CLINICAL STUDY PROTOCOL

Title: Phase 2, proof-of-concept, randomized, double-blinded, placebo-controlled, multicenter study to assess efficacy and safety of reparixin as add-on therapy to standard of care in adult patients with Acute Respiratory Distress Syndrome (RESPIRATIO)

Short title: Add-on reparixin in adult patients with ARDS

Study Number: REP0122

IND/EudraCT number: 161827 / 2022-001612-25

Investigational Product: Reparixin

Phase of the study: Phase 2 proof-of-concept

Protocol Version, Date: Version 3.0, February 8, 2024

Amendment number: Substantial Amendment # 2, Global

STUDY CONTACT INFORMATION

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COORDINATING INVESTIGATORS AND INVESTIGATIONAL SITES

Full list of coordinating investigators and investigational sites for each country involved will be kept in the Trial Master File. Updated versions will be filed chronologically. Copies will be provided to the sites.

AMENDMENT DETAILS

History of Amendments

Document	**Sponsor Approval Date** (dd/mmm/yyyy)	**Approximate {(#/%)} Enrolled**
Amendment #2 – Protocol version 3,0	08 FEB 2023	66, 100%
Amendment #1 – Protocol version 2.0	15 SEP 2022	20, 30%
Original Protocol version 1.0	26 APR 2022	0, 0%

Table of Contents

List of Abbreviations and Definitions of Terms

ABG	Arterial Blood Gas analysis
ADR	Adverse Drug Reaction
AE	Adverse Event
ALI	Acute Lung Injury
ALT	Alanine Aminotransferase
ARDS	Acute Respiratory Distress Syndrome
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BAL	Broncho-Alveolar Lavage
CBC	Complete Blood Count
CDE	Cat Dander Extract
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
Cmax	Maximum concentration
COVID	CoronaVirus Disease
CPAP	Continuous Positive Airway Pressure
CRO	Contract Research Organization
CRP	C-Reactive Protein
CSR	Clinical Study Report
Css	Concentration, steady state
CT	Computerized Tomography
CXR	Chest X-Rays
CYP	Cytochrome P450
DMC	Data Monitoring Committee
DMP	Data Management Plan
ECG	Electrocardiogram
ECMO	Extracorporeal Membrane Oxygenation
eCRF	electronic Case Record Form
EDC	Electronic Data Capture
eGFR	Estimated Glomerular Filtration Rate
FAS	Full Analysis Set
FDA	Food and Drug Administration
FIO_2	Fraction of inspiration oxygen
GCP	Good Clinical Practice
HR	Heart Rate
ICAM-1	Intercellular Adhesion Molecule-1
GI	Gastrointestinal
ICF	Informed Consent Form
ICU	Intensive Care Unit
IEC	Independent Ethics Committee
IL	Interleukin
IMP	Investigational Medicinal Product
IMV	Invasive Mechanical Ventilation
IRB	Institutional Review Board
IRS	Interactive Response System
ITT	Intention to Treat
IUS	Intrauterine hormone-releasing system
IV	Intravenous
KM	Kaplan-Meier
LD50	Lethal Dose 50%
LDH	Lactate Dehydrogenase
LR	Legal Representative
LTAC	Long Term Acute Care
MAP	Mean Arterial Pressure
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram (s)
ml	Milliliter (s)
mcg	Microgram (s)
ng	Nanogram (s)

NET	Neutrophils Extracellular Traps
NSAIDs	Non Steroidal Anti Inflammatory Drugs
OI	Oxygenation index
PaO_2	Partial pressure of oxygen
PaCO2	Partial pressure of carbon dioxide
PAI-1	Plasminogen Activator Inhibitor-1
PCR	Polymerase Chain Reaction
PEEP	Positive End Expiratory Pressure
PI	Principal Investigator
PIP	Peak Inspiratory Pressure
pg	Picogram (s)
PK	Pharmacokinetic
PMN	Polymorphonuclear cell (s)
PP	Per Protocol
RAGE	Receptor for Advanced Glycation End products
RALE	Radiographic Assessment of Lung Edema
RR	Respiratory Rate
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction
SAE	Serious Adverse Event
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SOFA	Sequential Organ Failure Assessment
SpO_2	Peripheral Capillary Oxygen Saturation
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment Emergent Adverse Event
TESAE	Treatment Emergent Serious Adverse Event
TID	Three times a day
TNFr1	Tumor Necrosis Factor receptor 1
TV	Tidal Volume
ULN	Upper Limit of Normal
VFDs	Ventilatory-free days

1. STUDY SYNOPSIS

CLINICAL STUDY SYNOPSIS:	
Study Code	REP0122
Title of Study	Phase 2, proof-of-concept, randomized, double-blinded, placebo-controlled, multicenter study to assess efficacy and safety of reparixin as add-on therapy to standard of care in adult patients with Acute Respiratory Distress Syndrome (RESPIRATIO)
Short title	Reparixin in Acute Respiratory Distress Syndrome (ARDS)
IND/EudraCT No.	161827/2022-001612-25
Study Centres (Country)	US, Germany, Italy
Development Phase	Phase 2, proof-of concept study
Objectives	1. To characterize the efficacy of reparixin in ameliorating lung injury and systemic inflammation and expediting clinical recovery and liberation from mechanical ventilation in adult patients with moderate to severe ARDS (PaO2/FIO2 ratio ≤ 200). Furthermore, to assess the effect of reparixin on systemic biomarkers linked to a hyper-inflammatory ARDS phenotype. 2. CCI CCI 3. To evaluate the safety of reparixin vs. placebo in patients enrolled in the study.
Design and Methodology	Phase 2, proof-of-concept, randomized, double-blinded, placebo-controlled, multicenter study. All patients will receive therapy in line with current standard-of-care as it pertains to ARDS management (protocolized ventilator management will be made available to all sites in accordance with currently accepted standard of care). Patients will be randomized (1:1) to either reparixin or placebo. Duration of treatment will be 14 days with the option to extend to 21 days if the patient is still intubated on Day 14.
Study procedures	The study will consist of 4 study periods: **Screening** **Randomization and Baseline assessments** **Treatment** (14 days, with the option of extension up to 21 days if the patient is still intubated on Day 14) **Follow-up** (up to 28 days and hospital discharge if later than 28 days; and then up to day 60). **Screening**: Potential study patients with confirmed ARDS will be identified from those hospitalized at the participating clinical sites for disease management. Screening will be performed in consented patients for assessment of eligibility and will include: • Demographic data, past medical history, allergies to medications, disease-specific clinical information • Concomitant medications • History of side effects related to prior use of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) such as gastrointestinal (GI) bleeding or other form of bleeding, peptic ulcer disease • Blood sampling for measurement of hematology/biochemistry (Safety Laboratory Tests) and derived renal [Estimated Glomerular Filtration rate (eGFR) evaluation by 2021 CKD-EPI] and hepatic function [ALT/AST and bilirubin determination] (unless already collected for clinical care) • Pregnancy test (urine test is acceptable) in women of childbearing

	potential; positive urine test results will be confirmed with a serum pregnancy test ● 12-lead ECG performed using local equipment (unless already collected for clinical care) ● PaO_2 and FIO_2 ● Chest imaging [either chest x-ray (CXR) or computerized tomography (CT) scan] **Randomization and Baseline assessments**: Patients successfully completing the screening process and confirmed eligible for the study will be randomized to either reparixin or placebo within 24 hours and as soon as possible from screening assessment. They then will proceed to the following baseline assessments, before start of treatment (day 1): ● PaO_2, $PaCO_2$ and FIO_2 ● Vital signs: respiratory rate (RR), heart rate (HR), mean arterial pressure (MAP) ● Invasive mechanical ventilation (IMV) parameters [tidal volume (TV), positive end expiratory pressure (PEEP), mean airway pressure, plateau airway pressure) ● Use of neuromuscular blocking agents and inhaled vasodilators ● Prone positioning ● Vasoactive medications (drug and dose calculated in norepinephrine equivalents) ● Sequential organ failure assessment (SOFA) score ● Blood sampling for measurement of IL-6, IL-8, PAI-1, TNFr-1, ICAM-1, RAGE ● CXR (screening values, if any, will be considered if screening was within 24 hours) **Treatment and post-treatment hospital stay assessments**: After the baseline assessments have been completed, patients will start receiving the IMP. The following data will be collected daily from day 1 until extubation: ● PaO_2, $PaCO_2$ and FIO_2 ● Vital signs: RR, HR, MAP ● IMV parameters (TV, PEEP, mean airway pressure, plateau airway pressure) ● Use of neuromuscular blocking agents and inhaled vasodilators ● Prone positioning ● Transition to Extracorporeal membrane oxygenation (ECMO) ● Vasoactive medications (drug and dose calculated in norepinephrine equivalents) ● SOFA score ● Number of attempts at weaning defined as continuous positive airway pressure (CPAP) trial (CPAP 5 cmH2O plus PEEP 5 cmH2O) of 2 hours duration If an initial extubation is followed by reintubation, the above parameters will be collected daily for each subsequent re-intubation period. At days 2(±8h), 3(±8h), 7±1 and 14±2 a CXR will be performed [CXR performed for routine clinical care in intensive care unit (ICU) patients will be accepted].

