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DEVELOPMENT SAFETY UPDATE REPORT

DSUR Number:	01
IND Numbers:	131383 (SN0021) 149585 (SN0004)
Investigational Drug:	Rejuveinix (RJX)
Reporting Period:	October 7, 2019 – October 6, 2020
Date of Report:	October 7, 2020
Sponsor Name and Address:	Reven, LLC 800 Corporate Circle, Suite D Golden, CO 80401

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LIST OF ACRONYMS AND ABBREVIATIONS

AEs	Adverse Events
ALI	Acute Lung Injury
API	Active Pharmaceutical Ingredient
ARDS	Acute respiratory distress syndrome
$AUC_{0\text{-last}}$	The area under the plasma drug concentration–time curve up to the last quantifiable time-point
BAL	Bronchoalveolar Lavage
BMI	Body Mass Index
CAT	Catalase
CI	Confidence Interval
CLI	Critical Limb Ischemia
C_{max}	The maximum observed plasma concentration determined directly from the raw concentration–time data
COVID-19	Coronavirus Disease 2019
CRP	C-reactive Protein
CRS	Cytokine Release Syndrome
DLTs	Dose-limiting Toxicities
DSMB	Data Safety Monitoring Board
DSUR	Development Safety Update Report
ECG	Electrocardiogram
ENaC	Epithelial Sodium Channel
GCP	Good Clinical Practice
GI	Gastrointestinal
GSH-Px	Glutathione Peroxidase
IB	Investigator's Brochure
ICH	International Council for Harmonisation
ICU	Intensive Care Unit
IL	Interleukin
IND	Investigational New Drug
ITT	Intent to Treat
IV	Intravenous
LD	Lethal Dose
LDH	Lactate Dehydrogenase
LPS-GalN	Lipopolysaccharide-galactosamine
LQTS	Long QT Syndrome
MAD	Multiple Ascending Dose
MDA	Malondialdehyde
NIPPV	Non-invasive Positive Presssure Ventilation
PK	Pharmacokinetic
QRS	Q, R, S waves

QTc	Corrected QT Interval
QTcF	QT interval corrected by the Fridericia correction formula
RJX	Rejuveinix
ROS	Reactive Oxygen Species
RP2D	Recommended Phase 2 Dose
SAD	Single Ascending dose
SAEs	Serious Adverse Events
SD	Standard Deviation
SOD	Superoxide Dismutase
SRC	Safety Review Committee
TdP	Torsades de pointes
TEAEs	Treatment-emergent Adverse Events
TGF-β	Transforming Growth Factor Beta
TNF-α	Tumor Nerosis Factor Alpha
ULN	Upper Limit of Normal
URI	Upper Respirtory infection
USP	United States Pharmacopeia
VT	Ventricular Tachycardia

1. EXECUTIVE SUMMARY

Rejuveinix (RJX) is an intravenous formulation of anti-oxidants and anti-inflammatory agents. Its active ingredients include ascorbic acid, cyanocobalamin, thiamine hydrochloride, riboflavin 5' phosphate, niacinamide, pyridoxine hydrochloride, and calcium D-pantothenate. RJX is being developed as an anti-inflammatory and anti-oxidant treatment platform for (1) patients with critical limb ischemia as well as (2) patients with an inflammatory disorder, such as sepsis, including COVID-19 patients with viral sepsis, multi-system inflammation, cytokine release syndrome (CRS), shock, acute respiratory distress syndrome (ARDS), and multi-organ failure.

Since 2018, Reven has an active IND to develop RJX for treatment of patients with critical limb ischemia (IND 131383, assigned to the Division of Cardiovascular and Nephrology, CDER-FDA) (Original submission: November 10, 2016, IND Submission No. 0001). Reven is also planning to submit an original IND application for treatment of COVID-19 with RJX based on guidance in the advice letter from the FDA on 6/18/2020 (viz.: ADVICE LETTER from Dr. Norman Stockbridge of FDA to Peter Pacult of Reven including answers to questions contained in the 6/5/20 letter from Reven (Reference ID 4626949) in response to its preIND briefing package and additional information submitted under PIND149585). The new IND application will cross-reference Module 3 (CMC Section) of IND131383. The clinical safety profile and pharmacokinetics of RJX was examined in a recently completed Phase I clinical study in healthy volunteers (RPI003; ClinicalTrials.gov Identifier: NCT03680105).

This is the 1st Development Safety Update Report (DSUR) for Rejuveinix (RJX). This DSUR covers the reporting period from October 7, 2019 through October 6, 2020, and it provides a summary of all relevant safety data received by Reven. It is being submitted to IND131383 for the CLI indication, and to IND 149585 for COVID-19 indication. Reven has prepared no other DSURs for RJX other than this DSUR, which will be submitted, as per 21 CFR Part 312.33, to IND 131383 as SN0021, and to IND149585 as SN0004.

No clinical trials were ongoing in the time period covered by this report. There were no IND safety reports submitted during the reporting period. Additional safety and pharmacokinetics information from the completed Phase I study is included in Section 11.

There has been no detrimental change in the risk/benefit relationship, and further study of RJX is warranted due to the anticipated benefits in patients with life-threatening conditions, such as CLI and COVID-19.

Reven has not applied for marketing approval for RJX for any indication in any country.

2. INTRODUCTION

This is the 1st Development Safety Update Report (DSUR) for Rejuveinix (RJX). This DSUR covers the reporting period from October 7, 2019 through October 6, 2020, and it provides a summary of all relevant safety data received by Reven. It is being submitted to IND131383 for the CLI indication, and to IND 149585 for COVID-19 indication. Reven has prepared no other DSURs for RJX other than this DSUR, which will be submitted, as per 21 CFR Part 312.33, to IND 131383 as SN0021, and to IND149585 as SN0004.

2.1. Overview of Rejuveinix (RJX)

Rejuveinix (RJX) is an intravenous formulation of anti-oxidants and anti-inflammatory agents. Its active ingredients include ascorbic acid, cyanocobalamin, thiamine hydrochloride, riboflavin 5' phosphate, niacinamide, pyridoxine hydrochloride, and calcium D-pantothenate. RJX is being developed as an anti-inflammatory and anti-oxidant treatment platform for (1) patients with critical limb ischemia as well as (2) patients with an inflammatory disorder, such as sepsis, including COVID-19 patients with viral sepsis, multi-system inflammation, cytokine release syndrome (CRS), shock, ARDS, and multi-organ failure.

Table 1: Quantitative Composition of IP

Vial	Component	% Content	Content mg/10 mL
Vial A	**API (designated by numbers)**		
	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
	Water for Injection USP (diluent)	79.8425	7984.25
	Sodium Chloride USP	0.001	0.1
Vial B	Sodium Bicarbonate USP	8.40	840.00
	Water for Injection USP (diluent)	91.599	9159.90
	Sodium Chloride USP	0.001	0.1

Abbreviations: USP = United States Pharmacopeia, API = Active Pharmaceutical Ingredient

A Phase I, double-blind, placebo-controlled, randomized, two-part, ascending dose-escalation study was performed in participating 76 healthy volunteer human subjects in compliance with the ICH(E6) good clinical practice (GCP) guidelines to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of RJX (Protocol No. RPI003; ClinicalTrials.gov Identifier: NCT03680105). RJX was overall well tolerated and no participant developed serious adverse events (SAEs) or Grade 3-Grade 4 adverse events (AEs) or prematurely discontinued participation from the study.

RJX exhibited potent and dose-dependent protective activity, decreased the inflammatory cytokine responses (IL-6, TNF-α, TGF-β), and improved survival in the LPS-GalN mouse model of sepsis, cytokine release syndrome, ARDS, and multi-organ failure. Histopathological examinations showed that RJX attenuated the LPS-GalN induced acute lung injury (ALI) and pulmonary edema as well as liver damage. These findings suggest that RJX has the potential to favorably affect the clinical course of high-risk COVID-19 by preventing ARDS and its complications. RJX also reversed the inflammatory cytokine response and improved the survival outcome of LPS-GalN challenged mice if the treatment was delayed until after the onset of the inflammatory cytokine response and ALI. These results provide direct evidence that low dose RJX is capable of preventing fatal cytokine storm and reduce the mortality rate of LPS/GalN-induced systemic inflammation when the treatment is delayed until after the onset of a systemic inflammatory cytokine response and oxidative stress in the lungs.

2.2. Overview of Clinical Investigations Covered in this Report

No clinical trials were ongoing in the time period covered by this report. There were no IND safety reports submitted during the reporting period. Additional safety and pharmacokinetics information from the completed Phase I study RPI003 (ClinicalTrials.gov Identifier: NCT03680105) is included in Section 11.

3. WORLDWIDE MARKETING APPROVAL STATUS

RJX has not been submitted for approval, nor has it been approved for marketing in any country.

4. ACTIONS TAKEN IN THE REPORTING PERIOD FOR SAFETY REASONS

There was no action taken in the reporting period for safety reasons.

5. CHANGES TO REFERENCE SAFETY INFORMATION

A COVID-19 specific IB document including relevant safety information from the Phase I study was prepared and submitted under IND149585 (Original IND application; SN0003).

Likewise, an updated Investigator's Brochure (IB) Edition Number V4.0 for IND131383 including relevant safety information from the Phase I study is being prepared and will supersede IB Edition number V3.0_17 February 2020. We anticipate the completion in Q1 of 2021.

These IBs will serve as the updated reference documents for the expectedness assessment of SARs in the projected studies under IND149585 and IND131383, respectively.

6. INVENTORY OF CLINICAL TRIALS ONGOING AND COMPLETED DURING THE REPORTING PERIOD

No clinical trials were ongoing or completed in the time period covered by this report.

The Phase I study, RPI003, under IND131383 was completed in February 2019. The safety data were reported in the Annual IND Submission (Sequence Number 0015). The clinical study report was filed as Sequence Number 0017 in May 2020.

The anticipated tentative start date for the projected Phase I/II COVID-19 study, RPI015, under IND149585 is November 15, 2020.

7. ESTIMATED CUMULATIVE EXPOSURE

7.1. Cumulative Human Subject Exposure in the Development Program

The Phase I study RPI003 under IND131383 was a double-blind, placebo-controlled, randomized, two-part, ascending dose-escalation study that was performed to evaluate the safety, tolerability, and pharmacokinetics of RJX in healthy volunteers (Protocol No. RPI003; ClinicalTrials.gov Identifier: NCT03680105). The study ended in February 2019. The safety data were reported in the Annual IND Submission (Sequence Number 0015). The clinical study report was filed as Sequence Number 0017 in May 2020.

In Part 1, 39 subjects were treated with RJX. In Part 2, 18 subjects were treated with RJX. Thus, a total of 57 human subjects, all healthy volunteers, were treated with RJX. None of these patients were treated during the covered reporting period for this DSUR.

In the projected Phase I/II study RPI015 under IND149585 study, a total of 12 patients with COVID-19 will be treated in Part 1, Safety-Lead in Phase of the study. A total of 120 patients with COVID-19 will be treated with RJX during the randomized Part 2 of the study. Thus, an estimated total number of 132 COVID-19 patients will be treated in the proposed RPI015 study.

7.2. Patient Exposure from Marketing Experience

No patient exposure data are available for RJX as this drug has not been submitted for marketing approval in any country.

8. DATA IN LINE LISTINGS AND SUMMARY TABULATIONS

8.1. Reference Information

No patients were treated with RJX or were being monitored for AEs during the reporting period.

8.2. Line Listings of Serious Adverse Reactions during the Reporting Period

There have been no serious adverse reactions reported during this reporting period.

8.3. Cumulative Summary Tabulations of Treatment Related Serious Adverse Events

To date, no serious adverse events (SAEs) were reported for any human subject treated with RJX. Cumulative summary tabulations for all AEs were provided under IND131383, Sequence number 0015.

9. SIGNIFICANT FINDINGS FROM CLINICAL TRIALS DURING THE REPORTING PERIOD

9.1. Ongoing and Completed Clinical Trials

No patients were treated with RJX or were being monitored for AEs during the reporting period. There have been no new safety signals during the reporting period.

9.2. Other Therapeutic Use of Investigational Drug

During the reporting period for this DSUR, Reven did not provide RJX for any expanded access programs, compassionate use programs, particular patient use, single patient INDs, or treatment INDs.

10. SAFETY FINDINGS FROM NONINTERVENTIONAL STUDIES

Reven has not provided RJX for use in any non-interventional studies.

11. OTHER CLINICAL TRIAL/STUDY SAFETY INFORMATION

We have recently completed a careful review of the Phase I clinical study information regarding the safety, tolerability, and pharmacokinetics of RJX in healthy volunteer subjects. The salient features of the dataset were summarized in a manuscript that was published in Frontiers in Pharmacology (Section 23 Appendix) and include the following findings:

A Phase I, double-blind, placebo-controlled, randomized, two-part, ascending dose-escalation study was performed to evaluate the safety, tolerability, and pharmacokinetics of RJX in healthy volunteers (Protocol No. RPI003; ClinicalTrials.gov Identifier: NCT03680105) (Table 2, Table 3). The dose levels ranged from 0.024 mL/kg to 0.759 mL/kg in Part 1 and from 0.240 mL/kg to 0.759 mL/kg in Part 2. The treatment-emergent AEs (TEAEs) are presented in Table 4-Table 7. In Part 1, no deaths or SAEs were reported, none of the 39 RJX-treated subjects experienced Grade 3 or

4 AEs, and no AEs led to discontinuation of RJX (Table 7A). In Part 2, no SAEs and no Grade 3 or 4 AEs were encountered by any of the 18 RJX-treated subjects. One subject in Cohort 1 developed a mild upper respiratory infection (URI); 3 subjects in Cohort 2 had mild infusion site discomfort, pain or reaction; and 3 subjects on Cohort 3 experienced mild-moderate pain (mild headache = 1; mild pain in extremity = 1, moderate back pain = 1). The TEAEs in 2 of the subjects were considered possibly or probably related to RJX. Both patients were in Cohort 2: One patient had a mild infusion site discomfort on day 6 considered possibly related to RJX and another patient had a mild infusion site pain on days 6 and 7 considered probably related to RJX infusion (Table 6, Table 7). These TEAEs did not require additional treatment, and they did not cause discontinuation of the dosing or study. All AEs considered possibly or probably related to RJX infusions have recovered/resolved with no sequelae. There were no clinically significant abnormal findings in 12-lead safety ECGs, and no notable changes were detected compared with baseline. For both study parts, there were no clinically meaningful changes in laboratory values identified in observed values or mean changes from baseline compared with placebo or evaluated by increasing the RJX dose level. Based on its tolerability, the 0.500 ml/kg dose level was selected as the recommended Phase 2 dose (RP2D) level for future studies.

A summary of RJX plasma PK parameters by cohort following single ascending dose IV infusion is presented in Table 8-Table 12**.** Plasma concentration-time profiles of baseline-adjusted ascorbic acid (A), cyanocobalamin (B), niacinamide (C) and thiamine (D) for the RJX dose level of 0.5 mL/kg (Cohort 6) following single-dose IV infusion of RJX are presented in Figure 1. The mean baseline plasma concentrations of ascorbic acid ranged from 7.99 to 10.12 µg/mL for all cohorts. Following the single ascending dose administration of RJX in healthy subjects, baseline-adjusted ascorbic acid C_{max} and $AUC_{0\text{-last}}$ increased with increasing doses of RJX (Table 8). The mean C_{max} ranged from 5.40 µg/mL (0.024 mL/kg [1.079 mg/kg of ascorbic acid]) to 152.69 µg/mL (0.759 mL/kg [34.130 mg/kg of ascorbic acid]), increasing 28-fold while the mean $AUC_{0\text{-last}}$ ranged from 32.36 h*µg/mL to 467.10 h*µg/mL, increasing 14-fold for a 32-fold increase in dose. At the RJX dose level of 0.5 mL/kg (22.483 mg/kg of ascorbic acid), C_{max} and $AUC_{0\text{-last}}$ increased by 1.2 to 1.3-fold in elderly subjects when compared to healthy subjects. The plasma RJX PK parameters for cyanocobalamin, magnesium sulfate, niacinamide, and thiamine by cohort following single-dose IV infusion are presented in Table 9-Table 12**.** At the dose level of 0.5 mL/kg (0.048 mg/kg of cyanocobalamin; 2.970 mg/kg of niacinamide; 1.246 mg/kg of thiamine), the C_{max} and the $AUC_{0\text{-last}}$ of cyanocobalamin, niacinamide, and thiamine increased by 1.3-fold, 1.7-fold, and 1.3-fold, respectively, in elderly subjects in Cohort 6 when compared to younger healthy subjects in Cohort 4. At the same dose level, the C_{max} and the $AUC_{0\text{-last}}$ of magnesium increased by 1.2 and 1.6-fold, respectively, in elderly subjects when compared to younger subjects.

The plasma concentration-time profiles of baseline-adjusted ascorbic acid, cyanocobalamin, niacinamide, and thiamine for the RJX dose level of 0.5 mL/kg (Cohort 6) following single-dose IV infusion of RJX are presented in Figure 1. The terminal phase following single-dose administration of ascorbic acid and magnesium could not be adequately characterized. The half-life ranged from 5 to 28 h, 0.4 to 1.5 h, and 2 to 10 h, for cyanocobalamin, niacinamide, and thiamine, respectively. There was no clinically significant accumulation of these compounds following once-daily IV infusion for 7 days in the part 2 MAD cohorts, as demonstrated by the PK profiles on Day 7 that were overall similar to those observed on Day 1 (Table 13-Table 17).

In cardio-dynamic ECG assessments, we examined the potential effects of RJX on the QT interval corrected by the Fridericia correction formula (QTcF). RJX at the studied doses caused mild QTc prolongation but it did not have a clinically relevant effects on any other ECG parameter, i.e., heart rate, PR and QRS intervals. In the by-time point analysis of QTcF, mean ΔQTcF on RJX generally followed the placebo pattern across post-dose time points in both parts of the study. For the pooled data from both parts of the study (SAD plus MAD), a full model including ascorbic acid, cyanocobalamin, magnesium, niacinamide, thiamine and their 1-by-1 interaction terms was initially fitted and a model selection procedure was performed. The model with ascorbic acid and magnesium alone was selected as the primary model and represented the data reasonably well. The estimated slopes of the concentration-QTc relationship were 0.012 ms per mg/L (90% CI: −0.0114 to 0.0346) for ascorbic acid and 0.34 ms per mg/L (90% CI: 0.081 to 0.606) for magnesium, of which the slope of magnesium was statistically significant. The treatment effect-specific intercept was −5.06 ms (90% CI: −10.244 to 0.126) and not statistically significant. The predicted placebo-corrected QT effects (ΔΔQTcF) were predicted to be 7.12 ms (90% CI: 4.54 to 9.70) for the SAD 0.759 mL/kg group on Day 1 and 9.13 ms (90% CI: 6.08 to 12.19) for the MAD 0.759 mL/kg group on Day 7 where the highest levels of plasma concentrations were observed for each part (Table 18). The prediction results from other models with each analyte alone were similarto the results from the primary model. The predicted, placebo-corrected ΔΔQTcF values at the geometric mean peak concentration are provided for the concentration-QTc models with each analyte alone in Table 19-Table 23 and Figure 2-Figure 6. Based on these concentration-QTc analyses, an effect on ΔΔQTcF exceeding 10 ms can be excluded within the observed ranges of plasma concentrations of ascorbic acid, cyanocobalamin, magnesium, niacinamide, and thiamine up to 166 mg/L, 362 µg/L, 4.0 mg/dL, 7570 µg/L, and 4890 µg/L, respectively. Such plasma concentrations were only observed at the 0.759 ml/kg dosing condition, thus an effect on the QTc interval > 10 ms cannot be excluded at this dose level.

Table 2. Summary of Participant Disposition (ITT Population)

		Part 1, Cohort						
	Placebo (N = 13)	1 0.024 mL/kg (N = 6)	2 0.076 mL/kg (N = 6)	3 0.240 mL/kg (N = 6)	4 0.500 mL/kg (N = 6)	5 0.759 mL/kg (N = 6)	6 0.500 mL/kg (N = 9)	Total (N = 52)
Randomized (ITT Population)	13	6	6	6	6	6	9	52
Dosed (Safety Analysis Population)	13	6	6	6	6	6	9	52
Dosed with Placebo	13	0	0	0	0	0	0	13
Dosed with RJX	0	6	6	6	6	6	9	39
Completed Study (N [%] of Participants)	13 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	9 (100.0)	52 (100.0)
Discontinued Early from the Study	0	0	0	0	0	0	0	0

		Part 2, Cohort			
	Placebo (N = 6)	1 0.240 mL/kg (N = 6)	2 0.500 mL/kg (N = 6)	3 0.759 mL/kg (N = 6)	Total (N = 24)
Randomized (ITT Population)	6	6	6	6	24
Dosed (Safety Analysis Population)	6	6	6	6	24
Dosed with Placebo	6	0	0	0	6
Dosed with RJX	0	6	6	6	18
Completed Study (N [%] of Participants)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	24 (100.0)
Discontinued Early from the Study	0	0	0	0	0

Abbreviations: ITT = Intent to Treat; N = number; RJX = Rejuveinix.

Table 3. Demographic and Baseline Characteristics of Subjects on Protocol No. RPI003; ClinicalTrials.gov Identifier: NCT03680105).

	Part 1: Single Ascending Dose (SAD)								Part 2: Multiple Ascending Dose (MAD)				
		Cohort								Cohort			
Parameter (units)	**Placebo (N = 13)**	**1 0.024 mL/kg (N = 6)**	**2 0.076 mL/kg (N = 6)**	**3 0.240 mL/kg (N = 6)**	**4 0.500 mL/kg (N = 6)**	**5 0.759 mL/kg (N = 6)**	**6 0.500 mL/kg (N = 9)**	**Total (N = 52)**	**Placebo (N = 6)**	**1 0.240 mL/kg (N = 6)**	**2 0.500 mL/kg (N = 6)**	**3 0.759 mL/kg (N = 6)**	**Total (N = 24)**
Age (years), Mean (SD)	34.8 (13.5)	28.0 (7.0)	32.3 (8.5)	32.5 (9.6)	33.7 (5.6)	27.3 (8.8)	54.6 (3.5)	35.9 (12.6)	36.2 (10.3)	37.2 (8.1)	37.3 (6.7)	35.8 (12.8)	36.6 (9.1)
Median	29.0	27.5	35.5	34.5	35.0	24.0	54.0	34.0	36.0	38.5	40.0	34.5	39.0
Sex (n [%]) Male	6 (46.2)	2 (33.3)	3 (50.0)	2 (33.3)	2 (33.3)	3 (50.0)	4 (44.4)	22 (42.3)	4 (66.7)	6 (100.0)	3 (50.0)	2 (33.3)	15 (62.5)
Female	7 (53.8)	4 (66.7)	3 (50.0)	4 (66.7)	4 (66.7)	3 (50.0)	5 (55.6)	30 (57.7)	2 (33.3)	0	3 (50.0)	4 (66.7)	9 (37.5)
Ethnicity (n [%])													
Hispanic or Latino	5 (38.5)	5 (83.3)	2 (33.3)	3 (50.0)	2 (33.3)	3 (50.0)	6 (66.7)	26 (50.0)	2 (33.3)	2 (33.3)	3 (50.0)	2 (33.3)	9 (37.5)
Not Hispanic or Latino	8 (61.5)	1 (16.7)	4 (66.7)	3 (50.0)	4 (66.7)	3 (50.0)	3 (33.3)	26 (50.0)	4 (66.7)	4 (66.7)	3 (50.0)	4 (66.7)	15 (62.5)
Race (n [%]) Black or African American	8 (61.5)	1 (16.7)	1 (16.7)	2 (33.3)	3 (50.0)	2 (33.3)	2 (22.2)	19 (36.5)	2 (33.3)	2 (33.3)	0	5 (83.3)	9 (37.5)
White	5 (38.5)	5 (83.3)	5 (83.3)	4 (66.7)	3 (50.0)	4 (66.7)	7 (77.8)	33 (63.5)	4 (66.7)	3 (50.0)	6 (100.0)	1 (16.7)	14 (58.3)
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	1 (16.7)	0	0	1 (4.2)
Weight (kg) Mean (SD)	79.01 (16.95)	66.48 (14.87)	75.47 (11.25)	72.48 (10.00)	69.28 (4.67)	72.25 (15.27)	77.58 (13.11)	74.25 (13.50)	90.77 (19.17)	83.52 (7.96)	67.08 (7.47)	72.60 (9.78)	78.49 (14.68)
Median	82.60	65.20	75.15	69.50	67.80	72.45	75.90	71.20	92.80	85.10	68.50	77.55	77.65
Height (cm) Mean (SD)	167.15 (9.30)	161.40 (9.54)	170.73 (7.51)	166.33 (8.43)	168.42 (9.45)	167.28 (12.69)	166.19 (10.21)	166.80 (9.40)	169.53 (13.25)	175.00 (5.84)	163.63 (10.56)	164.70 (6.70)	168.22 (10.04)
Median	166.50	158.95	172.30	165.25	168.35	167.40	164.20	166.60	169.05	175.00	164.10	166.00	168.40
BMI (kg/m^2) Mean (SD)	27.79 (4.01)	25.27 (3.46)	25.87 (3.93)	26.15 (4.27)	24.47 (3.60)	25.58 (4.15)	27.81 (4.40)	26.45 (3.98)	31.07 (2.52)	27.15 (2.34)	24.88 (3.13)	26.58 (2.42)	27.42 (3.36)
Median	28.60	23.90	27.40	26.20	24.25	27.15	27.20	26.25	31.50	27.10	24.05	26.70	27.05

Abbreviations: BMI = body mass index; ITT = Intent to Treat; N or n = number; Max = maximum; Min = minimum; RJX = Rejuveinix; SD = standard deviation

Table 4. Part 1 Summary of Treatment-emergent Adverse Events by System Organ Class and Preferred Term (Safety Analysis Population)

	Cohort															
			1		2		3		4		5		6			
	Placebo (N = 13)		0.024 mL/kg (N = 6)		0.076 mL/kg (N = 6)		0.240 mL/kg (N = 6)		0.500 mL/kg (N = 6)		0.759 mL/kg (N = 6)		0.500 mL/kg (N = 9)		Overall (N = 52)	
Category	No. Particip. (%)	No. Events (%)	No. Particip. (%)	No. Events (%)	No. Particip. (%)	No. Events (%)	No. Particip. (%)	No. Events (%)	No. Particip. (%)	No. Events (%)	No. Particip. (%)	No. Events (%)	No. Particip. (%)	No. Events (%)	No. Particip. (%)	No. Events (%)
Any TEAE	2 (15.4)	2	1 (16.7)	2	2 (33.3)	3	0	0	3 (50.0)	3	2 (33.3)	3	0	0	10 (19.2)	13
Nervous system disorders	0	0	1 (16.7)	1	1 (16.7)	1	0	0	2 (33.3)	2	2 (33.3)	3	0	0	6 (11.5)	7
Headache	0	0	1 (16.7)	1	0	0	0	0	1 (16.7)	1	2 (33.3)	2	0	0	4 (7.7)	4
Dizziness	0	0	0	0	1 (16.7)	1	0	0	0	0	1 (16.7)	1	0	0	2 (3.8)	2
Delayed sleep phase	0	0	0	0	0	0	0	0	1 (16.7)	1	0	0	0	0	1 (1.9)	1
Musculo-skeletal and connective tissue disorders	1 (7.7)	1	0	0	1 (16.7)	2	0	0	0	0	0	0	0	0	2 (3.8)	3
Back pain	1 (7.7)	1	0	0	1 (16.7)	1	0	0	0	0	0	0	0	0	2 (3.8)	2
Musculo-skeletal chest pain	0	0	0	0	1 (16.7)	1	0	0	0	0	0	0	0	0	1 (1.9)	1
Gastro-intestinal disorders	0	0	0	0	0	0	0	0	1 (16.7)	1	0	0	0	0	1 (1.9)	1
Nausea	0	0	0	0	0	0	0	0	1 (16.7)	1	0	0	0	0	1 (1.9)	1

Investigations	0	0	1 (16.7)	1	0	0	0	0	0	0	0	0	0	0	1 (1.9)	1
Blood pressure systolic decreased	0	0	1 (16.7)	1	0	0	0	0	0	0	0	0	0	0	1 (1.9)	1
Vascular disorders	1 (7.7)	1	0	0	0	0	0	0	0	0	0	0	0	0	1 (1.9)	1
Hypotension	1 (7.7)	1	0	0	0	0	0	0	0	0	0	0	0	0	1 (1.9)	1

Abbreviations: No. = number; Particip. = participants; RJX = Rejuveinix; TEAE = treatment-emergent adverse event.
Note: Participants with multiple events within a system organ class/preferred term are counted only once in the system organ class/preferred term. For the number of events, participants are counted more than once.

Table 5. Part 2 Summary of Treatment-emergent Adverse Events by System Organ Class and Preferred Term (Safety Analysis Population)

	Cohort									
			1		2		3			
	Placebo (N = 6)		0.240 mL/kg (N = 6)		0.500 mL/kg (N = 6)		0.759 mL/kg (N = 6)		Total (N = 24)	
Category	No. Particip. (%)	No. Events (%)	No. Particip. (%)	No. Events (%)	No. Particip. (%)	No. Events (%)	No. Particip. (%)	No. Events (%)	No. Particip. (%)	No. Events (%)
Any TEAE	1 (16.7)	4	1 (16.7)	1	3 (50.0)	5	3 (50.0)	4	8 (33.3)	14
General disorders and administration site conditions	0	0	0	0	3 (50.0)	4	0	0	3 (12.5)	4
Infusion site discomfort	0	0	0	0	1 (16.7)	1	0	0	1 (4.2)	1
Infusion site pain	0	0	0	0	1 (16.7)	2	0	0	1 (4.2)	2
Infusion site reaction	0	0	0	0	1 (16.7)	1	0	0	1 (4.2)	1
Musculoskeletal and connective tissue disorders	1 (16.7)	1	0	0	0	0	2 (33.3)	2	3 (12.5)	3
Back pain	0	0	0	0	0	0	1 (16.7)	1	1 (4.2)	1
Myalgia	1 (16.7)	1	0	0	0	0	0	0	1 (4.2)	1
Pain in extremity	0	0	0	0	0	0	1 (16.7)	1	1 (4.2)	1
Nervous system disorders	1 (16.7)	2	0	0	1 (16.7)	1	1 (16.7)	1	3 (12.5)	4
Headache	1 (16.7)	1	0	0	0	0	1 (16.7)	1	2 (8.3)	2

	Cohort									
			1		2		3			
	Placebo (N = 6)		0.240 mL/kg (N = 6)		0.500 mL/kg (N = 6)		0.759 mL/kg (N = 6)		Total (N = 24)	
Category	No. Particip. (%)	No. Events (%)	No. Particip. (%)	No. Events (%)	No. Particip. (%)	No. Events (%)	No. Particip. (%)	No. Events (%)	No. Particip. (%)	No. Events (%)
Clonus	0	0	0	0	1 (16.7)	1	0	0	1 (4.2)	1
Dizziness	1 (16.7)	1	0	0	0	0	0	0	1 (4.2)	1
Blood and lymphatic system disorders	0	0	0	0	0	0	1 (16.7)	1	1 (4.2)	1
Anaemia	0	0	0	0	0	0	1 (16.7)	1	1 (4.2)	1
Infections and infestations	0	0	1 (16.7)	1	0	0	0	0	1 (4.2)	1
Upper respiratory tract infection	0	0	1 (16.7)	1	0	0	0	0	1 (4.2)	1
Investigations	1 (16.7)	1	0	0	0	0	0	0	1 (4.2)	1
C-reactive protein increased	1 (16.7)	1	0	0	0	0	0	0	1 (4.2)	1

Abbreviations: No. = number; Particip. = participants; RJX = Rejuveinix; TEAE = treatment-emergent adverse event.
Note: Participants with multiple events within a system organ class/preferred term are counted only once in the system organ class/preferred term. For the number of events, participants are counted more than once.

