** *In vivo* treatment activity of low-dose RJX, standard-dose DEX and their combination in the LPS-GaIN mouse model of fatal cytokine storm, sepsis, systemic inflammation, ARDS and multiorgan failure. BALB/c mice were treated with intraperitoneal injections of RJX (n = 20, sixfold diluted, 4.2 ml/kg, 0.5 ml/mouse), DEX (n = 10, 0.6 mg/kg, 0.5 ml/mouse), RJX + DEX (n = 20, 0.5 ml/mouse) or vehicle (NS, 0.5 ml/mouse) 2 h post-injection of LPS-GaIN. Except for untreated control mice (control, n = 10), each mouse received 0.5 ml of LPS-GaIN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) intraperitoneally. The cumulative proportion of mice remaining alive (survival, %) is shown as a function of time after the LPS-GaIN challenge. Depicted are the Kaplan–Meier survival curves (A) and survival data with statistical analysis (B & C) of the different treatment groups. ARDS: Acute respiratory distress syndrome; DEX: Dexamethasone; LPS: Lipopolysaccharide; NS: Normal saline; RJX: Rejuveinix.

organ damage in lungs and liver from mice treated with monotherapy (RJX or DEX alone) versus combination therapy. Notably, initiation of combination therapy after onset of the inflammatory cytokine responses and systemic inflammation resulted within 24 h in a near-complete recovery of very severe organ damage in the lungs that was caused by LPS-GaIN-induced sepsis.

Glucocorticoids, including DEX, have multiple effects on the immune system and exhibit potent anti-inflammatory activity [47]. DEX has been shown to improve the survival outcome of patients with ARDS [34,48–52]. Furthermore, recent clinical studies, including the open-label randomized RECOVERY trial, have demonstrated

that the standard anti-inflammatory drug DEX improves the survival outcome of hospitalized high-risk COVID-19 patients [49,50].

Our preliminary data from the clinical phase I study showed that the majority of high-risk COVID-19 patients had a rapid clinical recovery with resolution of the hyperinflammatory response and oxygen therapy requirements. Additional studies in a randomized setting are needed to confirm the reported preliminary findings and assess any potential benefit from the addition of RJX to the SOC that includes DEX. RJX will be evaluated in a randomized, placebo-controlled study in hospitalized COVID-19 patients with viral sepsis to test the hypothesis that it will contribute to a faster resolution of respiratory failure and a reduced case mortality rate when combined with DEX and other components of the SOC. A randomized phase II proof-of-concept study has also been designed to determine if RJX plus DEX combination can reduce the mortality rate of high-risk bacterial sepsis.

## Conclusion

The combination of RJX plus DEX immediately and profoundly decreased the inflammatory cytokine (IL-6, TNF- $\alpha$ ) responses to LPS-GaIN, mitigated the inflammatory tissue damage in the lungs and liver, and prevented a fatal outcome. Although the treatments were started after the onset of fulminant cytokine storm characterized by markedly elevated serum IL-6 and TNF- $\alpha$  levels and systemic inflammation as well as severe lung damage, a near-complete recovery of the inflammatory lung injury was achieved within 24 h, and the survival outcome was improved. Our preliminary data from the clinical phase I study showed that the majority of high-risk COVID-19 patients treated with RJX in combination with DEX had a rapid clinical recovery with resolution of the hyperinflammatory response and oxygen therapy requirements. Additional studies in a randomized setting are needed to confirm the reported preliminary findings and assess any potential benefit from the addition of RJX to the SOC that includes DEX.