	On days 3(±8h), 7±1, 14±2 blood samples will be obtained for measurement of the following plasma biomarkers: IL-6, IL-8, PAI-1, TNFr-1, ICAM-1, RAGE Between extubation and day 28 or hospital discharge (if it occurs before day 28) the following data will be collected every 48±8 hours: • Vital signs: RR, HR, MAP • PaO_2 (or SpO_2) and FIO_2 • Type of oxygen supportive therapies used, if any • Need for reintubation (after initial extubation) CCI CCI CCI At day 28 and at hospital discharge, the following data will be collected: • PaO_2 (or SpO_2) and FIO_2 • Type of oxygen supportive therapies used, if any • Duration of IMV • ICU length of stay • Need for reintubation (after initial extubation) • Hospital length of stay • Performance of tracheostomy • Transfer to long term acute care (LTAC) facility **Safety**: The following safety assessments will be performed on day 3(±8h), 7±1, 14±2, 21±2 (if reparixin is still administered on day 21) and at day 28±2 and hospital discharge • Safety Laboratory Tests (Hematology/biochemistry) • Estimated Glomerular Filtration rate (eGFR) evaluation • Hepatic function evaluation (ALT/AST and bilirubin) • 12 lead ECG and rhythm analysis **Post-hospital discharge follow-up**: On day-60, the patients will be contacted by phone for evaluation of vital status, AEs.
Number of patients	60 evaluable patients. Assuming that 10% of enrolled patients will not be evaluable for primary analysis, approximately 66 patients will be enrolled

Diagnosis and Main Criteria for Inclusion/Exclusion	Inclusion criteria 1. Signed Informed Consent, according to local guidelines and regulations. 2. Male and female adults (≥18 years old) 3. Mechanically ventilated (invasive) patients with PaO_2/FIO_2 ratio ≤200 in the presence of PEEP of ≥5 cm H_2O. 4. Respiratory failure not fully explained by cardiac failure or fluid overload (if acute Congestive Heart Failure exacerbation is identified as part of the clinical picture, this should be addressed effectively and as soon as possible before the patient can be enrolled). 5. Bilateral radiologic opacities consistent with pulmonary edema on the frontal chest x-ray (CXR) radiograph, or bilateral ground glass opacities on a chest CT scan. 6. ≤48 hours from fulfilling above ARDS criteria (if a patient is transferred from a non-participating hospital to a participating site, a 12-hour period beyond the 48 hours is allowed) 7. Females of child-bearing potential who are sexually active must be willing not to get pregnant within 30 days after the last Investigational Medicinal Product (IMP) dose and must agree to at least one of the following reliable methods of contraception: • Hormonal contraception, systemic, implantable, transdermal, or injectable contraceptives from at least 2 months before the screening visit until 30 days after the last IMP dose; • A sterile sexual partner; • Abstinence. For patients unable to personally consent to the above, due to complications of acute illness and/or its treatment, assurances for the above must be given by LR and reiterated by the patient when/if she is able to do so. Female participants of non-child-bearing potential or in post-menopausal status for at least 1 year will be admitted. For all female subjects with child-bearing potential, pregnancy test results must be negative before first drug intake.

	Exclusion criteria 1. Moderate-Severe chronic hepatic disease (as verified by a previously known Child-Pugh score ≥7). If baseline Child-Pugh score is not known, it should not be calculated while the patient is acutely ill. In that case, the patient is excluded on the basis of: ALT/AST ≥ 3x ULN and total bilirubin > 2x ULN or ALT/AST ≥ 5x ULN. 2. Severe chronic renal dysfunction: eGFR (2021 CKD-EPI) < 30 mL/min/1.73m^2. If baseline (chronic) renal function is not known, the patient is only excluded if in need of acute renal replacement therapy (currently on RRT or to be imminently placed on RRT). 3. Participation in another interventional clinical trial. 4. Patients that are clinically determined to have a high likelihood of death within the next 24 hours based on PI's estimation. 5. Currently receiving ECMO or high frequency oscillatory ventilation. 6. Anticipated extubation within 24 hours of screening. (In such cases, re-screening is allowed if the patient is within the enrollment window). 7. Evidence of GI dysmotility as demonstrated by presence of all the following: persistent gastric distention and enteral feeding intolerability and persistent gastric residuals >500 ml). 8. Anticipated transfer to a hospital not participating in the trial within 72 hours of screening. 9. Decision to withhold or withdraw life-sustaining treatment (patients may still be eligible however if they are committed to full support except cardiopulmonary resuscitation if cardiac arrest occurs). 10. History of: a) Documented allergy/hypersensitivity to sulfonamides, ibuprofen and other COX-1 and 2 inhibitors, and to the study product and/or its excipients. b) Lactase deficiency, galactosemia or glucose-galactose malabsorption. c) History of peptic ulcer, GI bleeding or perforation due to previous NSAID therapy. 11. Active bleeding (excluding menses) from an uncontrolled site that cannot be definitively resolved prior to enrollment. 12. Pregnant or lactating women. 13. Women of childbearing potential and fertile men who do not agree to use at least one primary form of contraception during the study and up to 30 days after the last IMP dose. For patients unable to personally consent to above due to complications of acute illness and/or its treatment, assurances for the above must be given by LR and reiterated by the patient when/if he/she is able to do so.

Test Product, Dosage and Mode of Administration	Reparixin 600 mg tablets, administered crushed through nasogastric tube at the dose of 1200 mg TID (2 tablets TID) as add-on to the standard of care. After extubation and if the patient can swallow, reparixin may be administered orally
Duration of Treatment	14 days with the option to continue up to 21 days, if the patient is still intubated on Day 14
Reference product, Dosage and Mode of Administration	Placebo tablets. Administered crushed through nasogastric tube with the same schedule as reparixin as add-on to the standard of care. After extubation and if the patient can swallow placebo may be administered orally
Primary efficacy endpoints	- Change in oxygenation index (OI) from baseline to day 7 of treatment. The OI is defined as: % mean airway pressure x FIO_2/PaO_2 - Ventilator free days (VFD) at day 28
Secondary endpoints	- Change in OI from baseline to day 4 - Acute lung injury score [composite of PaO_2/FIO_2 ratio, PEEP, lung compliance (plateau airway pressure minus PEEP/TV) and extent of pulmonary infiltrates] at 2, 3, 7, 14 days (if still intubated) - SOFA scores at 2, 3, 7, 14 days (if still intubated) - Ventilatory ratio (product of minute ventilation and $PaCO_2$) at 2, 3, 7, 14 days (if still intubated) - Incidence of ECMO by day 14 - Use of vasoactive medications by day 14 - CXR assessment of pulmonary edema by "radiographic assessment of lung edema" (RALE) score at 2, 3, 7, 14 days - Percentage of patients achieving pressure support ventilation equal to 5 cm H_2O with PEEP equal to 5 cm H_2O for 2 hours (measure of weaning) by day 28 and at hospital discharge - ICU-free days by day 28 and hospital discharge - Hospital-free days by day 28 and hospital discharge - Incidence of tracheostomies by day 28 and hospital discharge - Incidence of transfer to long term acute care (LTAC) facility by day 28 and hospital discharge - All-cause mortality by day 28 - Hospital discharge by day 28 - All-cause mortality by day 60 - Change from baseline to day 3, 7 and day 14 in plasma levels of IL- 6, IL-8, PAI-1, TNFr-1, ICAM-1 RAGE
CCI	CCI [redacted]
Safety endpoints	• Incidence of Treatment Emergent AEs (TEAEs) and SAEs (TESAEs) from the beginning of study treatment to up to the end of study participation. • Hematology/biochemistry values change from screening to day 3(±8h), 7±1, 14±2, 21±2 (if still receiving reparixin), 28±2 and hospital discharge. • eGFR, absolute value and change from screening to day 3(±8h), 7±1, 14±2, 21±2 (if still receiving reparixin), 28±2 and hospital discharge. • Hepatic function (ALT/AST, total/direct bilirubin), absolute