Table 6A. Summary of Treatment-Emergent Adverse Events in Part 1 by Relationship to RJX (Safety Analysis Population)

		Placebo (N=13)		Cohort 1 0.024 mL/kg (N=6)		Cohort 2 0.076 mL/kg (N=6)		Cohort 3 0.240 mL/kg (N=6)	
System Organ Class Preferred Term	Relationship	No. of Subjects (%)	No. of Events	No. of Subjects (%)	No. of Events	No. of Subjects (%)	No. of Events	No. of Subjects (%)	No. of Events
Any TEAE	Unrelated	0	0	0	0	0	0	0	0
	Unlikely Related	2 (15.4)	2	1 (16.7)	1	1 (16.7)	2	0	0
	Possibly Related	0	0	1 (16.7)	1	1 (16.7)	1	0	0
	Probably Related	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0
Nervous system disorders	Unrelated	0	0	0	0	0	0	0	0

	Unlikely Related	0	0	1 (16.7)	1	0	0	0	0
	Possibly Related	0	0	0	0	1 (16.7)	1	0	0
	Probably Related	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0
Headache	Unrelated	0	0	0	0	0	0	0	0
	Unlikely Related	0	0	1 (16.7)	1	0	0	0	0
	Possibly Related	0	0	0	0	0	0	0	0
	Probably Related	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0
Dizziness	Unrelated	0	0	0	0	0	0	0	0
	Unlikely Related	0	0	0	0	0	0	0	0
	Possibly Related	0	0	0	0	1 (16.7)	1	0	0
	Probably Related	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0
Delayed sleep phase	Unrelated	0	0	0	0	0	0	0	0
	Unlikely Related	0	0	0	0	0	0	0	0
	Possibly Related	0	0	0	0	0	0	0	0
	Probably Related	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0
Musculoskeletal and connective tissue disorders	Unrelated	0	0	0	0	0	0	0	0
	Unlikely Related	1 (7.7)	1	0	0	1 (16.7)	2	0	0
	Possibly Related	0	0	0	0	0	0	0	0
	Probably Related	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0
Investigations	Unrelated	0	0	0	0	0	0	0	0
	Unlikely Related	0	0	0	0	0	0	0	0
	Possibly Related	0	0	1 (16.7)	1	0	0	0	0
	Probably Related	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0
Syst. BP decreased	Unrelated	0	0	0	0	0	0	0	0
	Unlikely Related	0	0	0	0	0	0	0	0
	Possibly Related	0	0	1 (16.7)	1	0	0	0	0
	Probably Related	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0

		Cohort 4 **0.500 mL/kg** (N=6)		**Cohort 5** **0.759 mL/kg** (N=6)		**Cohort 6** **0.500 mL/kg** (N=9)		**Overall** (N=52)	

System Organ Class Preferred Term	Relationship	No. of Subjects (%)	No. of Events	No. of Subjects (%)	No. of Events	No. of Subjects (%)	No. of Events	No. of Subjects (%)	No. of Events
Any TEAE	Unrelated	0	0	0	0	0	0	0	0
	Unlikely Related	1 (16.7)	1	1 (16.7)	1	0	0	6 (11.5)	7
	Possibly Related	2 (33.3)	2	2 (33.3)	2	0	0	6 (11.5)	6
	Probably Related	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0
Nervous system disorders	Unrelated	0	0	0	0	0	0	0	0
	Unlikely Related	0	0	1 (16.7)	1	0	0	2 (3.8)	2
	Possibly Related	2 (33.3)	2	2 (33.3)	2	0	0	5 (9.6)	5
	Probably Related	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0
Headache	Unrelated	0	0	0	0	0	0	0	0
	Unlikely Related	0	0	1 (16.7)	1	0	0	2 (3.8)	2
	Possibly Related	1 (16.7)	1	1 (16.7)	1	0	0	2 (3.8)	2
	Probably Related	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0
Dizziness	Unrelated	0	0	0	0	0	0	0	0
	Unlikely Related	0	0	0	0	0	0	0	0
	Possibly Related	0	0	1 (16.7)	1	0	0	2 (3.8)	2
	Probably Related	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0
Delayed sleep phase	Unrelated	0	0	0	0	0	0	0	0
	Unlikely Related	0	0	0	0	0	0	0	0
	Possibly Related	1 (16.7)	1	0	0	0	0	1 (1.9)	1
	Probably Related	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0
Musculoskeletal and connective tissue disorders	Unrelated	0	0	0	0	0	0	0	0
	Unlikely Related	0	0	0	0	0	0	2 (3.8)	3
	Possibly Related	0	0	0	0	0	0	0	0
	Probably Related	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0
Investigations	Unrelated	0	0	0	0	0	0	0	0
	Unlikely Related	0	0	0	0	0	0	0	0
	Possibly Related	0	0	0	0	0	0	1 (1.9)	1
	Probably Related	0	0	0	0	0	0	0	0

	Highly Probably Related	0	0	0	0	0	0	0	0
BP, syst., decreased	Unrelated	0	0	0	0	0	0	0	0
	Unlikely Related	0	0	0	0	0	0	0	0
	Possibly Related	0	0	0	0	0	0	1 (1.9)	1
	Probably Related	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0

Table 6B. Summary of Treatment-Emergent Adverse Events in Part 2 by Relationship to RJX (Safety Analysis Population)

		Placebo (N=6)		**Cohort 1 0.240 mL/kg** (N=6)		**Cohort 2 0.500 mL/kg** (N=6)		**Cohort 3 0.759 mL/kg** (N=6)		**Overall** (N=24)	
System Organ Class Preferred Term	**Relationship**	**No. of Subject** (%)	**No. of Events**	**No. of Subjects** (%)	**No. of Events**	**No. of Subjects** (%)	**No. of Events**	**No. of Subjects** (%)	**No. of Events**	**No. of Subject** (%)	**No. of Events**
Any TEAE	Unrelated	0	0	1 (16.7)	1	1 (16.7)	1	1 (16.7)	1	3 (12.5)	3
	Unlikely Related	1 (16.7)	1	0	0	1 (16.7)	1	3 (50.0)	3	5 (20.8)	5
	Possibly Related	1 (16.7)	3	0	0	1 (16.7)	1	0	0	2 (8.3)	4
	Probably Related	0	0	0	0	1 (16.7)	2	0	0	1 (4.2)	2
	Highly Probably Related	0	0	0	0	0	0	0	0	0	0
General disorders and administration site conditions	Unrelated	0	0	0	0	1 (16.7)	1	0	0	1 (4.2)	1
	Unlikely Related	0	0	0	0	0	0	0	0	0	0
	Possibly Related	0	0	0	0	1 (16.7)	1	0	0	1 (4.2)	1
	Probably Related	0	0	0	0	1 (16.7)	2	0	0	1 (4.2)	2
	Highly Probably Related	0	0	0	0	0	0	0	0	0	0
Infusion site discomfort	Unrelated	0	0	0	0	0	0	0	0	0	0
	Unlikely Related	0	0	0	0	0	0	0	0	0	0
	Possibly Related	0	0	0	0	1 (16.7)	1	0	0	1 (4.2)	1
	Probably Related	0	0	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0	0	0
Infusion site pain	Unrelated	0	0	0	0	0	0	0	0	0	0
	Unlikely Related	0	0	0	0	0	0	0	0	0	0
	Possibly Related	0	0	0	0	0	0	0	0	0	0
	Probably Related	0	0	0	0	1 (16.7)	2	0	0	1 (4.2)	2
	Highly Probably Related	0	0	0	0	0	0	0	0	0	0

Musculoskeletal and connective tissue disorders	Unrelated	0	0	0	0	0	0	1 (16.7)	1	1 (4.2)	1
	Unlikely Related	0	0	0	0	0	0	1 (16.7)	1	1 (4.2)	1
	Possibly Related	1 (16.7)	1	0	0	0	0	0	0	1 (4.2)	1
	Probably Related	0	0	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0	0	0
Myalgia	Unrelated	0	0	0	0	0	0	0	0	0	0
	Unlikely Related	0	0	0	0	0	0	0	0	0	0
	Possibly Related	1 (16.7)	1	0	0	0	0	0	0	1 (4.2)	1
	Probably Related	0	0	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0	0	0
Nervous system disorders	Unrelated	0	0	0	0	0	0	0	0	0	0
	Unlikely Related	0	0	0	0	1 (16.7)	1	1 (16.7)	1	2 (8.3)	2
	Possibly Related	1 (16.7)	2	0	0	0	0	0	0	1 (4.2)	2
	Probably Related	0	0	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0	0	0
Headache	Unrelated	0	0	0	0	0	0	0	0	0	0
	Unlikely Related	0	0	0	0	0	0	1 (16.7)	1	1 (4.2)	1
	Possibly Related	1 (16.7)	1	0	0	0	0	0	0	1 (4.2)	1
	Probably Related	0	0	0	0	0	0	0	0	0	0
	Highly Probably Related	0	0	0	0	0	0	0	0	0	0

TEAE: Treatment-emergent adverse events; RJX: Rejuveinix. PT: Preferred term. AEs were coded using MeDRA Version 21.1. Subjects with multiple events within a system organ class/preferred term with the relationship to RJX. For the number of events, subjects were counted more than once.

Table 7A. Summary of Treatment-Emergent Adverse Events in Part 1 by Severity Grade (Safety Analysis Population)

		Placebo (N=13)		**Cohort 1** 0.024 mL/kg (N=6)		**Cohort 2** **0.076 mL/kg** (N=6)		**Cohort 3** **0.240 mL/kg** (N=6)	
System Organ Class Preferred Term [1]	*Severity*	*No. of Subjects (%)*	*No. of Events*	*No. of Subjects (%)*	*No. of Events*	*No. of Subjects (%)*	*No. of Events*	*No. of Subjects (%)*	*No. of Events*
Any TEAE	Mild	2 (15.4)	2	1 (16.7)	2	2 (33.3)	3	0	0
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Nervous system disorders	Mild	0	0	1 (16.7)	1	1 (16.7)	1	0	0
	Moderate	0	0	0	0	0	0	0	0

	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Headache	Mild	0	0	1 (16.7)	1	0	0	0	0
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Dizziness	Mild	0	0	0	0	1 (16.7)	1	0	0
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Delayed sleep phase	Mild	0	0	0	0	0	0	0	0
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Musculoskeletal and connective tissue disorders	Mild	1 (7.7)	1	0	0	1 (16.7)	2	0	0
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Back pain	Mild	1 (7.7)	1	0	0	1 (16.7)	1	0	0
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Chest pain	Mild	0	0	0	0	1 (16.7)	1	0	0
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Investigations	Mild	0	0	1 (16.7)	1	0	0	0	0
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
BP, syst., decreased	Mild	0	0	1 (16.7)	1	0	0	0	0
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Vascular disorders	Mild	1 (7.7)	1	0	0	0	0	0	0
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Hypotension	Mild	1 (7.7)	1	0	0	0	0	0	0
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Gastrointestinal disorders	Mild	0	0	0	0	0	0	0	0
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0

Nausea	Mild	0	0	0	0	0	0	0	0
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0

		Cohort 4 0.500 mL/kg (N=6)		**Cohort 5** 0.759 mL/kg (N=6)		**Cohort 6 0.500 mL/kg** (N=9)		**Overall** (N=52)	
System Organ Class Preferred Term [1]	*Severity*	*No. of Subjects (%)*	*No. of Events*	*No. of Subjects (%)*	*No. of Events*	*No. of Subjects (%)*	*No. of Events*	*No. of Subjects (%)*	*No. of Events*
Any TEAE	Mild	2 (33.3)	2	1 (16.7)	2	0	0	8 (15.4)	11
	Moderate	1 (16.7)	1	1 (16.7)	1	0	0	2 (3.8)	2
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Nervous system disorders	Mild	1 (16.7)	1	1 (16.7)	2	0	0	4 (7.7)	5
	Moderate	1 (16.7)	1	1 (16.7)	1	0	0	2 (3.8)	2
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Headache	Mild	0	0	1 (16.7)	1	0	0	2 (3.8)	2
	Moderate	1 (16.7)	1	1 (16.7)	1	0	0	2 (3.8)	2
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Dizziness	Mild	0	0	1 (16.7)	1	0	0	2 (3.8)	2
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Delayed sleep phase	Mild	1 (16.7)	1	0	0	0	0	1 (1.9)	1
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Musculoskeletal and connective tissue disorders	Mild	0	0	0	0	0	0	2 (3.8)	3
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Back pain	Mild	0	0	0	0	0	0	2 (3.8)	2
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Chest pain	Mild	0	0	0	0	0	0	1 (1.9)	1
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Investigations	Mild	0	0	0	0	0	0	1 (1.9)	1
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0

BP, syst., decreased	Mild	0	0	0	0	0	0	1 (1.9)	1
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Vascular disorders	Mild	0	0	0	0	0	0	1 (1.9)	1
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Hypotension	Mild	0	0	0	0	0	0	1 (1.9)	1
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Gastrointestinal disorders	Mild	1 (16.7)	1	0	0	0	0	1 (1.9)	1
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0
Nausea	Mild	1 (16.7)	1	0	0	0	0	1 (1.9)	1
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0

Table 7B. Summary of Treatment-Emergent Adverse Events in Part 2 by Severity Grade (Safety Analysis Population)

		Placebo (N=6)		**Cohort 1 0.240 mL/kg** (N=6)		**Cohort 2 0.500 mL/kg** (N=6)		**Cohort 3 0.759 mL/kg** (N=6)		**Overall** (N=24)	
System Organ Class Preferred Term [1]	*Severity*	*No. of Subjects (%)*	*No. of Events*	*No. of Subjects (%)*	*No. of Events*	*No. of Subjects (%)*	*No. of Events*	*No. of Subjects (%)*	*No. of Events*	*No. of Subjects (%)*	*No. of Events*
Any TEAE	Mild	0	2	1 (16.7)	1	3 (50.0)	5	2 (33.3)	3	6 (25.0)	11
	Moderate	1 (16.7)	2	0	0	0	0	1 (16.7)	1	2 (8.3)	3
	Severe	0	0	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0	0	0
General disordersand administration site conditions	Mild	0	0	0	0	3 (50.0)	4	0	0	3 (12.5)	4
	Moderate	0	0	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0	0	0
Infusion site discomfort	Mild	0	0	0	0	1 (16.7)	1	0	0	1 (4.2)	1
	Moderate	0	0	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0	0	0
Infusion site pain	Mild	0	0	0	0	1 (16.7)	2	0	0	1 (4.2)	2
	Moderate	0	0	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0	0	0
Infusion site reaction	Mild	0	0	0	0	1 (16.7)	1	0	0	1 (4.2)	1

	Moderate	0	0	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0	0	0
Musculoskeletal and connective tissue disorders	Mild	0	0	0	0	0	0	1 (16.7)	1	1 (4.2)	1
	Moderate	1 (16.7)	1	0	0	0	0	1 (16.7)	1	2 (8.3)	2
	Severe	0	0	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0	0	0
Back pain	Mild	0	0	0	0	0	0	0	0	0	0
	Moderate	0	0	0	0	0	0	1 (16.7)	1	1 (4.2)	1
	Severe	0	0	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0	0	0
Myalgia	Mild	0	0	0	0	0	0	0	0	0	0
	Moderate	1 (16.7)	1	0	0	0	0	0	0	1 (4.2)	1
	Severe	0	0	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0	0	0
Pain in extremity	Mild	0	0	0	0	0	0	1 (16.7)	1	1 (4.2)	1
	Moderate	0	0	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0	0	0
Nervous system disorders	Mild	0	1	0	0	1 (16.7)	1	1 (16.7)	1	2 (8.3)	3
	Moderate	1 (16.7)	1	0	0	0	0	0	0	1 (4.2)	1
	Severe	0	0	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0	0	0
Headache	Mild	0	0	0	0	0	0	1 (16.7)	1	1 (4.2)	1
	Moderate	1 (16.7)	1	0	0	0	0	0	0	1 (4.2)	1
	Severe	0	0	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0	0	0
Clonus	Mild	0	0	0	0	1 (16.7)	1	0	0	1 (4.2)	1
	Moderate	0	0	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0	0	0
Dizziness	Mild	1 (16.7)	1	0	0	0	0	0	0	1 (4.2)	1
	Moderate	0	0	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0	0	0
Blood and lymphatic system disorders	Mild	0	0	0	0	0	0	1 (16.7)	1	1 (4.2)	1
	Moderate	0	0	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0	0	0
Anemia	Mild	0	0	0	0	0	0	1 (16.7)	1	1 (4.2)	1
	Moderate	0	0	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0	0	0
Infections and infestations	Mild	0	0	1 (16.7)	1	0	0	0	0	1 (4.2)	1
	Moderate	0	0	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0	0	0

	Life Threatening	0	0	0	0	0	0	0	0	0	0
URI	Mild	0	0	1 (16.7)	1	0	0	0	0	1 (4.2)	1
	Moderate	0	0	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0	0	0
	Life Threatening	0	0	0	0	0	0	0	0	0	0

Table 8. Summary Statistics of Plasma Pharmacokinetic Parameters of Baseline-Adjusted Ascorbic Acid After Single Ascending Dose of IV Infusion of RJX in Healthy Adult and Elderly Volunteers

		Day 1 (Single Ascending Dose of Rejuveinix)					
		Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5	Cohort 6
Parameter		RJX 0.024 mL/kg (1.079 mg/kg of Ascorbic Acid) in Healthy Subjects (n=6)[a]	RJX 0.076 mL/kg (3.417 mg/kg of Ascorbic Acid) in Healthy Subjects (n=6)[a]	RJX 0.240 mL/kg (10.792 mg/kg of Ascorbic Acid) in Healthy Subjects (n=6)[b]	RJX 0.500 mL/kg (22.483 mg/kg of Ascorbic Acid) in Healthy Subjects (n=6)[b]	RJX 0.759 mL/kg (34.130 mg/kg of Ascorbic Acid) in Healthy Subjects (n=6)[c]	RJX 0.500 mL/kg (22.483 mg/kg of Ascorbic Acid) in Elderly (n=9)[d]
C_{max} (µg/mL)	Mean (SD)	5.40 (2.02)	17.45 (2.74)	47.97 (8.56)	108.63 (14.82)	152.69 (30.17)	132.95 (22.10)
	CV (%)	37.3	15.7	17.8	13.6	19.8	16.6
$AUC_{0\text{-last}}$ (h*µg/mL)	Mean (SD)	32.36 (7.30)	80.44 (45.90)	190.01 (50.82)	322.68 (33.45)	467.10 (51.07)	406.53 (97.07)
	CV (%)	22.6	57.1	26.7	10.4	10.9	23.9
$t_{1/2}$ (h)	Mean (SD)	3.64 (NC)	2.65 (NC)	NC (NC)	NC (NC)	16.43 (NA)	21.27 (10.65)
	CV (%)	NC	NC	NC	NC	NA	50.1
Cl ((L/h)/kg)	Mean (SD)	0.04 (NC)	0.12 (NC)	NC (NC)	NC (NC)	0.06 (NA)	0.05 (0.01)
	CV (%)	NC	NC	NC	NC	NA	19.2
V (L/kg)	Mean (SD)	0.19 (NC)	0.45 (NC)	NC (NC)	NC (NC)	1.38 (NA)	1.42 (0.69)
	CV (%)	NC	NC	NC	NC	NA	48.3
t_{max} (h)	Min	0.52	0.75	0.75	0.75	0.75	0.75
	Max	1.25	0.75	0.80	0.80	1.10	0.90

NA : Not Applicable, NC : Not Calculated
[a] n=1 for $t_{1/2}$; [b] n=0 for $t_{1/2}$; [c] n=2 for $t_{1/2}$; [d] n=4 for $t_{1/2}$

Table 9. Summary Statistics of Plasma Pharmacokinetic Parameters of Baseline-Adjusted Cyanocobalamin After Single Ascending Dose of IV Infusion of RJX in Healthy Adult and Elderly Volunteers

		Day 1 (Single Ascending Dose of RJX)					
		Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5	Cohort 6
Parameter		RJX 0.024 mL/kg (0.002 mg/kg of Cyanocobalamin) in Healthy Subjects (n=6)[a]	RJX 0.076 mL/kg (0.007 mg/kg of Cyanocobalamin) in Healthy Subjects (n=6)[a]	RJX 0.240 mL/kg (0.023 mg/kg of Cyanocobalamin) in Healthy Subjects (n=6)	RJX 0.500 mL/kg (0.048 mg/kg of Cyanocobalamin) in Healthy Subjects (n=6)	RJX 0.759 mL/kg (0.073 mg/kg of Cyanocobalamin) in Healthy Subjects (n=6)	RJX 0.500 mL/kg (0.048 mg/kg of Cyanocobalamin) in Elderly (n=9)
C_{max} (ng/mL)	Mean (SD)	11.414 (1.151)	42.549 (8.363)	109.124 (20.470)	242.030 (25.013)	368.629 (75.827)	326.587 (44.513)
	CV (%)	10.1	19.7	18.8	10.3	20.6	13.6
$AUC_{0\text{-last}}$ (h*ng/mL)	Mean (SD)	42.636 (12.638)	119.652 (20.671)	296.432 (41.684)	685.949 (68.733)	959.464 (89.802)	899.686 (174.992)
	CV (%)	29.6	17.3	14.1	10.0	9.4	19.5
$t_{1/2}$ (h)	Mean (SD)	28.24 (11.22)	24.35 (13.03)	7.24 (3.31)	5.25 (0.55)	4.84 (0.84)	5.14 (0.53)
	CV (%)	39.7	53.5	45.7	10.5	17.2	10.2
Cl ((L/h)/kg)	Mean (SD)	0.035 (0.015)	0.044 (0.013)	0.076 (0.009)	0.070 (0.007)	0.076 (0.007)	0.055 (0.010)
	CV (%)	44.0	28.6	12.2	10.5	9.0	18.6
V (L/kg)	Mean (SD)	1.252 (0.363)	1.381 (0.484)	0.791 (0.374)	0.526 (0.044)	0.528 (0.074)	0.408 (0.099)
	CV (%)	29.0	35.0	47.3	8.4	14.1	24.2
t_{max} (h)	Min	0.75	0.75	0.75	0.75	0.75	0.75
	Max	0.77	0.75	0.80	0.80	1.10	0.90

[a] n=4 for $t_{1/2}$

Table 10. Summary Statistics of Serum Pharmacokinetic Parameters of Baseline-Adjusted Magnesium After Single Ascending Dose of IV Infusion of RJX in Healthy Adult and Elderly Volunteers

		Day 1 (Single Ascending Dose of RJX)					
		Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5	Cohort 6
Parameter		RJX 0.024 mL/kg (0.096 mg/kg of Magnesium) in Healthy Subjects (n=5)[a]	RJX 0.076 mL/kg (0.303 mg/kg of Magnesium) in Healthy Subjects (n=6)[b]	RJX 0.240 mL/kg (0.956 mg/kg of Magnesium) in Healthy Subjects (n=6)[a]	RJX 0.500 mL/kg (1.992 mg/kg of Magnesium) in Healthy Subjects (n=6)[a]	RJX 0.759 mL/kg (3.024 mg/kg of Magnesium) in Healthy Subjects (n=6)[c]	RJX 0.500 mL/kg (1.992 mg/kg of Magnesium) in Elderly (n=9)[a]
C_{max}	Mean (SD)	0.171 (0.053)	0.283 (0.044)	0.443 (0.103)	0.797 (0.241)	1.316 (0.310)	0.938 (0.207)
(mg/dL)	CV (%)	30.9	15.6	23.2	30.2	23.6	22.1
$AUC_{0\text{-last}}$	Mean (SD)	2.393 (1.142)	3.688 (1.231)	4.998 (2.313)	4.694 (3.399)	9.378 (3.589)	7.541 (2.406)
(h*mg/dL)	CV (%)	47.7	33.4	46.3	72.4	38.3	31.9
$t_{1/2}$	Mean (SD)	8.86 (NC)	NC (NC)	13.24 (NC)	19.96 (NC)	9.46 (5.78)	4.50 (NC)
(h)	CV (%)	NC	NC	NC	NC	61.1	NC
Cl	Mean (SD)	0.002 (NC)	NC (NC)	0.008 (NC)	0.028 (NC)	0.034 (0.018)	0.028 (NC)
((L/h)/kg)	CV (%)	NC	NC	NC	NC	52.2	NC
V	Mean (SD)	0.027 (NC)	NC (NC)	0.150 (NC)	0.820 (NC)	0.361 (0.041)	0.181 (NC)
(L/kg)	CV (%)	NC	NC	NC	NC	11.4	NC
t_{max}	Min	8.75	1.25	0.75	0.75	0.75	0.75
(h)	Max	12.75	12.75	4.73	1.82	1.27	0.90

NC : Not Calculated
[a] n=1 for $t_{1/2}$; [b] n=0 for $t_{1/2}$; [c] n=4 for $t_{1/2}$

Table 11. Summary Statistics of Plasma Pharmacokinetic Parameters of Baseline-Adjusted Niacinamide After Single Ascending Dose of IV Infusion of RJX in Healthy Adult and Elderly Volunteers

		Day 1 (Single Ascending Dose of RJX)					
		Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5	Cohort 6
Parameter		RJX 0.024 mL/kg (0.143 mg/kg of Niacinamide) in Healthy Subjects (n=6)[a]	RJX 0.076 mL/kg (0.451 mg/kg of Niacinamide) in Healthy Subjects (n=6)[b]	RJX 0.240 mL/kg (1.426 mg/kg of Niacinamide) in Healthy Subjects (n=6)[c]	RJX 0.500 mL/kg (2.970 mg/kg of Niacinamide) in Healthy Subjects (n=6)[d]	RJX 0.759 mL/kg (4.508 mg/kg of Niacinamide) in Healthy Subjects (n=6)[e]	RJX 0.500 mL/kg (2.970 mg/kg of Niacinamide) in Elderly (n=9)
C_{max}	Mean (SD)	90.1 (12.8)	495.6 (142.3)	1290.8 (313.8)	3472.2 (1038.5)	7072.5 (2475.3)	5862.9 (1529.2)
(ng/mL)	CV (%)	14.2	28.7	24.3	29.9	35.0	26.1
$AUC_{0\text{-last}}$	Mean (SD)	111.5 (72.1)	542.2 (218.7)	1507.8 (578.5)	4770.6 (2159.0)	14087.7 (6257.0)	7910.5 (3108.3)
(h*ng/mL)	CV (%)	64.7	40.3	38.4	45.3	44.4	39.3
$t_{1/2}$	Mean (SD)	NC (NC)	0.50 (NA)	0.41 (0.13)	0.59 (0.38)	1.51 (1.49)	0.59 (0.26)
(h)	CV (%)	NC	NA	32.9	63.8	99.1	43.3
Cl	Mean (SD)	NC (NC)	0.6 (NA)	0.9 (0.3)	0.7 (0.3)	0.4 (0.2)	0.4 (0.1)
((L/h)/kg)	CV (%)	NC	NA	36.2	41.1	49.1	34.0
V	Mean (SD)	NC (NC)	0.4 (NA)	0.5 (0.1)	0.5 (0.1)	0.8 (0.7)	0.3 (0.2)
(L/kg)	CV (%)	NC	NA	11.6	15.0	92.2	43.9
t_{max}	Min	0.25	0.50	0.75	0.75	0.57	0.75
(h)	Max	0.77	0.75	0.80	0.80	1.10	0.90

NA : Not Applicable, NC : Not Calculated
[a] n=0 for $t_{1/2}$; [b] n=2 for $t_{1/2}$; [c] n=4 for $t_{1/2}$; [d] n=3 for $t_{1/2}$; [e] n=5 for $t_{1/2}$

Table 12. Summary Statistics of Plasma Pharmacokinetic Parameters of Baseline-Adjusted Thiamine After Single Ascending Dose of IV Infusion of RJX in Healthy Adult and Elderly Volunteers

		Day 1 (Single Ascending Dose of RJX)					
		Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5	Cohort 6
Parameter		RJX 0.024 mL/kg (0.060 mg/kg of Thiamine) in Healthy Subjects (n=6)[a]	RJX 0.076 mL/kg (0.189 mg/kg of Thiamine) in Healthy Subjects (n=6)[b]	RJX 0.240 mL/kg (0.598 mg/kg of Thiamine) in Healthy Subjects (n=6)	RJX 0.500 mL/kg (1.246 mg/kg of Thiamine) in Healthy Subjects (n=6)[b]	RJX 0.759 mL/kg (1.891 mg/kg of Thiamine) in Healthy Subjects (n=6)	RJX 0.500 mL/kg (1.246 mg/kg of Thiamine) in Elderly (n=9)
C_{max}	Mean (SD)	46.89 (15.72)	280.79 (77.24)	887.69 (150.60)	2468.83 (352.15)	3695.59 (958.75)	3108.77 (378.29)
(ng/mL)	CV (%)	33.5	27.5	17.0	14.3	25.9	12.2
$AUC_{0\text{-last}}$	Mean (SD)	53.71 (23.41)	300.06 (88.33)	1164.07 (234.10)	3432.48 (291.45)	5367.91 (1154.18)	4393.07 (961.61)
(h*ng/mL)	CV (%)	43.6	29.4	20.1	8.5	21.5	21.9
$t_{1/2}$	Mean (SD)	1.99 (2.46)	2.59 (1.55)	10.41 (6.00)	7.54 (0.71)	7.98 (2.44)	8.40 (1.74)
(h)	CV (%)	123.8	59.8	57.6	9.4	30.6	20.7
Cl	Mean (SD)	1.02 (0.33)	0.69 (0.26)	0.52 (0.11)	0.35 (0.03)	0.37 (0.10)	0.29 (0.06)
((L/h)/kg)	CV (%)	32.5	37.6	21.2	7.7	27.1	20.7
V	Mean (SD)	2.12 (1.70)	2.18 (0.68)	8.06 (5.44)	3.85 (0.45)	4.07 (1.17)	3.58 (1.20)
(L/kg)	CV (%)	80.2	31.2	67.4	11.7	28.8	33.4
t_{max}	Min	0.50	0.50	0.75	0.75	0.57	0.75
(h)	Max	0.77	0.75	0.80	0.80	1.10	0.90

[a] n=4 for $t_{1/2}$; [b] n=5 for $t_{1/2}$

Table 13. Summary Statistics of Plasma Pharmacokinetic Parameters of Baseline-Adjusted Ascorbic Acid After Single and Multiple Ascending Dose of IV Infusion of RJX in Healthy Adult Volunteers