## Summary points

- The primary objective of the present study was to compare the effects of Rejuveinix (RJX), the standard anti-inflammatory drug dexamethasone (DEX) and their combination on the severity of sepsis and survival outcome in an animal model that employs LPS plus D-galactosamine (LPS-GaIN) to induce fatal sepsis.
- We hypothesized that RJX, especially when combined with DEX, would improve the survival outcome of mice who develop systemic inflammation following injection with a lethal dose of LPS-GaIN.
- We demonstrate that RJX – at a dose level > ten-times lower than its clinical maximum tolerated dose – exhibits potent single-agent anti-inflammatory activity and is as effective as DEX at standard dose levels in this model of fatal sepsis.
- The combination of RJX plus DEX immediately and profoundly decreased the inflammatory cytokine (IL-6, TNF- $\alpha$ ) responses to LPS plus D-galactosamine, mitigated the inflammatory tissue damage in the lungs and liver, and prevented a fatal outcome.
- Although the treatments were started after the onset of fulminant cytokine storm characterized by markedly elevated serum IL-6 and TNF- $\alpha$  levels and systemic inflammation as well as severe lung damage, a near-complete recovery of the inflammatory lung injury was achieved within 24 h, and the survival outcome was improved.
- Preliminary data from a clinical phase I study indicated that RJX plus DEX and other components of the standard of care were well tolerated.

## Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: [www.futuremedicine.com/doi/suppl/10.2217/fmb-2022-0044](http://www.futuremedicine.com/doi/suppl/10.2217/fmb-2022-0044)

## Author contributions

Each author has made significant and substantive contributions to the study, reviewed and revised the manuscript, provided final approval for submission of the final version. FM Uckun conceived the study, designed the evaluations reported in this paper, directed the data compilation and analysis, analyzed the data and prepared the initial draft of the manuscript. K Sahin directed the nonclinical study. K Sahin and C Orhan collected the nonclinical data and performed their analysis. IH Ozercan performed the necropsies and histopathologic examinations on mice. A Shibli, M Saeed, M Awili, A Skolnick, A Prusmack, CIP Barrera and J Varon assisted in clinical study design, served as site investigators, screened and recruited participants, administered treatments, assessed adverse events and disease responses, collected safety and efficacy data, met regularly to review study data during the study and reviewed the manuscript; FM Uckun, S Qazi and C Lee analyzed and validated clinical data, and performed statistical analyses; M Volk assisted FM Uckun in study design and evaluations.

### Financial & competing interests disclosure

This study was funded by Reven Pharmaceuticals, LLC, a wholly owned subsidiary of Reven Holdings Inc. Author FM Uckun is employed by Ares Pharmaceuticals, and he serves as a consultant for Reven Pharmaceuticals as well as Oncotelic Therapeutics. Author M. Volk serves as a consultant to Reven Pharmaceuticals. Author C Lee is employed by Oncotelic Therapeutics, and she served as a consultant to Reven Pharmaceuticals. The sponsor did not participate in the collection of data. The funder had the following involvement with the study: 1) Provided funding to clinical research organizations for the operationalization of the study by clinical monitoring, medical monitoring, pharmacovigilance and data management; 2) provided site awards to the investigative sites to compensate the sites for their clinical research expenses; and 3) sponsored the Investigational New Drug (IND), funded the regulatory affairs and quality assurance activities to ensure compliance with the regulations of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) relative to Good Clinical Practice (GCP). The sponsor did not participate in the safety or efficacy assessments of the investigators. Among the authors, FM Uckun and C Lee, who participated in the analysis and decision to submit the manuscript for publication, were consultants of Reven Pharmaceuticals. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

### Ethical conduct of research

The nonclinical research project was approved by the Animal Care and Use Committee of Firat University (no. 03092020-391-047; Ethics Committee no. 420629). The clinical study protocol was approved by the WCG-Central Institutional Review Board (IRB) (OHRP/FDA Parent Organization no. IORG0000432; OHRP/FDA IRB registration no. IRB00000533). The Central IRB-approved study/protocol number was RPI015 (IRB tracking no. 20203418). The study was performed in compliance with the International Conference on Harmonization guidelines for Good Clinical Practice (ICH6/GCP). Each patient provided a signed informed consent form (ICF) prior to enrollment.