	values and change from screening to day 3(±8h), 7±1, 14±2, 21±2 (if still receiving reparixin), 28±2 and hospital discharge. ● ECG/rhythm analysis changes from screening to day 3±8h, 7±1, 14±2, 21±2 (if still receiving reparixin), 28±2 and hospital discharge. ● Incidence of secondary infections by day 28±2 or hospital discharge.
Statistical Methods	A sample size of 60 evaluable subjects is considered adequate for descriptively assessing the difference between groups in primary endpoints, i.e. change from baseline in OI at day 7 and VFD at day 28 Assuming equal size groups (1:1), the given sample size will provide a reasonable precision in the estimation of group differences in primary endpoints to an expected margin of error for a 95% one-sided confidence interval of at most 46.5% the standard deviation, whatever it is. Consequently, assuming a standard deviation of 11.5 points for OI and of 8 days for VFD (based on literature), the 95% one-sided confidence intervals for the differences between groups will have a width of 5.35 points and 3.75 days, respectively. Assuming that 10% of enrolled patients will not be evaluable for primary analysis, the total number of patients to be enrolled will be approximately 66. Appropriate descriptive statistics will be produced by treatment arms according to the nature of the variable. For continuous data, number of observations, mean, standard deviation, median, range (minimum and maximum) and 95% confidence intervals will be presented. For categorical data, frequency distributions and percentages with 95% confidence intervals (Wilson method) per category will be presented. The Safety and the Full Analysis Set (FAS) populations will consist of all patients who will be randomized and received at least one dose of the investigational product. Safety population will be analyzed according to the actual treatment received; FAS population will be analyzed according to the Intention To Treat principle, i.e. by treatment allocation. All secondary endpoints will be analyzed at each available time point by means of summary statistics and by appropriate parametric or non-parametric tests depending on the nature of the variable and its distribution. All analyses will be descriptive in nature. TEAEs and TESAEs will be presented in terms of the number and incidence of TEAEs and TESAEs. Other safety parameters will be summarized by treatment at each available time point by means of descriptive statistics. The Study Statistical Analysis Plan with more technical and detailed elaboration of the principal features of statistical analyses will be finalized before database lock. Any deviation from the original statistical plan will be described in the Clinical Study Report.

2. SCHEDULE OF EVALUATION

The grid below summarizes the study schedule of the trial. For all measurements, the actual date and time of assessment, including date of sampling, will be recorded in the Source Document and/or eCRFs. Timeframe for each assessment is also shown in the grid below.

Days (windows)	Screening (day-1 or 1)	Randomization and baseline assessments[1]	Day 1	Day 2 (±8h)	Day 3 (±8h)	Day7 (±1)	Day 14 (±2)	End of Treatment[21]	Extubation to Hospital Discharge (every 48h±8h)	Day 28 (±2) and Hospital Discharge[19]	Day 60
ELIGIBILITY											
Informed consent signed	X										
Demo-graphics, Medical History, Disease-Specific Information, allergies	X										
Pregnancy test[2]	X										
STUDY INTERVENTION											
Randomization		X									
IMP administration[3]			Daily through day 14 with the option to extend to day 21								
STUDY PROCEDURES											
Vital signs (RR, HR, MAP)		Daily through extubation							X		
PaO2 (or SaO2), FIO2	X	Daily through extubation							X	X	
PaCO2		Daily through extubation									
Mechanical ventilation parameters[4]		Daily through extubation									
SOFA score		Daily through extubation									
Neuromuscular blocking agents, inhaled vasodilators[6]		Daily through extubation									
Prone positioning[6]		Daily through extubation									
Vasoactive medications[7]		Daily through extubation									
ECMO[8]		Daily through extubation									
CPAP attempts [9]		Daily through extubation									
Chest imaging[10]	X	X		X	X	X	X				
Biomarkers[11]		X			X	X	X				
Reintubation[12]										X	
Other clinical events[13]										X	
Follow up[14]											X
SAFETY TESTS											
Adverse events	◄----------										----------►
Concomitant medications	◄----------										----------►
Safety hematology and	X				X	X	X	X		X	

Days (windows)	Screening (day-1 or 1)	Randomization and baseline assessments[1]	Day 1	Day 2 (±8h)	Day 3 (±8h)	Day 7 (±1)	Day 14 (±2)	End of Treatment[21]	Extubation to Hospital Discharge (every 48h±8h)	Day 28 (±2) and Hospital Discharge[19]	Day 60
biochemistry[15]											
eGFR[16]	X				X	X	X	X		X	
Hepatic function[17]	X				X	X	X	X			
ECG	X				X	X	X	X		X[21]	
CCI [redacted]											

1. Baseline and initiation of IMP (day1) may coincide
2. In females of childbearing potential, only date will be required
3. Study product will be administered every 8 hours (q8h) for 14 days, and optionally up to 21 days
4. Mode of ventilation, TV, mean airway pressure, plateau airway pressure, PEEP
5. Use or not of neuromuscular blocking medications and/or inhaled vasodilators
6. Use or not of the modality of prone positioning
7. Type and dose of vasoactive medications measured in norepinephrine equivalents
8. Transition or not to ECMO
9. Defined as use of pressure support ventilation equal to 5 cm H_2O with PEEP equal to 5 cm H_2O for 2 hours
10. Chest imaging at screening will be in the form of CXR or CT scan based on institutional preferences and resources. CXR will be performed at other timepoints. If no more than 24 hours have elapsed from a screening CXR, baseline CXR will be deferred.
11. IL-6, IL-8, PAI-1, TNFr-1, ICAM-1, RAGE
12. Denotes repeat intubation at least 24 hours after a successful extubation
13. Denotes: Duration of IMV, ICU length of stay, need for reintubation (after initial extubation, over length of hospitalization), Hospital length of stay, performance of tracheostomy, transfer to LTAC facility*
14. Verification of vital status and inquiry about AE (phone interview)
15. Includes Hematology (RBB, hematocrit, hemoglobin, WBC, neutrophils and lymphocytes absolute count, platelets count) and Biochemistry (sodium, potassium, chloride, calcium, glucose, creatinine, albumin, AST, ALT, total and direct bilirubin). Results obtained at the hospital in the previous 48 hours can be accepted as screening values.
16. Estimated glomerular filtration rate (eGFR) determined by 2021 CKD-EPI
17. Hepatic function is evaluated based on ALT/AST and total/direct bilirubin levels – day 14 assessment required if treatment is prolonged up to day 21

CC
CCI [redacted]
CCI [redacted]
CCI [redacted]
CCI [redacted]
CCI

19. If hospital discharge occurs <28d, a 28-d follow-up in-person visit should be performed. If this is not feasible, a phone follow-up will be planned to collect: Duration of IMV, ICU length of stay, need for reintubation, Hospital length of stay, performance of tracheostomy, transfer to LTAC* facility, or mortality.

CCI [redacted]

21. Only at hospital discharge

*An LTAC is a Long Term Acute Care Hospital, i.e., a facility that specializes in the treatment of patients with serious medical conditions, including patients with ongoing needs for mechanical ventilation, but who no longer require intensive care or extensive diagnostic procedures. The patients in LTAC are transferred there directly from the intensive care unit because they require more care than they can receive in a rehabilitation center, skilled care facility or at home.

2.1. BACKGROUND INFORMATION

Reparixin is a specific inhibitor of the two receptors for IL-8 (CXCL8), i.e., CXCR1 and CXCR2, stemming from a program of drug design of molecules intended to modulate chemokine activity. Reparixin is the first low molecular weight blocker of IL-8 biological activity in clinical development. It has been shown to inhibit polymorphonuclear leukocytes (PMN) chemotaxis in animal and human studies. Early, pre-clinical characterization of reparixin was specifically targeted on the prevention of ischemia/reperfusion injury during solid organ transplantation which is known to be due to PMN recruitment in the transplanted organ. In this field, reparixin received orphan drug designation in EU in September 2001 and in USA in January 2003 for prevention of delayed graft dysfunction after solid organ transplantation. More recently, orphan drug designation was granted in EU (September 2011) for the "prevention of graft loss in pancreatic islet transplantation" and in the US (September 2012) for the "prevention of graft loss in islet cell transplantation".

The recent COVID-19 epidemic provided a unique opportunity to evaluate the role of IL-8 inhibition in severe lung injury induced by SARS-COV2. Initial compassionate use of reparixin, in the form of IV infusion, in 4 patients with COVID-19 induced Acute Respiratory Distress Syndrome (ARDS) led to clinical improvement and was subsequently followed by phase II and phase III studies which verified the salutary effect of reparixin in preventing disease progression. The insights gained from these studies will be further expanded in our proposed study of reparixin in ARDS.

2.2. RELEVANT NON-CLINICAL STUDIES

Reparixin is a potent and specific inhibitor of CXCL8 biological activity. *In vitro* experiments have shown that reparixin inhibits CXCL8-induced chemotaxis of human PMN in the low nanomolar range. Studies to elucidate the mechanism of action have shown that reparixin is a non-competitive allosteric inhibitor of the CXCL8 receptors CXCR1 and CXCR2. Interaction of reparixin with CXCL8 receptors inhibits the intracellular signal transduction events activated by binding of CXCL8 to CXCR1 and CXCR2 [1,2].