		Day 1 (Single Ascending Dose of RJX)			Day 7 (Multiple Ascending Dose of RJX)		
		Cohort 1	Cohort 2	Cohort 3	Cohort 1	Cohort 2	Cohort 3
Parameter		**RJX 0.240 mL/kg (10.792mg/kg of Ascorbic Acid) (n=6)**	**RJX 0.500 mL/kg (22.483 mg/kg of Ascorbic Acid) (n=6)**	**RJX 0.759 mL/kg (34.130 mg/kg of Ascorbic Acid (n=6)**	**RJX 0.240 mL/kg (10.792 mg/kg of Ascorbic Acid) (n=6)**	**RJX 0.500 mL/kg (22.483 mg/kg of Ascorbic Acid) (n=6)**	**RJX 0.759 mL/kg (34.130 mg/kg of Ascorbic Acid) (n=6)**
C_{max}	Mean (SD)	51.10 (7.51)	112.70 (14.31)	166.52 (19.41)	53.32 (11.06)	120.90 (12.61)	204.96 (23.18)
(µg/mL)	CV (%)	14.7	12.7	11.7	20.7	10.4	11.3
AUC_{0-12}	Mean (SD)	156.54 (25.92)	271.03 (27.08)	436.27 (53.66)	124.50 (13.87)	331.03 (43.19)	531.77 (88.53)
(h*µg/mL)	CV (%)	16.6	10.0	12.3	11.1	13.0	16.6
AUC_{0-last}	Mean (SD)	156.54 (25.92)	324.23 (39.52)	524.55 (86.68)	NA	NA	NA
(h*µg/mL)	CV (%)	16.6	12.2	16.5	NA	NA	NA
$t_{1/2}$	Mean (SD)	3.52 (1.42)	34.66 (NC)	29.85 (NC)	NA	NA	NA
(h)	CV (%)	40.3	NC	NC	NA	NA	NA
Cl	Mean (SD)	0.06 (0.02)	0.04 (NC)	0.03 (NC)	0.08 (0.01)	0.06 (NA)	0.05 (NA)
((L/h)/kg)	CV (%)	24.3	NC	NC	10.7	NA	NA
V	Mean (SD)	0.31 (0.08)	1.94 (NC)	1.43 (NC)	0.29 (0.06)	1.51 (NA)	3.41 (NA)
(L/kg)	CV (%)	25.7	NC	NC	21.8	NA	NA
t_{max}	Min	0.75	0.75	0.50	0.75	0.75	0.75
(h)	Max	0.77	0.75	0.77	1.47	0.75	0.80

Table 14. Summary Statistics of Plasma Pharmacokinetic Parameters of Baseline-Adjusted Cyanocobalamin After Single and Multiple Ascending Dose of IV Infusion of RJX in Healthy Adult Volunteers

		Day 1 (Single Ascending Dose of RJX)			Day 7 (Multiple Ascending Dose of RJX)		
		Cohort 1	Cohort 2	Cohort 3	Cohort 1	Cohort 2	Cohort 3
Parameter		RJX 0.240 mL/kg (0.023 mg/kg of Cyanocobalamin) in Healthy Subjects (n=6)	RJX 0.500 mL/kg (0.048 mg/kg of Cyanocobalamin) in Healthy Subjects (n=6)	RJX 0.759 mL/kg (0.073 mg/kg of Cyanocobalamin) in Healthy Subjects (n=6)	RJX 0.240 mL/kg (0.023 mg/kg of Cyanocobalamin) in Healthy Subjects (n=6)	RJX 0.500 mL/kg (0.048 mg/kg of Cyanocobalamin) in Healthy Subjects (n=6)[a]	RJX 0.759 mL/kg (0.073 mg/kg of Cyanocobalamin) in Healthy Subjects (n=6)
C_{max} (ng/mL)	Mean (SD)	114.572 (13.026)	278.088 (41.173)	379.213 (49.753)	133.060 (23.018)	264.051 (30.942)	395.782 (47.718)
	CV (%)	11.4	14.8	13.1	17.3	11.7	12.1
AUC_{0-12} (h*ng/mL)	Mean (SD)	313.084 (13.976)	605.606 (56.374)	913.259 (87.995)	347.938 (32.154)	622.783 (51.232)	965.143 (77.843)
	CV (%)	4.5	9.3	9.6	9.2	8.2	8.1
AUC_{0-last} (h*ng/mL)	Mean (SD)	313.094 (13.984)	632.991 (62.623)	957.728 (94.345)	NA	NA	NA
	CV (%)	4.5	9.9	9.9	NA	NA	NA
$t_{1/2}$ (h)	Mean (SD)	3.26 (1.19)	5.76 (0.90)	5.51 (1.16)	NA	NA	NA
	CV (%)	36.6	15.6	21.1	NA	NA	NA
Cl ((L/h)/kg)	Mean (SD)	0.070 (0.003)	0.076 (0.007)	0.076 (0.008)	0.061 (0.006)	0.073 (0.007)	0.072 (0.007)
	CV (%)	3.9	9.5	10.7	9.6	8.9	9.5
V (L/kg)	Mean (SD)	0.331 (0.119)	0.629 (0.118)	0.612 (0.188)	0.735 (0.040)	0.582 (0.160)	0.583 (0.141)
	CV (%)	36.1	18.7	30.8	5.5	27.5	24.1
t_{max} (h)	Min	0.75	3.082	3.554	0.75	0.75	0.50
	Max	0.77	5.403	8.570	1.47	0.75	0.80

Table 15. Summary Statistics of Serum Pharmacokinetic Parameters of Baseline-Adjusted Magnesium After Single and Multiple Ascending Dose of IV Infusion of RJX in Healthy Adult Volunteers

		Day 1 (Single Ascending Dose of RJX)			Day 7 (Multiple Ascending Dose of RJX)		
		Cohort 1	Cohort 2	Cohort 3	Cohort 1	Cohort 2	Cohort 3
Parameter		RJX 0.240 mL/kg (0.956 mg/kg of Magnesium) in Healthy Subjects (n=6)	RJX 0.500 mL/kg (1.992 mg/kg of Magnesium) in Healthy Subjects (n=6)	RJX 0.759 mL/kg (3.024 mg/kg of Magnesium) in Healthy Subjects (n=6)	RJX 0.240 mL/kg (0.956 mg/kg of Magnesium) in Healthy Subjects (n=6)	RJX 0.500 mL/kg (1.992 mg/kg of Magnesium) in Healthy Subjects (n=6)	RJX 0.759 mL/kg (3.024 mg/kg of Magnesium) in Healthy Subjects (n=6)
C_{max}	Mean (SD)	0.455 (0.139)	0.967 (0.174)	1.543 (0.154)	0.466 (0.063)	1.127 (0.182)	1.596 (0.159)
(mg/dL)	CV (%)	30.6	18.0	10.0	13.6	16.1	10.0
AUC_{0-12}	Mean (SD)	3.008 (1.163)	4.634 (1.030)	6.695 (0.985)	1.964 (0.854)	6.017 (1.239)	5.076 (1.365)
(h*mg/dL)	CV (%)	38.7	22.2	14.7	43.5	20.6	26.9
AUC_{0-last}	Mean (SD)	3.009 (1.165)	6.570 (2.744)	9.324 (1.236)	NA	NA	NA
(h*mg/dL)	CV (%)	38.7	41.8	13.3	NA	NA	NA
$t_{1/2}$	Mean (SD)	NC (NC)	7.72 (NA)	20.28 (18.26)	NA	NA	NA
(h)	CV (%)	NC	NA	90.0	NA	NA	NA
Cl	Mean (SD)	NC (NC)	0.028 (NA)	0.020 (0.005)	0.034 (NC)	0.024 (NA)	0.046 (0.008)
((L/h)/kg)	CV (%)	NC	NA	23.4	NC	NA	18.2
V	Mean (SD)	NC (NC)	0.310 (NA)	0.501 (0.267)	0.137 (NC)	0.294 (NA)	0.265 (0.058)
(L/kg)	CV (%)	NC	NA	53.2	NC	NA	21.8
t_{max}	Min	0.062	0.75	0.133	0.50	0.75	0.75
(h)	Max	0.409	0.75	0.391	1.75	0.75	0.80

Table 16. Summary Statistics of Plasma Pharmacokinetic Parameters of Baseline-Adjusted Niacinamide After Single and Multiple Ascending Dose of IV Infusion of RJX in Healthy Adult Volunteers

		Day 1 (Single Ascending Dose of RJX)			Day 7 (Multiple Ascending Dose of RJX)		
		Cohort 1	Cohort 2	Cohort 3	Cohort 1	Cohort 2	Cohort 3
Parameter		RJX 0.240 mL/kg (1.426 mg/kg of Niacinamide) in Healthy Subjects (n=6)	RJX 0.500 mL/kg (2.970 mg/kg of Niacinamide) in Healthy Subjects (n=6)	RJX 0.759 mL/kg (4.508 mg/kg of Niacinamide) in Healthy Subjects (n=6)	RJX 0.240 mL/kg (1.426 mg/kg of Niacinamide) in Healthy Subjects (n=6)	RJX 0.500 mL/kg (2.970 mg/kg of Niacinamide) in Healthy Subjects (n=6)	RJX 0.759 mL/kg (4.508 mg/kg of Niacinamide) in Healthy Subjects (n=6)
C_{max}	Mean (SD)	1554.5 (464.5)	4891.6 (1014.6)	7735.1 (811.0)	1584.0 (417.7)	4611.9 (1114.6)	8145.5 (571.3)
(ng/mL)	CV (%)	29.9	20.7	10.5	26.4	24.2	7.0
AUC_{0-12}	Mean (SD)	1857.1 (558.8)	6322.7 (1435.7)	11245.1 (1876.6)	1939.4 (542.6)	6845.7 (1430.9)	12349.8 (1686.3)
(h*ng/mL)	CV (%)	30.1	22.7	16.7	28.0	20.9	13.7
AUC_{0-last}	Mean (SD)	1774.3 (452.2)	6305.0 (1418.2)	11228.3 (1815.1)	NA	NA	NA
(h*ng/mL)	CV (%)	25.5	22.5	16.2	NA	NA	NA
$t_{1/2}$	Mean (SD)	0.57 (0.33)	0.58 (0.27)	0.70 (0.35)	NA	NA	NA
(h)	CV (%)	58.1	46.3	50.7	NA	NA	NA
Cl	Mean (SD)	0.9 (0.4)	0.5 (0.2)	0.4 (0.1)	0.8 (0.3)	0.5 (0.1)	0.3 (0.0)
((L/h)/kg)	CV (%)	42.3	32.0	16.1	39.6	28.5	11.0
V	Mean (SD)	0.6 (0.3)	0.4 (0.3)	0.4 (0.2)	1.2 (1.4)	0.3 (0.1)	0.3 (0.1)
(L/kg)	CV (%)	52.6	69.0	41.0	109.9	22.6	22.6
t_{max}	Min	0.75	0.75	0.75	0.75	0.75	0.50
(h)	Max	0.77	0.75	0.77	1.47	0.75	0.80

Table 17. Summary Statistics of Plasma Pharmacokinetic Parameters of Baseline-Adjusted Thiamine After Single and Multiple Ascending Dose of IV Infusion of RJX in Healthy Adult Volunteers

		Day 1 (Single Ascending Dose of RJX)			Day 7 (Multiple Ascending Dose of RJX)		
		Cohort 1	Cohort 2	Cohort 3	Cohort 1	Cohort 2	Cohort 3
Parameter		**RJX 0.240 mL/kg (0.598 mg/kg of Thiamine) in Healthy Subjects (n=6)**	**RJX 0.500 mL/kg (1.246 mg/kg of Thiamine) in Healthy Subjects (n=6)**	**RJX 0.759 mL/kg (1.891 mg/kg of Thiamine) in Healthy Subjects (n=6)**	**RJX 0.240 mL/kg (0.598 mg/kg of Thiamine) in Healthy Subjects (n=6)**	**RJX 0.500 mL/kg (1.246 mg/kg of Thiamine) in Healthy Subjects (n=6)[a]**	**RJX 0.759 mL/kg (1.891 mg/kg of Thiamine) in Healthy Subjects (n=6)[b]**
C_{max}	Mean (SD)	980.04 (206.67)	2765.17 (409.76)	4527.05 (646.70)	1160.17 (262.00)	2725.79 (495.77)	4728.21 (759.21)
(ng/mL)	CV (%)	21.1	14.8	14.3	22.6	18.2	16.1
AUC_{0-12}	Mean (SD)	1266.44 (247.23)	3608.88 (730.57)	5681.88 (877.99)	1591.73 (290.06)	3802.97 (552.19)	6086.58 (1034.46)
(h*ng/mL)	CV (%)	19.5	20.2	15.5	18.2	14.5	17.0
AUC_{0-last}	Mean (SD)	1266.46 (247.25)	3683.37 (767.06)	5759.70 (907.59)	NA	NA	NA
(h*ng/mL)	CV (%)	19.5	20.8	15.8	NA	NA	NA
$t_{1/2}$	Mean (SD)	3.46 (2.61)	8.65 (1.82)	8.48 (1.60)	NA	NA	NA
(h)	CV (%)	75.3	21.0	18.9	NA	NA	NA
Cl	Mean (SD)	0.48 (0.11)	0.34 (0.06)	0.33 (0.05)	0.32 (0.03)	0.32 (0.04)	0.31 (0.06)
((L/h)/kg)	CV (%)	22.2	17.2	14.0	10.4	14.1	30.1
V	Mean (SD)	2.16 (1.22)	4.35 (1.38)	4.09 (1.20)	6.89 (0.65)	5.55 (1.58)	4.08 (1.40)
(L/kg)	CV (%)	56.5	31.7	29.3	9.5	28.5	34.4
t_{max}	Min	0.5	0.75	0.50	0.75	0.75	0.50
(h)	Max	0.77	0.75	0.75	1.47	0.75	0.80

Table 18. Predicted ΔΔQTcF Interval At Geometric Mean Peak Concentration For Ascorbic Acid And Magnesium (SAD and MAD) (PK/QTc Analysis Set)

Treatment	Geometric Mean (mg/L) (90% CI) C_{max} of ascorbic acid	Geometric Mean (mg/L) (90% CI) C_{max} of magnesium	ΔΔQTcF Estimate (ms) (90% CI)
SAD 0.024 mL/kg	13.0 (9.19; 18.27)	18.0 (17.20; 18.87)	1.28 (-0.84, 3.40)
SAD 0.076 mL/kg	26.2 (23.98; 28.71)	20.5 (18.90; 22.29)	2.30 (0.21, 4.38)
SAD 0.240 mL/kg	54.8 (45.18; 66.51)	21.2 (20.55; 21.78)	2.84 (0.76, 4.92)
SAD 0.5 mL/kg	117.9 (105.54; 131.65)	24.8 (23.24; 26.51)	4.83 (2.50, 7.16)
SAD Elderly 0.5 mL/kg	141.4 (129.32; 154.64)	27.3 (25.25; 29.54)	5.96 (3.51, 8.41)
SAD 0.759 mL/kg	158.4 (134.35; 186.78)	30.1 (27.78; 32.69)	7.12 (4.54, 9.70)
MAD 0.240 mL/kg	66.1 (57.71; 75.64)	23.0 (21.48; 24.55)	3.59 (1.50, 5.68)
MAD 0.5 mL/kg	129.9 (116.18; 145.16)	29.1 (28.23; 30.06)	6.45 (4.03, 8.86)
MAD 0.759 mL/kg	211.2 (193.85; 230.02)	34.2 (32.71; 35.79)	9.13 (6.08, 12.19)

Table 19. Predicted ΔΔQTcF Interval At Geometric Mean Peak Concentration For Ascorbic Acid (SAD And MAD) (PK/QTc Analysis Set)

Treatment	Geometric Mean (mg/L) (90% CI) C_{max} of ascorbic acid	ΔΔQTcF Estimate (ms) (90% CI)
SAD 0.024 mL/kg	13.0 (9.19; 18.27)	1.51 (-0.90, 3.91)
SAD 0.076 mL/kg	26.2 (23.98; 28.71)	2.08 (-0.28, 4.44)
SAD 0.240 mL/kg	54.8 (45.18; 66.51)	3.31 (1.00, 5.62)
SAD 0.5 mL/kg	117.9 (105.54; 131.65)	6.03 (3.51, 8.55)
SAD Elderly 0.5 mL/k	141.4 (129.32; 154.64)	7.05 (4.35, 9.74)
SAD 0.759 mL/kg	158.4 (134.35; 186.78)	7.78 (4.93, 10.63)
MAD 0.240 mL/kg	66.1 (57.71; 75.64)	3.80 (1.48, 6.12)
MAD 0.5 mL/kg	129.9 (116.18; 145.16)	6.55 (3.94, 9.15)
MAD 0.759 mL/kg	211.2 (193.85; 230.02)	10.05 (6.63, 13.48)
10 ms Threshold	147	7.29 (4.54, 10.03)

Based on a linear mixed-effects model with ΔQTcF as the dependent variable, time-matched ascorbic acid plasma concentration as an explanatory variate, centered baseline as an additional covariate, treatment (active = 1 or placebo = 0) and time as fixed effects, and a random intercept and slope per subject.

Table 20. Predicted ΔΔQTcF Interval At Geometric Mean Peak Concentration For Cyanocobalamin (SAD and MAD)

Treatment	Geometric Mean (µg/L) (90% CI) C_{max} of cyanocobalamin	ΔΔQTcF Estimate (ms) (90% CI)
SAD 0.024 mL/kg	10.6 (9.42; 11.96)	1.64 (-0.73, 4.01)
SAD 0.076 mL/kg	41.9 (35.78; 49.05)	2.20 (-0.13, 4.52)
SAD 0.240 mL/kg	107.5 (91.86; 125.79)	3.36 (1.08, 5.64)
SAD 0.5 mL/kg	241.0 (221.56; 262.08)	5.72 (3.26, 8.19)
SAD Elderly 0.5 mL/k	323.9 (297.62; 352.49)	7.19 (4.45, 9.93)
SAD 0.759 mL/kg	362.1 (305.31; 429.50)	7.87 (4.97, 10.77)
MAD 0.240 mL/kg	131.1 (111.82; 153.82)	3.78 (1.49, 6.06)
MAD 0.5 mL/kg	262.4 (236.25; 291.37)	6.10 (3.58, 8.63)
MAD 0.759 mL/kg	393.4 (356.26; 434.47)	8.43 (5.39, 11.46)
10 ms Threshold	328	7.27 (4.51, 10.02)

Based on a linear mixed-effects model with ΔQTcF as the dependent variable, time-matched cyanocobalamin plasma concentration as an explanatory variate, centered baseline as an additional covariate, treatment (active = 1 or placebo = 0) and time as fixed effects, and a random intercept and slope per subject.

Table 21. Predicted ΔΔQTcF Interval At Geometric Mean Peak Concentration For Magnesium (SAD and MAD)

Treatment	Geometric Mean (mg/dL) (90% CI) C_{max} of magnesium	ΔΔQTcF Estimate (ms) (90% CI)
SAD 0.024 mL/kg	1.8 (1.72; 1.89)	1.30 (-0.83, 3.43)
SAD 0.076 mL/kg	2.1 (1.89; 2.23)	2.43 (0.35, 4.51)
SAD 0.240 mL/kg	2.1 (2.05; 2.18)	2.71 (0.64, 4.79)
SAD 0.5 mL/kg	2.5 (2.32; 2.65)	4.37 (2.21, 6.52)
SAD Elderly 0.5 mL/k	2.7 (2.53; 2.95)	5.49 (3.21, 7.77)
SAD 0.759 mL/kg	3.0 (2.78; 3.27)	6.76 (4.27, 9.25)
MAD 0.240 mL/kg	2.3 (2.15; 2.45)	3.53 (1.43, 5.63)
MAD 0.5 mL/kg	2.9 (2.82; 3.01)	6.31 (3.90, 8.71)
MAD 0.759 mL/kg	3.4 (3.27; 3.58)	8.59 (5.71, 11.47)
10 ms Threshold	3	7.42 (4.80, 10.04)

Based on a linear mixed-effects model with ΔQTcF as the dependent variable, time-matched magnesium plasma concentration as an explanatory variate, centered baseline as an additional covariate, treatment (active = 1 or placebo = 0) and time as fixed effects, and a random intercept per subject.

Table 22. Predicted ΔΔQTcF Interval At Geometric Mean Peak Concentration For Niacinamide (SAD and MAD) (PK/QTc Analysis Set)

Treatment	Geometric Mean (µg/L) (90% CI) C_{max} of niacinamide	ΔΔQTcF Estimate (ms) (90% CI)
SAD 0.024 mL/kg	114.4 (94.27; 138.96)	1.99 (-0.34, 4.32)
SAD 0.076 mL/kg	511.7 (390.97; 669.70)	2.30 (0.00, 4.60)
SAD 0.240 mL/kg	1282.3 (1022.38; 1608.39)	2.91 (0.65, 5.17)
SAD 0.5 mL/kg	3383.9 (2657.47; 4309.03)	4.56 (2.24, 6.89)
SAD Elderly 0.5 mL/kg	5694.3 (4773.12; 6793.26)	6.38 (3.74, 9.03)
SAD 0.759 mL/kg	6748.1 (5059.28; 9000.60)	7.21 (4.35, 10.07)
MAD 0.240 mL/kg	1569.2 (1242.00; 1982.62)	3.13 (0.88, 5.39)
MAD 0.5 mL/kg	4528.8 (3706.58; 5533.52)	5.46 (3.01, 7.92)
MAD 0.759 mL/kg	8151.0 (7695.03; 8634.04)	8.32 (5.12, 11.51)
10 ms Threshold	6680	7.16 (4.31, 10.00)

Based on a linear mixed-effects model with ΔQTcF as the dependent variable, time-matched niacinamide plasma concentration as an explanatory variate, centered baseline as an additional covariate, treatment (active = 1 or placebo = 0) and time as fixed effects, and a random intercept and slope per subject.

Table 23. Predicted ΔΔQTcF interval At Geometric Mean Peak Concentration For Thiamine (SAD and MAD) (PK/QTc Analysis Set)

Treatment	Geometric Mean (µg/L) (90% CI) C_{max} of thiamine	ΔΔQTcF Estimate (ms) (90% CI)
SAD 0.024 mL/kg	42.2 (28.26; 63.09)	2.17 (-0.16, 4.49)
SAD 0.076 mL/kg	269.9 (206.38; 353.05)	2.42 (0.12, 4.72)
SAD 0.240 mL/kg	875.0 (745.24; 1027.24)	3.10 (0.83, 5.36)
SAD 0.5 mL/kg	2449.6 (2187.76; 2742.79)	4.85 (2.39, 7.32)
SAD Elderly 0.5 mL/kg	3088.5 (2865.16; 3329.36)	5.57 (2.91, 8.22)
SAD 0.759 mL/kg	3596.5 (2920.21; 4429.46)	6.13 (3.30, 8.97)
MAD 0.240 mL/kg	1132.5 (921.39; 1392.10)	3.38 (1.11, 5.65)
MAD 0.5 mL/kg	2688.3 (2313.14; 3124.24)	5.12 (2.59, 7.65)
MAD 0.759 mL/kg	4676.1 (4081.25; 5357.60)	7.34 (4.04, 10.64)
10 ms Threshold	4270	6.89 (3.77, 10.00)

Based on a linear mixed-effects model with ΔQTcF as the dependent variable, time-matched thiamine plasma concentration as an explanatory variate, centered baseline as an additional covariate, treatment (active = 1 or placebo = 0) and time as fixed effects, and a random intercept and slope per subject.

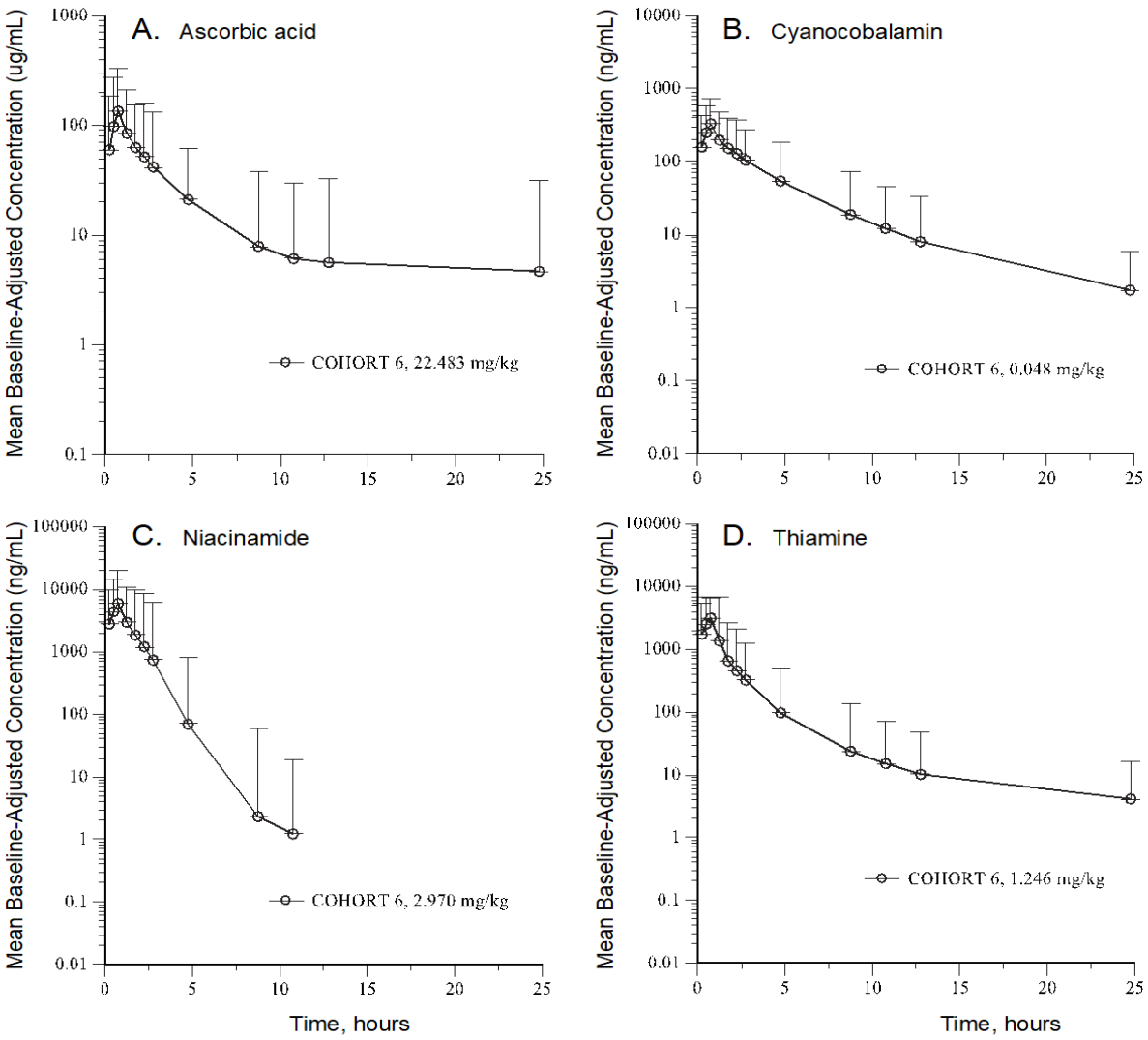

Figure 1. Pharmacokinetics of the Vitamin Components of RJX. Plasma concentration-time profiles of baseline-adjusted ascorbic acid (A), cyanocobalamin (B), niacinamide (C) and thiamine (D) for the RJX dose level of 0.5 mL/kg (Cohort 6) following single-dose IV infusion of RJX are presented on a semi-logarithmic scale.

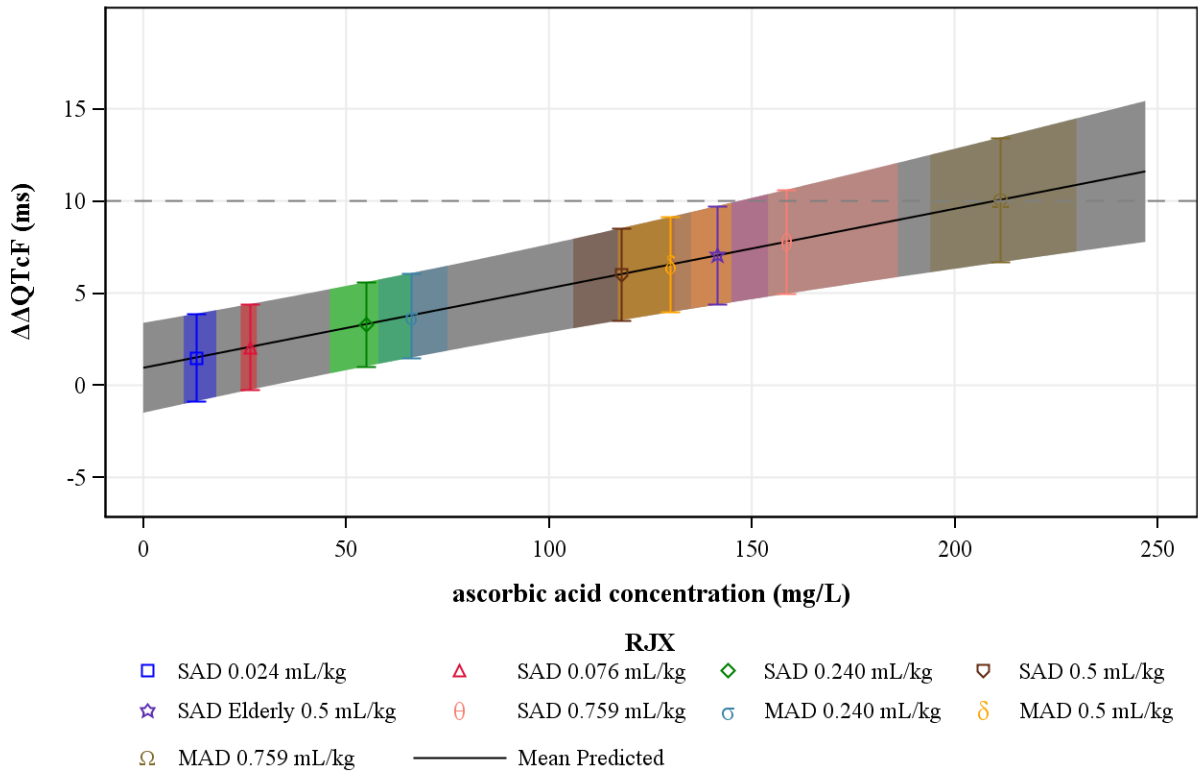

Figure 2. Predicted ΔΔQTcF interval at geometric mean peak concentrations for ascorbic acid (SAD and MAD) (PK/QTc analysis set). The solid black line with gray shaded area denotes the model-predicted mean (90% CI) ΔΔQTcF, which is calculated from the equation ΔΔQTcF = 0.95 + 0.043 × ascorbic acid. The non-gray shaded areas denote the estimated mean (90% CI) ΔΔQTcF with plotted points at the geometric mean (90% CI) C_{max} of ascorbic acid.

