

# Clinical impact potential of Rejuveinix (RJX) for prevention of fatal Acute Respiratory Distress Syndrome (ARDS) and multi-organ failure in COVID-19 patients

## Abstract

RJX is an Intravenous (IV) formulation of known physiologically compatible compounds that is being developed for more effective supportive therapy of patients with sepsis, including COVID-19 patients with viral sepsis and Acute Respiratory Distress Syndrome (ARDS). The RJX formulation is a solution of buffered acid products, electrolyte components, and vitamins, including ascorbic acid, cyanocobalamin, thiamine hydrochloride, riboflavin 5' phosphate, niacinamide, pyridoxine hydrochloride, and calcium d-pantothenate, and magnesium sulfate heptahydrate, a mineral with a negative oxidation-reduction potential. The components of RJX exhibited promising activity in clinical studies involving ARDS patients and/or non-clinical studies in animal models of ARDS. The published data from these clinical and non-clinical studies provided the medical-scientific rationale for our clinical development strategy for RJX and a clinical study in COVID-19 patients.

**Keywords:** sepsis • pneumonia • ARDS • cytokine release syndrome • COVID-19

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## Introduction

### Sepsis and ARDS

Sepsis is the consequence of a systemic host inflammatory response to an infection with bacteria, fungi, or viruses, and is associated with a complex metabolic dysfunction [1-9]. Severe sepsis is frequently associated with septic shock leading to Acute Respiratory Distress Syndrome (ARDS) and a potentially fatal Multiple Organ Dysfunction Syndrome (MODS) with acute renal failure, disseminated intravascular coagulopathy, and several poor prognostic circulatories, cellular, and metabolic abnormalities [1-9]. Immunocompromised patients such as patients with cancer, Acquired Immunodeficiency Syndrome (AIDS), organ

transplant recipients, as well as patients receiving immune-suppressive therapies for an inflammatory disorder (e.g. inflammatory bowel disease, autoimmune diseases) receiving chemotherapy, are at an increased risk for developing fatal ARDS and MODS as a result of severe sepsis. The case mortality rate of patients with septic shock is 40-80% and can be higher in elderly individuals [6]. A study of sepsis prevalence among 7 million inpatients from 409 hospitals in the US documented between 2009 and 2014 recognized a persistently high annual incidence of 6% during hospitalizations of adult patients [2]. Approximately 50 million incident cases of sepsis and 11 million sepsis-related deaths were recorded globally in 2017. The highest rates of sepsis

Fatih M. Uckun<sup>1,2\*</sup>, Jim Ervin<sup>3</sup>, Kazim Sahin<sup>4</sup>, Joy Powell<sup>3</sup>, Natalie Pizzimenti<sup>3</sup>, Jackie Earabino<sup>3</sup>, Hendrik van Wyk<sup>3</sup>, Mariette van Wyk<sup>3</sup>, Peter Pacult<sup>3</sup>, Taha Koray Sahin<sup>5</sup>, Kristina Cabala<sup>3</sup>

<sup>1</sup>COVID-19 Task Force, Worldwide Clinical Trials, Wayne, USA

<sup>2</sup>Department of Developmental Therapeutics, Immunology, and Integrative Medicine, Ares Pharmaceuticals, St. Paul, USA

<sup>3</sup>Drug Discovery Program, Reven Pharmaceuticals, Golden, USA

<sup>4</sup>Departments of Veterinary Medicine and Pathology, Firat University, Turkey

<sup>5</sup>Department of Internal Medicine, Hacettepe University, Turkey

\*Author for correspondence: fatih.uckun@worldwide.com

and sepsis-related mortality were observed in Sub-Saharan Africa, Oceania, South Asia, East Asia, and Southeast Asia [3].