### Data sharing statement

The clinical registration number for the phase I study is NCT04708340. De-identified patient data are presented in the Supplemental Information. Study protocol as well as updated safety and pharmacodynamic data will be available upon publication of the article and 2 years thereafter. Data can be requested via email from the corresponding author.

### References

- Kadri SS, Rhee C, Strich JR *et al.* Estimating ten-year trends in septic shock incidence and mortality in United States academic medical centers using clinical data. *Chest* 151(2), 278–285 (2017).
- Rhee C, Dantes R, Epstein L *et al.* Incidence and trends of sepsis in US hospitals using clinical vs claims data, 2009–2014. *JAMA* 318(13), 1241 (2017).
- Rudd KE, Johnson SC, Agesa KM *et al.* Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the global burden of disease study. *Lancet* 395(10219), 200–211 (2020).
- Anand V, Zhang Z, Kadri SS, Klompas M, Rhee C. Epidemiology of quick sequential organ failure assessment criteria in undifferentiated patients and association with suspected infection and sepsis. *Chest* 156(2), 289–297 (2019).
- Rhodes A, Evans LE, Alhazzani W *et al.* Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: 2016. *Intensive Care Med.* 43(3), 304–377 (2017).
- Kaukonen K-M, Bailey M, Pilcher D, Cooper DJ, Bellomo R. Systemic inflammatory response syndrome criteria in defining severe sepsis. *N. Engl. J. Med.* 372(17), 1629–1638 (2015).
- Meyer N, Harhay MO, Small DS *et al.* Temporal trends in incidence, sepsis-related mortality, and hospital-based acute care after sepsis. *Crit. Care Med.* 46(3), 354–360 (2018).
- Seymour CW, Kennedy JN, Wang S *et al.* Derivation, validation, and potential treatment implications of novel clinical phenotypes for sepsis. *JAMA* 321(20), 2003 (2019).
- Li H, Liu L, Zhang D *et al.* SARS-CoV-2 and viral sepsis: observations and hypotheses. *Lancet* 395(10235), 1517–1520 (2020).
- Uckun FM. Reducing the fatality rate of COVID-19 by applying clinical insights from immuno-oncology and lung transplantation. *Front. Pharmacol.* 11, 796 (2020).
- Wu C, Chen X, Cai Y *et al.* Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Intern. Med.* 180(7), 934 (2020).
- Lian J, Jin X, Hao S *et al.* Analysis of epidemiological and clinical features in older patients with coronavirus disease 2019 (COVID-19) outside Wuhan. *Clin. Infect. Dis.* 71(15), 740–747 (2020).