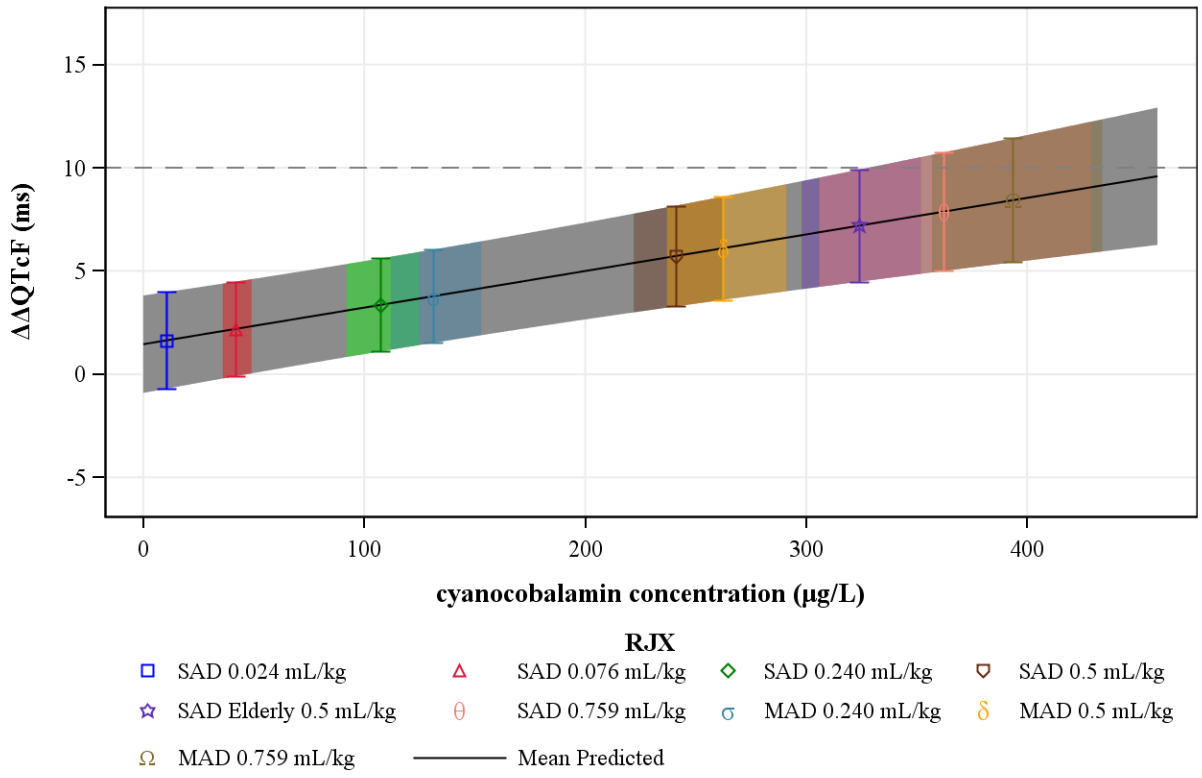

Figure 3. Predicted ΔΔQTcF interval at geometric mean peak concentrations for cyanocobalamin (SAD and MAD) (PK/QTc analysis set). The solid black line with gray shaded area denotes the model-predicted mean (90% CI) ΔΔQTcF, which is calculated from the equation ΔΔQTcF = 1.45 + 0.018 × cyanocobalamin. The non-gray shaded areas denote the estimated mean (90% CI) ΔΔQTcF with plotted points at the geometric mean (90% CI) C_{max} of cyanocobalamin.

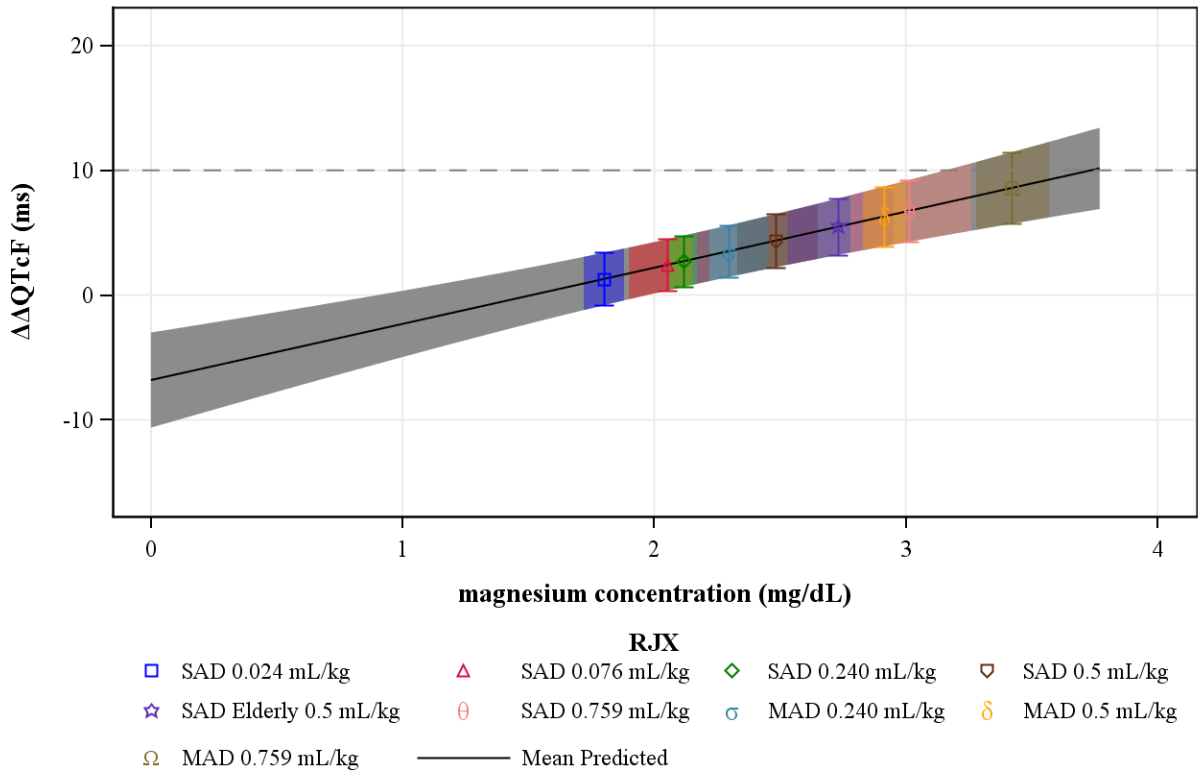

Figure 4. Predicted ΔΔQTcF interval at geometric mean peak concentrations for magnesium (SAD and MAD) (PK/QTc analysis set). The solid black line with gray shaded area denotes the model-predicted mean (90% CI) ΔΔQTcF, which is calculated from the equation ΔΔQTcF = −6.81 + 4.50 × magnesium. The non-gray shaded areas denote the estimated mean (90% CI) ΔΔQTcF with plotted points at the geometric mean (90% CI) C_{max} of magnesium.

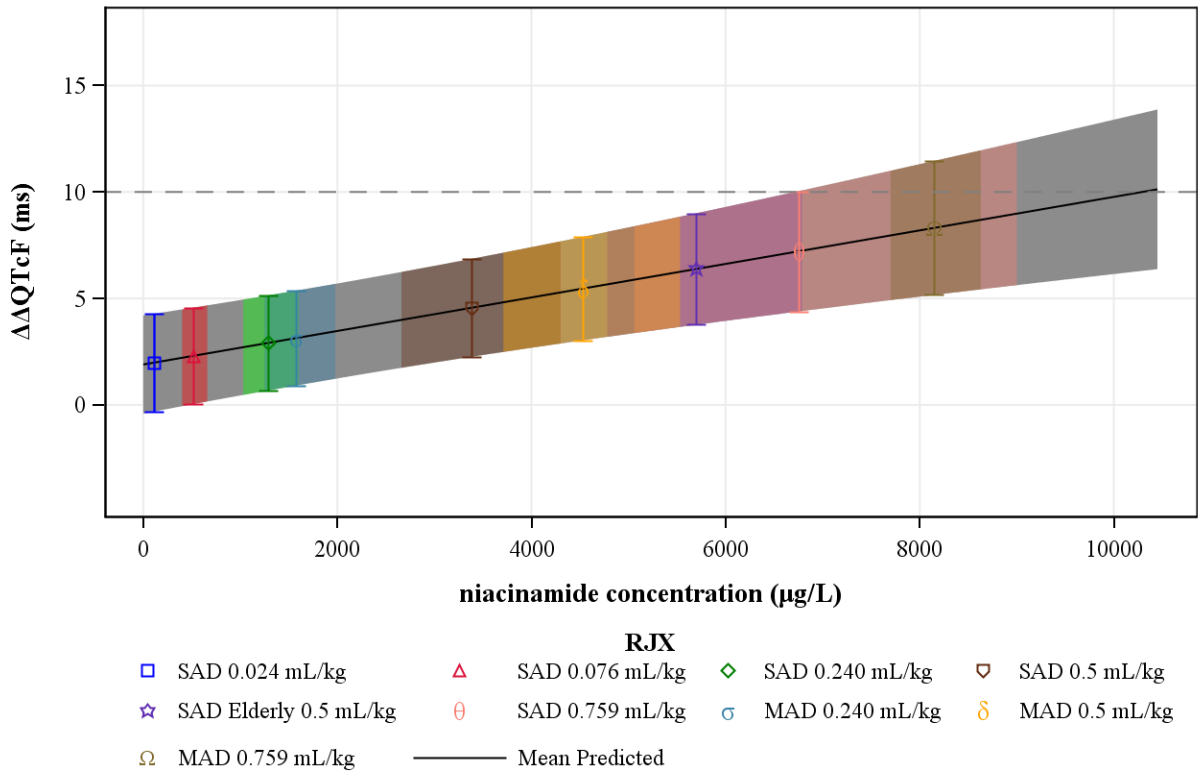

Figure 5. Predicted ΔΔQTcF interval at geometric mean peak concentrations for niacinamide (SAD and MAD) (PK/QTc analysis set). The solid black line with gray shaded area denotes the model-predicted mean (90% CI) ΔΔQTcF, which is calculated from the equation ΔΔQTcF = 1.90 + 0.00079 × niacinamide. The non-gray shaded areas denote the estimated mean (90% CI) ΔΔQTcF with plotted points at the geometric mean (90% CI) C_{max} of niacinamide.

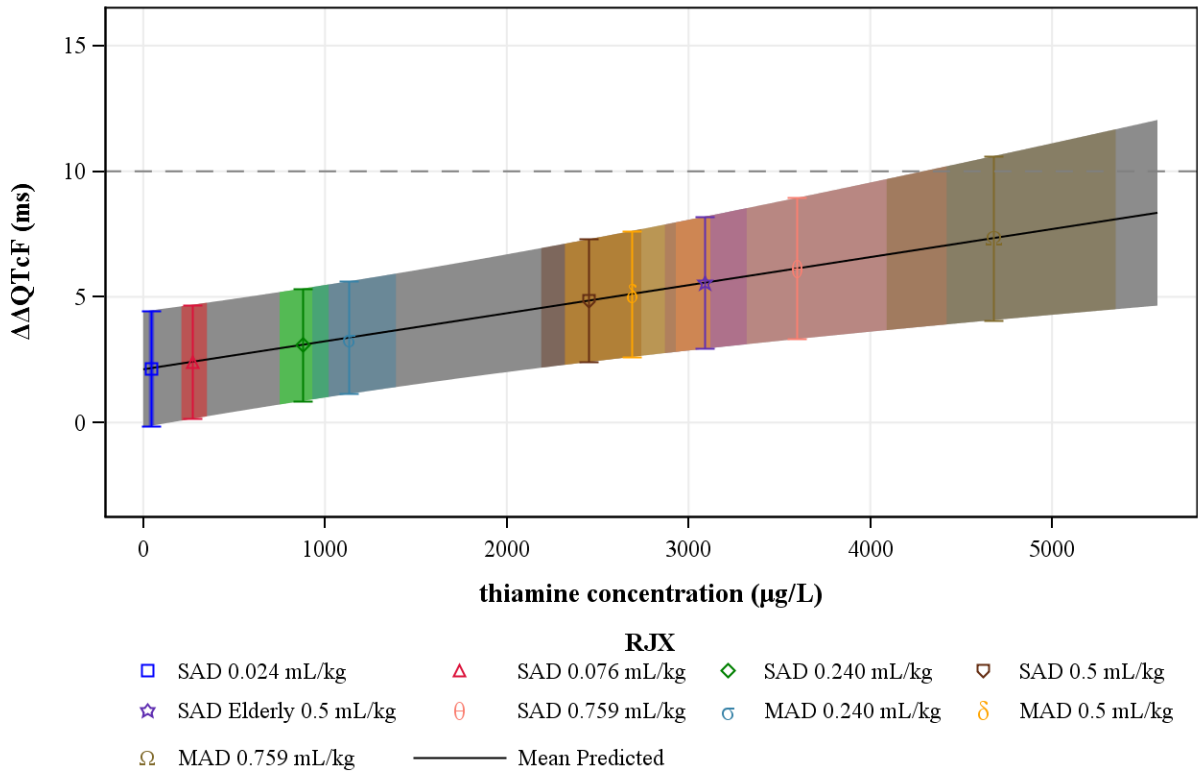

Figure 6. Predicted ΔΔQTcF interval at geometric mean peak concentrations for thiamine (SAD and MAD) (PK/QTc analysis set. The solid black line with gray shaded area denotes the model-predicted mean (90% CI) ΔΔQTcF, which is calculated from the equation ΔΔQTcF = 2.12 + 0.0011 × thiamine. The non-gray shaded areas denote the estimated mean (90% CI) ΔΔQTcF with plotted points at the geometric mean (90% CI) C_{max} of thiamine.

12. SAFETY FINDINGS FROM MARKETING EXPERIENCE

RJX has not been submitted for marketing application approval nor has it been approved for marketing in any country; therefore, no additional safety data is available from marketing experience.

13. OTHER DSURS

The Sponsor has prepared no other DSURs for RJX other than this DSUR, which will be submitted to IND131383 as SN0021, and to IND149585 as SN0004.

14. LACK OF EFFICACY

No data has been received during the reporting period that indicates a lack of efficacy for RJX relative to established therapies.

15. SUMMARY OF U.S. IND SAFETY REPORTS SUBMITTED DURING THE REPORTING PERIOD

No IND Safety Reports were submitted during the reporting period.

16. LIST OF SUBJECTS WHO DIED DURING THE REPORTING PERIOD

No human subject died during the reporting period in any clinical study of RJX.

17. SIGNIFICANT MANUFACTURING CHANGES

There were no significant manufacturing changes during the reporting period.

18. DRUG PRODUCT STABILITY UPDATE

Updated stability data for RJX was provided in a recent CMC Update to Module 3 of IND131383 (SN0019 and SN0020).

Batches of Vial A assessed for stability were stored under frozen conditions (-15°C to -25°C) until tested.

Table 24. Stability Testing Vial A

cGMP Batch #/Certificate of Analysis (COAs)	Retest Date	Test Methods
PPP.18.1031*	June 2020	M-4987 M-4866
PPP.18.1051*		
PPP.18.1053*		
PPP.18.1055*		

*Stability testing was initiated November 2018 and is ongoing.

Batches of Vial B assessed for stability were stored under controlled ambient (20°C to 25°C) conditions until tested.

Table 25. Stability Testing Vial B

cGMP Batch #/Certificate of Analysis (COAs)	Retest Date	Test Methods
PPP.18.994*	June 2020	M-4898
PPP.18.998*		
PPP.18.1011*		
PPP.18.1019*		

*Stability testing initiated November 2018 and is ongoing.

The two-vial system with a frozen 'Vial A' (Table 26, Table 27, Table 28, Table 29), and ambient temperature 'Vial B' (Table 30, Table 31, Table 32, Table 33) has successfully demonstrated stability of all components (± 10% label claim) through the 18-month timepoint with testing ongoing.

Table 26. Vial A Frozen (-15°C to -25°C) Stability Data for Batch PPP.18.1031

Batch: PPP.18.1031			**Stability Interval (Months)**									
Date of Initiation	**November 2018**											
Test	**Specification**		**Initial Release (T=0)**		**1**		**3**		**12**		**18**	
Appearance	Brownish orange/no particulate		conforms		conforms		conforms		conforms		conforms	
pH	2.2 - 3.8		3.0		2.9		3.0		3.0		3.0	
Sterility	USP <71>		NT		NT		NT		Sterile		Sterile	
Endotoxin	USP <85>, NMT 17.5 EU/mL – MVD = 1:3500		NT		NT		NT		<2.5000 EU/mL		<2.5000 EU/mL	
Particulate Matter	USP <788>, The average number of particles present in the units tested does not exceed 6000 per container ≥ 10 µm and does not exceed 600 per container ≥ 25 µm		NT		NT		NT		≥ 10 µm: 55 particles/container; ≥ 25 µm: 0 particles/container		≥ 10 µm: 12 particles/container; ≥ 25 µm: 1 particle/container	
Assay	Lower Limit (% content by weight)	Upper Limit (% content by weight)	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content
Ascorbic Acid	8.094	9.893	8.737	97.2	8.782	97.7	8.934	99.3	8.823	98.1	8.981	99.9
Niacinamide	1.069	1.307	1.179	99.2	1.174	98.8	1.184	99.7	1.186	99.8	1.193	100.4
Pyridoxine HCl	1.069	1.307	1.177	99.1	1.176	99.0	1.188	100.0	1.184	99.7	1.200	101.0
Riboflavin 5' Phosphate	0.023	0.028	0.025	98.8	0.025	98.8	0.026	102.8	0.025	98.8	0.025	98.8
Thiamine HCl	0.570	0.697	0.619	97.7	0.614	97.0	0.612	96.6	0.609	96.2	0.625	98.7
Calcium Pantothenate	0.026	0.032	0.029	99.0	0.029	99.0	0.028	95.6	0.028	95.6	0.028	95.6
Cyanocobalamin	0.017	0.021	0.018	93.3	0.019	98.4	0.020	103.6	0.018	93.3	0.019	98.4
Magnesium Sulfate	7.272	8.888	8.170	101.1	8.111	100.4	8.229	101.8	8.151	100.9	8.151	100.9

NT = not tested at this interval

Table 27. Vial A Frozen (-15°C to -25°C) Stability Data for Batch PPP.18.1051

Batch: PPP.18.1051			**Stability Interval (Months)**									
Date of Initiation	**November 2018**											
Test	**Specification**		**Initial Release (T=0)**		**1**		**3**		**12**		**18**	
Appearance	Brownish orange/no particulate		conforms		conforms		conforms		conforms		conforms	
pH	2.2 - 3.8		3.0		2.9		3.0		3.0		3.0	
Sterility	USP <71>		NT		NT		NT		Sterile		Sterile	
Endotoxin	USP <85>, NMT 17.5 EU/mL – MVD = 1:3500		NT		NT		NT		<2.5000 EU/mL		<2.5000 EU/mL	
Particulate Matter	USP <788>, The average number of particles present in the units tested does not exceed 6000 per container ≥ 10 µm and does not exceed 600 per container ≥ 25 µm		NT		NT		NT		≥ 10 µm: 123 particles/container; ≥ 25 µm: 1 particle/container		≥ 10 µm: 15 particles/container; ≥ 25 µm: 0 particles/container	
Assay	Lower Limit (% content by weight)	Upper Limit (% content by weight)	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content
Ascorbic Acid	8.094%	9.893%	8.792	97.8	8.855	98.5	8.975	99.8	8.868	98.6	9.011	100.2
Niacinamide	1.069%	1.307%	1.170	98.5	1.171	98.6	1.18	99.3	1.177	99.1	1.186	99.8
Pyridoxine HCl	1.069%	1.307%	1.169	98.4	1.172	98.7	1.184	99.7	1.178	99.2	1.191	100.3
Riboflavin 5' Phosphate	0.023%	0.028%	0.025	98.8	0.025	98.8	0.025	98.8	0.024	94.9	0.025	98.8
Thiamine HCl	0.570%	0.697%	0.628	99.2	0.638	100.7	0.638	100.7	0.631	99.6	0.643	101.5
Calcium Pantothenate	0.026%	0.032%	0.029	99.0	0.029	99.0	0.028	95.6	0.028	95.6	0.028	95.6
Cyanocobalamin	0.017%	0.021%	0.019	98.4	0.019	98.4	0.020	103.6	0.018	93.3	0.019	98.4
Magnesium Sulfate	7.272%	8.888%	8.120	100.5	8.159	101.0	8.109	100.4	8.121	100.5	8.176	101.2

NT = not tested at this interval

Table 28. Vial A Frozen (-15°C to -25°C) Stability Data for Batch PPP.18.1053

Batch: PPP.18.1053			**Stability Interval (Months)**									
Date of Initiation	**November 2018**											
Test	**Specification**		**Initial Release (T=0)**		**1**		**3**		**12**		**18**	
Appearance	Brownish orange/no particulate		conforms		conforms		conforms		conforms		conforms	
pH	2.2 - 3.8		3.0		2.9		3.0		3.0		3.0	
Sterility	USP <71>		NT		NT		NT		Sterile		Sterile	
Endotoxin	USP <85>, NMT 17.5 EU/mL – MVD = 1:3500		NT		NT		NT		<2.5000 EU/mL		<2.5000 EU/mL	
Particulate Matter	USP <788>, The average number of particles present in the units tested does not exceed 6000 per container ≥ 10 µm and does not exceed 600 per container ≥ 25 µm		NT		NT		NT		≥ 10 µm: 131 particles/container; ≥ 25 µm: 1 particle/container		≥ 10 µm: 11 particles/container; ≥ 25 µm: 0 particles/container	
Assay	Lower Limit (% content by weight)	Upper Limit (% content by weight)	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content
Ascorbic Acid	8.094	9.893	8.818	98.1	8.833	98.2	8.938	99.4	8.901	99.0	8.989	100.0
Niacinamide	1.069	1.307	1.175	98.9	1.173	98.7	1.179	99.2	1.183	99.6	1.191	100.3
Pyridoxine HCl	1.069	1.307	1.171	98.6	1.168	98.3	1.18	99.3	1.178	99.2	1.195	100.6
Riboflavin 5' Phosphate	0.023	0.028	0.025	98.8	0.025	98.8	0.025	98.8	0.025	98.8	0.025	98.8
Thiamine HCl	0.570	0.697	0.623	98.4	0.620	97.9	0.619	97.7	0.616	97.3	0.629	99.3
Calcium Pantothenate	0.026	0.032	0.029	99.0	0.029	99.0	0.029	99.0	0.028	95.6	0.028	95.6
Cyanocobalamin	0.017	0.021	0.019	98.4	0.019	98.4	0.02	103.6	0.018	93.3	0.018	93.3
Magnesium Sulfate	7.272	8.888	8.086	100.1	8.142	100.8	8.082	100.0	8.115	100.4	8.147	100.8

NT = not tested at this interval

Table 29. Vial A Frozen (-15°C to -25°C) Stability Data for Batch PPP.18.1055

Batch: PPP.18.1055			**Stability Interval (Months)**									
Date of Initiation	**November 2018**											
Test	**Specification**		**Initial Release (T=0)**		**1**		**3**		**12**		**18**	
Appearance	Brownish orange/no particulate		conforms		conforms		conforms		conforms		conforms	
pH	2.2 - 3.8		3.0		2.9		3.0		3.1		3.0	
Sterility	USP <71>		NT		NT		NT		Sterile		Sterile	
Endotoxin	USP <85>, NMT 17.5 EU/mL – MVD = 1:3500		NT		NT		NT		<2.5000 EU/mL		<2.5000 EU/mL	
Particulate Matter	USP <788>, The average number of particles present in the units tested does not exceed 6000 per container ≥ 10 µm and does not exceed 600 per container ≥ 25 µm		NT		NT		NT		≥ 10 µm: 133 particles/container; ≥ 25 µm: 1 particle/container		≥ 10 µm: 17 particles/container; ≥ 25 µm: 1 particle/container	
Assay	Lower Limit (% content by weight)	Upper Limit (% content by weight)	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content
Ascorbic Acid	8.094	9.893	8.788	97.7	8.813	98.0	8.94	99.4	8.876	98.7	8.998	100.1
Niacinamide	1.069	1.307	1.167	98.2	1.170	98.5	1.177	99.1	1.178	99.2	1.184	99.7
Pyridoxine HCl	1.069	1.307	1.165	98.1	1.171	98.6	1.18	99.3	1.177	99.1	1.189	100.1
Riboflavin 5' Phosphate	0.023	0.028	0.025	98.8	0.025	98.8	0.025	98.8	0.025	98.8	0.025	98.8
Thiamine HCl	0.570	0.697	0.619	97.7	0.620	97.9	0.617	97.4	0.616	97.3	0.627	99.0
Calcium Pantothenate	0.026	0.032	0.029	99.0	0.029	99.0	0.028	95.6	0.028	95.6	0.028	95.6
Cyanocobalamin	0.017	0.021	0.019	98.4	0.019	98.4	0.018	93.3	0.018	93.3	0.019	98.4
Magnesium Sulfate	7.272	8.888	8.080	100.0	8.093	100.2	8.127	100.6	8.108	100.3	8.161	101.0

NT = not tested at this interval

Table 30. Vial B Controlled Ambient Temperature (20°C to 25°C) Stability Data for Batch PPP.18.994

Batch: PPP.18.994			**Stability Interval (Months)**									
Date of Initiation	**November 2018**											
Test	**Specification**		**Initial Release (T=0)**		**1**		**3**		**12**		**18**	
Appearance	Clear, colorless, free of particulate		conforms		conforms		conforms		conforms		conforms	
pH	7.0 - 8.6		8.3		8.1		8.3		8.4		8.3	
Sterility	USP <71>		NT		NT		NT		Sterile		Sterile	
Endotoxin	USP <85>, NMT 17.5 EU/mL – MVD = 1:3500		NT		NT		NT		<0.0500 EU/mL		<0.0500 EU/mL	
Particulate Matter	USP <788>, The average number of particles present in the units tested does not exceed 6000 per container ≥ 10 µm and does not exceed 600 per container ≥ 25 µm		NT		NT		NT		≥ 10 µm: 50 particles/container; ≥ 25 µm: 2 particles/container		≥ 10 µm: 17 particles/container; ≥ 25 µm: 4 particles/container	
Assay	Lower Limit (% content by weight)	Upper Limit (% content by weight)	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content
Sodium Bicarbonate	7.560	9.240	8.82	98.1	8.83	98.2	8.81	98.0	8.80	97.9	8.76	97.4

NT = not tested at this interval

Table 31. Vial B Controlled Ambient Temperature (20°C to 25°C) Stability Data for Batch PPP.18.998

Batch: PPP.18.998			**Stability Interval (Months)**									
Date of Initiation	**November 2018**											
Test	**Specification**		**Initial Release (T=0)**		**1**		**3**		**12**		**18**	
Appearance	Clear, colorless, free of particulate		conforms		conforms		conforms		conforms		conforms	
pH	7.0 - 8.6		8.3		8.1		8.3		8.4		8.3	
Sterility	USP <71>		NT		NT		NT		Sterile		Sterile	
Endotoxin	USP <85>, NMT 17.5 EU/mL – MVD = 1:3500		NT		NT		NT		<0.0500 EU/mL		<0.0500 EU/mL	
Particulate Matter	USP <788>, The average number of particles present in the units tested does not exceed 6000 per container ≥ 10 µm and does not exceed 600 per container ≥ 25 µm		NT		NT		NT		≥ 10 µm: 38 particles/container; ≥ 25 µm: 1 particle/container		≥ 10 µm: 10 particles/container; ≥ 25 µm: 3 particles/container	
Assay	Lower Limit (% content by weight)	Upper Limit (% content by weight)	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content
Sodium Bicarbonate	7.560	9.240	8.81	98.0	8.81	98.0	8.79	97.7	8.82	98.1	8.80	97.9

NT = not tested at this interval

Table 32. Vial B Controlled Ambient Temperature (20°C to 25°C) Stability Data for Batch PPP.18.1011

Batch: PPP.18.1011			**Stability Interval (Months)**									
Date of Initiation	**November 2018**											
Test	**Specification**		**Initial Release (T=0)**		**1**		**3**		**12**		**18**	
Appearance	Clear, colorless, free of particulate		conforms		conforms		conforms		conforms		conforms	
pH	7.0 - 8.6		8.3		8.1		8.3		8.4		8.4	
Sterility	USP <71>		NT		NT		NT		Sterile		Sterile	
Endotoxin	USP <85>, NMT 17.5 EU/mL – MVD = 1:3500		NT		NT		NT		<0.0500 EU/mL		<0.0500 EU/mL	
Particulate Matter	USP <788>, The average number of particles present in the units tested does not exceed 6000 per container ≥ 10 µm and does not exceed 600 per container ≥ 25 µm		NT		NT		NT		≥ 10 µm: 25 particles/container; ≥ 25 µm: 0 particles/container		≥ 10 µm: 3 particles/container; ≥ 25 µm: 0 particles/container	
Assay	Lower Limit (% content by weight)	Upper Limit (% content by weight)	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content
Sodium Bicarbonate	7.560	9.240	8.81	98.0	8.80	97.9	8.82	98.1	8.81	98.0	8.83	98.2

NT = not tested at this interval

Table 33. Vial B Controlled Ambient Temperature (20°C to 25°C) Stability Data for Batch PPP.18.1019

Batch: PPP.18.1019			**Stability Interval (Months)**									
Date of Initiation	**November 2018**											
Test	**Specification**		**Initial Release (T=0)**		**1**		**3**		**12**		**18**	
Appearance	Clear, colorless, free of particulate		conforms		conforms		conforms		conforms		conforms	
pH	7.0 - 8.6		8.3		8.1		8.4		8.6		8.3	
Sterility	USP <71>		NT		NT		NT		Sterile		Sterile	
Endotoxin	USP <85>, NMT 17.5 EU/mL – MVD = 1:3500		NT		NT		NT		<0.0500 EU/mL		<0.0500 EU/mL	
Particulate Matter	USP <788>, The average number of particles present in the units tested does not exceed 6000 per container ≥ 10 µm and does not exceed 600 per container ≥ 25 µm		NT		NT		NT		≥ 10 µm: 45 particles/container; ≥ 25 µm: 1 particle/container		≥ 10 µm: 8 particles/container; ≥ 25 µm: 0 particles/container	
Assay	Lower Limit (% content by weight)	Upper Limit (% content by weight)	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content	Measured % content	% of Specified Target Content
Sodium Bicarbonate	7.560	9.240	8.87	98.6	8.87	98.6	8.87	98.6	8.87	98.6	8.85	98.4

NT = not tested at this interval

19. DESCRIPTION OF THE GENERAL INVESTIGATION PLAN FOR THE COMING YEAR

Currently, there are no active clinical trials with RJX under IND131383. The company is continuing discussions to secure a partner or otherwise obtain additional capital prior to initiating additional clinical trials for the CLI indication.

We are planning to start a Phase I/II study, RPI015, under IND149585 aimed at evaluating the safety, tolerability, and efficacy of RJX in COVID-19 patients. The study design is described in the clinical protocol for RI015 that was submitted as part of the original IND submission for IND149585 (SN0003).