Ischemia/reperfusion

In vivo, in a rat model of lung transplantation, reparixin improved graft oxygenation, decreased pulmonary oedema, and significantly reduced neutrophil infiltration into the transplanted lung. Moreover, reparixin prevented PMN infiltration and tissue damage in other animal models of ischemia/reperfusion injury of liver, brain, intestine, heart and spinal cord. In these models, *in vivo* inhibition of PMN recruitment ranged from 40 to 90%, and inhibition of tissue damage ranged from 50 to 80%.

LPS induced Acute Lung Injury (ALI)

In a murine model of LPS-induced ALI, reparixin (15 µg/g) reduced neutrophil recruitment in the interstitial pulmonary compartment by approximately 50% and ameliorated vascular permeability. Both prophylactic and therapeutic application of reparixin improved gas exchange and reduced neutrophil recruitment and vascular permeability in a clinically relevant model of acid-induced ALI[3].

Allergen induced airway inflammation

In the cat dander extract (CDE) single challenge model, administration of reparixin (15 mg/kg) suppressed neutrophil recruitment into the lungs. In the CDE multiple challenge model, reparixin decreased eosinophils, neutrophils, and total cell numbers in bronchoalveolar lavage (BAL) fluid, serum levels of total IgE and CDE-specific IgE, airway epithelial mucin secretion, levels of Th2 inflammation associated genes periostin and muc5ac, and BAL fluid levels of IL- 4, IL- 13, IL- 33, and TSLP. Pharmacological inhibition of CXCR1/2-axis by administration of reparixin inhibited allergen induced airway inflammation in mice[4].

Bleomycin model of pulmonary fibrosis

In a murine model of pulmonary fibrosis induced by exposure to a combination of bleomycin and particulate matter, inhibition of CXCR2 with reparixin reduced neutrophil number and neutrophil elastase concentration in BAL fluid. Moreover, reparixin improved lung function and reduced extent of pulmonary fibrosis, as assayed by total lung collagen content and histochemical stains of fibrosis markers, on day 14 lung tissues[5].

Pneumococcal and influenza pneumonia

The role of CXCR1/2 inhibition during influenza, pneumococcal, and post-influenza pneumococcal infections was also investigated. Most experiments were conducted using DF2162, a reparixin analogue, belonging to the same family of non-competitive allosteric inhibitors of CXCR1 and CXCR2. Reparixin and DF2162 exhibit similar potency in the inhibition of the target receptors CXCR1 and CXCR2 (IC50s for IL-8-induced chemotaxis in the range of 1 nM). The molecular mechanism of action has been characterized by point-mutagenesis studies on CXCR1 and CXCR2 showing that reparixin and DF2162 bind the receptors in the same allosteric site of the trans-membrane region, which is highly conserved between the two receptor subtypes. DF2162 was used to assess the role of CXCR1/2 in influenza (IAV), pneumococcal, and post-influenza pneumococcal infections in mice. Mice were infected with IAV or *Streptococcus pneumoniae* and then treated daily with DF2162. To simulate secondary pneumococcal infection, mice were infected with a sublethal inoculum of IAV followed by infection with *S. pneumoniae* 14 days later. DF2162 was given in a therapeutic schedule from day 3 to day 6 after influenza infection. Lethality, weight loss, inflammation, viral/bacterial counts, and lung injury were assessed. CXCL1 and CXCL2 (the functional murine analogues of CXCL8) were produced at high levels during IAV infection. DF2162 treatment decreased morbidity, and this was associated with decreased lung neutrophil infiltration and reduced pulmonary damage and viral titers. During *S. pneumoniae* infection, DF2162 treatment decreased neutrophil recruitment, pulmonary damage, and mortality, without affecting bacterial burden. Treatment with DF2162 during sublethal IAV infection followed by infection with *S. pneumoniae* reduced the morbidity associated with the viral infection and also decreased the magnitude of inflammation, lung damage, and bacterial titers in mice subsequently infected with *S. pneumoniae*. These data suggest that modulation of the inflammatory response through blockade of CXCR1/2 improves disease outcome during

influenza and pneumococcal infections, without compromising the ability of the murine host to deal with infection[6]. To determine if reparixin would replicate the effect of DF2162 in influenza infection, mice were infected with 1×10^4 PFU of IAV and then treated three times a day (from day 0 i.e., time of infection, to day 5 post-infection) with reparixin at 15 mg/kg. As with DF2162, treatment with reparixin decreased morbidity, as seen by the reduction in weight loss, suppressed the pro-inflammatory cytokines TNF-α and CXCL1 as well as the leukocytic, and in particular, neutrophilic infiltration into the airways and improved the histopathologic lung injury score.

<u>COVID-19</u>

During COVID-19 neutrophils undergo IL-8-induced degranulation that drives a systemic prothrombotic phenotype. Therapeutic blockade of IL-8 by reparixin reduced COVID-19-associated human neutrophil activation *in vitro* and attenuated pulmonary capillary micro-thrombosis *in vivo* in the transgenic hACE2 mouse model of COVID-19 associated respiratory failure where reparixin treatment led to decreased fibrinogen binding by intravascular neutrophils[7].

2.3. SUMMARY OF TOXICOLOGY DATA

Reparixin was tested for toxicity in rodent and non-rodent animal species after single and repeated IV doses. The repeated dose administration studies were conducted by IV continuous infusion, according to the intended human administration route. The general toxicological profile of IV reparixin, in the studies conducted to date, is characterized by a low toxicity after single or repeated dose administrations in rats CCI [redacted]. Continuous IV infusion of reparixin to male and female rats at dose levels of up to CCI [redacted] did not have any significant adverse effects on mating performance and fertility. Reparixin poses no genotoxic hazard for humans.

Reparixin lysine salt, at doses in excess of those intended for use in humans, has a safe pharmacology profile in the renal, cardiovascular and respiratory systems of rats and dogs. The local tolerability of reparixin lysine salt was assayed in the rabbit ear lateral vein. The compound was well tolerated in concentrations up to CCI [redacted] infused over one minute. In order to provide evidence of the safety of DF2243Y, the main metabolite of reparixin excreted in urine in humans, safety, pharmacology, and toxicity studies have been performed at doses 2 to 3 times higher than those reached in man, as may occur during the treatment of patients receiving kidney transplantation.

In the preclinical setting, reparixin has shown an excellent safety profile as demonstrated with very comprehensive toxicological studies *in vitro* and in animal species.

2.4. PHARMACOKINETICS AND PRODUCT METABOLISM

Pharmacokinetic (PK) studies by IV injection revealed that reparixin is very rapidly eliminated in rats and humans (T1/2 0.5-3 hrs and 1.0-1.5 hrs, respectively) whereas elimination is slower in dogs (12-28 hrs). The PK of reparixin was linear in rats and in dogs, but linearity was less evident in humans. Reparixin undergoes complete metabolism (oxidation + conjugation) in all the species tested. The *in vitro* human hepatic, phase I metabolism of reparixin is catalyzed by CYP2C9 and to a lesser extent by CYP2C19. DF2243Y, DF2188Y, methanesulfonamide and ibuprofen are the metabolites detected in human plasma and urine, with DF2243Y being the major metabolite. The plasma levels of ibuprofen after administration of reparixin 2.77 mg/kg/h for 48 hrs (the highest dose tested in humans) were similar or lower to those obtained after a standard therapeutic single dose of ibuprofen (300mg). Preliminary PK data obtained in a few patients undergoing islet transplantation showed that plasma levels of reparixin (total and unbound) and its major metabolite DF2243Y were within the expected range according to the dose administered. Due to extensive metabolism, unchanged reparixin was minimally or not at all observed in the urine of rats, dogs and humans, suggesting that the PK profile of reparixin is not influenced by renal impairment. *In vitro* protein binding of [14C]- reparixin showed that reparixin is highly bound (approximately 99%) to plasma proteins in rats, dogs, rabbits, cynomolgus monkeys and humans. Albumin is likely to be the major binding protein in plasma in all species, accounting for 99.2% in humans.

In clinical trials with oral tablets, reparixin was administered for 21 consecutive days followed by 7 days of drug holiday before the next cycle. Reparixin was rapidly absorbed (median T_{max} 1 hr). Reparixin systemic exposure (C_{max} and AUC_{last}) did not change from day 1 to day 21, indicating the absence of accumulation over the dosing period. Also, $T_{1/2}$ did not change from day 1 to day 21, with a median value of about 2 hrs. Once absorbed, reparixin is highly bound to proteins as only <0.1% to 0.2% of total reparixin is available as unbound (free) drug. Reparixin was rapidly metabolized to DF2243Y, DF2188Y and ibuprofen. For all three metabolites systemic exposure was similar on both day 1 and day 21 within the observed inter-subject variability. The $T_{1/2}$ of all three metabolites appeared to remain about the same from day 1 to day 21.