13. Zhou F, Yu T, Du R *et al.* Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 395(10229), 1054–1062 (2020).
14. Carver C, Jones N. Are there risk factors and preventive interventions for acute respiratory distress syndrome (ARDS) in COVID-19? *Centre for Evidence-Based Medicine* (2020). [www.cebm.net/covid-19/are-there-risk-factors-and-preventative-interventions-for-acute-respiratory-distress-syndrome-ards-in-covid-19/](http://www.cebm.net/covid-19/are-there-risk-factors-and-preventative-interventions-for-acute-respiratory-distress-syndrome-ards-in-covid-19/)
15. Zheng Z, Peng F, Xu B *et al.* Risk factors of critical & mortal COVID-19 cases: a systematic literature review and meta-analysis. *J. Infect.* 81(2), e16–e25 (2020).
16. Kuderer NM, Choueiri TK, Shah DP *et al.* Clinical impact of COVID-19 on patients with cancer (CCC19): a cohort study. *Lancet* 395(10241), 1907–1918 (2020).
17. Uckun FM, Carlson J, Orhan C *et al.* Rejuveinix shows a favorable clinical safety profile in human subjects and exhibits potent preclinical protective activity in the lipopolysaccharide-galactosamine mouse model of acute respiratory distress syndrome and multi-organ failure. *Front. Pharmacol.* 11, 594321 (2020).
18. Fowler AA, Truweit JD, Hite RD *et al.* Effect of vitamin C infusion on organ failure and biomarkers of inflammation and vascular injury in patients with sepsis and severe acute respiratory failure. *JAMA* 322(13), 1261 (2019).
19. Uckun FM, Ozercan IH, Orhan C, Gitterle M, Sahin K. Rejuveinix reverses severe inflammatory lung damage in a mouse model of fatal sepsis. *BioRxiv* doi:10.1101/2020.12.31.424986 (2021) (Epub ahead of print).
20. Hager DN, Hooper MH, Bernard GR *et al.* The vitamin C, thiamine and steroids in sepsis (VICTAS) protocol: a prospective, multi-center, double-blind, adaptive sample size, randomized, placebo-controlled, clinical trial. *Trials* 20(1), 197 (2019).
21. Uckun FM, Orhan C, Powell J *et al.* Non-clinical safety profile and pharmacodynamics of two formulations of the anti-sepsis drug candidate Rejuveinix (RjX). *Biomed. Pharmacother.* 141, 111823 (2021).
22. Li J. Evidence is stronger than you think: a meta-analysis of vitamin C use in patients with sepsis. *Crit. Care* 22(1), 258 (2018).
23. Uckun FM, Volk MA, Lange P *et al.* Pharmaceutical compositions and methods for prevention and/or treatment of inflammation. US patent application. Publication no. 20220096538 (2022). <https://patents.justia.com/patent/20220096538>
24. Putzu A, Daems A-M, Lopez-Delgado JC, Giordano VF, Landoni G. The effect of vitamin C on clinical outcome in critically ill patients. *Crit. Care Med.* 47(6), 774–783 (2019).
25. Iglesias J, Vassallo AV, Patel VV, Sullivan JB, Cavanaugh J, Elbaga Y. Outcomes of metabolic resuscitation using ascorbic acid, thiamine, and glucocorticoids in the early treatment of sepsis. *Chest* 158(1), 164–173 (2020).
26. Obi J, Pastores SM, Ramanathan LV, Yang J, Halpern NA. Treating sepsis with vitamin C, thiamine, and hydrocortisone: exploring the quest for the magic elixir. *J. Crit. Care* 57, 231–239 (2020).
27. Shan M, Zhou S, Fu C *et al.* vitamin B6 inhibits macrophage activation to prevent lipopolysaccharide-induced acute pneumonia in mice. *J. Cell. Mol. Med.* 24(5), 3139–3148 (2020).
28. Noormandi A, Khalili H, Mohammadi M, Abdollahi A. Effect of magnesium supplementation on lactate clearance in critically ill patients with severe sepsis: a randomized clinical trial. *Eur. J. Clin. Pharmacol.* 76(2), 175–184 (2020).
29. Woolf SH, Chapman DA, Lee JH. COVID-19 as the leading cause of death in the United States. *JAMA* 325(2), 123 (2021).
30. Faust JS, Krumholz HM, Du C *et al.* All-cause excess mortality and COVID-19-related mortality among US adults aged 25–44 years, March–July 2020. *JAMA* 325(8), 785 (2021).
31. Woolf SH, Chapman DA, Sabo RT, Weinberger DM, Hill L, Taylor DDH. Excess deaths from COVID-19 and other causes, March–July 2020. *JAMA* 324(15), 1562 (2020).
32. Villar J, Ferrando C, Martínez D *et al.* Dexamethasone treatment for the acute respiratory distress syndrome: a multicentre, randomised controlled trial. *Lancet Respir. Med.* 8(3), 267–276 (2020).
33. Sterne JAC, Murthy S, Diaz JV *et al.* Association between administration of systemic corticosteroids and mortality among critically ill patients with COVID-19. *JAMA* 324(13), 1330 (2020).
34. Tomazini BM, Maia IS, Cavalcanti AB *et al.* Effect of dexamethasone on days alive and ventilator-free in patients with moderate or severe acute respiratory distress syndrome and COVID-19. *JAMA* 324(13), 1307 (2020).
35. Moskowitz A, Huang DT, Hou PC *et al.* Effect of ascorbic acid, corticosteroids, and thiamine on organ injury in septic shock. *JAMA* 324(7), 642 (2020).
36. Fujii T, Luethi N, Young PJ *et al.* Effect of vitamin C, hydrocortisone, and thiamine vs hydrocortisone alone on time alive and free of vasopressor support among patients with septic shock. *JAMA* 323(5), 423 (2020).
37. Byerly S, Parreco JP, Soe-Lin H *et al.* Vitamin C and thiamine are associated with lower mortality in sepsis. *J. Trauma Acute Care Surg.* 89(1), 111–117 (2020).
38. Pasrija R, Naime M. The deregulated immune reaction and cytokines release storm (CRS) in COVID-19 disease. *Int. Immunopharmacol.* 90, 107225 (2021).
39. Conti P, Caraffa A, Gallenga CE *et al.* Coronavirus-19 (SARS-CoV-2) induces acute severe lung inflammation via IL-1 causing cytokine storm in COVID-19: a promising inhibitory strategy. *J. Biol. Regul. Homeost. Agents* 34(6), 1971–1975 (2020).