20. LOG OF OUTSTANDING BUSINESS WITH RESPECT TO THE IND131383 AND IND149585.

There is currently no outstanding business with respect to the IND131383. The initial IND for IND149585 was submitted on October 6, 2020.

21. LATE-BREAKING INFORMATION

No important safety findings have occurred while the DSUR was in preparation (between the end of the reporting period and the submission of this report).

22. OVERALL SAFETY ASSESSMENT; SUMMARY OF DATA AND GUIDANCE FOR THE INVESTIGATOR

The COVID-19 specific IB document for IND149585 provides detailed information regarding the safety and risk-benefit considerations regarding RJX. The updated IB for IND131383 is in preparation and will provide overall safety assessment, risk-benefit considerations, and guidance regarding the use of RJX in CLI patients:

22.1. Summary of Efficacy of RJX in Clinical Trials

The efficacy of RJX in the prevention or treatment of COVID-19 associated pulmonary inflammation or ARDS has not been evaluated in a clinical trial setting.

22.2. Summary of Safety and Tolerability of RJX in Human Subjects and Reference Safety Information (RSI)

A placebo-controlled, randomized Phase I dose escalation study was performed to evaluate the safety and tolerability of RJX in healthy volunteer subjects (Protocol No. RPI003; ClinicalTrials.gov Identifier: NCT03680105). In the second part of the study, participants were treated with RJX or placebo every day for 7 days using the same administration schedule as proposed in the Phase I/II study in COVID-19 patients. The highest dose that was used in the study was 0.759 mL/kg which was higher than the proposed 0.5 mL/kg dose for the COVID-19 study which was also evaluated as one of the 3 dose levels in the NCT03680105 study.

Overall, RJX was well tolerated:

- No participant discontinued early from the study or from dosing, and safety and tolerability were demonstrated in healthy participants through escalating single and multiple IV infusions at all dose levels of RJX.

- No deaths or other SAEs were reported and no AEs led to discontinuation of study or IP. There were no severe AEs.

- The infusion-related AEs of interest did not require additional treatment. They were not SAEs and did not cause participants to discontinue the study or dosing. All of the infusion-related AEs recovered/resolved with no sequelae.

- Single and multiple doses of RJX did not result in any clinically significant changes in the levels of bicarbonate, nor result in any clinically significant changes in the blood pH or the urinary pH environments in healthy participants, from a safety perspective.

- There were no 12-lead safety ECG abnormal clinically significant findings and no notable changes compared with baseline.

- The only neurological and physical examination finding that was considered clinically significant in 1 participant treated with 0.500 mL/kg RJX with post-dose 4+ hyperreflexia with sustained ankle clonus [TEAE preferred term of clonus, verbatim term ankle clonus]), was observed on Day 12 (follow-up) with no other associated symptoms. This was a mild TEAE considered unlikely related to RJX.

- There were no clinically meaningful changes identified in observed laboratory values or changes from baseline when compared with placebo or evaluated by increasing dose level. Individual post-dose clinically significant laboratory values were considered unlikely related to placebo or RJX.

- RJX at the studied doses caused mild QTc prolongation on the ECGs and an effect exceeding 10 msec can be excluded at plasma concentrations of ascorbic acid, cyanocobalamin, magnesium, niacinamide, and thiamine up to 166 mg/L, 362 µg/L, 4.0 mg/dL, 7570 µg/L, and 4890 µg/L, respectively. Such plasma concentrations were only observed at the 0.759 mL/kg dosing condition. As such concentrations were not observed at the dose levels that will be used in our COVID-19 Phase I/II trial, we do not anticipate any clinically meaningful effect on the QTc of the COVID-19 patients. However, risk mitigation measures will be used to monitor QTc prolongation. RJX did not have any clinically relevant effects regarding safety on any other ECG parameter, i.e., heart rate, PR and QRS intervals.

- The safety of RJX has not been evaluated in COVID-19 patients. The proposed study will be the first evaluation of RJX in COVID-19 patients. COVID-19 patients may be more vulnerable to potential side effects of RJX, including the transient GI and neurotoxicities observed in non-clinical studies. Therefore, it is prudent that risk mitigation measures aimed at maximizing patient safety be strictly followed.

Reference Safety Information (RSI)

There were no related SAEs observed in previous clinical trials with RJX.

There are no expected serious adverse reactions (SARs) to RJX.

22.3. Proposed Indication

The proposed indication is for the treatment of COVID-19 patients at high risk for ARDS and multiorgan failure. Such patients include COVID-19 patients without hypoxemia who have the following high risk characteristics:

- Age ≥65 years AND type 2 diabetes, or
- Abnormal blood tests with C-reactive protein (CRP) >50 mg/L PLUS at least 1 of the following biomarkers: (a) D-dimer >1,000 ng/mL, (b) Ferritin >500 mcg/L, (c) High sensitivity cardiac troponin >2 x upper limit of normal (ULN), (c) lactate dehydrogenase (LDH) >245 U/L.

Additional target patients also include COVID-19 patients with hypoxemia without ARDS who are receiving either non-invasive positive pressure ventilation (NIPPV) or high-flow oxygen.

22.4. Contraindications

There are no known contraindications for RJX based on nonclinical data generated in nonclinical trials or clinical data obtained in a Phase I study in healthy volunteers. Patients with known allergies to any of the components of RJX will be excluded from the proposed COVID-19 study. Likewise, several exclusion criteria will limit patient eligibility in order to maximize patient safety.

22.5. General Precautions and Risk/Benefit Analysis

Potential risks associated with RJX. In the nonclinical studies, RJX toxicities involved the GI and CNS systems in rats and dogs. While RJX was very well tolerated in healthy subjects, COVID-19 patients may be at higher risk to experience GI and nervous system toxicity due to disease-related complications and organ dysfunction affecting these organ systems. For these reasons, specific monitoring guidelines have been specified in the protocol to mitigate the adverse effect profile.

There will be a Data Safety Monitoring Board (DSMB) with scheduled meetings to provide safety oversight of the patients, review dose-limiting toxicities (DLTs), and determine the appropriateness of the initiation of the Phase II portion of the study. The DSMB will meet after all patients in each cohort of the Lead-in Phase are at least 14 days post their first dose of RJX. The DSMB will also conduct unscheduled meetings on an as needed basis to review the results of routine signal detection efforts, unexpected toxicities, and/or other information that may be relevant to the conduct of this study or safety of the patients. DSMB will review safety data from all patients enrolled into Part 1, and then provide recommendations to open Part 2 of each cohort at the end of the Part 1 data review meeting. For Part 2, DSMB will perform scheduled data reviews and safety evaluations will be performed according to its charter as well as periodically as it deems necessary.

In the Phase I study performed in healthy volunteers, RJX treatment at a higher dose level than the dose level proposed for the COVID-19 trial was associated with mild QTc prolongation. RJX did not have any clinically relevant effects regarding safety on any other ECG parameter, i.e., heart rate,

PR and QRS intervals. Nevertheless, risk mitigation measures in the COVID-19 protocol include ECGs and monitoring measures for QTc prolongation as well as arrhythmias to maximize patient safety.

The cardiodynamic evaluation performed by ERT is presented in the ERT cardiodynamics report. RJX at the studied doses caused mild QTc prolongation and an effect exceeding 10 ms can be excluded at plasma concentrations of ascorbic acid, cyanocobalamin, magnesium, niacinamide, and thiamine up to 166 mg/L, 362 µg/L, 4.0 mg/dL, 7570 µg/L, and 4890 µg/L, respectively. Such plasma concentrations were only observed at the 0.759 mL/kg dosing condition. Thus, an effect on the QTc interval > 10 ms cannot be excluded at this dose level. As such concentrations were not observed at the 0.5 mL/kg dose level that will be used in our COVID-19 Phase I trial, we do not anticipate any clinically meaningful effect on the QTc of COVID-19 patients. That being said, risk mitigation measures will be used to monitor QTc prolongation. RJX did not have any clinically relevant effects regarding safety on any other ECG parameter, i.e., heart rate, PR and QRS intervals.

Torsades de pointes (TdP) is a form of polymorphic ventricular tachycardia (VT) that occurs in the setting of acquired or congenital QT interval prolongation. TdP is usually short-lived and terminates spontaneously. However, most patients experience multiple episodes of the arrhythmia, and episodes can recur in rapid succession, potentially degenerating to ventricular fibrillation and sudden cardiac death. Acquired long QT syndrome (LQTS) is commonly associated with bradycardia and pauses, in contrast to some forms of congenital LQTS in which arrhythmias often follow a sudden adrenergic surge. Drugs that prolong the QT interval include antiarrhythmic drugs (classes IA and III), macrolide antibiotics, and certain psychotropic medications.

Risk factors for drug-induced TdP include high concentrations, concurrent use of other drugs that can prolong the QT interval or that slow drug metabolism due to inhibition of cytochrome P450 enzymes, concurrent intake of grapefruit juice, baseline QT prolongation or T wave lability, development of marked QT prolongation or T wave changes during therapy, bradycardia, electrolyte disturbances (particularly hypokalemia and hypomagnesemia), impaired hepatic and/or renal function, underlying heart disease, recent conversion from atrial fibrillation, and female sex. Exclusion criteria take these facts into consideration and patients with QTc prolongation at screening/baseline or on medications that cause marked QT prolongations will not be eligible for the proposed study. Further, drug withholding and discontinuation criteria have been established to stop or discontinue RJX if a given patient develops QTc prolongation while on study or develops RJX related bradycardia or arrythmias.

There are some risks associated with the components of RJX:

Thiamine and **cyanocobalamin** are well characterized and known vitamins. There are no known overdose limits for cyanocobalamin. For thiamine, average parenteral doses of 100 mg to 500 mg singly have been administered without toxic effects. No syndrome of excess thiamine has been identified. It is believed that toxic levels are unlikely because the kidneys can rapidly clear almost all excess thiamine and because (like most water-soluble vitamins) thiamine is not stored. However, dosages exceeding 30 mg three (3) times a day are not utilized effectively. When the body tissues are saturated with thiamine, it is excreted in the urine as pyrimidine. As the intake of thiamine is further increased, it appears unchanged in the urine.

- RJX contains 6.333 mg/mL of thiamine and 0.193 mg/mL of cyanocobalamin. While there are no known overdose limits for cyanocobalamin, the highest dose exposure anticipated for both thiamine and cyanocobalamin in the COVID-19 study will be well within the range of parenteral dose levels that have been previously administered safely resulting in no toxic effects.

- A small percentage of the population has, or can develop, a sensitivity to thiamine or cyanocobalamin. In certain circumstances, this can lead to anaphylaxis. If a study subject has a history of sensitivity or allergy to any compound in RJX, or if there is concern that a study subject is experiencing a hypersensitivity to thiamine and/or cyanocobalamin specifically, then that subject will either not be enrolled or if enrolled removed from the study.

Pyridoxine overdose causes a sensory neuropathy characterized by poor coordination, numbness, and decreased sensation to touch, temperature, and vibration. Symptoms include paraesthesia, hyperaesthesia, bone pains, muscle weakness, numbness and fasciculation. Therefore, clinical site staff will be advised to watch for signs of increased sensitivity or pain.

Ascorbic acid toxicity is associated with diarrhea, nausea, vomiting, heartburn, abdominal cramps, headache, and insomnia.

No adverse effects have been reported after ingestion of high doses of **riboflavin**. However, this may be because excessive amounts of riboflavin are usually not absorbed due to the limited water-solubility and the inability of the human gastrointestinal tract to absorb toxic doses of the compound. The lethal dose (LD)50 of riboflavin in rats following intraperitoneal administration is 560 mg per kg. Death occurs within 2 to 5 days with signs of anuria and azotemia and is due to obstruction of the kidney by concretions.

Niacinamide can render allergies more severe because it may contribute to histamine release. Niacinamide may also make gallbladder disease worse. Large amounts of niacinamide could trigger gout. Taking niacinamide may increase the risk of low blood-platelet levels in persons with kidney failure who are on dialysis. Niacinamide can also increase liver damage. Niacinamide can also make ulcers worse. Niacinamide might interfere with blood sugar control during and after surgery. Therefore, patients with scheduled elective surgical interventions should not receive RJX at least 14 days before their scheduled surgery.

The approved human prescription drug label for 50% **magnesium sulfate** (as magnesium sulfate heptahydrate) injection USP provides the following information regarding the dosage to be employed: The rate of IV injection should generally not exceed 150 mg/minute. Solutions for IV infusion must be diluted. The diluents commonly used are 5% Dextrose Injection, USP and 0.9% Sodium Chloride Injection, USP. A 20 mL volume of RJX drug therapy provides **magnesium sulfate heptahydrate** at a dose of 808 mg per treatment. In the nonclinical safety pharmacology and toxicology studies, animals in the high dose group received 5.0 mL/kg. This is equivalent to 5.18 mg/kg/minute of magnesium sulfate heptahydrate administered as an IA bolus injection under 5 minutes. In the SAD portion of the Phase I clinical study, participants received from 0.45 mg/kg/minute to 1.50 mg/kg/minute of magnesium sulfate heptahydrate diluted in 100 mL 0.9% saline administered intravenously. As such, human subjects received 8% the level of magnesium sulfate heptahydrate that the rats and dogs received on a mg/kg/minute basis per treatment. In the proposed COVID-19 study, patients will receive ~5.3% the amount of magnesium sulfate heptahydrate that the rats and

dogs received on a mg/kg/minute basis per treatment and ~75% of the dose given to healthy volunteer subjects in the completed Phase I study.

The potential for magnesium to affect QT is broadly recognized since extracellular Mg^{2+} (and Ca^{2+}) can block the hERG channel in a voltage- and time-dependent manner, to disrupt the efflux of K+ at the ion channel gate. Excessive magnesium presentation therefore creates potential for hERG disturbances.

Table 34. RJX Administration Rate is Below Recommended Infusion Rate for Products Containing Magnesium Sulfate Heptahydrate

	Dosed In 30 min (Nominal – 10 min)	**Dosed in 40 min (Nominal)**	**Dosed In 50 min (Nominal + 10 min)**
Magnesium Sulfate Heptahydrate per 20 mL dose of RJX	808 mg	808 mg	808 mg
Rate of magnesium sulfate heptahydrate infusion	26.9 mg/min	20.2 mg/min	16.2 mg/min
Magnesium sulfate administration not to exceed 150 mg/minute[1]	18% of recommended rate	13.5% of recommended rate	11% of recommended rate

[1]As per approved package insert for 50% Magnesium Sulfate Heptahydrate Injection.

22.6. Potential Benefits

Several of the API components of RJX, especially niacinamide, ascorbic acid, thiamine, pyridoxine, and magnesium sulfate have exhibited promising activity in clinical studies involving sepsis/septic shock and ARDS patients and/or non-clinical studies in animal models of sepsis/septic shock and ARDS. The published data from these clinical and non-clinical studies, provided the medical-scientific rationale for our proposed clinical Phase I/II study in COVID-19 patients with hypoxemic respiratory failure.

Older age is one of the most important risk factors in COVID-19. Notably, patients ≥65 years of age had a ~3.3-fold increased risk of developing ARDS than patients <65 years of age. Importantly, once patients with ≥65 years of age develop ARDS, their risk of death is 6.2-fold higher than the risk of younger patients. Notably, older COVID-19 patients with mild or moderate disease have a 3.6-fold higher risk of progression to ARDS. Thus, older patient with high-risk COVID-19 are in urgent need for treatment platforms capable of preventing the disease progression and/or reducing the case mortality rate in the high-risk COVID-19 patients by stopping or reversing the inflammatory process that causes ARDS and multi-organ failure after development of the pulmonary inflammation. The associated side effects of available treatment platforms for treatment of ARDS and CRS, such as the antibodies to the IL-6 receptor, GM-CSF, VEGF, and complement component C5 require extensive risk mitigation measures to avoid potentially life-threatening complications and maximize safety in older high-risk COVID-19 patients, such as those receiving chemotherapy for advanced cancer. Therefore, the favorable safety and tolerability profile of RJX as observed in healthy volunteer subjects, if confirmed for COVID-19 patents as well, could be particularly attractive for its use in older patients with high-risk COVID-19.

TGF-β genes are up-regulated in COVID-19 and TGF-β is a key molecule, along with IL-6 and TNFα in the pathogenesis of severe COVID-19. TGF-β has been implicated as an important pro-

inflammatory cytokine in the pathophysiology of acute lung injury and ARDS. The upregulation of TGF-β in ARDS causes an ENaC trafficking defect with marked reduction in the cell-surface abundance of ENaC on lung epithelial cells, thereby rapidly and substantially impairing the alveolar fluid reabsorption in ARDS patients and contributing to the persistence of their pulmonary edema. Notably, a significant negative correlation existed between TGF-β levels in bronchoalveolar lavage fluid (BAL) samples from ARDS patients and ventilator-free days and ICU-free days. Furthermore, lower TGF-β levels correlated with better survival outcome indicating that patients with higher TGF-β levels may have a higher and faster case mortality. TGF-β also stimulates platelet activation and formation of thrombi and may contribute to the coagulopathy in COVID-19 associated ARDS. Pulmonary micro-thrombi has been a consistent finding in COVID-19 autopsies.

Notably, our results demonstrate that RJX treatments result in a marked reduction of not only IL-6 and TNF-α, but also in TGF-β levels in the lungs as well as liver of mice challenged with LPS-GalN in a dose-dependent fashion. We postulate that the RJX-mediated suppression of TGF-β expression in the lungs will accelerate the resolution of pulmonary inflammation of COVID-19 patients by reducing the contributions of TGF-β to ARDS and its complications. TGF-β is also involved in the pathogenesis of lung tissue remodeling and lung fibrosis that follows ARDS. Specifically, TGF-β contributes to the development of lung fibrosis by stimulating the proliferation/differentiation of lung fibroblasts, accumulation of collagen and other extracellular matrix proteins in the pulmonary interstitial and alveolar space, leading to the occurrence and development of pulmonary fibrosis. Therefore, inhibition of the TGF-β signaling pathway by RJX also has the potential to prevent development of pulmonary fibrosis following ARDS and improve the pulmonary healing process.

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. It is the main signature cytokine implicated in COVID-19 associated CRS and ARDS. Anti-IL6 receptor antibodies tocilizumab (ClinicalTrials.gov Identifiers: NCT04317092, NCT04306705, NCT04310228) and sarilumab (ClinicalTrials.gov Identifier: NCT04315298) are currently being evaluated for their efficacy in COVID-19 patients in both open-label as well as randomized clinical trials. TNF-α is another proinflammatory cytokine implicated in the pathophysiology of CRS and ARDS in COVID-19.

RJX treatments appeared to have a statistically significant and dose-dependent lowering effect on the levels of IL-6 as well as TNF-α in the blood, lung tissue and liver. The observed robust effect of RJX on serum and tissue cytokine levels significantly contributed to its ability to reduce acute lung injury and pulmonary inflammation, while preventing the development of pulmonary edema. It also contributed to its ability to reduce the liver damage that was accompanied by destruction of the liver parenchyma, severe hepatic inflammation, massive hepatic necrosis, and hepatic dysfunction in LPS-GalN challenged mice. These effects ultimately translated into improved survival outcomes of RJX-treated mice. 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.

Hydroxyl radicals are produced from superoxide anion radicals and/or hydrogen peroxide. Both superoxide radicals and hydroxyl radicals can individually act on lipid membranes to promote the formation of lipid radicals which in the presence of oxygen are capable of generating lipid peroxy

radicals that can cause damaging lipid peroxidation of cell membranes. Lipid peroxides contribute to liver injury by increasing production of TNF-α, increasing the influx of inflammatory cells and neutrophils, impairing DNA and protein synthesis and depleting protective cellular antioxidants such as glutathione.

Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) are three pivotal anti-oxidant defense enzymes and their levels are altered by the level of oxidative stress. SOD converts superoxide anion radicals into hydrogen peroxide and oxygen, whereas CAT catalyzes the production of water and oxygen from hydrogen peroxide. CAT is absent in the mitochondria, hence the reduction of H_2O_2 to water the reduction of lipid peroxides to their corresponding alcohols is carried out by GSH-Px. GSH-Px plays a more crucial role of inhibiting lipid peroxidation process, and therefore protects cells from oxidative stress.

In agreement with the established role of ROS generated via the mitochondrial energy production pathway in LPS-GalN induced lung and liver injury, the lung/liver MDA levels, owing to increased lipid peroxidation, were markedly elevated and the levels of the anti-oxidant enzymes SOD, CAT, GSH-Px, as well as ascorbic acid were reduced in LPS-GalN treated mice, consistent with severe oxidative stress. RJX suppressed the levels of pro-inflammatory cytokines in the lung/liver, decreased the lung/liver MDA levels and normalized in a dose-dependent manner the reduced levels of the anti-oxidant enzymes SOD, CAT, GSH-Px and ascorbic acid.

These results establish RJX as a potent anti-oxidant with strong anti-inflammatory activity, and / or as an agent reducing production of oxidative species from metabolic processes. RJX could therefore favorably affect the patient journey in COVID-19 at multiple stages, including (i) prevention of progression of mild disease, (ii) faster resolution of ARDS, prevention of multi-organ failure, and reduction of case mortality during respiratory failure, and (iii) improved long-term healing after recovery.

It is our central working hypothesis that RJX will prevent the progression of disease from mild to moderate or severe in hospitalized COVID-19 patients. We further postulate that RJX may shorten the time to resolution of the hypoxemic respiratory failure and reduce the case mortality rate when used in combination with standard of care in COVID-19 by eliminating the contributions of IL-6, TNF-α and TGF-β to CRS and ARDS, thereby reducing acute lung injury and lung inflammation in COVID-19 patients with imminent or early-stage ARDS.

22.7. Risk Mitigation Measures to Maximize Patient Safety

The combined datasets from non-clinical studies, especially the toxicology and safety pharmacology studies in dogs, taken together with the data from recent clinical studies with the API components of RJX as well as the Phase I study of RJX in healthy volunteers, have informed the development of the proposed clinical study design in Phase I/II study RPI015 for COVID-19 patients. The findings from nonclinical studies with RJX, with the exception of transient neurotoxicity, aligned well with the expected safety profile based on previous studies with ascobic acid and other vitamins that are API components.

The dose level for the Safety Lead-in Phase (Part 1 for both cohorts) of RPI015 represents a dose level that was well tolerated in rats and dogs as well as healthy volunteer human subjects. Well-

defined rules for withholding, withdrawal, and discontinuation of study drug will be followed to maximize patient safety.

There will be a Data Safety Monitoring Board (DSMB) serving as a safety review committee (SRC) with scheduled meetings among the Sponsor and participating sites to provide safety oversight of the patients, determine dose-limiting toxicities (DLTs), and to guide de-escalation and determine the appropriateness of the initiation of the Phase II portion of the study. The DSMB/SRC will meet after all patients in the Lead-in Phase have had a study visit at least 14 days after receiving their first dose of RJX. The DSMB/SRC will also conduct unscheduled meetings on an as needed basis to review the results of routine signal detection efforts, unexpected toxicities, and/or other information that may be relevant to the conduct of this study or safety of the patients. The DSMB/SRC will determine whether to proceed with the Phase II portion of the study based on DLT frequency other safety information.

22.8. Drug interactions

No dedicated studies to evaluate potential drug interactions have been conducted with RJX. There are no known drug contraindications to RJX or its components. Reven will continue to monitor for contraindications in future clinical studies.

22.9. Carcinogenicity, Reproductive/Developmental Toxicity and Impairment of Fertility

The components of RJX are well characterized and are included in human daily diet recommendations. There are no genotoxicity risks known to be associated with these components. A preliminary GLP reverse mutation assay (i.e., Ames Assay) was conducted in *Salmonella typhimurium* (*S. typhimurium*) and *Escherichia coli* (*E. coli*). Study 17-00178-G1 investigated the potential of RJX to induce reverse mutations in histidine and tryptophan genes in *S. typhimurium* and *E. coli*. The results showed that RJX exposure was not associated with mutagenicity or any toxicity. Repeat testing is pending.

In vivo clastogenicity or carcinogenicity studies have not been performed with RJX. However, based on the nonmutagenic results from an *in vitro* reverse mutation assay, and lack of genotoxicity observed for these well characterized components, no carcinogenicity is anticipated.

Formal reproductive and/or developmental toxicity studies have not been performed on RJX. However, given that RJX is composed of known and well characterized components, no reproductive risks are anticipated. That being said, clinical evaluation of RJX will exclude women who are pregnant or breastfeeding and stipulate the use of birth control during the study for both men and woman to prevent pregnancy during the study.

22.10. Pregnancy

Since reproductive and developmental toxicity studies have not yet been performed, the potential effects of RJX on human fertility and embryonic development are unknown. In the absence of the reproductive and developmental toxicity package for RJX, male and female subjects of childbearing potential who are enrolled into clinical studies must take adequate contraceptive measures as outlined

in the study protocols. Pregnant or breast feeding women will not be eligible to participate in clinical trials of RJX until more information is available.

22.11. Nursing Mothers

No studies have been conducted to evaluate the potential of RJX to be excreted in human milk.

22.12. Overdose

There is currently no information available regarding potential overdosing of RJX.

23. APPENDIX

23.1. Peer-Reviewed Publications

1. Uckun FM, Ervin J, Jim Ervin, et al. Clinical Impact Potential Of Rejuveinix (RJX) For Prevention Of Fatal Acute Respiratory Distress Syndrome (ARDS) And Multi-Organ Failure In Covid-19 Patients. Clinical Investigation (London). 2020;10(2):177-184.
2. Cabala K, Earabino J, Pacult P, Uckun FM. Rationale For A Randomized, Placebo-Controlled, Phase 2 Study Of Rejuveinix (RJX) in COVID-19 Patients With Acute Lung Injury And Hypoxemic Respiratory Failure. *Clinical Investigation (London)* 2020;10(2): 185-189.
3. Uckun FM, Carlson J, Orhan C, et al. Rejuveinix shows a favorable clinical safety profile in human subjects and exhibit potent preclinical protective activity in the LPS-GalN mouse model of acute respiratory distress syndrome (ARDS). Front. Pharmacol. 2020; | doi: 10.3389/fphar.2020.594321.

Review Article

CLINICAL INVESTIGATION

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

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

COVID-19 and ARDS

Approximately 1/3rd to 1/5th 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 MODS 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-α), Transforming Growth Factor-β (TGF-β) 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-β in the immunopathology of ARDS, Uckun and Trieu have recently proposed the use of TGF-β 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 and 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 β-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]. β-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^+), 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^+ 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 (ClinicalTrial.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-α 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-α and IL-1β 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 herein above, 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
API (designated by numbers) Contained in Vial A		
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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Mini Review

CLINICAL INVESTIGATION

Rationale for a randomized, placebo-controlled, Phase 2 study of Rejuveinix (RJX) in COVID-19 patients with acute lung injury and hypoxemic respiratory failure

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Abstract

New drug products that can effectively improve the ability of supportive care to reduce the risk of ARDS or its mortality rate in COVID-19 patients are urgently needed. Rejuveinix (RJX) is a formulation of physiologically compatible active ingredients, including several vitamins, such as ascorbic acid, cyanocobalamin, thiamine hydrochloride, riboflavin 5′ phosphate, niacinamide, pyridoxine hydrochloride, calcium d-pantothenate that exhibited promising anti-ARDS activity in animal models of sepsis and ARDS as well as clinical studies in patients with sepsis, septic shock, and ARDS. RJX could favorably affect the patient journey in COVID-19 at multiple stages, including (i) prevention of progression of mild disease, (ii) faster resolution of ARDS, prevention of multi-organ failure, and reduction of case mortality during respiratory failure, and (iii) improved long-term healing after recovery. Here we are reporting the critical design elements for a two-cohort, two-part placebo-controlled, and double-blind Phase II study of RJX in COVID-19 patients.

Keywords: COVID-19 • SARS-CoV-2 • acute respiratory distress syndrome • Rejuveinix

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Introduction

COVID-19 patients are at risk of developing Acute Respiratory Distress Syndrome (ARDS) with a high fatality rate especially if they are older and have comorbidities [1-6]. Proinflammatory cytokines, especially Interleukin-6 (IL-6), contribute to the development, progression, and severity of ARDS as well as its complications [2,6]. Immunocompromised COVID-19 patients such as patients with cancer, 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 multi-organ failure as a result of severe viral sepsis caused by SARS-CoV-2. Despite the implementation of the best available supportive care measures, the fatality rate for COVID-19 associated ARDS remains high [7]. Therefore, new drug products that can effectively improve the ability of supportive care to reduce the risk of ARDS or its mortality rate in COVID-19 patients are urgently needed.

Rejuveinix (RJX) [8] is a formulation of several vitamins, including ascorbic acid, cyanocobalamin, thiamine hydrochloride, riboflavin 5’ phosphate, niacinamide, pyridoxine hydrochloride, calcium d-pantothenate that exhibited promising anti-ARDS activity in animal models of sepsis and ARDS as well as clinical studies in patients with sepsis, septic shock and ARDS [9]. Notably, RJX exhibited potent and dose-dependent activity in the LPS-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. RJX could favorably affect the patient journey in COVID-19 at multiple stages, including (i) prevention of progression of mild disease, (ii) faster resolution of ARDS, prevention of multi-organ failure, and reduction of case mortality during respiratory failure, and (iii) improved long-term healing after recovery.

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). It is our central working hypothesis that RJX will prevent the progression of the disease from mild to moderate or severe in hospitalized COVID-19 patients. We further postulate that RJX may shorten the time to resolution of the hypoxemic respiratory failure and reduce the case mortality rate when used in combination with standard of care in COVID-19 by eliminating the contribution of Interleukin-6 (IL-6) and Tumor Necrosis Factor-Alpha (TNF-α) to Cytokine Release Syndrome (CRS) and Acute Respiratory Distress Syndrome (ARDS) and thereby reducing acute lung injury and lung inflammation in COVID-19 patients with imminent or early-stage ARDS. These observations and considerations have provided the medical-scientific rationale for our clinical development strategy for RJX and a clinical study in COVID-19 patients.

Clinical Development Strategy for COVID-19 Patients

Based on the clinical safety profile and PK of RJX in healthy volunteers and the aforementioned medical-scientific rationale, we have designed our study in COVID-19 patients as a two-cohort, two-part study, placebo-controlled, double-blind Phase II study. The two cohorts in this study will be comprised of hospitalized COVID-19 patients allocated to one of the two cohorts based on their stage of disease according to an 8-point ordinal scale. Cohort 1 in the study will be hospitalized COVID-19 patients without hypoxemia who are either not receiving any oxygen therapy or are receiving supplemental oxygen via mask or nasal prongs (namely, clinical status score 4 or 5 on an 8-point ordinal scale). Cohort 1 patients will be required to have the following high risk characteristics: (i) Age ≥ 65 years AND type 2 diabetes, or (ii) abnormal blood tests with CRP >50 mg/L PLUS at least 1 of the following biomarkers: (a) D-dimer >1,000 ng/mL, (b) Ferritin >500 mcg/L, (c) High sensitivity cardiac troponin >2 × Upper Limit of Normal (ULN), (c) Lactate Dehydrogenase (LDH) >245 U/L. By comparison, Cohort 2 will be comprised of hospitalized COVID-19 patients with hypoxemia without ARDS who are receiving either NIPPV or high flow oxygen (namely, clinical status score 3 on 8-point ordinal scale).