### COVID-19 and ARDS

Approximately 1/3<sup>rd</sup> to 1/5<sup>th</sup> of patients infected with the new coronavirus, SARS-CoV-2, the causative agent of COVID-19, develops viral pneumonia that causes an Acute Lung Injury (ALI) and rapidly progresses to viral sepsis and ARDS [10-13]. A systemic inflammatory response syndrome also referred to as cytokine storm or Cytokine Release Syndrome [CRS], is generally thought to be the driving force behind the ARDS and often irreversible multi-organ dysfunction associated with the severe-critical forms of COVID-19 [10-13]. It is characterized by a rapid and substantial increase in the production of pro-inflammatory cytokines, such as Interleukin-6 (IL-6), Tumor Necrosis Factor-Alpha (TNF- $\alpha$ ), Transforming Growth Factor- $\beta$  (TGF- $\beta$ ) and Interleukin 10 (IL-10) [10-13]. Older patients with comorbidities such as cancer, diabetes, cardiovascular diseases, and inflammatory disorders are at increased risk for a fulminant course of COVID-19 [10-13]. The standard supportive care for ARDS patients is highly variable based on institutional preferences, and the fatality rate remains high with contemporary supportive care alone [10]. Treatments that can effectively reduce the risk of ARDS or its mortality rate in high-risk patients with SARS-CoV-2 viremia or COVID-19 pneumonia are urgently needed [10].

### Therapeutic landscape for patients with severe to critical COVID-19

IL-6 is a pro-inflammatory cytokine that contributes to the development, progression, and severity of CRS as well as its complications, including Disseminated Intravascular Coagulopathy (DIC) and multi-organ failure [10-14]. It is the main signature cytokine implicated in COVID-19 associated CRS and ARDS [10]. In an open-label Phase 2 study (ClinicalTrials.gov Identifier: NCT04317092), Tocilizumab is being studied in patients with COVID-19 pneumonia and in a related randomized study (ClinicalTrials.gov Identifier: CT04306705), it is being evaluated for its efficacy in CRS associated with COVID-19. It is also being assessed in combination with Favipiravir (ClinicalTrials.gov Identifier: NCT04310228). Likewise, Sarilumab (Kevzara), another monoclonal antibody to the IL-6 receptor, is being evaluated in a Phase 2/3 study in hospitalized

COVID-19 patients (ClinicalTrials.gov Identifier: NCT04315298). Based on the role of TGF- $\beta$  in the immunopathology of ARDS, Uckun and Trieu have recently proposed the use of TGF- $\beta$  inhibitors for the treatment of COVID-19 pneumonia and ARDS [15,16]. The use of convalescent plasma containing virus-specific antibodies has been effective in some patients with COVID-19 [17-19]. Also, Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) contributes to the severity of CRS and neutralizing antibodies to GM-CSF, such as lenzilumab, are being explored as part of the treatment algorithms for COVID-19 associated ARDS [20]. Likewise, complement inhibitors, such as Eculizumab (Soliris), are being used in an attempt to reduce the mortality in COVID-19 patients with ARDS (ClinicalTrials.gov Identifier: NCT04288713) [20].

The macrolide antibiotic azithromycin has immune-modulatory effects and reduces the Interleukin-17 (IL-17) response that has been implicated in the development of a steroid-refractory neutrophilic inflammation in the lung and ARDS after acute lung injury [10,21-23]. Azithromycin was associated with more ICU-free days in severe sepsis patients with and without pneumonia in a multicenter study involving 218 ARDS patients on mechanical ventilation [10,21,22]. Likewise, early use of macrolide antibiotic therapy decreased time to come off the mechanical ventilation and improved the survival outcome in another study on patients with ARDS [10,23].

### Clinical activity of vitamins in prevention and treatment of sepsis-associated ards and multi-organ dysfunction

**Niacinamide/Nicotinamide:** Niacinamide (also known as Nicotinamide or Vitamin [Vit.] B3) has been shown to effectively abrogate the ALI caused by ischemia/reperfusion or endotoxin exposure by inhibition of production free radicals and pro-inflammatory cytokines with the restoration of adenosine triphosphate [24,25]. Acute Kidney Injury (AKI) that can develop during septic shock, has a high mortality rate and poor prognosis [26]. In rats, a severe systemic inflammatory response with a high mortality rate is induced by zymosan administration. Nicotinamide decreased mortality after the zymosan challenge [27]. Vit. B3 inhibits the Poly ADP Ribose Polymerase (PARP) [28]. Because the increased activity of PARP elevates the inflammatory cytokines

that contribute to the cytokine storm, Vit. B3 therapy may reduce cytokine storm in COVID-19 [28].