To investigate the PD characteristics of reparixin, the effect of the drug in inhibiting IL-8- mediated Neutrophils Extracellular Traps (NET) release by human PMN in the whole blood of healthy volunteers was assessed. Reparixin blocked IL-8-induced NET formation in a concentration dependent manner, with the inhibition (40%) becoming statistically significant at 5 µg/mL and reaching near completeness (about 90%) at 25 µg/mL which corresponds to the total blood concentration (equivalent to a free unbound concentration of 100 nM) that is reached at the steady state (Css) by IV infusion or by repeated oral administration.

Reparixin has some potential *in vitro* for a non-competitive inhibition of the human hepatic enzyme CYP3A4 that is involved in the metabolism of cyclosporine A, tacrolimus and rapamycin. However, since inhibition

is evident at concentrations far higher than the free plasma concentration of reparixin at steady state in humans, it is predicted that the clinical relevance of such inhibition is minor. Indeed, reparixin does not affect, to a clinically relevant extent, the activity of CYP3A4 and CYP2C9 (enzyme involved in reparixin metabolism), as revealed by an interaction study where the PK of midazolam and tolbutamide (probe substrates for these enzymes) was evaluated in healthy subjects receiving single oral doses of the probes alone or in combination with reparixin.

2.5. SUMMARY OF CLINICAL DATA

A total of 1.050 subjects have been treated in phase 1, phase 2 and phase 3 completed (CSR issued) clinical studies. Among these, 666 have been exposed to reparixin.
During the phase 1 studies a total of 166 subjects of whom 103 normal volunteers (including 3 females) and 17 patients with different grade of renal impairment (including 5 females), 16 patients undergoing cardiopulmonary bypass (including 6 females) and 30 patients with metastatic breast cancer were exposed to reparixin. A total of 65 subjects were treated with placebo.
In phase 2 and 3 studies, a total of 282 patients, 46 undergoing lung transplantation (23 M and 23 F), 48 patients undergoing kidney transplant (including 17 females), 20 patients with early breast cancer (20 F), 61 female patients with metastatic TNBC, 85 patients undergoing intrahepatic islet transplantation (32 M and 53 F) and 22 (18 M and 4 F) undergoing orthotopic liver transplantation, were treated with reparixin. In the islet transplantation studies, 22 patients received reparixin twice. A total of 212 subjects were treated with placebo.

Out of the 166 subjects exposed to reparixin in phase 1 studies, 30 female patients with metastatic breast cancer received reparixin oral tablets in combination with paclitaxel in a phase 1b clinical trial. In the phase 2 early breast cancer study, 20 female patients received reparixin oral tablets alone. In the randomized, phase 2 study 61 metastatic TNBC patients received reparixin oral tablets in combination with paclitaxel.

In all studies, reparixin showed a good safety profile with both IV and oral administration.

2.5.1. Efficacy in patients with acute lung injury due to COVID-19

To date 3 studies were performed with reparixin in COVID-19 patients. These were:

1. Compassionate use with reparixin IV infusion
2. Phase II open-label study (CCI)
3. Phase III double-blind study (CCI

In particular:

1. In March 2020, reparixin was used in IRCCS Ospedale San Raffaele, a reference center for COVID-19 in Italy, under a compassionate use application. Four patients with ARDS caused by COVID-19 pneumonia were treated with reparixin IV infusion CCI via high-flow central vein for five days. One out of the 4 patients was never intubated and discharged from the hospital, whereas among the 3 who were intubated 1 was in stable condition and 2 were gradually improving with concomitant improvement or stabilization of inflammatory markers (C-reactive protein, procalcitonin, and ferritin) and tissue damage markers (LDH, ALT, AST). As per the last update of March 2021, all 3 patients weresuccessfully extubated and discharged from the hospital.
2. Phase II open-label study CCI . In 2020 Dompé concluded a Phase II randomized, controlled multicentre study on the efficacy and safety of reparixin in the treatment of hospitalized patients with COVID-19 pneumonia. Fifty-six patients were randomized 2:1 to receive 1200 mg (2x 600 mg tablets) oral reparixin three times daily (37 pts) or standard of care (19 pts) for up to 21 days. Results suggested that reparixin may prevent the progression to severe disease, as it significantly decreased the incidence of at least one clinical event of the composite endpoint including supplemental oxygen requirement, invasive mechanical ventilation use, admission to Intensive Care Unit (ICU), and use of a rescue medication for any reason. Treatment with reparixin was well tolerated in terms of treatment-emergent adverse events.
3. Phase III double-blind study CCI The above results informed the design of a subsequent double-blind, placebo-controlled clinical study (CCI approved in Italy (EudraCT Number: CCI

CCI and in the US although, due to late activation of the three centers in US that coincided with a decline of new COVID-19 infections, the study enrolled only in Italy. CCI was CCI [redacted]" and its objective was to evaluate efficacy and safety of oral reparixin as compared to placebo (both on top of standard treatment). From February 2021 to June 2021, patients with RT-PCR-confirmed severe COVID-19 were randomized in a 2:1 fashion to receive oral reparixin 1200 mg (2x 600 mg tablets) three times a day (TID) or placebo for 21 days. The treatment schedule and regimen were consistent with the previous trial. Follow-up information on the patients' clinical condition and survival were collected until day 90. Briefly, patients were considered for inclusion if they had evidence of respiratory failure requiring oxygen support (i.e., PaO2/FiO2 ratio between 100 and 300, SpO2 <95% at room air, or respiratory rate (RR) ≥24 breaths/min at room air), abnormal chest imaging, and laboratory signs of inflammation (LDH >normal range, CRP ≥100 mg/L or IL-6 ≥40 pg/mL, serum ferritin ≥900 ng/mL, XDP >20 mcg/mL). The primary efficacy outcome was the proportion of patients alive and with no respiratory failure at day 28, defined as no need for invasive mechanical ventilation (IMV), extracorporeal membrane oxygenation (ECMO), or admission to the intensive care unit (ICU) due to worsening respiratory status. Among 279 randomized patients, all enrolled in Europe, 182/185 (98.4%) in the Reparixin group and 88/94 (93.6%) in the placebo group received at least one dose of the investigational product. All participants had a positive RT-PCR assay for SARS-CoV-2 RNA from the upper respiratory tract. At baseline, the cohort was well-balanced across the two groups: mean age 60.8 (SD=11.9) years, 73% were males, 27% were of Hispanic origin, and 30% had a BMI>30 Kg/m^2. All participants had the typical features of acute respiratory failure i.e., need for oxygen therapy either in the form of low flow supplemental oxygen or high flow nasal cannula (NHNC)/non-invasive ventilation (49.2% in the reparixin and 50.0% in the placebo group required HFNC/NIV), with a mean PaO2/FIO2 ratio of 200.2 (SD=67.5). The treatment was discontinued more frequently in the placebo group (34 patients [18.4%] vs. 20 [21.3%]), with good compliance to study medication during treatment in both arms (median 97.22% in the reparixin group and 96.85% in the placebo group). According to the Intention-to-Treat (ITT) principle, all randomized and treated patients were considered for the efficacy analysis, using retrieved dropouts' information to handle missing data at Day 28. CCI [redacted] The mortality at 28 days was CCI in the placebo group and CCI in the reparixin group (OR 0.468, 95% CI: 0.158-1.386, p=0.170). The incidence of ICU admission due to deterioration of respiratory failure (followed by IMV, or ECMO, or death) was: 10 patients (6.1%) in the reparixin group vs. 10 (12.2%) in the placebo group (OR 0.429, 95% CI: 0.167-1.100, p=0.078). A statistically significantly difference in the PaO_2/FIO_2 ratio between groups, in favour of the reparixin group, was observed at Day 3 (p = 0.002), when the mean (± SD) changes from baseline were 13.377 ± 36.568 mmHg in the reparixin group and -5.274 ± 41.103 mmHg in the placebo group. Once again, there was a strong safety signal with the number of adverse events being steadily higher in the control group.

2.5.2. Safety

In all studies, reparixin has shown a good safety profile with both IV and oral administration.