For each cohort, there will be an open-label Safety Lead-in (Part 1) Figure 1 and a placebo-controlled, randomized; double-blind portion (Part 2) Figure 2. In Part 1 and the active treatment arm of Part 2 for both cohorts, RJX will be administered as a 40 ± 10 min IV infusion daily for seven days.

The primary objective in Part 1 of this study is to

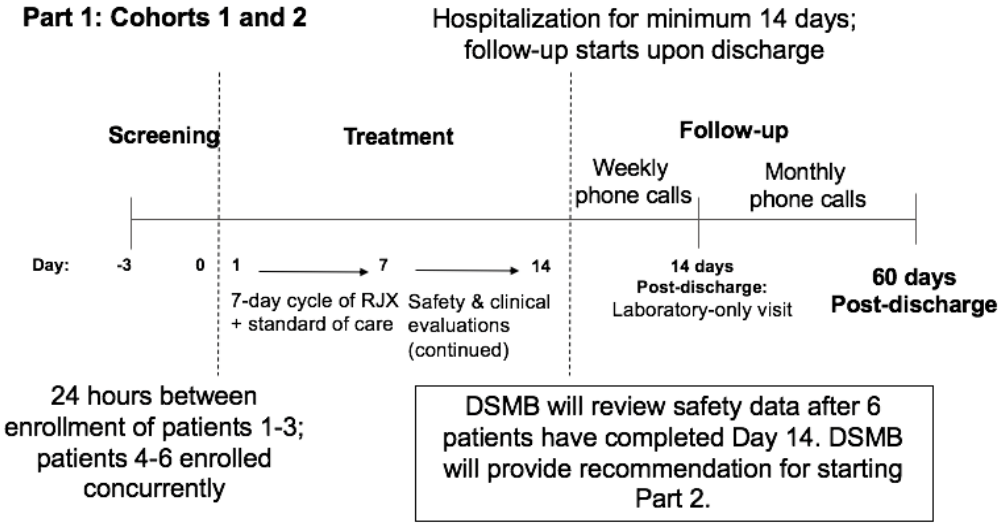

Figure 1: Safety Lead-in Phase (Part 1) of the COVID-19 Study.

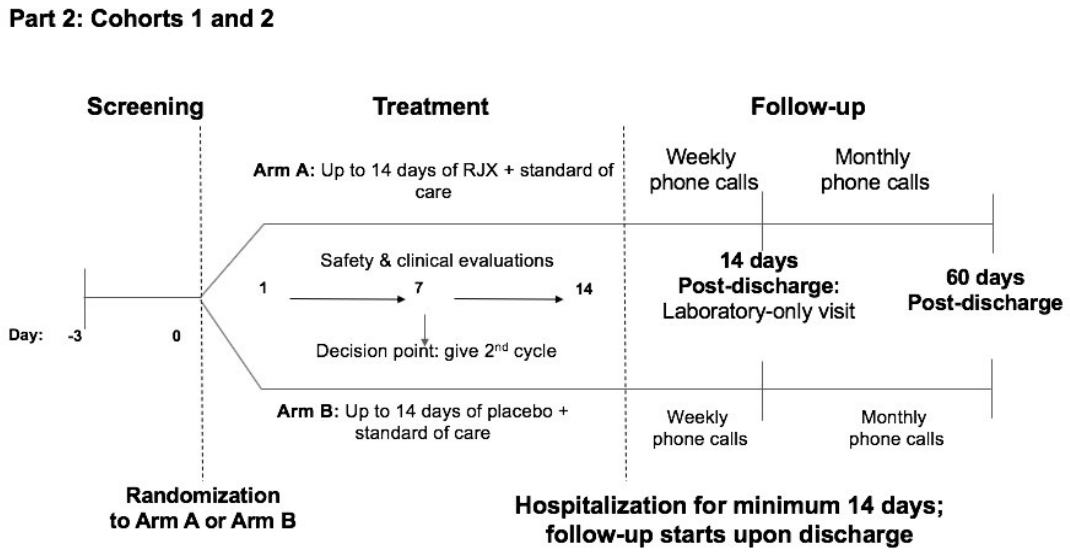

Figure 2: Randomized portion of the COVID-19 Study.

evaluate the safety and tolerability of RJX when used in combination with standard of care in hospitalized COVID-19 patients who have certain high-risk features but no hypoxemia (Cohort 1) or patients who have hypoxemia without ARDS (Cohort 2). Patients in Part 1 are allowed to receive only one, 7-day cycle of RJX. Eligible patients will be entered in a staggered fashion with a minimum of 24 hours between the first 3 patients in each cohort.

If a patient develops any Treatment- Emergent Adverse Event (TEAE) that is a Grade 3 or Grade 4 Adverse Event (AE) related or possibly related to RJX, the Medical Monitor and investigator will review the case and approve the enrollment of the next patient if medically appropriate. A safety follow up period will begin at Day 14, or or when treatment is discontinued and will continue for approximately 60 days post-discharge.

An independent Data Safety Monitoring Board (DSMB) will oversee subject safety during the study. Specific withholding and discontinuation criteria will be used for risk mitigation to maximize patient safety regarding the continuation of RJX for the full 7-day cycle for any patient who develops a Dose-Limiting Toxicity (DLT) or a Grade 3/4 AE that is related or possibly related to RJX. Treatment with RJX will be discontinued for any patient in Cohort 2 who progresses to moderate ARDS according to the Berlin criteria [10]. Patients will be monitored for AEs throughout the study. Treatment/standard of care will be discontinued upon resolution of the patient's respiratory distress and discharge from the hospital. For patients who are discharged before Day 14, follow-up phone calls (3 × weekly) will be used until Day 14 to collect safety data. After Day 14, patients will move into the safety follow-up period as described above. Part 2 will be initiated if RJX is not associated with drug-related SAE in more than 1 of 6 patients during the 7 day treatment period in Part 1.

In Part 2, an Interactive Voice Response/ Interactive Web Response (IVRS/IWR)-based randomization procedure will be applied to assign patients to 1 of the 2 treatment groups. Dynamic randomization will be used to allow an increase in the recruitment target to prevent an underpowered trial. Patients will be stratified based on their age, prior therapy, and absolute lymphocyte count. The primary objective in Part 2 of this study is to evaluate the clinical activity of RJX when used in combination with standard of care in patients hospitalized with COVID-19 who have certain high-risk features but no hypoxemia (Cohort 1); patients who have hypoxemia without ARDS (Cohort 2). The primary endpoints of this study are: (i) For Part 1, Cohorts 1 and 2: Cumulative incidence of Dose-Limiting Toxicities (DLTs) and drug-related Serious Adverse Events (SAEs); (ii) For Part 2, Cohort 1: Time to progression to severe disease with status change on 8-point ordinal scale from a clinical status score of 4 or 5 to a clinical status score of 3, 2, or 1) and (or alternatively reduction of the risk of progression to severe disease within 2 weeks by 50 percent)(iii)For

Part 2, Cohort 2: Time to resolution of respiratory failure, with status change on 8-point ordinal scale from a clinical status score of 3 to a clinical status score of ≥ 4.

In both Part 1 and Part 2, patients will remain hospitalized for a minimum of 14 days and will subsequently move into the safety follow-up period of the study after discharge. After Day 14, patients will be followed with weekly follow-up phone calls for two weeks after discharge and then monthly phone calls for approximately 60 days post-discharge to document their survival status, interim hospitalization history, medical care since discharge from the hospital, and oxygen requirements. The follow-up period also includes one laboratory only visit, which will occur 14 days after discharge.

In Part 2, all patients and clinical staff will be blinded to the assigned treatment. Study treatments (RJX and placebo) will be labeled and coded in a manner that protects the blind. The investigator will be ultimately responsible for ensuring that the integrity of the blind is maintained throughout the study and will be required to document and notify the sponsor in the event of any breaking of the blind for any reason.

If an SAE occurs during the study that meets the criteria for expedited reporting (i.e., is a Suspected Unexpected Serious Adverse Reaction), the patient will be unblinded by the contract research organization.

In an emergency situation, the investigator may unblind the study patient's treatment assignment. When possible, the medical monitor should be notified before unblinding. The investigator should inform the medical monitor of the unblinding if there is no notification before the unblinding. Unblinding procedures will be provided to each site.

Conclusion

RJX could favourably affect the patient journey in COVID-19 at multiple stages, including (i) prevention of progression of mild disease, (ii) faster resolution of ARDS, prevention of multi-organ failure, and reduction of case mortality during respiratory failure, and (iii) improved long-term healing after recovery.

Executive summary

New drug products that can effectively improve the ability of supportive care to reduce the risk of ARDS or its mortality rate in COVID-19 patients are urgently needed. Rejuveinix (RJX) is a formulation of physiologically compatible active ingredients, including several vitamins, such as ascorbic acid, cyanocobalamin, thiamine hydrochloride, riboflavin 5' phosphate, niacinamide, pyridoxine hydrochloride, calcium d-pantothenate, that exhibited promising anti-ARDS activity in animal models of sepsis and ARDS as well as clinical studies in patients with sepsis, septic shock, and ARDS. RJX could favorably affect the patient journey in COVID-19 at multiple stages, including (i) prevention of progression of mild disease, (ii) faster resolution of ARDS, prevention of multi-organ failure, and reduction of case mortality during respiratory failure, and (iii) improved long-term healing after recovery. Here we are reporting the critical design elements for a two-cohort, two-part placebo-controlled, double-blind Phase II study of RJX in COVID-19 patients.

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REJUVEINIX SHOWS A FAVORABLE CLINICAL SAFETY PROFILE IN HUMAN SUBJECTS AND EXHIBITS POTENT PRECLINICAL PROTECTIVE ACTIVITY IN THE LPS-GALN MOUSE MODEL OF ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)

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Abstract: Background. New treatment platforms that can prevent acute respiratory distress syndrome (ARDS) or reduce its mortality rate in high-risk COVID-19 patients, such as those with an underlying cancer, are urgently needed. Rejuveinix (RJX) is an intravenous formulation of anti-oxidants and anti-inflammatory agents. Its active ingredients include ascorbic acid, cyanocobalamin, thiamine hydrochloride, riboflavin 5' phosphate, niacinamide, pyridoxine hydrochloride, and calcium D-pantothenate. RJX is being developed as an anti-inflammatory and anti-oxidant treatment platform for patients with sepsis, including COVID-19 patients with viral sepsis and ARDS. Here, we report its clinical safety profile in a Phase 1 clinical study (ClinicalTrials.gov Identifier: NCT03680105) and its potent protective activity in the LPS-GalN mouse model of ARDS. Methods. A Phase 1, double-blind, placebo-controlled, randomized, two-part, ascending dose-escalation study was performed in participating 76 healthy volunteer human subjects in compliance with the ICH(E6) good clinical practice (GCP) guidelines to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of RJX (Protocol No. RPI003; ClinicalTrials.gov Identifier: NCT03680105). The ability of RJX to prevent fatal shock, ARDS, and multi-organ failure

was examined in the well-established lipopolysaccharide (LPS)-Galactosamine (GalN) mouse model of sepsis and ARDS. Standard methods were employed for the statistical analysis of data in both studies. Findings. In the Phase 1 clinical study, no participant developed serious adverse events (SAEs) or Grade 3-Grade 4 adverse events (AEs) or prematurely discontinued participation from the study. In the non-clinical study, RJX exhibited potent and dose-dependent protective activity, decreased the inflammatory cytokine responses (IL-6, TNF-α, TGF-β), and improved survival in the LPS-GalN mouse model of ARDS. Histopathological examinations showed that RJX attenuated the LPS-GalN induced acute lung injury (ALI) and pulmonary edema as well as liver damage. Conclusion. RJX showed a very favorable safety profile and tolerability in human subjects. It show potential to favorably affect the clinical course of high-risk COVID-19 by preventing ARDS and its complications.

Keywords: Cancer, Sepsis, Pneumonia, ARDS, Acute lung injury (ALI), Multi-organ dysfunction (MODS), Cytokine release syndrome (CRS), COVID-19

1. Introduction

A significant minority of patients infected with the new coronavirus, SARS-CoV-2, the causative agent of coronavirus disease 2019 (COVID-19), develop viral pneumonia that causes an ALI capable of rapid progression to viral sepsis and ARDS with a high fatality rate especially if they are older and have comorbidities [1-8]. A systemic inflammatory response syndrome, also referred to as cytokine storm or cytokine release syndrome (CRS), contributes to the development of ARDS and often irreversible multi-organ dysfunction syndrome (MODS) associated with the severe-critical forms of COVID-19 [1]. Approximately 20% of COVID-19 patients with mild-moderate disease progress to severe-critical disease, and this percentage increases to 40% for the high-risk subgroup ≥65 years of age with comorbidities or laboratory parameters indicative of systemic inflammation, such as high levels of C-reactive protein (CRP), lactate dehydrogenase (LDH), and Ferritin or dysfunction of the coagulation system, as evidenced by elevated D-dimer levels [8]. High-risk patients not only have a higher incidence of ARDS owing to severe viral sepsis caused by SARS-CoV-2, but they also progress faster and have a significantly higher case mortality rate [1-8]. COVID-19 patients with an underlying cancer, especially if they are undergoing chemotherapy, are at an augmented risk for developing potentially fatal ARDS and multi-organ failure [7,8]. Therefore, treatment platforms capable of preventing the disease progression and/or reducing the case mortality rate in such high-risk COVID-19 patients are urgently needed [7,8].

Rejuveinix (RJX) [9] is a formulation of several vitamins, including ascorbic acid (Vitamin C), cyanocobalamin (Vitamin B12), thiamine hydrochloride (Vitamin B1), riboflavin 5' phosphate

(Vitamin B2), niacinamide (Vitamin B3), pyridoxine hydrochloride (Vitamin B6), calcium D-pantothenate, and magnesium sulphate as a potent calcium antagonist, representing components that have been studied in animal models of septic shock and ARDS as well as clinical studies in septic patients [10-20]. Here we are reporting for the first time the primary safety and pharmacokinetics data from a Phase 1 clinical study of RJX and the results of a nonclinical pharmacodynamics study that evaluated its activity in a mouse model of sepsis, ARDS, and multi-organ failure. The main objective of the present study was to evaluate the clinical tolerability of RJX in a double-blind, placebo-controlled Phase 1 clinical trial in healthy volunteer subjects (ClinicalTrials.gov Identifier: NCT03680105). We show that RJX has a very favorable safety profile in human subjects. In the nonclinical pharmacodynamic study we sought to determine if RJX can improve the survival outcome of mice challenged with an otherwise invariably fatal dose of LPS-GalN in a model of sepsis, ARDS and multi-organ failure. RJX exhibited potent protective anti-CRS and anti-ARDS activity in the LPS-GalN model at clinically safe low dose levels.

2. Materials and Methods

2.1. RJX

RJX is a proprietary composition of naturally occurring anti-oxidants and anti-inflammatory agents which, in combination, provide potent and immediate tissue protection. Its ingredients include ascorbic acid, magnesium sulfate heptahydrate, cyanocobalamin, thiamine hydrochloride, riboflavin 5' phosphate, niacinamide, pyridoxine hydrochloride, and calcium D-pantothenate. RJX is a two-vial system and A and B are each of the two vials. Vial A contains the active ingredients and minerals whereas Vial B contains the buffer, sodium bicarbonate as the Vial A content is acidic. The components of each of the vials are shown in **Table S1**. RJX is being developed as an anti-inflammatory and anti-oxidant treatment platform for patients with sepsis, including COVID-19 patients with viral sepsis and ARDS [9].

2.2. Ethics Statement, Approval, and Conduct of the Clinical Phase 1 Study

The safety profile, pharmacokinetics, and pharmacodynamics of RJX were evaluated in a placebo-controlled, double-blind Phase 1 dose-escalation study in healthy volunteers (ClinicalTrials.gov Identifier: NCT03680105). This was a 2 part study with part 1 single asending doses (n=52) and part 2 multiple ascending doses (n=24) (**Table S2**). The study was performed at the clinical facility of the ICON Early Phase Services, LLC, 8307 Gault Lane, San Antonio, TX 78209 USA, in compliance with the ICH(E6) good clinical practice (GCP) guidelines, in adherence to the ethical principles based on the Declaration of Helsinki, and the applicable national and local laws and regulatory requirements. Date of first enrollment: 8/27/2018; Date of last completed: 2/16/2019.

The study was approved by the IntegReview IRB (3815 S. Capital of Texas Hwy, Suite 320, Austin, TX 78704, IRB Registration Numbers: IRB IRB00008463, IRB00003657, IRB00004920, IRB00001035, IRB00006075). Each subject provided a written informed consent. A safety review committee (SRC) reviewed the safety data for each cohort prior to escalation to the next cohort. The details of the subject demographics, safety assessments, pharmacokinetics (PK) analyses, and cardio-dynamic studies are detailed in the **Supplemental Material**.

The starting dose was based on the results of animal toxicity studies. The no observed adverse effect level (NOAEL) was >5 mL/kg in both Sprague Dawley rats (when administered via intra-arterial route daily for 21 days) and Beagle dogs (when administered via intra-arterial route daily x 28 days). The HED of the >5 mL/kg NOAEL in rats is 0.8 mL/kg and the HED of the ≥5 mL/kg NOAEL in dogs is 2.7 mL/kg. The starting dose of 0.024 mL/kg in the Phase 1 study represents the HED in the most sensitive species divided by a safety factor of 30 (i.e., 33-times lower than the NOAEL in rats and >112.5-fold lower than the NOAEL in dogs).

Part 1 was a single ascending dose (SAD) escalation study in 52 participants, 6 cohorts in total. Cohorts 1 to 5 included 8 participants per cohort (6 RJX: 2 placebo) aged 18 to 50 years, inclusive (**Table S3**). Cohort 6 investigated an older population of 12 participants (9 RJX: 3 placebo) aged 51 to 70 years, inclusive (**Table S3**). Part 2 of the study was a multiple ascending dose (MAD) escalation study in 24 participants, 3 cohorts of 8 participants (6 RJX: 2 placebo).The MAD arm of the study commenced in parallel with Part 1, Cohort 6, following completion and review of the safety and PK findings for Part 1, Cohorts 1 through 5. Each patient in the SAD cohort received a single intravenous infusion of either placebo or RJX at escalating doses. The MAD cohort subjects were randomly assigned to receive RJX at one of 3 dose levels or placebo (6 RJX:2 placebo) every day for 7 days. The assigned RJX dose was administered intravenously in a total volume of 100 mL normal saline over 45 minutes (± 5 minutes).

Placebo was used in both Part 1 (SAD study) and Part 2 (MAD study). The placebo was normal saline (i.e., an aqueous solution of 0.9% NaCl). In the Phase 1 Clinical trial, RJX was given intravenously at ascending doses for SAD of (cohort 1) 0.024 mL/kg, (cohort 2) 0.076 mL/kg, (cohort 3) 0.240 mL/kg, (cohort 4) 0.500 mL/kg, (cohort 5) 0.759 mL/kg, and the elderly cohort (cohort 6) 0.500 mL/kg RJX. In the MAD group, RJX was given intravenously at doses of 0.240 mL/kg, 0.500 mL/kg, and 0.759 mL/kg (**Table S2**). Each patient in the SAD cohort received a single intravenous infusion of either placebo or RJX at escalating doses. The MAD cohort subjects were randomly assigned to receive RJX at one of 3 dose levels or placebo (6 RJX:2 placebo) every day for 7 days. The appropriate RJX dose was calculated based on mL/kg, and it was prepared for intravenous administration by adding the appropriate dose of RJX to normal saline in a total volume of 100 mL. 100 mL normal saline served as the Placebo. Placebo was administered as 0.9% IV saline in a 100 mL total volume over 45 minutes (± 5 minutes). In Part 1

(SAD), RJX was administered as a 100 mL IV infusion over 45 minutes (± 5 minutes) on a single occasion on Day 1. In Part 2 (MAD), RJX was administered as a 100 mL IV infusion over 45 minutes (± 5 minutes), as in Part 1, every day for 7 days.

The pharmacokinetic blood sampling in Part 1 (SAD study) was done at a total of 15 time points, including 3 time points prior to the RJX infusion (i.e., within 90±2 min prior to, within 60±2 min prior to and 30±2 min prior to RJX), plus 3 time points after start of the RJX infusion (i.e., 15±2 min post start, 30 ±2 min post start, and 45±2 min post start), plus 9 time points after the end of the RJX infusion (30±2 min post-end, 1 h ± 2 min post-end, 1.5 h ± 2 min post-end, 2 h ± 2 min post-end, 4 h ± 2 min post-end, 8 h ± 5 min post-end, 10 h ± 5 min post-end, 12 h ± 10 min post-end, and 24 h ± 10 min post-end). In Part 2 (MAD study), the same 15 time points were used on day 1 and day 7. In addition, blood samples were also obtained on days 4 and 6 within 30 ± 2 min prior to the RJX infusion.

2.3. Statistical Analyses for Clinical Study

Statistical analysis of data was performed using standard methods. Mean, median and standard deviation were reported along with n's of categorical data (see supplemental tables). Clinical laboratory safety parameters, vital sign measurements, and 12-lead safety electrocardiogram (ECG) parameters were listed and summarized using descriptive statistics for each treatment, timepoint, and changes from baseline. The bioanalytical studies were performed at Altasciences Company Inc. in Laval, Quebec, Canada. Concentration-time profiles of ascorbic acid, cyanocobalamin, niacinamide and thiamine in plasma and magnesium in serum were analyzed by noncompartmental methods using Phoenix® WinNonlin® (version 8.0) (Cetara), model 200-202 for intravenous injection. Pharmacokinetic (PK) parameters calculated for each active ingredient (except riboflavin 5' phosphate, pyridoxine hydrochloride, calcium D-pantothenate) included t_{max}, Cmax, $AUC_{0\text{-last}}$, and $t_{1/2}$.

2.4. LPS-GalN Model of Fatal Cytokine Storm and ARDS

The ability of RJX to prevent fatal shock, ARDS, and multi-organ failure was examined in the well-established LPS-GalN model, as described in detail in the Supplemental Material. In brief, LPS was combined with GalN, which further sensitizes mice to LPS-induced systemic inflammatory syndrome and multi-organ failure [21]. The care and treatment of the animals were in accordance with the *Guide for the Care and Use of Laboratory Animals*. They were approved by the Animal Care and Use Committee of Firat University (Project No. 04052020-391-046). BALB/c mice were randomly divided into 6 treatment groups with 10 mice in each group. All mice were genetically identical, of the same age, and the LPS-GalN challenged mice in Groups 2-6 were injected with the same amount of LPS-GalN. This statistical equivalency of mice allowed the use of a pseudo-

randomization convenience allocation to assign mice to identified cages. For random treatment allocation, cages were randomly selected to receive one of the specified treatments. We applied concealment of treatment allocation and blind outcome assessment to reduce the risk of bias in our conclusions. Health care assessments were performed by animal care technicians not involved in the treatment assignments or treatments. Investigators did not participate in individual health status or outcome assessments.

In the LPS-GalN mouse model, mice were treated via intraperitoneal injections of 6-fold diluted (vehicle = normal saline) RJX (0.5, 1.05, 2.1, 4.2 mL/kg, 0.5 mL/mouse) or normal saline 2 hours before and 2 hours post-injection of LPS-GalN (see also Supplemental Methods). Untreated normal control mice (Group 1) did not receive any treatments. Vehicle control mice (Group 2) were treated with 500 µL normal saline (NS), i.e., an aqueous solution of 0.9% NaCl with no RJX. NS was administered intraperitoneally (*i.p*) 2 hours before and 2 hours after the *i.p* injection of LPS-GalN. Test mice (Groups 3-6) received the designated RJX dose (500 µL/mouse) at the indicated dose levels (0.5, 1.05, 2.1, 4.2 mL/kg of a 6-fold diluted RJX solution) 2 hours before and 2 hours after the *i.p* injection of LPS-GalN (see also Supplemental Methods). Safety pharmacology labs, serum and tissue cytokine levels, tissue activity levels of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), tissue ascorbic acid levels, as well as tissue concentrations of malondialdehyde (MDA) were determined to examine the effects of RJX on the inflammatory process caused by LPS-GaN. IL-6 and TNF-α levels in mouse serum samples were measured by quantitative enzyme-linked immunosorbent assays (ELISA) using the commercially available “Mouse IL-6 Quantikine ELISA Kit” (Sensitivity for mouse IL-6: 1.6 pg/mL, Assay Range: 7.8 - 500 pg/mL) and “Mouse TNFa Quantikine ELISA Kit” (Sensivity for mouse TNFa: 7.21 pg/mL, Assay Range: 10.9 - 700 pg/mL), respectively, and an absorbance microplate reader (Bio-Tek Elx800 Universal Microplate Reader, Bio-Tek Instruments, Inc, Winooski, USA) according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN, USA). Samples and standards were analyzed in duplicate. The Kaplan-Meier method, log-rank chi-square test was used to analyze the 48 h survival outcomes of mice in the different treatment groups. At the time of death, lungs, heart, kidneys, liver, and brain from 6 mice per group were harvested, fixed in 10% buffered formalin, and processed for histopathologic examination. In another experiment, treatments with vehicle (NS) or RJX were delayed until 2 hours post LPS-GalN. Groups of 6 mice were treated with i.p. injections of 4.2 mL/kg NS or 6-fold diluted RJX at 2 hours and 3 hours (total volume: 500 µL/mouse). Mice were monitored for survival and serum cytokine levels as well as lung MDA levels were measured at the time of death or elective termination at 24 h post LPS-GalN injection. The Kaplan-Meier method, log-rank chi-square test was used to analyze the 24 h survival outcomes of mice in the two treatment groups. This experimental design was basd on a pilot experiment, in which serum cytokine

levels and lung MDA levels were documented to be markedly elevated at 2 hours after LPS-GalN injection in 6 of 6 mice, when compared to the levels measured in 6 control untreated mice.

2.5. Statistical Analyses for Non-Clinical Studies in the LPS-GalN Mouse Model of Cytokine Storm, ARDS and Multi-organ Failure

The Decision statistical structure includes analysis of variance (ANOVA) using SPSS statistical program (IBM, SPPS Version 21) and/or covariance (ACOVA), nonparametric analysis of variance, simple t-tests, Tukey's Test for homogeneity of variance, and pairwise tests by the Dunnett's test for parametric and nonparametric data. The Tukey's multiple comparisons detected alterations among groups. Furthermore, the Kaplan–Meier method, log-rank chi-square test, was used to investigate survival and fatality in each group.

3. Results

3.1. Clinical Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Rejuveinix (RJX)

A Phase 1, double-blind, placebo-controlled, randomized, two-part, ascending dose-escalation study was performed to evaluate the safety, tolerability, and pharmacokinetics of RJX in healthy volunteers (Protocol No. RPI003; ClinicalTrials.gov Identifier: NCT03680105) (**Table S2, Table S3**). The dose levels ranged from 0.024 mL/kg to 0.759 mL/kg in Part 1 and from 0.240 mL/kg to 0.759 mL/kg in Part 2. The treatment-emergent AEs (TEAEs) are presented in **Tables S4-S7**. In Part 1, no deaths or SAEs were reported, none of the 39 RJX-treated subjects experienced Grade 3 or 4 AEs, and no AEs led to discontinuation of RJX (**Table S7A**). In Part 2, no SAEs and no Grade 3 or 4 AEs were encountered by any of the 18 RJX-treated subjects. One subject in Cohort 1 developed a mild upper respiratory infection (URI); 3 subjects in Cohort 2 had mild infusion site discomfort, pain or reaction; and 3 subjects on Cohort 3 experienced mild-moderate pain (mild headache = 1; mild pain in extremity = 1, moderate back pain = 1). The TEAEs in 2 of the subjects were considered possibly or probably related to RJX. Both patients were in Cohort 2: One patient had a mild infusion site discomfort on day 6 considered possibly related to RJX and another patient had a mild infusion site pain on days 6 and 7 considered probably related to RJX infusion (**Table S6, Table S7**). These TEAEs did not require additional treatment, and they did not cause discontinuation of the dosing or study. All AEs considered possibly or probably related to RJX infusions have recovered/resolved with no sequelae. There were no clinically significant abnormal findings in 12-lead safety ECGs, and no notable changes were detected compared with baseline. For both study parts, there were no clinically meaningful changes in laboratory values identified in observed values or mean changes from baseline compared with placebo or evaluated by increasing

the RJX dose level. Based on its tolerability, the 0.500 ml/kg dose level was selected as the recommended Phase 2 dose (RP2D) level for future studies.

A summary of RJX plasma PK parameters by cohort following single ascending dose IV infusion is presented in **Table S8-Table S12.** Plasma concentration-time profiles of baseline-adjusted ascorbic acid (A), cyanocobalamin (B), niacinamide (C) and thiamine (D) for the RJX dose level of 0.5 mL/kg (Cohort 6) following single-dose IV infusion of RJX are presented in **Figure S1** and box plots for $AUC_{0\text{-last}}$ are shown in **Figure 1**. The mean baseline plasma concentrations of ascorbic acid ranged from 7.99 to 10.12 µg/mL for all cohorts. Following the single ascending dose administration of RJX in healthy subjects, baseline-adjusted ascorbic acid C_{max} and $AUC_{0\text{-last}}$ increased with increasing doses of RJX (**Table S8**). The mean C_{max} ranged from 5.40 µg/mL (0.024 mL/kg [1.079 mg/kg of ascorbic acid]) to 152.69 µg/mL (0.759 mL/kg [34.130 mg/kg of ascorbic acid]), increasing 28-fold while the mean $AUC_{0\text{-last}}$ ranged from 32.36 h*µg/mL to 467.10 h*µg/mL, increasing 14-fold for a 32-fold increase in dose. At the RJX dose level of 0.5 mL/kg (22.483 mg/kg of ascorbic acid), C_{max} and $AUC_{0\text{-last}}$ increased by 1.2 to 1.3-fold in elderly subjects when compared to healthy subjects. The plasma RJX PK parameters for cyanocobalamin, magnesium sulfate, niacinamide, and thiamine by cohort following single-dose IV infusion are presented in **Table S9-Table S12.** At the dose level of 0.5 mL/kg (0.048 mg/kg of cyanocobalamin; 2.970 mg/kg of niacinamide; 1.246 mg/kg of thiamine), the C_{max} and the $AUC_{0\text{-last}}$ of cyanocobalamin, niacinamide, and thiamine increased by 1.3-fold, 1.7-fold, and 1.3-fold, respectively, in elderly subjects in Cohort 6 when compared to younger healthy subjects in Cohort 4. At the same dose level, the C_{max} and the $AUC_{0\text{-last}}$ of magnesium increased by 1.2 and 1.6-fold, respectively, in elderly subjects when compared to younger subjects.