40. Khadke S, Ahmed N, Ahmed N *et al.* Harnessing the immune system to overcome cytokine storm and reduce viral load in COVID-19: a review of the phases of illness and therapeutic agents. *Virol. J.* 17(1), 154 (2020).
41. McGonagle D, Sharif K, O'Regan A, Bridgewood C. The role of cytokines including Interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome-like disease. *Autoimmun. Rev.* 19(6), 102537 (2020).
42. Berlin DA, Gulick RM, Martinez FJ. Severe Covid-19. *N. Engl. J. Med.* 383(25), 2451–2460 (2020).
43. Li X, Ma X. Acute respiratory failure in COVID-19: is it “typical” ARDS? *Crit. Care* 24(1), 198 (2020).
44. Torres Acosta MA, Singer BD. Pathogenesis of COVID-19-induced ARDS: implications for an ageing population. *Eur. Respir. J.* 56(3), (2020).
45. Montenegro F, Unigarro L, Paredes G *et al.* Acute respiratory distress syndrome (ARDS) caused by the novel coronavirus disease (COVID-19): a practical comprehensive literature review. *Expert Rev. Respir. Med.* 15(2), 183–195 (2021).
46. Tan LY, Komarasamy TV, Rmt Balasubramaniam V. Hyperinflammatory immune response and COVID-19: a double edged sword. *Front. Immunol.* 12, 742941 (2021).
47. Coutinho AE, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Mol. Cell. Endocrinol.* 335(1), 2–13 (2011).
48. RECOVERY Collaborative Group. Dexamethasone in hospitalized patients with Covid-19. *N. Engl. J. Med.* 384(8), 693–704 (2021).
49. RECOVERY Collaborative Group. Effect of dexamethasone in hospitalized patients with COVID-19 – preliminary report. *MedRxiv* doi.org/10.1101/2020.06.22.20137273 (2020).
50. Noreen S, Maqbool I, Madni A. Dexamethasone: therapeutic potential, risks, and future projection during COVID-19 pandemic. *Eur. J. Pharmacol.* 894, 173854 (2021).
51. Angus DC, Derde L, Al-Beidh F *et al.* Effect of hydrocortisone on mortality and organ support in patients with severe COVID-19. *JAMA* 324(13), 1317 (2020).
52. Jeronimo CMP, Farias MEL, Val FFA *et al.* Methylprednisolone as adjunctive therapy for patients hospitalized with coronavirus disease 2019 (COVID-19; Metcovid): a randomized, double-blind, phase iib, placebo-controlled trial. *Clin. Infect. Dis.* 72(9), e373–e381 (2021).