The patient population exposed to the IV formulation includes 103 adult healthy subjects (100M/3F), 17 patients with different grades of renal impairment (12M/5F), 16 patients undergoing cardiopulmonary by- pass (10M/6F), 46 patients undergoing lung transplantation (23M/23F), 48 patients undergoing kidney transplantation (31M/17F), 22 undergoing liver transplantation (18M/4F) and 85 receiving intrahepatic pancreatic islet infusion (32M/53F), with 22 patients in this group receiving reparixin twice. Exposure included short or prolonged IV infusion up to CCI over 30 min or CCI over 48h and, in pancreatic islet and liver transplantation studies, CCI continuous infusion for 7 days. Overall, reparixin was safe and well tolerated in both healthy subjects and critically ill patients. In phase 1 studies, no deaths, Serious Adverse Events (SAE) or Adverse Events (AE)-related withdrawals were reported. The majority of AEs reported were of mild intensity. All subjects recovered completely or had ongoing adverse events of mild intensity when they were discharged. The safety of reparixin was also confirmed in patients with different grades of renal impairment. In the interaction study no safety concerns were raised during co-administration of midazolam/tolbutamide with reparixin. During phase II and III

studies, AE and SAE profile was similar for both placebo and reparixin groups and no particular safety concerns were raised. Data obtained in the trials in islet transplantation further support the safety profile of the proposed dose, even after a 7-day administration, repeated CCI in several patients. Most frequent Adverse Drug Reactions (ADRs) were nausea, headache, and vomiting; the great majority of these were mild to moderate in nature and none required discontinuation of the investigational product. Tachycardia occurred in one patient from Day 5 to 38 after the 1st islet infusion which was judged to be probably related to the investigational product. Vomiting, nausea and headache on Day 5 and 6 after the 2nd islet infusion in one patient and erythema, nausea and headache on Days 2 to 6 after the 1st islet infusion in another patient were judged as very likely related to the investigational product. Nausea, vomiting and severe gastrointestinal bleeding associated with anemia developed early after the beginning of reparixin infusion in a female patient who received a dose of reparixin 3 times as high as that foreseen in the protocol (medical error). These events were assessed as serious by the investigator and by the Sponsor.

The most frequent (>10%) ADRs observed in studies on IV formulation were:

Gastrointestinal disorders (about 28%), including abdominal pain lower, abdominal pain not otherwise specified, abdominal pain upper, constipation, diarrhea, dyspepsia, flatulence, gastroesophageal reflux disease, gastrointestinal hemorrhage, intra-abdominal hemorrhage, nausea and vomiting.

Nervous system disorders (about 16.5%), including headache, dizziness, hypoaesthesia, somnolence.

General disorders and administration site conditions (about 15%), including IV site insertion reactions including hemorrhage, thrombosis and localized edema, fatigue, lethargy, malaise, peripheral oedema, and pyrexia.

The patient population exposed to reparixin oral tablets in the oncological program, consisted of 111 female patients receiving either single agent reparixin (CCI operable breast cancer: 20 patients) or the combination of reparixin and weekly paclitaxel in metastatic breast cancer (CCI phase Ib: 30 patients; REP0114, randomized phase II: 61 patients). In these studies, reparixin was generally well tolerated at all doses studied. Overall, 505 ADRs were reported in 78 patients in the safety population: 70.9% of the ADRs were grade 1 (mild), 22.9% were grade 2 (moderate) and 4.3% were grade 3 (severe). One grade 4 ADR was reported overall (CCI In addition, one patient in clinical trial REP0114 experienced serious ADRs including grade 4 peritonitis and grade 5 intestinal perforation.

The most frequent (>10%) ADRs observed in the the oncological program were:

Gastrointestinal disorders (32.0%), including nausea, vomiting, constipation, diarrhea and dyspepsia

General disorders and administration site conditions (19.5%), including fatigue and peripheral oedema.

Nervous system disorders (9.7%) including headache as most frequent ADR.

Further data can be found in the Investigator's Brochure.

Furthermore, the patient population exposed to reparixin oral tablets in the Covid-19 studies includes 218 patients (36 in the Phase 2/3 CCI study and 182 in the Phase 3 CCI trial).

Safety data from study CCI, show that treatment with reparixin was well tolerated in terms of treatment-emergent adverse events, laboratory tests and vital signs parameters. Serious TEAEs (all unrelated) were reported in 2 patients (5.6%) in the reparixin group (2 TEAEs) and in 4 (21.1%) in the standard of care group (4 TEAEs): among these, death occurred in 1 patient (2.8%) in the reparixin group and in 3 (15.8%) in the standard of care group. No ADR was reported.

In the study CCI the Safety population included 182 and 88 patients in reparixin and placebo arm, respectively. Treatment with oral reparixin was well tolerated in terms of TEAEs safety laboratory tests and vital signs parameters. Serious TEAEs were reported in 20 patients (11.0%) in the reparixin group (23 TEAEs) and in 13 (14.8%) in the placebo group (16 TEAEs) and none of these was related to treatment. Ten TEAEs leading to death were reported in 10 patients (5.5%) in the reparixin group and 7 TEAEs leading to death were reported in 7 patients (8.0%) in the placebo group. A total of 13 ADRs were reported in 10 patients (5.5%) in the reparixin group and 11 ADRs were reported in 8 patients (9.1%) in the placebo group on treatment or follow-up period. In summary, treatment with reparixin was associated with lower proportions of patients that reported TEAEs (serious, severe, leading to discontinuation of IMP or having a fatal outcome).

The SOC more frequently (>10%) involved in ADRs observed with oral tablets in COVID-19 pneumonia studies were:

Infections and infestations (about 31% of the overall reported ADRs), including bacterial sepsis, lower respiratory tract infection fungal and pneumonia bacterial.

Gastrointestinal disorders (15%), including abdominal pain upper and diverticulum intestinal haemorrhagic.

Hepatobiliary disorders (15%), including hypertransaminasemia.

2.6. DISEASE REVIEW AND STUDY RATIONALE

ARDS is a heterogeneous syndrome of acute respiratory failure first described in 1967[8] and more recently defined by the clinical criteria of bilateral pulmonary opacities on chest radiograph, arterial hypoxemia (PaO2/FiO2 ratio < 300), on positive end-expiratory pressure (PEEP) ≥5 cm H_2O, and exclusion of cardiac failure as the primary etiology of the syndrome[9]. The pathophysiology of the syndrome is characterized by pulmonary edema due to excessive alveolocapillary permeability associated with inflammatory injury to the alveolocapillary barrier, generated either locally in the lungs or systemically from extra- pulmonary sites. Examples of such injuries include pneumonia due to viral, bacterial or fungal pathogens, sepsis, aspiration of gastric contents, smoke inhalation, acute pancreatitis, severe trauma, drug overdose and ischemia-reperfusion injury[10]. By products generated by invading microorganisms called pathogen associated molecular patterns, in combination with damage associated molecular patterns released by damaged or dying cells interact with pattern recognition receptors on pulmonary vascular endothelial cells, epithelial cells, smooth muscle cells and resident innate immune cells leading to the release, from these cells, of inflammatory cytokines[11,12] some of which act as chemoattractants for leukocytes (chemokines). Leucocyte chemokines are classified into several families based on the position of cysteine (C) residues: CXC, CC, C and CX3C[13]. CXC chemokines, and mainly CXCL8 (interleukin 8) in humans and its orthologs in mice are considered the prototypical neutrophil-specific chemokines that regulate neutrophil migration[14]. Neutrophil influx into the extravascular compartments of the lungs is a defining characteristic of ARDS[15]. Neutrophils retained within the pulmonary capillaries or migrating through the alveolocapillary barrier into the airspaces release oxidants and proteases whose primary role is the killing of pathogens[16]. However, when excessive, intrapulmonary accumulation and activation of neutrophils can cause disproportionate tissue injury. In fact, disease severity and mortality in ARDS correlates with the extent of neutrophilia in the lung[15] as well as with the levels of CXC chemokines both in patients with ARDS[17] and animal models of lung injury[18,19] . Two CXC chemokine receptors, CXCR1 and CXCR2, have been shown to mediate the response to CXC chemokines in human neutrophils. Whereas human CXCR1 binds to CXCL6 (IL-6) and CXCL8 (IL-8) with a high affinity, human CXCR2 binds also to IL-6 and IL-8 as well as several other CXC chemokines (GRO-α, GRO-β, GRO-γ, CXCL1, CXCL2, CXCL3), ENA-78 (CXCL5) and CXCL7[20]. Binding of IL-8 to its receptors on neutrophils mediates not only recruitment of these cells but also their activation. Upon their activation neutrophils release neutrophil extracellular traps (NET) which are networks of extracellular fibers, primarily composed of DNA, embedded with histones, myeloperoxidase and neutrophil elastase (NE)[21]. Neutrophils release NETs in response to both infectious and non-infectious systemic inflammatory diseases, including sepsis, acute ischemia-reperfusion injury of the liver, and transfusion-related or LPS-induced acute lung injury[22-24]. NET release is associated with induction of alveolar-capillary barrier damage, platelet aggregation, and cytokine production and inhibition of NETs through inhibition of NE ameliorates acute lung injury in mouse models[25,26]. Strategies that would prevent excessive neutrophil accumulation in the injured lung through inhibition of the main neutrophilic chemotactic cytokine, i.e., IL-8, are intuitively attractive and, in fact, have been pursued with promising results as neutralization of CXCL8 by polyclonal antibodies has been shown to reduce the severity of ARDS in animal models[27].