The plasma concentration-time profiles of baseline-adjusted ascorbic acid, cyanocobalamin, niacinamide, and thiamine for the RJX dose level of 0.5 mL/kg (Cohort 6) following single-dose IV infusion of RJX are presented in **Figure S1.** The terminal phase following single-dose administration of ascorbic acid and magnesium could not be adequately characterized. The half-life ranged from 5 to 28 h, 0.4 to 1.5 h, and 2 to 10 h, for cyanocobalamin, niacinamide, and thiamine, respectively. There was no clinically significant accumulation of these compounds following once-daily IV infusion for 7 days in the part 2 MAD cohorts, as demonstrated by the PK profiles on Day 7 that were overall similar to those observed on Day 1 (**Table S13**-**Table S17)**.

In cardio-dynamic ECG asessments, we examined the potential effects of RJX on the QT interval corrected by the Fridericia correction formula (QTcF). RJX at the studied doses caused mild QTc prolongation but it did not have a clinically relevant effects on any other ECG parameter, i.e., heart rate, PR and QRS intervals. In the by-time point analysis of QTcF, mean ΔQTcF on RJX generally followed the placebo pattern across post-dose time points in both parts of the study. For

the pooled data from both parts of the study (SAD plus MAD), a full model including ascorbic acid, cyanocobalamin, magnesium, niacinamide, thiamine and their 1-by-1 interaction terms was initially fitted and a model selection procedure was performed. The model with ascorbic acid and magnesium alone was selected as the primary model and represented the data reasonably well. The estimated slopes of the concentration-QTc relationship were 0.012 ms per mg/L (90% CI: −0.0114 to 0.0346) for ascorbic acid and 0.34 ms per mg/L (90% CI: 0.081 to 0.606) for magnesium, of which the slope of magnesium was statistically significant. The treatment effect-specific intercept was −5.06 ms (90% CI: −10.244 to 0.126) and not statistically significant. The predicted placebo-corrected QT effects (ΔΔQTcF) were predicted to be 7.12 ms (90% CI: 4.54 to 9.70) for the SAD 0.759 mL/kg group on Day 1 and 9.13 ms (90% CI: 6.08 to 12.19) for the MAD 0.759 mL/kg group on Day 7 where the highest levels of plasma concentrations were observed for each part (**Table S18**). The prediction results from other models with each analyte alone were similar to the results from the primary model. The predicted, placebo-conrrected ΔΔQTcF values at the geometric mean peak concentration are provided for the concentration-QTc models with each analyte alone in **Tables S19-S23** and **Figures S2-S6**. Based on these concentration-QTc analyses, an effect on ΔΔQTcF exceeding 10 ms can be excluded within the observed ranges of plasma concentrations of ascorbic acid, cyanocobalamin, magnesium, niacinamide, and thiamine up to 166 mg/L, 362 µg/L, 4.0 mg/dL, 7570 µg/L, and 4890 µg/L, respectively. Such plasma concentrations were only observed at the 0.759 ml/kg dosing condition, thus an effect on the QTc interval > 10 ms cannot be excluded at this dose level.

3.2. Dose-Dependent In Vivo Protective Activity of Prophylactic RJX Treatments in the LPS-GalN Model of ARDS and Multiorgan Failure

One hundred (100) percent (%) of untreated control mice remained alive throughout the experiment (Group 1). By comparison, 100% of LPS-GalN alone injected mice died at a median of 5.4 hours (Group 2) (**Figure 2**). RJX-treated mice had an improved survival outcome after being injected with LPS-GalN. RJX reduced the incidence of fatal outcome and delayed the time to death in a dose-dependent manner. In contrast to the invariably fatal treatment outcome of vehicle-treated control mice, which had a median survival time of 5.4 hours, 40% of mice treated with 4.2 mL/kg of 6-fold diluted RJX (N=10) remained alive and the median survival time was prolonged to 15.25 hours (Log-rank P=0.004; Z-score: -4.059, P<0.001) (**Figure 2**). Hence, RJX exhibited potent and dose-dependent activity in the LPS-GalN model of ARDS and multi-organ failure in mice. The most effective dose was the highest dose level, 4.2 mL/kg of a 6-fold diluted RJX, which corresponds to a human equivalent dose of 0.05 mL/kg (0.3 mL/kg:6), which is 10-times lower than the 0.5 mL/kg recommended Phase 2 dose (RP2D) level of RJX [22].

In LPS-GalN challenged control mice not receiving any RJX treatments, serum interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α) and LDH levels were drastically increased at the time of death which is consistent with a cytokine storm and marked systemic inflammation ($P<0.0001$ for IL-6, TNF-α, and LDH when compared to Group 1) (**Figure 3**). RJX treatments had a statistically significant and dose-dependent lowering effect on the serum levels of the inflammation markers (IL6, TNF-α, and LDH) (**Figure 3**). These results of mice that died within 24 hours after the LPS-GalN challenge demonstrate that RJX decreased the inflammatory cytokine responses of mice injected with LPS-GalN, and thereby improved the survival time of mice in a dose-dependent fashion.

Western blot analysis of the lung tissue pro-inflammatory cytokine levels in LPS-GalN injected control mice (Group 2) vs. untreated healthy control mice not injected with LPS-GalN (Group 1) showed a highly statistically significant ($P<0.0001$) 7.2±0.1-fold elevation for interleukin 1 beta (IL-1β), 12.8±0.1-fold elevation for IL-6, 3.3±0.2-fold elevation for TNF-α, and 3.7±0.8-fold elevation for transforming growth factor beta (TGF-β) (**Figure 4**). The lung malondialdehyde (MDA) levels measuring lipid peroxidation were markedly elevated (6.5±0.5 vs. 2.6±0.4 nmol/g, $P<0.0001$) and the levels of the antioxidant enzymes SOD (30.5±1.2 U/mg vs. 80.4±1.6 U/mg, $P<0.0001$), CAT (19.9±1.1 U/mg vs. 56.7±1.4 U/mg, $P<0.0001$), GSH-Px (54.2±3.1 U/mg vs. 126.4±4.1 U/mg, $P<0.0001$), as well as ascorbic acid (54.5±0.1 µg/g vs. 398.2±0.1 µg/g, $P<0.0001$) were markedly reduced in LPS-GalN treated mice (Group 2) when compared to healthy control mice (Group 1), consistent with severe oxidative stress in the lung tissue (**Figure 5**). Histopathological examination of hematoxylin-eosin (H/E)-stained lung tissues from LPS-GalN injected mice showed histological changes consistent with severe acute ALI, including alveolar hemorrhage, thickening of alveolar wall, edema/congestion, and leukocyte infiltration (**Figure 5A**, **Figure 6, Figure S7**). These changes were not observed in the lung tissues of mice in the control group that was not injected with LPS-GalN.

RJX suppressed the levels of pro-inflammatory cytokines in the lung (**Figure 4**), decreased the lung MDA levels, and increased in a dose-dependent manner the reduced levels of the antioxidant enzymes SOD, CAT, GSH-Px and ascorbic acid (**Figure 5**). Notably, RJX attenuated the LPS-GalN induced ALI in a dose-dependent fashion, as evidenced by significantly less damage in the lungs of RJX-treated mice that were treated at the 2.1 and 4.2 mL/kg dose levels and were terminated healthy at 48 h. The ALI scores depicted in **Figure 5A** show a dose-dependent protective effect of RJX with a highly statistically significant reduction of the ALI scores at the highest two dose levels of RJX that were tested. The alveolar wall thickness as a measure of pumnonary edema was substantially decreased to near normal values at these dose levels of RJX (Group 5 and 6) (**Figure 6, Figure S7**). Thus, RJX appeared to prevent the development of pulmonary edema in LPS-GalN challenged mice.

LPS-GalN caused significant liver damage and hepatic dysfunction in mice with a marked elevation of the liver enzymes as well as total bilirubin when compared to the enzyme levels in untreated healthy control mice: alanine aminotransferase (ALT) (1297.1±106.8 vs. 28.3±4.5; 46-fold elevation; P<0.0001), aspartate aminotransferase (AST) (1756.0±96.8 vs. 57.9±10.3; 30-fold elevation; P<0.0001), alkaline phosphatase (ALP) (491.2±33.9 vs. 69.9±6.7; 7-fold elevation; P<0.0001), total bilirubin (TBIL) (1.9±0.1vs. 0.3±0.1; 6-3 fold elevation; P<0001) (**Figure S8**). Western blot analysis of the liver tissue pro-inflammatory cytokine levels (IL-1β, TNF-α, IL-6, and TGF-β) in LPS-GalN injected control mice vs. untreated healthy control mice not injected with LPS-GalN showed a highly statistically significant (P<0.0001) elevation for each cytokine (**Figure S9**). Histopathologic examination of the livers from LPS-GalN treated mice showed massive generalized hepatocyte vacuolation and necrosis corresponding to >25% necrosis and destruction of the liver parenchyma, severe lobular inflammation involving >50% of the liver parenchyma, and severe portal inflammation involving more than >50% of portal tracts (**Figure 7**, **Figure S10, Figure S11**). The liver MDA levels measuring lipid peroxidation were markedly elevated and the levels of the antioxidant enzymes SOD, CAT, GSH-Px, as well as ascorbic acid were markedly reduced in LPS-GalN treated mice consistent with severe oxidative stress (**Figure S10**). RJX suppressed the levels of pro-inflammatory cytokines in the liver (**Figure S9**) as well as the LPS-GalN induced liver injury and inflammation noted in histological examination (**Figure S10A, Figure S11, Figure 7**) in a dose-dependent fashion. Furthermore, RJX decreased the liver MDA levels and normalized in a dose-dependent manner the reduced levels of the antioxidant enzymes SOD, CAT, and GSH-Px as well as ascorbic acid. Consequently, RJX reduced the mortality rate of LPS-GalN associated systemic inflammatory response, and RJX-treated mice that died following the LPS-GalN challenge had significantly lower, albeit still abnormal, levels for liver enzymes and TBIL (**Figure S8**).

3.3. In Vivo Protective Activity of Delayed-Onset RJX Treatments in the LPS-GalN Model of ARDS and Multiorgan Failure

We next sought to determine if RJX could also improve the survival outcome of LPS-GalN challenged mice if the treatment is delayed until after the onset of the inflammatory cytokine response. As shown in **Figure S12**, the serum IL-6 and TNF-α levels were markedly elevated in 6 of 6 control mice that were terminated at 2 hours after the i.p. LPS-GalN injection when compared to 6 untreated control mice. At the time of their termination, these LPS-GalN injected mice also had marked lipid peroxidation in the lungs as evidenced by dramatically elevated MDA levels in the lungs. Hence, at 2 hours post LPS-GalN, mice had evidence of a significant inflammatory response and oxidative stress in the lungs. In our proof of concept experiment aimed at evaluating RJX as a potential treatment for sepsis, we started treatments of mice with normal saline versus RJX at this time 2 h time point. Control mice (N=6) treated with 2 injections of vehicle (normal saline) at 2 hours

and 3 hours, respectively, post LPS-GalN challenge all rapidly died within 4 hours at a median survival time of 2.15 hours after the first injection of normal saline and 4.15 hours after the LPS-GalN challenge. At the time of death, their serum IL-6 and TNF-α levels as well lung tissue MDA levels were drastically elevated and even higher than the levels in untreated control mice that were terminated at 2 hours post LPS-GalN injection (**Figure S12**). Notably, treatment of mice with 6-fold diluted 4.2 mL/kg RJX at 2 hours and 3 hours post LPS-GalN injection resulted in improved survival outcome with 3 of 6 mice remaining alive at 24 hours post LPS-GalN injection (**Figure 8**). The median survival time of these mice was markedly longer than the median survival of normal saline-treated control mice (15.1 hours vs. 4.15 hours, P = 0.0098) (**Figure 8**). The serum levels of inflammatory cytokines IL-6 and TNF-α as well as lung MDA levels at the time of death (N=3) or lective termination at 24 hours (N=3) were lower than the baseline levels in mice sacrificed at the 2 hours post LPS-GalN challenge time point when RJX and normal saline treatments were initiated (**Figure S12**). These results provide direct evidence that low dose RJX is capable of preventing fatal cytokine storm and reduce the mortality rate of LPS/GalN-induced systemic inflammation when the treatment is delayed until after the onset of a systemic inflammatory cytokine response and oxidative stress in the lungs.

4. Discussion

In the Phase 1 clinical study of RJX in healthy volunteers, no participant developed SAEs or Grade 3-Grade 4 AEs or discontinued participation early. Thus, RJX showed a very favorable clinical safety profile. Following both single and multiple-dose IV infusion of RJX, all 5 API evaluated (ascorbic acid, cyanocobalamin, magnesium sulfate, niacinamide, and thiamine) rapidly appeared in the systemic circulation. There was no clinically significant accumulation of these compounds following once-daily IV infusion for 7 days, as demonstrated by the PK profiles on Day 7 that were overall similar to those observed on Day 1. Despite its tolerability, the 0.759 mL/kg dose level (viz.: dose level in Cohort 5 of Part 1 and Cohort 3 in Part 2 of the Phase 1 study) was therefore not selected for further evaluation in future studies, including the projected Phase 1-2 COVID-19 study due to the model fitted projections in the cardiodynamic evaluations. Specifically, the results of the concentration-QTc analysis of data informed our decision to select the 0.5 mL/kg dose level and not the 0.759 mL/kg dose level for the projected Phase 1 – 2 COVID-19 trial. In all concentration-QTc analyses, the upper bound of the 90% CI of the QTc effect was greater than 10 ms in the 0.759 mL/kg group for all component concentrations, except in the modeling of thiamine and magnesium alone for the SAD group. An effect exceeding 10 msec could be excluded at plasma concentrations of ascorbic acid, cyanocobalamin, magnesium, niacinamide, and thiamine up to 166 mg/L, 362 µg/L, 4.0 mg/dL, 7570 µg/L, and 4890 µg/L, respectively. Such plasma concentrations were only observed at the 0.759 ml/kg dose level and not at 0.5 mL/kg dose level. Therefore, the modeling study

indicated that a potential QTc interval prolongation of >10 msec could not be excluded at the 0.759 ml/kg dose level. Based on these predictions, a dose level of 0.500 mL/kg for which the above mentioned concentrations were not observed or predicted was selected as the tentative dose level for further clinical development of RJX in future studies. While we do not anticipate any clinically meaningful effect on the QTc of the COVID-19 patients who will not receive RJX at a dose exceeding 0.500 mL/kg, risk mitigation measures will be used to monitor patients for QTc prolongation. Serum potassium and magnesium levels will be required to be within normal limits, prior to RJX infusion and the protocol advises that medications with potential for QTc prolongation should ideally not be started within 24 hours of RJX administration. If a subject has a QTcF interval >500 msec on an ECG machine printout, it is recommended that the subject be placed on a cardiac monitor while cardiology consultation and review of the ECG is obtained. If the QTcF >500 msec is confirmed, the treating physician will follow the recommendations of the consulting cardiologist**.** Furthermore, the exclusion criteria include subjects with a history of congenital long QT syndrome or of Torsades de pointes; subjects with bradycardia (<60 bpm), heart block (excluding 1st degree block, being PR interval prolongation only); subjects with any of the following findings on electrocardiogram (ECG): QTc interval >470 msec in women OR >450 msec in men; subjects requiring any drugs known to prolong the QTc interval, including antiarrhythmic medications.

Older age is a widely recognized risk factor in COVID-19 [1-8]. Notably, patients ≥65 years of age had a 3.3 to 3.6-fold increased risk of developing ARDS than patients <65 years of age [2, 8, 23]. Importantly, once patients with ≥65 years of age develop ARDS, their risk of death is 6.2-fold higher than the risk of younger patients. Likewise, the risk of developing ARDS for patients ≥60 years of age was reportedly 3.1-fold higher than the risk for younger patients [3]. Whereas the reported median age for non-survivors was 69 years, the median age for survivors was 52 years (P<0.0001) [4]. A recent review of 17 clinical studies by Carver and Jones also confirmed that older age, diabetes, higher serum levels of inflammatory markers (LDH, CRP), and coagulopathy with higher serum D-dimer levels are associated with a higher risk for ARDS [5]. Thus, an older patient with high-risk COVID-19 is in urgent need for treatment platforms capable of preventing the disease progression and/or reducing the case mortality rate in the high-risk COVID-19 patients by stopping or reversing the inflammatory process that causes ARDS and multi-organ failure after the development of pulmonary inflammation. In our projected COVID-19 study, Cohort 1 will include older patients with at least one additional comorbidity (diabetes or hypertension). Therefore, it will be important to evaluate the safety and tolerability profile of RJX is in older patients with high-risk COVID-19 who are in urgent need for therapeutic innovations.

TGF-β genes are up-regulated in COVID-19 [24-26] and Stafford et al. postulated that TGFβ is a crucial molecule, along with IL-6 and TNFα in the pathogenesis of severe COVID-19 [27]. TGF-β has been implicated as an important pro-inflammatory cytokine in the pathophysiology of acute

lung injury and ARDS [28-33]. A recent study by Peters et al. demonstrated that TGF-β profoundly impacts alveolar ion and fluid transport through regulating the epithelial sodium channel (ENaC) activity and trafficking via a TGF-βR1-dedicated signaling pathway [34]. The upregulation of TGF-β in ARDS causes an ENaC trafficking defect with a marked reduction in the cell-surface abundance of ENaC on lung epithelial cells, thereby rapidly and substantially impairing the alveolar fluid reabsorption in ARDS patients and contributing to the persistence of their pulmonary edema [34]. Notably, a significant negative correlation existed between TGF-β levels in bronchoalveolar lavage fluid samples and ventilator-free/ICU-free days in ARDS patients. Furthermore, lower TGF-β levels correlated with better survival outcomes indicating that patients with higher TGF-β levels may have higher and faster case mortality [35]. TGF-β also stimulates platelet activation and formation of thrombi [20] and may contribute to the coagulopathy and pulmonary blood clot formation in COVID-19 associated ARDS [37]. A soluble recombinant TGF-β receptor protein capable of sequestering TGF-β has effectively attenuated the severity of pulmonary edema in experimental models of ARDS [38]. Notably, our results demonstrate that RJX treatments result in a marked reduction of not only IL-6 and TNF-α, but also in TGF-β levels in the lungs as well as liver of mice challenged with LPS-GalN in a dose-dependent fashion. We postulate that the RJX-mediated suppression of TGF-β expression in the lungs will accelerate the resolution of pulmonary inflammation of COVID-19 patients by reducing the contributions of TGF-β to ARDS and its complications. TGF-β is also involved in the pathogenesis of lung tissue remodeling and lung fibrosis that follows ARDS. Specifically, TGF-β contributes to the development of lung fibrosis by stimulating the proliferation/differentiation of lung fibroblasts, accumulation of collagen and other extracellular matrix proteins in the pulmonary interstitial and alveolar space, leading to the occurrence and development of pulmonary fibrosis [39, 40]. Therefore, inhibition of the TGF-β signaling pathway by RJX similarly has the potential to prevent the development of pulmonary fibrosis following ARDS and improve the pulmonary healing process [41].

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 [1, 42-45]. It is the main signature cytokine implicated in COVID-19 associated CRS and ARDS [10]. Anti-IL6 receptor antibodies tocilizumab (ClinicalTrials.gov Identifiers: NCT04317092, NCT04306705, NCT04310228) and sarilumab (ClinicalTrials.gov Identifier: NCT04315298) are being assessed for their therapeutic activity in COVID-19 patients in both open-label as well as randomized clinical trials. TNF-α is another pro-inflammatory cytokine implicated in the pathophysiology of CRS and ARDS in COVID-19 [1, 42-45]. RJX treatments appeared to have a statistically significant and dose-dependent lowering effect on the levels of IL-6 as well as TNF-α in the blood, lung tissue, and liver. We believe the observed robust effect of RJX

on serum and tissue cytokine levels significantly contributed to its ability to reduce the ALI, pulmonary inflammation and prevent the development of pulmonary edema, as well as its ability to reduce the liver damage that was accompanied by destruction of the liver parenchyma, severe hepatic inflammation, massive hepatic necrosis, and hepatic dysfunction in LPS-GalN challenged mice. Together these favorable effects ultimately translated into improved survival outcomes of RJX-treated mice.

Some pediatric patients with COVID-19 have developed Kawasaki disease (KD), a TGF-β mediated systemic immunoglobulin A (IgA) vasculitis causing cardiac complications, including acute myocardial infarction, myocarditis, left ventricular dysfunction, pericardial effusion and mitral valve insufficiency as well as a multi-system inflammatory syndrome [46-50]. Reminiscent of the cytokine profiles of COVID-19 patients with severe disease, acute KD is characterized by high levels of IL-6, TNF-α, and TGF-β [48]. Based on the aforementioned role of TGF-β in KD and the ability of RJX to suppress the production of IL-6, TNF-α, and TGF-β, we hypothesize that RJX will emerge as a clinically useful adjunct to the IV immunoglobulin therapy and best supportive care in pediatric COVID-19 patients who develop KD.

Hydroxyl radicals are produced from superoxide anion radicals and/or hydrogen peroxide. Both superoxide radicals and hydroxyl radicals can individually act on lipid membranes to promote the formation of lipid radicals, which in the presence of oxygen are capable of generating lipid peroxy radicals that can cause damaging lipid peroxidation of cell membranes [51-53]. Lipid peroxides contribute to liver injury by increasing production of TNF-α, increasing the influx of inflammatory cells and neutrophils, impairing DNA and protein synthesis, and depleting protective cellular antioxidants such as glutathione. SOD, CAT, and GSH-Px are three pivotal anti-oxidant defense enzymes and their levels are altered by the level of oxidative stress [51-53]. SOD converts superoxide anion radicals into hydrogen peroxide and oxygen, whereas CAT catalyzes the production of water and oxygen from hydrogen peroxide. CAT is absent in the mitochondria, hence the reduction of H_2O_2 to water the reduction of lipid peroxides to their corresponding alcohols is carried out by GSH-Px instead. GSH-Px plays a more crucial role in inhibiting the lipid peroxidation process and therefore is essential for protecting cells from oxidative stress [52]. In accordance with the published role of oxidative stress in LPS-GalN induced lung and liver injury [52], the lung/liver MDA levels, reflecting increased lipid peroxidation, were markedly elevated, and the levels of the antioxidant enzymes SOD, CAT, GSH-Px, as well as ascorbic acid were reduced in LPS-GalN treated mice consistent with severe oxidative stress. RJX suppressed the levels of pro-inflammatory cytokines in the lung/liver, decreased the lung/liver MDA levels, and increased in a dose-dependent manner the reduced levels of the antioxidant enzymes SOD, CAT, GSH-Px and ascorbic acid. These results establish RJX as a potent antioxidant with strong anti-inflammatory activity. A Phase

II study is designed to obtain the clinical proof of concept that RJX could favorably alter the clinical course and reduce the mortality rate of high-risk COVID-19 [54].

Rejuveinix (RJX) [9] is a formulation of several vitamins, including ascorbic acid (Vitamin C), cyanocobalamin (Vitamin B12), thiamine hydrochloride (Vitamin B1), riboflavin 5' phosphate (Vitamin B2), niacinamide (Vitamin B3), pyridoxine hydrochloride (Vitamin B6), calcium D-pantothenate, and magnesium sulphate as a potent calcium antagonist, representing components with reported, albeit controversial, protective activity in animal models of septic shock and ARDS as well as some of the clinical studies in septic patients [10]. Increased lactate levels contribute to the enhanced mortality of septic patients [18]. Two of the RJX ingredients, thiamine [20] and magnesium sulfate [17], accelerate the lactate clearance, which has been shown to improve survival outcomes [19]. While some preclinical, translational, early-stage, as well as late-stage clinical studies, have yielded promising positive data regarding the clinical impact potential of ascorbic acid, thiamine, riboflavin, niacinamide, and pyridoxine (Vit. B6) in the prevention and treatment of cytokine storm, CRS, coagulopathy, ALI, acute kidney injury (AKI), ARDS, and MODS in the context of sepsis [11-17], other studies have not shown any meaningful activity [55, 56]. For example, Moskowitz et al. recently reported the results of a 205-patient, randomized blinded, multicenter study of ascorbic acid, thiamine and steroids in patients with septic shock that was performed at 14 centers in the U.S. Patients were randomly assigned to receive parenteral ascorbic acid (1500 mg), hydrocortisone (50 mg), and thiamine (100 mg) every 6 hours for 4 days (n = 103) or placebo in matching volumes at the same time points (n = 102) (ClinicalTrials.gov Identifier: NCT03389555) [55]. The primary endpoint was change in the Sequential Organ Failure Assessment (SOFA) score (range, 0-24; 0 = best) between enrollment and 72 hours. There was no statistically significant interaction between time and treatment group with regard to SOFA score over the 72 hours after enrollment. Hence, the combination of ascorbic acid, corticosteroids, and thiamine, compared with placebo, did not result in a statistically significant reduction in SOFA score during the first 72 hours after enrollment. Similarly, Fujii et al. reported that treatment with intravenous vitamin C, hydrocortisone, and thiamine does not lead to a more rapid resolution of septic shock compared with intravenous hydrocortisone alone based on the results of a randomized trial in 216 patients with septic shock ((ClinicalTrials.gov Identifier: NCT03333278) [56]. In contrast to these studies, Iglesias et al reported that the combination of IV ascorbic acid, thiamine, and hydrocortisone significantly reduced the time to resolution of shock based on another randomized study ((ClinicalTrials.gov Identifier: NCT03422159) [15]. Likewise, Byerly et al. reported that ascorbic acid plus thiamine is associated with increased survival in septic ICU patients based on the results of 11,330 patients with sepsis and elevated lactate levels regarding the impact of thiamine and ascorbic acid on survival outcome [57]. After controlling for confounding factors, ascorbic acid (adjusted odds ratio [AOR], 0.69 [0.50-0.95]) and thiamine (AOR, 0.71 [0.55-0.93]) were

independently associated with survival [57]. Additional studies are needed to confirm these findings and assess any potential benefit from this treatment. RJX has no steroids and it contains niacinamide, pyridoxine, cyanocobalamine and Mg-sulfate in addition to ascorbic acid and thiamine. The clinical potential of RJX will be examined in COVID-19 patients under IND149585 with an emphasis on prevention of ARDS and multiorgan failure in COVID-19 patients at high risk for fatal viral sepsis rather than treatment of septic shock.

The selection of the tentative Phase 2 dose level was not based on a validated pharmacodynamic marker for the COVID-19 indication, but rather based on its safety profile in the Phase 1 clinical study in healthy volunteers (Protocol No. RPI003; ClinicalTrials.gov Identifier: NCT03680105) with no evidence of Grade 3 or 4 AEs, no SAEs and no deaths observed at this dose level. The dose level selected for the COVID-19 study is >1.6-fold lower than the NOAEL in rats and >5.4-fold lower than the NOAEL in dogs, and it is one dose level lower than the highest dose tested in the Phase 1 study. In the projected Phase 1 - 2 clinical study for COVID-19 patients, an open label Safety Lead in (Part 1) will be included aimed at further assessing its tolerability and safety in COVID-19 patients. The oxidative stress in peripheral blood mononuclear cells obtained from consenting COVID-19 patients before and after RJX infusion will be evaluated using flow cytometry with the standard DCF staining method. The effects of RJX on inflammatory biomarkers will also be evaluated within the confines of an open label Phase 1 study. The efficacy of RJX will be determined in the randomized, double blind, placebo-controlled Part 2 of the study following the review of the part 1 data by an independent Date and Safety Monitoring Board.

Disease progression from mild-moderate to severe-critical was systematically analyzed by Bi et al. [23]. Among the 417 patients who were classified as mild or moderate at the time of initial assessment, 21.6% (90/417) progressed to the severe stage. Those who progressed to the severe stage progressed within on average 9.5 days (95% confidence interval [CI]: 8.7–10.3) after symptom onset. They developed ARDS on average 11.0 days (95% CI: 9.7–12.3) after symptom onset. Patients with certain co-morbidities such as diabetes and hypertension at baseline and/or laboratory parameters such as high concentration of CRP, LDH, and high concentration of D-dimer formed a higher risk subgroup with a higher likelihood of progression to ARDS. In this high-risk group, Bi et al. estimated that 43% (95%CI: 35–52%) cases (i.e., twice as many as the 21.6% for the total population of mild-moderate patients) became severe and 9% (95%CI: 4–14%) required ICU admission within 14 days from symptom onset [23]. More information has recently become available regarding the patient characteristics, disease progression, and clinical outcomes for the COVID-19 patient population in the U.S. Gold et al. [58] reported the results on 305 patients hospitalized in Georgia in the month of March 2020 [0]. 37.6% of the 117 patients in the high-risk age category ≥65 years required high-flow oxygen, or noninvasive ventilation (NIV), whereas 41.0% developed ARDS and required mechanical ventilation (MV). By comparison, 25.6% of the total

population required high-flow oxygen or NIV, and 30.6% developed ARDS. In summary, approximately 20% of COVID-19 patients with mild-moderate disease progress to severe-critical disease and this percentage is 40% for the high-risk subgroup ≥65 years of age with co-morbidities or laboratory parameters indicative of systemic inflammation such as high levels of CRP, LDH, and ferritin or a dysfunction of the coagulation system as evidenced by elevated D-dimer levels [8]. We hypothesize that RJX treatments will reduce the progression for the high-risk subgroup in Cohort 1 from 40% down to 20%.

Our preliminary data in the mouse model of LPS-GalN induced sepsis, ARDS, and multi-organ failure indicate that RJX also shows potential for treatment of COVID-19 with evolving viral sepsis. Cohort 2 of the proposed COVID-19 study will be hospitalized COVID-19 patients with hypoxemia without ARDS receiving either NIPPV or high-flow oxygen. In the randomized Part 2 of the study, these Cohort 2 patients will be treated with Placebo + Standard of care or RJX + Standard of care. We hypothesize that RJX will contribute to a faster resolution of the respiratory failure and a reduced case mortality rate.

5. Conclusions

RJX could favorably affect the clinical course of high-risk COVID-19 and reduce its case mortality rate. RJX is anticipated to shorten the time to resolution of ARDS by eliminating the contributions pro-inflammatory cytokines IL-6, TNF-α and TGF-β to the systemic and pulmonary inflammatory process.

Supplementary Materials: The following are available online: Supplementary Methods, Supplementary Results, Tables S1-Table S23; Figures S1-S10

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Conflicts of Interest: F.M.U., J.C., J.P., N.P., H.V-W., and M.V are employees of Reven Pharmaceuticals. C.O., I.H.O., and K.S declare no current competing financial interests.