Lipopolysaccharide (LPS) is an endotoxin that induces a strong host immune response associated with a marked increase in the production and release of pro-inflammatory cytokines. Extracellular Nicotinamide Adenine Dinucleotide (NAD) attenuates the LPS-induced lung inflammation as well as ALI. Umapathy et al. examined the effects of  $\beta$ -NAD on LPS-induced lung Endothelial Cell (EC) barrier dysfunction and vascular leak affecting the lungs in a mouse model of ALI and ARDS [29].  $\beta$ -NAD treatment significantly attenuated the inflammatory response leading to decreased interstitial edema in the lung parenchyma consistent with decreased vascular leakage [29]. Nicotinamide riboside, the pyridine-nucleoside form of Vit. B3 that functions as a precursor to Nicotinamide Adenine Dinucleotide (NAD<sup>+</sup>), prevented ALI/ARDS and heart injury, and improved the survival of mice after the LPS challenge or sepsis caused by intraperitoneal injection of feces [30]. In LPS-challenged rats, the administration of nicotinamide prevented the LPS-induced decrease in mitochondrial respiration and intracellular NAD<sup>+</sup> levels in peritoneal macrophages and improved the contractility of the thoracic aorta *ex vivo* [31].

**Ascorbic acid (Vit. C) and thiamine:** Several studies have evaluated the clinical potential of Vit. C in the treatment of ALI and ARDS [32-34]. Fisher et al. used the LPS model of ALI and sepsis to determine in mice whether parenteral ascorbic acid is capable of favorably modulating the dysregulated pro-inflammatory, procoagulant state that leads to lung injury [35]. A time-delayed infusion protocol of both Vit. C and dehydroascorbic acid attenuated pro-inflammatory, procoagulant states that induce lung vascular injury and significantly prolonged survival [35]. Likewise, parenteral Vit. C infusion protected mice from developing fatal ALI and ARDS in a septic peritonitis model [36]. Besides, Vit. C prevented the formation of the Neutrophil Extracellular Traps (NETs) that contribute to the vascular damage in sepsis [37].

The safety of intravenously infused Vit. C was examined in patients with severe sepsis with ARDS and multi-organ dysfunction in a randomized, double-blind, placebo-controlled, phase I trial (ClinicalTrials.gov identifier NCT01434121). Vit. C reduced the pro-inflammatory biomarkers

C-reactive protein and procalcitonin, prevented an increase in thrombomodulin levels consistent with reduced vascular damage, and caused reductions in SOFA scores [38,39]. Thiamine deficiency impairs aerobic metabolism, potentially contributing to the development of fatal lactic acidosis. Thiamine administration during septic shock accelerates the clearance of lactate and improves the survival outcome in thiamine-deficient patients [40]. Thiamine also reduces the risk of Vit. C-associated nephropathy. The combination of intravenous Vit. C, thiamine, and hydrocortisone in severe sepsis and septic shock reduces the case mortality by almost a third [39]. Patients receiving this combination treatment did not develop multi-organ failure, their Sequential Organ Failure Assessment (SOFA) scores improved, and they could be taken off vasopressors much faster than the control patients [39]. Similarly, in the randomized, placebo-controlled double-blind CITRIS-ALI study (ClinicalTrials.gov Identifier: NCT02106975), administration of Vit. C for treatment of sepsis and ARDS resulted in fewer ICU days and a significant reduction of the 28-day mortality [41]. Recently, a new clinical trial (ClinicalTrials.gov identifier: NCT04264533) was initiated in Wuhan, China, to evaluate the clinical effects of a 7-day therapy with Vit. C in patients with severe COVID-19 pneumonia. Several studies are underway to further evaluate the efficacy of Vit. C alone or in combination with thiamine and steroids in the treatment of severe sepsis and septic shock, including the double-blind, randomized, placebo-controlled Vit. C, Thiamine and Steroids in Sepsis (VICTAS) trial (ClinicalTrials.gov Identifier: NCT03509350) [42-45].