Our experience with reparixin induced IL-8 inhibition in patients with ARDS due to COVID-19, obtained through the compassionate use of reparixin, as well as in the phase II and III trials in COVID-19 patients support the potential clinical efficacy of reparixin in ARDS of other etiologies. These findings, coupled with the safety shown in phase I to III clinical trials, provide strong rationale supporting further testing of reparixin in ARDS.

2.6.1. Alternative treatments

Currently the treatment of ARDS is mainly supportive and involves implementation of lung protective

ventilation, prone positioning, careful monitoring of fluid balance and treatment of the underlying etiologic pathology. All patients participating in the study will receive the standard of care for ARDS as described above.

2.6.2. Risk – benefit evaluation

2.6.2.1. Risk related to reparixin

Results from preclinical and clinical studies justify the level of drug exposure planned in this study. The safety of reparixin was confirmed in the phase II and III trials using the same schedule; indeed, no safety concerns were raised in COVID-19 patients. Any possible risk derived from the continuous administration of reparixin in the specific population involved in this study will be minimized by integrated monitoring which will include laboratory tests and daily clinical observations.

Even if reparixin marginally inhibits the enzyme CYP2C9 (IC50 79 µM) and slightly inhibits CYP3A4 (IC50 8 µM) *in vitro*, this effect does not translate into clinically significant inhibition of either enzyme as revealed by an interaction study where the PK of midazolam and tolbutamide (probe substrates for these enzymes) was evaluated in healthy subjects receiving single oral doses of the probes alone or in combination with reparixin (Reparixin Investigator's Brochure, 2021).

The ADRs most frequently reported for the oral formulation used in oncology studies (in patients with metastatic diseases, undergoing chemotherapy) were not different from SOC (hepatic enzymes elevation). In COVID-19 (protocol CCI), ADRs occurred in few subjects without any particular trend. With reference to cumulative ADRs (irrespectively of the seriousness), the SOCs more frequently involved were Gastrointestinal disorders (mainly nausea and vomiting), Nervous System Disorders (mainly headache) and General disorders and administration site conditions (fatigue and peripheral edema).

In regard to serious events in particular, the signal detection process for single Serious Adverse Events and reactions led to no important risk having been identified or suspected so far for patients treated with reparixin, regardless of the formulation and the indication for treatment. No SAR was reported in the Covid-19 studies.

2.6.2.2. Blood sampling

Measurement of study related parameters can generally be done from blood samples collected for routine care of ICU patients. Participation in the study might require additional blood samplings other than the routine ones. In particular:

- blood samples for inflammatory biomarkers (approx. 10 mL) will be collected at baseline as well as on days 3, 7 and 14
- CCI
  CCI
  CCI

Venipuncture might cause minor pain, bruising, inflammation or excessive bleeding at the venipuncture site and faintness and/or swelling, pain, redness, or infection (infection rarely happens) at the site where the needle is inserted. Rare risks include hematoma, infection, arterial puncture. The volume of blood above is an amount that the body can safely replace.

In the absence of an indwelling arterial catheter (which is however common in ICU patients) arterial puncture is required daily for assessment of PaO_2 and $PaCO_2$; a small syringe and small needle (topical 1% lidocaine, if available) will be utilized to withdraw approximately 1 mL (minimum volume 0.2 ml) of blood from an artery (preferably radial). Arterial puncture might cause local discomfort, hemorrhage, topical hematoma, topical infection and arterial occlusion leading to tissue hypoperfusion in the distribution of the artery.

2.6.2.3. Potential benefit

To the patients: half of the patients will be assigned to the placebo arm and will therefore obtain no benefit. Patients receiving reparixin treatment may possibly benefit, but this is to be ascertained.

To society: This study may identify a useful medication that may help limit disease progression in ARDS patients. Currently, ARDS is diagnosed in 10% of ICU patients and 23% of mechanically ventilated patients

and has a disease-associated mortality which ranges from 27% to 45% based on severity[28]. Regimens that can inhibit disease progression and improve severity of disease can have significant effects on quality of life and healthcare related costs.

In summary, given the cumulative knowledge for the safety profile of reparixin in patients with COVID-19 and acute illness, and the significant unmet need for a treatment of ARDS, the benefit/risk balance for this study is assessed to be favorable.

2.6.3. Description of the Investigational Product

In this study the Investigational Medicinal Product (IMP) will be either reparixin 600 mg tablet or matched placebo. The proposed dose in this clinical study is 1200 mg oral reparixin TID for 14 days with optional continuation of treatment up to 21 days, if the patient is still intubated on Day 14. Placebo will be administered with the same schedule.

The particular dose regimen has been selected based on the clinical data from previous clinical studies in patients with acute lung injury due to COVID-19.

The 1200 mg TID dosage is supported by both preclinical studies and efficacy/safety results from phase I and phase II clinical studies already conducted. The resulting average steady state plasma concentration of the reparixin unbound fraction should ensure full inhibition of PMN migration, considering that the *in vitro* IC_{50} is in the range of 1 ng/mL.

The toxicity studies conducted to date support the dose regimen proposed for this study, which has already been used for the previous phase II and phase III trials in COVID-19 adult patients.

3. OVERALL STUDY DESIGN AND INVESTIGATIONAL PLAN

3.1. STUDY OBJECTIVES

To characterize the efficacy of reparixin in ameliorating lung injury and systemic inflammation and expediting clinical recovery and liberation from mechanical ventilation of adult patients with moderate to severe ARDS (PaO_2/FIO_2 ratio ≤ 200). Furthermore, to characterize the PK of reparixin in this group of severely ill patients as well as the effect of reparixin on systemic biomarkers linked to a hyper-inflammatory ARDS phenotype[29-31].

Safety objectives: To evaluate the safety of reparixin versus placebo in the specific clinical setting, including the effect on the incidence of secondary infections.

3.2. STUDY ADMINISTRATIVE STRUCTURES, STAFF AND RESPONSIBILITY

This study will be performed at designated clinical spaces available for clinical studies, under the supervision and responsibility of the Principal Investigator (PI). The PI will be responsible for ensuring that the investigation is conducted according to the signed Investigator agreement, the protocol, Good Clinical practice (GCP) guidelines, institutional, federal, state and local regulations.

The PI will be responsible for the management of the study, which will consist of maintaining the study file and the patient records, reporting SAEs within required timelines, completing the electronic case report form (eCRF) and any other study document. PI at US sites should also correspond with the Institutional Review Board (IRB).

The PI is responsible for supervising any individual or party to whom (s)he delegates trial related duties and functions conducted at the trial site. The PI/institution should ensure that any individual or party that performs trial related duties and functions is qualified to perform those trial related duties and functions and should implement procedures to ensure the integrity of the trial related duties and functions performed and any data generated. Similarly, it is the responsibility of the PI to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study, including detailed knowledge of and training in all procedures to be followed.

The PI will maintain a list of delegated responsibility detailing the various study tasks to be performed by each member of his/her study staff. Each staff member should sign the delegation of authority/activity log for their performing each of the tasks delegated to them on the list. Where reference is made in this protocol to the PI, either the PI and/or one or more delegated members of his/her staff are meant, according to the list of delegated responsibility.

3.3. OVERALL STUDY DESIGN

The study is an international, multicenter, 1:1 randomized, double-blind, placebo-controlled, phase II proof-of-concept trial. It will enroll approximately 66 adult patients hospitalized with ARDS that meet the inclusion criteria, randomly assigned at 1:1 ratio to receive either reparixin 1200 mg (treatment group) through a nasogastric tube or matched placebo (control group) TID for 14 days with optional continuation of treatment up to 21 days, if the patient is still intubated on Day 14. The IMP may be administered orally after extubation and between extubation and day 14 should the patient be able to swallow. In such cases the IMP may be administered either intact or crushed and mixed with a vehicle as per speech swallow evaluation. All patients must receive standard and supportive care according to their clinical status and local guidelines during the whole study period. Although the ventilatory strategy in both groups is in the discretion of the PI, physicians are encouraged to comply with the following ventilator strategy: tidal volume of 4-6 ml/Kg of predicted body weight, a plateau pressure < 30 cm H_2O, driving pressure < 15 cm H_2O to maintain arterial pH > 7.2 and FIO_2/PEEP adjusted to keep $SpO_2 \geq 88\%$ or PaO2≥55 mmHg.