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FIGURES

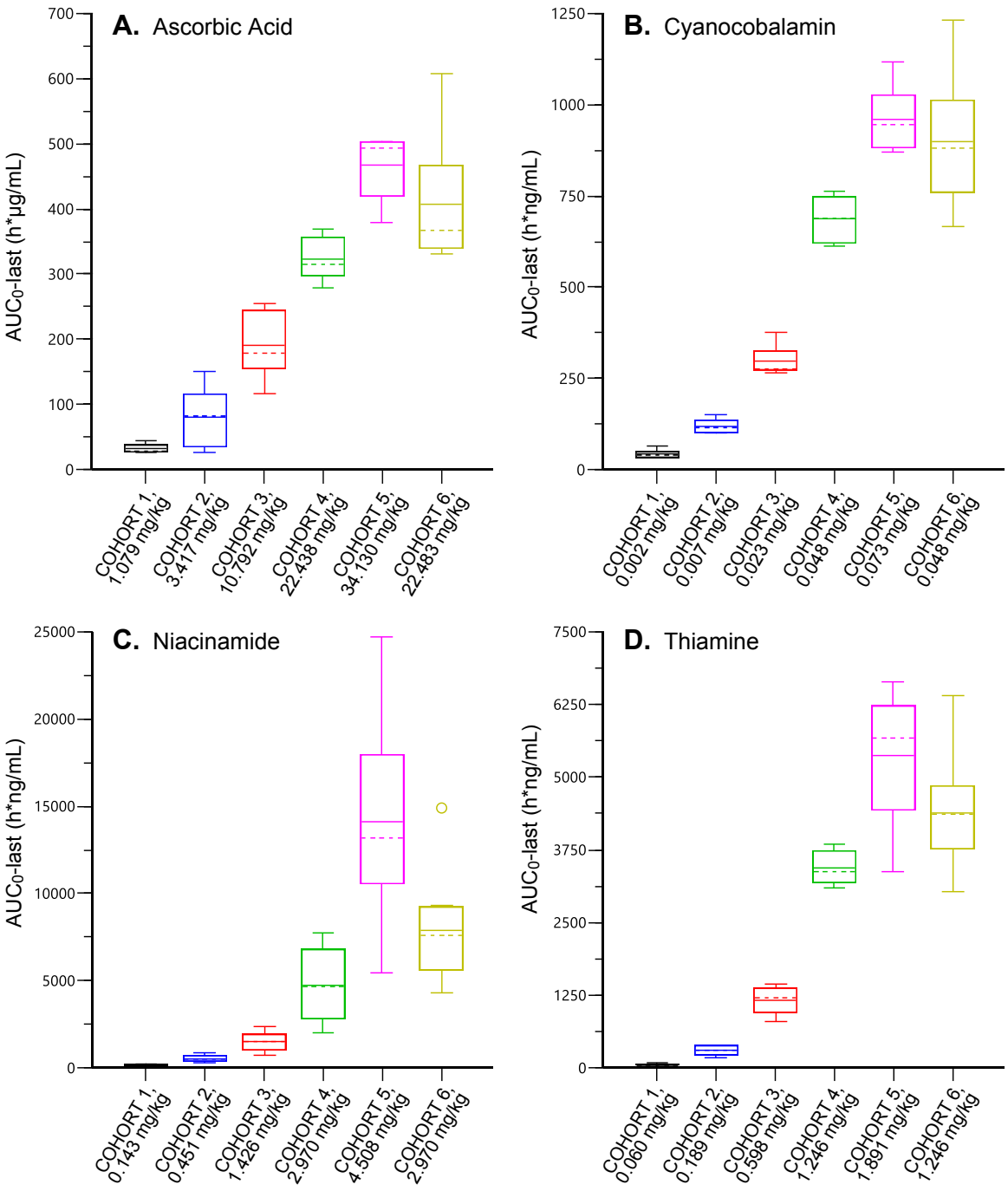
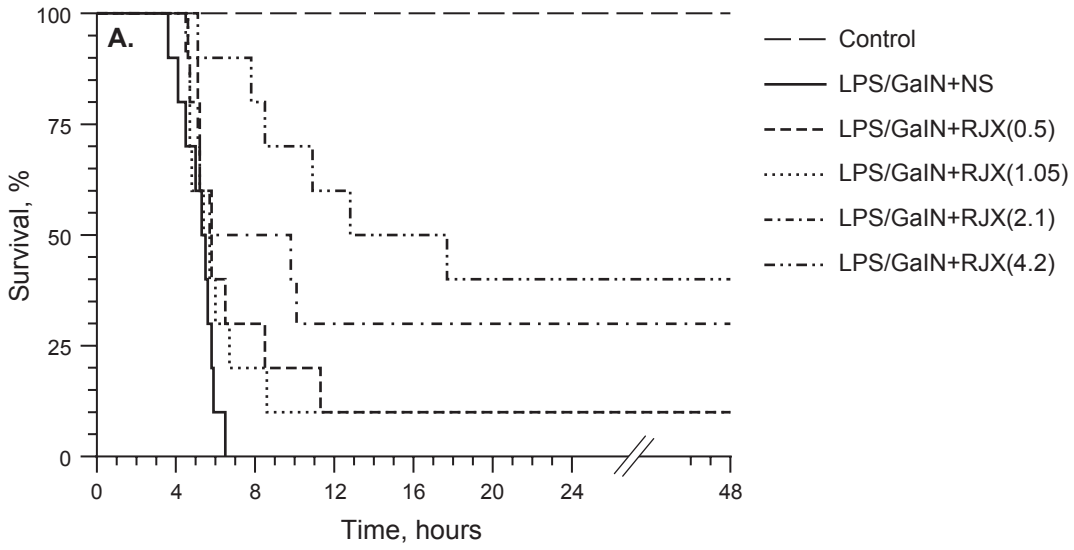


Figure 1. Plasma Pharmacokinetic Parameters for Ascorbic Acid, Cyanocobalamin, Niacinamide and Thiamine after Single Ascending Dose of IV Infusion of RJX in Healthy Adult and Elderly Volunteers. See text for discussion.



B. Survival data

Groups	Censored	Events	N	Proportion	Median
Untreated Control	10	0	10	0.0 %	-
NS +LPS/GalN	0	10	10	100.0 %	5.40
RJX (0.5) +LPS/GalN	1	9	10	90.0 %	5.80
RJX (1.05) +LPS/GalN	1	9	10	90.0 %	5.55
RJX (2.1) +LPS/GalN	3	7	10	70.0 %	7.75
RJX (4.2) + LPS/GalN	4	6	10	60.0 %	15.25

C. Log-rank tests

Groups being compared		v	SE	z	P
Control	NS + LPS/GalN	6.688	1.43	4.673	< 0.001
Control	RJX (0.5)+LPS/GalN	5.704	1.39	4.114	< 0.001
Control	RJX (1.05)+LPS/GalN	5.749	1.39	4.128	< 0.001
Control	RJX (2.1)+LPS/GalN	4.176	1.28	3.252	0.001
Control	RJX (4.2)+LPS/GalN	3.462	1.20	2.880	0.004
NS+LPS/GalN	RJX (0.5)+LPS/GalN	-3.388	1.90	-1.779	0.075
NS+LPS/GalN	RJX (1.05)+LPS/GalN	-2.771	2.00	-1.387	0.165
NS+LPS/GalN	RJX (2.1)+LPS/GalN	-3.828	1.87	-2.049	0.040
NS+LPS/GalN	RJX (4.2)+LPS/GalN	-6.198	1.53	-4.059	<0.001

Figure 2. Dose-Dependent In Vivo Protective Activity of Prophylactic RJX Treatments in the LPS-GalN Model of ARDS and Multiorgan Failure. Groups of 10 BALB/C mice were treated with i.p injections of RJX (0.5, 1.05, 2.1, 4.2 mL/kg, 0.5 ml/mouse) or vehicle (NS) 2 hours before or 2 hours 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) i.p. The cumulative proportion of mice remaining alive (Survival, %) is shown as a function of time after the LPS-GalN challenge. Depicted are the Kaplan Meier survival curves (Panel A) and survival data with statistical analysis (Panels B and C) of the different treatment groups. The RJX dose levels (in ml/kg) are indicated in parentheses.

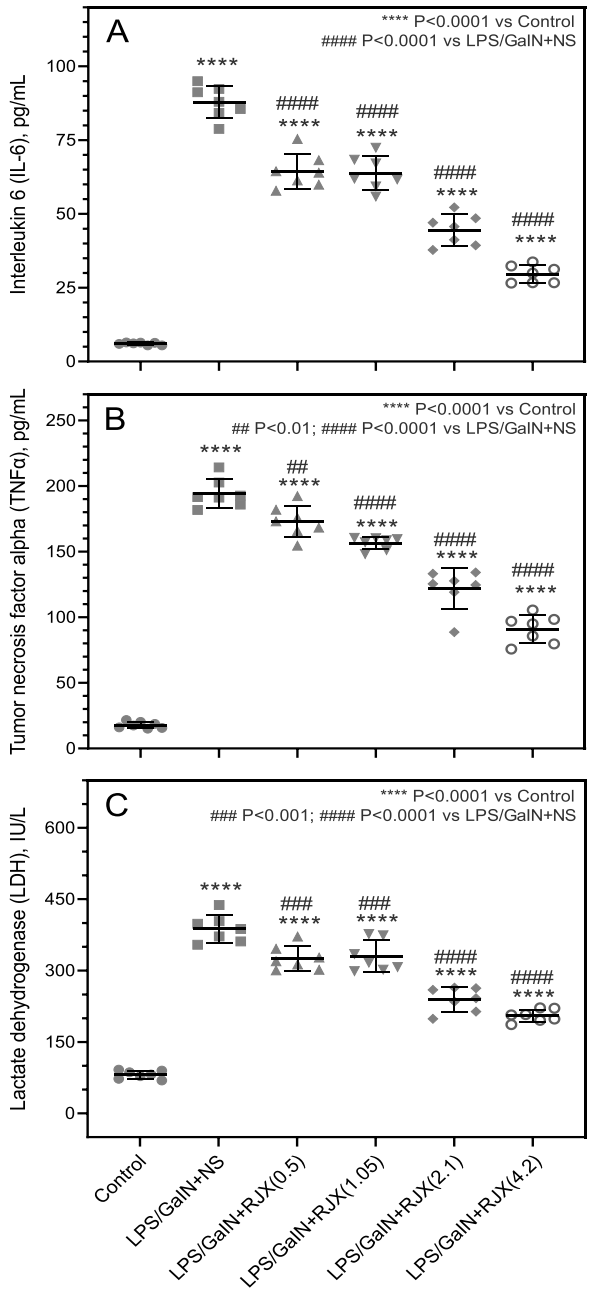


Figure 3. Rejuveinix (RJX) Prevents the Surge of Inflammatory Markers in the Blood Following LPS-GalN Challenge in a Mouse Model of ARDS and Multi-organ Failure. Mice were treated with i.p injections of RJX (0.5, 1.05, 2.1, 4.2 mL/kg, 0.5 ml/mouse) or NS 2 hours before and 2 hours post-injection of LPS-GalN. Except for untreated mice (Control), each mouse received 0.5 ml of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) i.p. The RJX dose levels (in ml/kg) are indicated in parentheses. See text for discussion of results. The depicted lines represent the mean and standard deviation for the indicated inflammatory markers, namely serum IL-6, TNF-α and LDH levels. ANOVA and Tukey's post-hoc test were used for comparing the results among different treatment groups. Statistical significance between groups is shown by: **** $P < 0.0001$ compared as control group and, # $P < 0.05$; ## $P < 0.01$; #### $P < 0.0001$ compared as LPS/GalN+NS group).

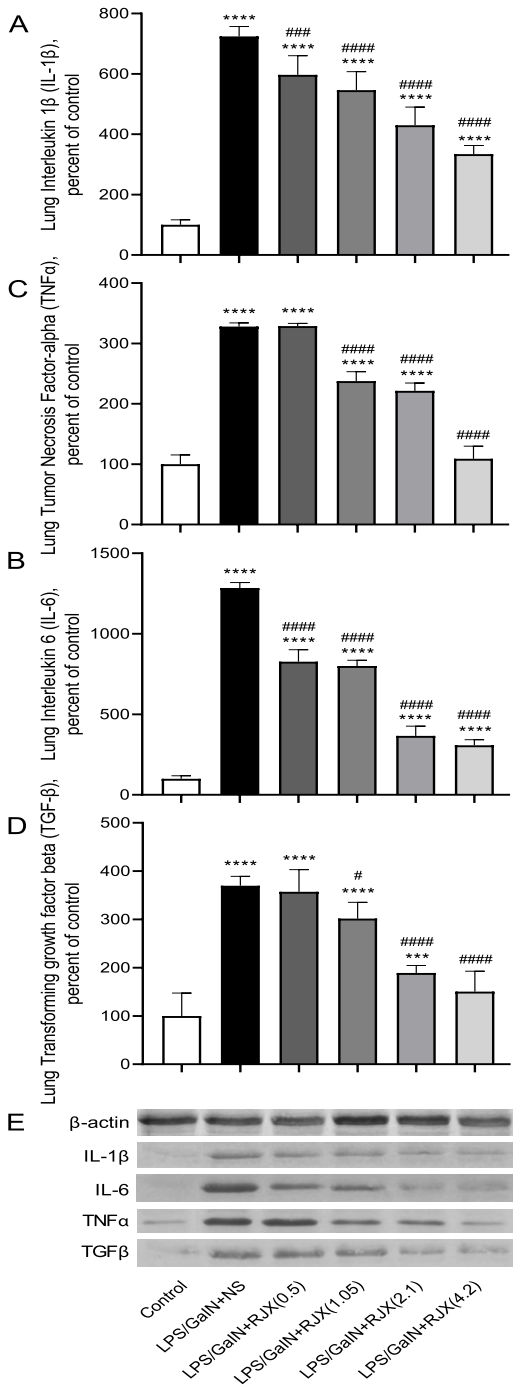


Figure 4. Rejuveinix (RJX) Prevents Pro-inflammatory Cytokine Response in the Lungs of Mice Challenged with LPS-GalN. Mice were treated with i.p injections of RJX (0.5, 1.05, 2.1, 4.2 mL/kg, 0.5 ml/mouse) or NS 2 hours before and 2 hours post-injection of LPS-GalN. Except for untreated mice (Control), each mouse received 0.5 ml of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) i.p. The RJX dose levels (in ml/kg) are indicated in parentheses. See text for discussion of results. Depicted are the results of Western blot analyses of cytokine expression in the pooled lung tissue samples from mice in various treatment groups. Results are expressed as percent of control with the expression level in the lung tissue sample from untreated control mice taken as 100% for comparisons. The bar represents mean and standard deviation. Immunoblotting with an anti-actin antibody was used to ensure equal protein loading. (ANOVA and Tukey's post-hoc test. Statistical significance between groups is shown by: *** $P < 0.001$; **** $P < 0.0001$ compared as control group and, # $P < 0.05$; ### $P < 0.001$; #### $P < 0.0001$ compared as LPS/GalN+NS group).

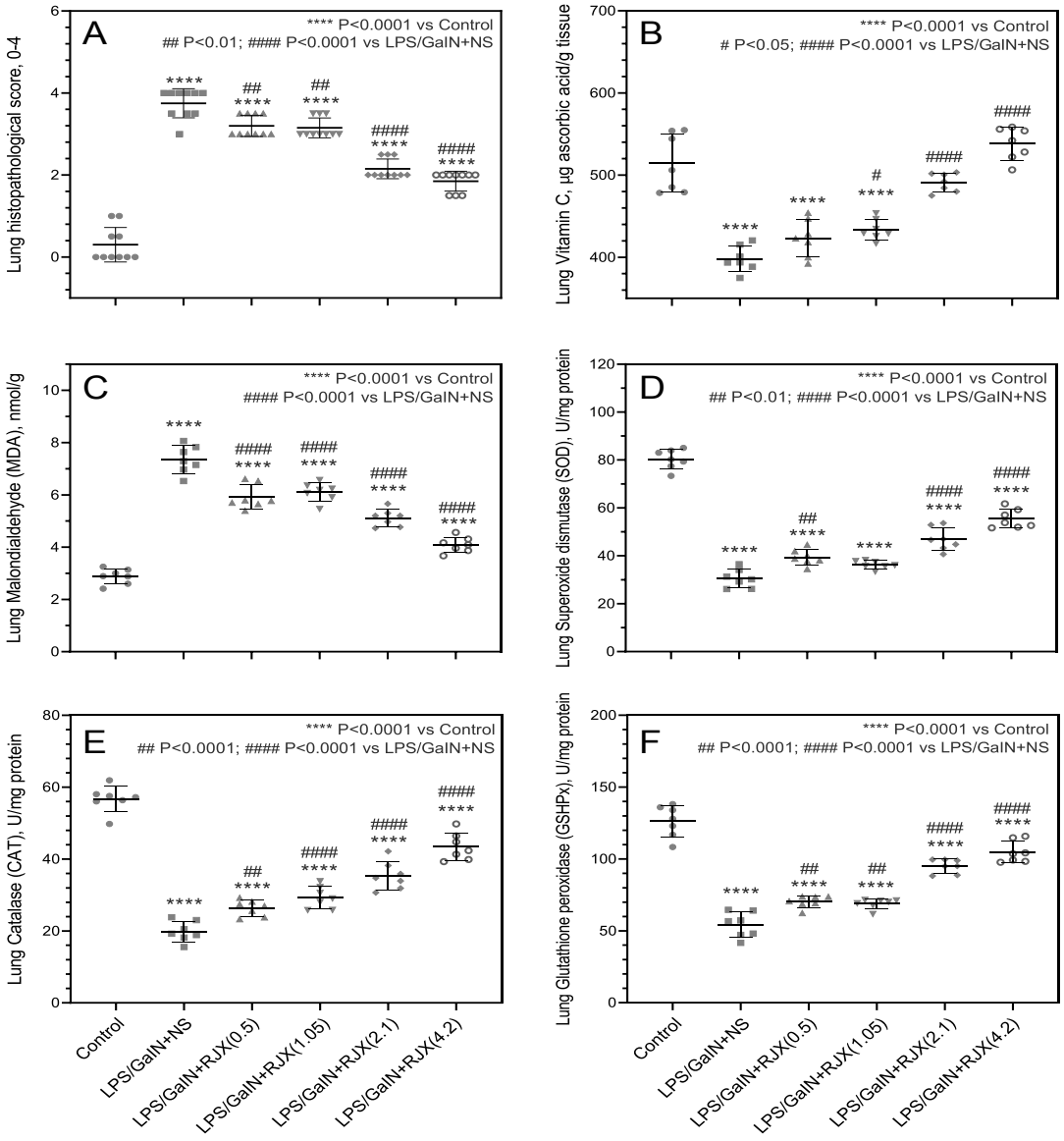


Figure 5. Tissue-Level In Vivo Anti-Inflammatory and Anti-Oxidant Activity of Rejuveinix (RJX) in the LPS-GalN Mouse Model of ARDS and Multi-organ Failure. Mice were treated with i.p injections of RJX (0.5, 1.05, 2.1, 4.2 mL/kg, 0.5 ml/mouse) or NS 2 hours before and 2 hours post-injection of LPS-GalN. Except for untreated mice (Control), each mouse received 0.5 ml of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) i.p. The RJX dose levels (in ml/kg) are indicated in parentheses. See text for discussion of results. These assays were performed on the lungs of mice that died after the LPS-GalN challenge. The lungs of the mice were harvested at the time of death which occurred within 24 hours for all mice. The depicted lines represent the mean and standard deviation for the indicated parameters. In [A], the score was graded according to a 5-point scale from 0 to 4 as follows: 0, I, 2, 3 and 4 represented no damage, mild damage, moderate damage, severe damage and very severe damage, respectively. Statistical significance between groups is shown by: **** P < 0.0001 compared as control group and, ## P < 0.01; #### P < 0.0001 compared as LPS/GalN+NS group, Kruskal-Wallis test and Mann Whitney test. In [B]-[F], ANOVA 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 compared as control group and, # P < 0.05; ## P < 0.01; #### P < 0.0001 compared as LPS/GalN+NS group).

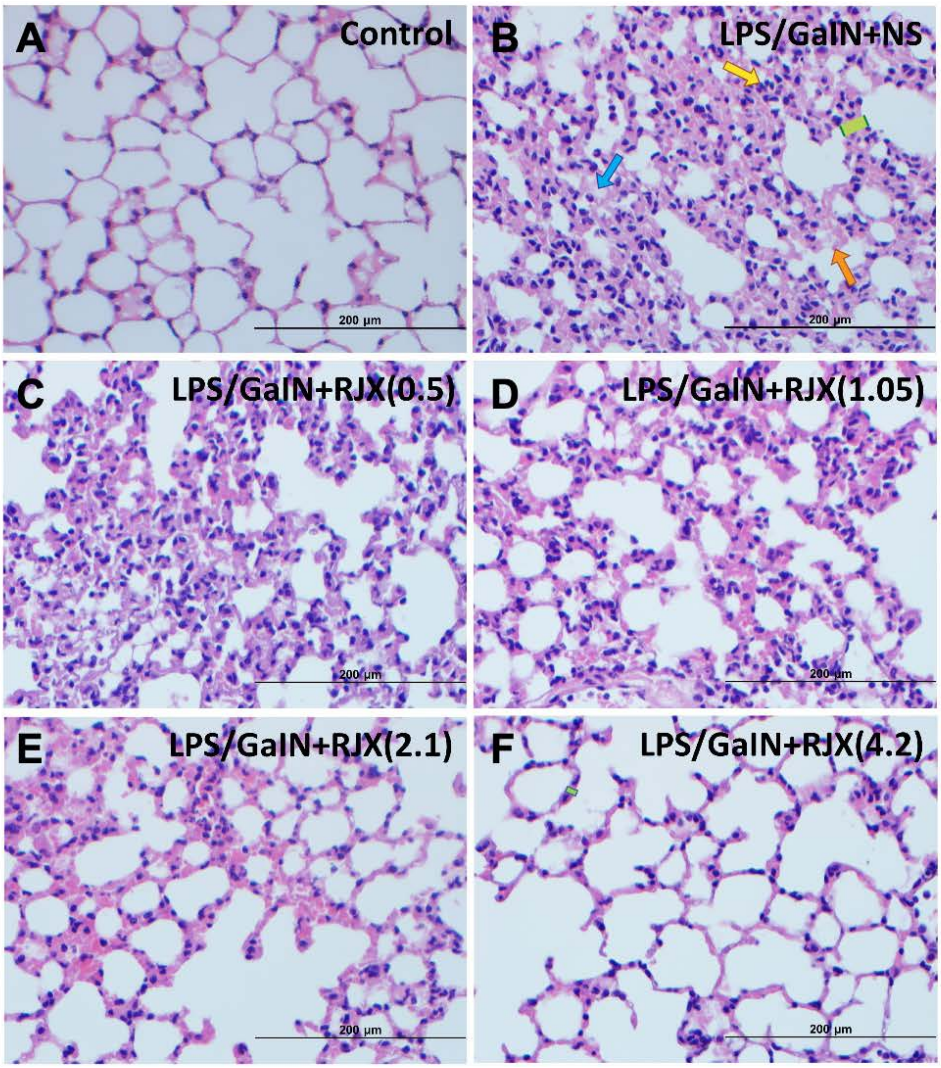


Figure 6. Rejuveinix (RJX) Prevents Acute Lung Injury and Inflammation in the LPS-GalN Mouse Model of ARDS and Multi-organ Failure. Mice were treated with i.p injections of RJX (0.5, 1.05, 2.1, 4.2 mL/kg, 0.5 ml/mouse) or NS 2 hours before and 2 hours post-injection of LPS-GalN. Except for untreated mice (Control), each mouse received 0.5 ml of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) i.p. The RJX dose levels (in ml/kg) are indicated in parentheses. Yellow arrow: inflammatory cell infiltration; Blue arrow: Exudate; Orange arrow: hemorrhage; Green block: thickness of alveolar wall. Panel B: Blue arrow: Exudate (see B1 of Figure S7 for an enlarged inset); Yellow arrow: inflammatory cell infiltration (see B2 of Figure S7 for an enlarged inset); Green block: thickness of alveolar wall (see B3 of Figure S7 for an enlarged inset). Orange arrow: hemorrhage (see B4 of Supplemental Figure S7 for an enlarged inset); H&E X400

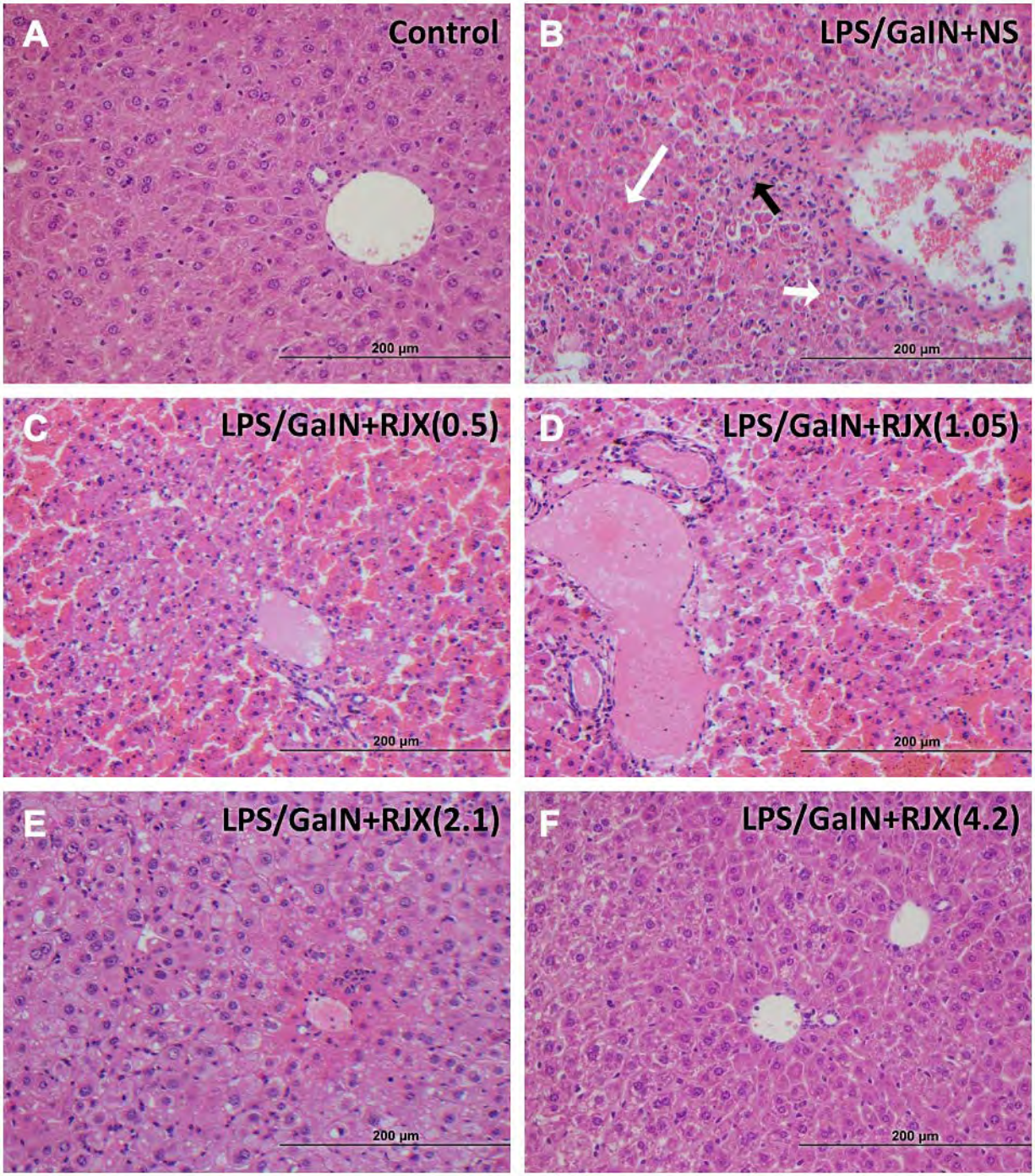


Figure 7. Rejuveinix (RJX) Prevents Acute Liver Injury and Inflammation in the LPS-GalN Mouse Model of ARDS and Multi-organ Failure. Mice were treated with i.p injections of RJX (0.5, 1.05, 2.1, 4.2 mL/kg, 0.5 ml/mouse) or NS 2 hours before and 2 hours post-injection of LPS-GalN. Except for untreated mice (Control), each mouse received 0.5 ml of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) i.p. The RJX dose levels (in ml/kg) are indicated in parentheses. Panel B: White arrow (long): necrosis (see B1 of Figure S11 for an enlarged inset); Black arrow: inflammatory cell infiltration (see B2 of Figure S11 for an enlarged inset); White arrow (short): hemorrhage (see B3 of Figure S11 for an enlarged inset). See text for a discussion of the findings. H&E 400x

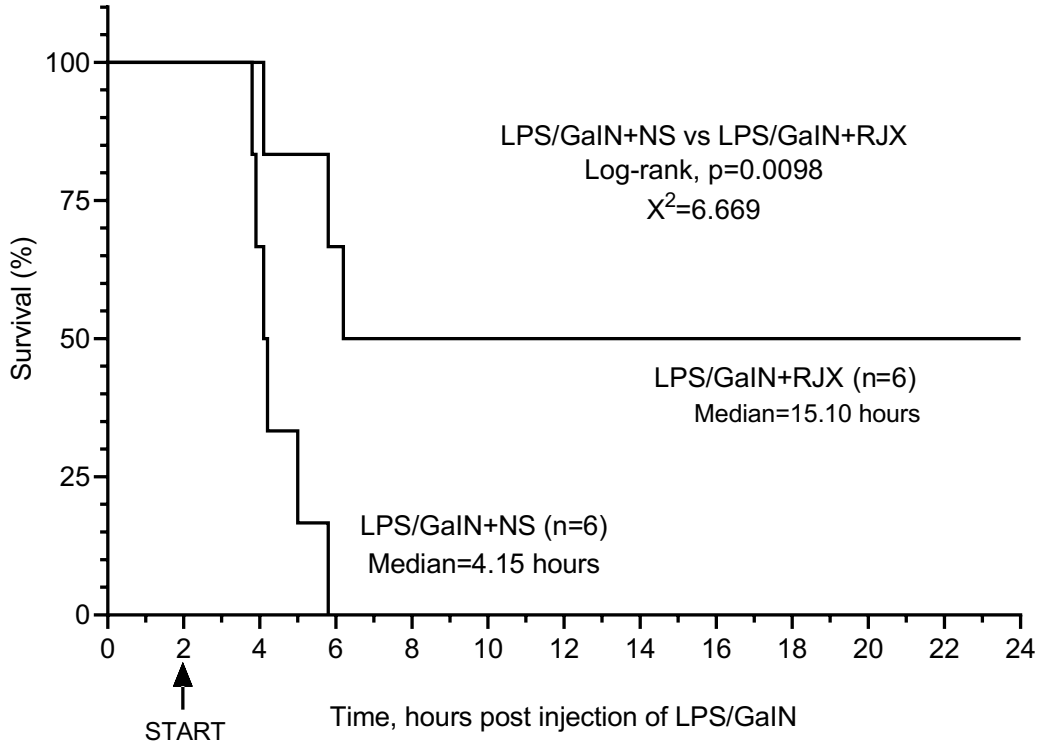


Figure 8. In Vivo Protective Activity of Delayed-Onset RJX Treatments in the LPS-GaIN Model of ARDS and Multiorgan Failure. Groups of 6 BALB/C mice were treated with i.p injections of 6-fold diluted RJX (4.2 mL/kg, 0.5 ml/mouse) or vehicle (NS) 2 and 3 hours post-injection of LPS-GaIN. Each mouse received 0.5 ml of LPS-GaIN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) i.p. Percent (%) survival for each treatment group is shown as a function of time after the LPS-GaIN challenge. Depicted are the survival curves for each group along with the median survival times and the log-rank P-value for the comparison of LPS-GaIN+RJX group versus the LPS/GaIN+NS group.