A randomized, double-blind, placebo-controlled trial conducted from February 2018 to June 2019 assessing the combination of Vit. C, thiamine, and hydrocortisone showed that the combination therapy results in faster resolution of shock [44]. Importantly, treatment with Vit. C and thiamine were associated with improved clinical outcomes in patients who present with increased inflammatory markers [46], and it was associated with lower in-hospital mortality rates in patients with high SOFA scores [47]. Hydrocortisone-Vit. C-Thiamine use was associated with lower mortality in pediatric septic shock [48].

**Riboflavin:** Riboflavin (Vit. B2) has been shown to reduce the production and release of pro-inflammatory cytokines in adipocyte-macrophage

co-cultures *in vitro* [49,50]. In an LPS mouse model, treatment with Vit. B2 led to a significant reduction in IL-6 and NO [49]. Macrophage Inflammatory Protein (MIP)-2, a pro-inflammatory cytokine, was also significantly reduced in mice [49]. Because of its anti-inflammatory activity, Vit. B2 has been proposed as a potentially active vitamin for treating sepsis, septic shock, and ARDS [51]. In animal models of systemic inflammation, the combination of Vit. B2 and thiamine have been shown to potentiate the anti-inflammatory activity of dexamethasone and reduce the production of TNF- $\alpha$  and IL-6 [52]. Early multivitamin supplementation, including thiamine, Vit. B2, niacin was associated with lower overall mortality in patients with Ebola Virus Disease (EVD) [53].

**Vitamin B6/Pyridoxine:** Vit. B6 exhibits anti-inflammatory activity and in mouse models of ARDS, it suppressed pulmonary inflammation by inhibiting macrophage activation and reduced productions of TNF- $\alpha$  and IL-1 $\beta$  *in vivo* [54]. Notably, Vit. B6 protected mice from lethal endotoxic shock after a challenge with LPS [55]. The utilization of Vit. B6 rapidly increases under inflammatory conditions, and COVID-19 patients probably may have Vit. B6 deficiency [56] suggesting Vit. B6 may have clinical utility in mitigating their immune dysfunction and coagulopathy.

#### Clinical activity of magnesium sulfate

Magnesium is a mineral involved in the balance of other electrolytes, storage and transfer of energy, synthesis of proteins and nucleic acids, and inflammatory processes. Magnesium is also a co-factor that through activated Vit. B1 facilitates the conversion of pyruvate to acetyl coenzyme A for aerobic metabolism to occur. It is common in critically

ill patients to have a deficiency in magnesium, which can lead to an increase in lactate production. In a recently published, randomized, placebo-controlled study, magnesium supplementation for three days facilitated lactate clearance in critically ill patients with severe sepsis and significantly shortened the median length of ICU stay [57]. Elevated levels of lactate dehydrogenase have been noted in COVID-19 patients, and magnesium supplementation as a component of RJX could potentially aid in the clearance of the high levels [12,58].

#### Rejuveinix and its clinical impact potential in the treatment of COVID-19

We are developing RJX as a new treatment platform that can be used to improve the effectiveness of the available supportive care measures for COVID-19. RJX has clinical impact potential for the prevention of imminent ARDS in the context of sepsis, including viral sepsis associated with COVID-19. RJX is a formulation of known physiologically compatible active ingredients, including ascorbic acid, magnesium sulfate heptahydrate, cyanocobalamin, thiamine hydrochloride, riboflavin 5' phosphate, niacinamide, pyridoxine hydrochloride, calcium d-pantothenate, and sodium bicarbonate, combined in specific ratios Table 1 and dissolved in an aqueous acidic solution (electrolyzed sodium chloride and water for injection) [59]. RJX components are divided into a 2-vial system to enhance stability and shelf-life and diluted in saline to present with a negative Oxidative Reduction Potential (ORP) as administered. As discussed in Section 4 hereinabove, several of the Active Pharmaceutical Ingredient (API) components of RJX, especially niacinamide, ascorbic acid, thiamine, riboflavin, pyridoxine, and magnesium sulfate exhibited promising activity in clinical studies involving ARDS patients and/or non-

**Table 1.** Quantitative Composition of RJX

| Component   | % Content | Content mg/10 mL |
|---|-----------|------------------|
| <b>API (designated by numbers) Contained in Vial A</b>  |           |                  |
| 1) Ascorbic Acid USP  | 8.9933    | 899.33           |
| 2) Thiamine HCl USP   | 0.6333    | 63.33            |
| 3) Magnesium Sulfate Heptahydrate USP   | 8.080     | 808.00           |
| 4) Cyanocobalamin Crystalline USP   | 0.0193    | 1.93             |
| 5) Niacinamide USP  | 1.188     | 118.80           |
| 6) Pyridoxine HCl USP   | 1.188     | 118.80           |
| 7) Riboflavin 5' Phosphate USP  | 0.0253    | 2.53             |
| 8) Calcium D-Pantothenate USP   | 0.0293    | 2.93             |
| RJX drug product is stored in a two-vial system. Vial B contains Sodium Bicarbonate. The diluent for Vial A and Vial B is comprised of Water For Injection USP and Sodium Chloride (<0.1% mg/10 mL) |           |                  |
| Abbreviations: USP: United States Pharmacopeia; API: Active Pharmaceutical Ingredient   |           |                  |

clinical studies in animal models of ARDS [15-58]. The clinical tolerability of RJX was confirmed in a recently completed double-blind, placebo-controlled Phase 1 dose-escalation study in healthy volunteers (ClinicalTrials.gov Identifier: NCT03680105). Notably, RJX exhibited potent and dose-dependent activity in the LPS- Galactosamine (GalN) model of ARDS in mice. RJX-treated mice had a significantly improved survival outcome after being challenged with an otherwise invariably fatal dose of LPS-GalN. Additionally, decreases in key cytokines were observed and the alveolar wall thickening was substantially reduced in histopathological lung sections of RJX treated mice versus controls.

The clinical development plan for RJX includes a Phase II study to evaluate its tolerability and activity in mild-moderate COVID-19 patients without hypoxemia as well as severe-critical COVID-19

patients with hypoxemic respiratory failure receiving either Non-Invasive Positive Pressure Ventilation (NIPPV) or Mechanical Ventilation (MV) [60]. The combined datasets from clinical and non-clinical studies with the individual API components of RJX and the Phase 1 study of RJX in healthy volunteers and mouse ARDS models have informed the development of the study design for the projected COVID-19 study.

## Conclusion

It is hoped that RJX will reduce ALI and lung inflammation in COVID-19 patients with hypoxemic respiratory failure receiving either NIPPV or MV thereby shortening the time to resolution of the hypoxemic respiratory failure and reducing the case mortality rate when used in combination with standard of care.

## Executive summary

RJX is an Intravenous (IV) formulation of known physiologically compatible compounds that are being developed for more effective supportive therapy of patients with sepsis, including COVID-19 patients with viral sepsis and Acute Respiratory Distress Syndrome (ARDS). The RJX formulation is a solution of buffered acid products, electrolyte components, and vitamins, including ascorbic acid, cyanocobalamin, thiamine hydrochloride, riboflavin 5' phosphate, niacinamide, pyridoxine hydrochloride, and calcium d-pantothenate, and magnesium sulfate heptahydrate, a mineral with a negative oxidation-reduction potential. The components of RJX exhibited promising activity in clinical studies involving ARDS patients and/or non-clinical studies in animal models of ARDS. The published data from these clinical and non-clinical studies provided the medical-scientific rationale for our clinical development strategy for RJX and a clinical study in COVID-19 patients.

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