Sedation, use of neuromuscular blocking agents or nitric oxide and other inhaled vasodilators, prone positioning, and use of ECMO are left to the PI's discretion and are registered daily on the study's electronic case report form.

Each patient will be involved in the study for up to 60 days. This period consists of informed consent acquisition and screening, randomization and baseline assessments, a post-randomization study period of up to 28 days from the first IMP dose or hospital discharge and a follow-up period of up to day-60. Patients will be assessed daily while hospitalized up to extubation from first IMP administration. The end of this study is defined as the end of follow up.

3.3.1. Rationale for Selection of dose, control group and treatment schedule in the study

The dose and dose regimen have been selected according to the rationale reported in Section 2.6.3.

A double-blind, randomized study design including a placebo group is being adopted as the gold standard to minimize systematic bias and increase baseline comparability between treatment groups. Both groups of patients will receive the current standard of care for ARDS.

3.4. STUDY TIME_TABLE

Overall planned study timelines are reported below:

Study period First Patient First Visit: February 2023

Last Patient First Visit: December 2024

Last Patient Last Visit: February 2025

4. SELECTION OF STUDY POPULATION

Number of patients: approximately 66 adult patients hospitalized with ARDS will be enrolled to target 60 evaluable patients. A patient is considered enrolled if, after signature of consent and screening, it is determined that (s)he fully meets all the Inclusion Criteria and none of the Exclusion Criteria described in Sections 4.1. and 4.2. below.

4.1. INCLUSION CRITERIA

To be eligible for inclusion into this study, each patient must fulfill the following inclusion criteria:

1. Signed Informed Consent, according to local guidelines and regulations.
2. Male and female adults (≥18 years old).
3. Mechanically ventilated (invasive) patients with PaO_2/FIO_2 ratio ≤200 in the presence of PEEP of ≥5 cm H_2O.
4. Respiratory failure not fully explained by cardiac failure or fluid overload (if acute Congestive Heart Failure exacerbation is identified as part of the clinical picture this should be addressed effectively and as soon as possible before the patient can be enrolled).
5. Bilateral radiologic opacities consistent with pulmonary edema on the frontal chest x-ray (CXR) radiograph, or bilateral ground glass opacities on a chest CT scan.
6. ≤48 hours from fulfilling above ARDS criteria (if a patient is transferred from a non-participating hospital to a participating site, a 12-hour period beyond the 48 hours is allowed)
7. Females of child-bearing potential who are sexually active must be willing not to get pregnant within 30 days after the last Investigational Medicinal Product (IMP) dose and must agree to at least one of the following reliable methods of contraception:
   - Hormonal contraception, systemic, implantable, transdermal, or injectable contraceptives from at least 2 months before the screening visit until 30 days after the last IMP dose;
   - A sterile sexual partner;
   - Abstinence.

   For patients unable to personally consent to the above, due to complications of acute illness and/or its treatment, assurances for the above must be given by LR and reiterated by the patient when/if she is able to do so.

   Female participants of non-child-bearing potential or in post- menopausal status for at least 1 year will be admitted. For all female subjects with child-bearing potential, pregnancy test results must be negative before first drug intake.

4.2. EXCLUSION CRITERIA

1. Moderate-Severe chronic hepatic disease (as verified by a previously known Child-Pugh score ≥7). If baseline Child-Pugh score is not known, it should not be calculated while the patient is acutely ill. In that case, the patient is excluded on the basis of: ALT/AST ≥ 3x ULN and total bilirubin > 2x ULN or ALT/AST ≥ 5x ULN.
2. Severe chronic renal dysfunction: eGFR (2021 CKD-EPI) < 30 mL/min/1.73m^2. If baseline (chronic) renal function is not known the patient is only excluded if in need of acute renal replacement therapy (currently on RRT or to be imminently placed on RRT).
3. Participation in another interventional clinical trial.
4. Patients that are clinically determined to have a high likelihood of death within the next 24 hours based on PI's estimation.
5. Currently receiving ECMO or high frequency oscillatory ventilation.
6. Anticipated extubation within 24 hours of screening. (In such cases, re-screening is allowed if the patient is within the enrollment window).
7. Evidence of GI dysmotility as demonstrated by presence of all the following: persistent gastric distention and enteral feeding intolerability and persistent gastric residuals >500 ml).
8. Anticipated transfer to a hospital not participating in the trial within 72 hours of screening.
9. Decision to withhold or withdraw life-sustaining treatment (patients may still be eligible however if they

are committed to full support except cardiopulmonary resuscitation if cardiac arrest occurs).

10. History of:
    a) Documented allergy/hypersensitivity to sulfonamides, ibuprofen and other COX-1 and 2 inhibitors, and to the study product and/or its excipients.
    b) Lactase deficiency, galactosemia or glucose-galactose malabsorption.
    c) History of peptic ulcer, GI bleeding or perforation due to previous NSAID therapy.
11. Active bleeding (excluding menses) from an uncontrolled site that cannot be definitively resolved prior to enrollment.
12. Pregnant or lactating women.
13. Women of childbearing potential and fertile men who do not agree to use at least one primary form of contraception during the study and up to 30 days after the last IMP dose.

    For patients non able to personally consent to above due to complications of acute illness and/or its treatment assurances for the above must be given by LR and reiterated by the patient when/if he/she is able to do so.

4.3. ASSIGNMENT OF PATIENT NUMBER

At screening, each patient will be assigned a unique sequential screening number. If the patient is randomized, the randomization number will be assigned in a sequential manner. This number will be used for identification throughout the study and will not be used for any other participant.

If a patient is dropped from the study for any reason, the patient's randomization number will not be reassigned.

5. STUDY MEDICATION

5.1. PRESENTATION, PACKAGING AND LABELING, SUPPLY, AND STORAGE OF THE INVESTIGATIONAL MEDICINAL PRODUCT

5.1.1. Presentation of the Investigational Medicinal Product

In this study the IMP will be either reparixin or matched placebo, which will be provided in the form of tablets to be disintegrated for naso-gastric tube administration or administered orally after extubation.

A tablet of reparixin has the following composition:

Composition of each reparixin unit (tablet)

Ingredient	Amount per tablet (mg)	Function	Quality standard
Reparixin (DF 1681Y)	600.0	Drug substance	In-house specification
[redacted]	[redacted]	CCI [redacted]	[redacted]
CCI [redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	---	---

Composition of each placebo unit (tablet)

Names of ingredients	Formula %	Amount per tablet	Function of ingredient	Reference to quality standards
Reparixin (DF 1681Y)	---	---	Drug substance	Internal monograph
CCI [redacted]				

Batch release certificate will be provided together with the IMP. Placebo tablets are identical in appearance to the active formulation.

5.1.2. Manufacturing, Packaging and Labeling of IMP

Tablets will be manufactured by either PPI or PPI PPI Primary packaging will be performed by either PPI or PPI Secondary packaging and labeling will be performed by PPI PPI or, alternatively, by PPI

The study medication will be provided as a **Patient Kit**, containing CCI [redacted]

All labels will be prepared to meet local regulatory requirements. Details of packaging and labeling are reported in **Appendix 14.3**.

5.1.3. Supply, Storage and Handling of IMP

An appropriate number of packages will be initially sent to the site as soon as all essential documents and regulatory/ethics approvals have been obtained. IMP re-supply will be planned on demand, according to enrolment rate.

The IMP must be kept at a temperature not exceeding 30°C and must not be frozen.

A temperature probe will accompany the drug on shipment. Temperature range reached during shipment will be verified on receipt at site, so that potential stability concerns during shipment can be investigated and appropriate action taken.

Once received at the site, the Pharmacist (or designee) will check the package for accurate delivery and acknowledge receipt; any deviations from expected package content (inconsistency, damages) should be immediately reported to Dompé (or appointed CRO) and the use of the drug suspended until authorization for its continued use has been given by Dompé (or appointed CRO).

The IMP must be stored at site in a secure location, in a temperature-controlled room. Temperature records must be available for the clinical research associate (CRA) to review at monitoring visits; any deviations from the recommended storage conditions should be immediately reported to Dompé (or appointed CRO) and the use of the drug suspended until authorization for its continued use has been given by Dompé (or appointed CRO).

5.1.4. Unblinding

Appearance, including packaging and labeling, of the IMP will not allow recognition of the treatment arm (either reparixin or placebo).

For each randomized subject, individual code breaks will be accessible in the event of a medical emergency requiring knowledge of the treatment assigned to the subject. Only the responsible investigator, or authorized delegates, can break the code via the Interactive Response System (IRS).

Investigators will be allowed to unblind study medication directly through the IRT system; any unblinding must be notified to the CRO's medical monitor. Training is provided to investigators prior to authorization to use the IRT system and the unblinding function is outlined in the study specific user guide.

Dompé Pharmacovigilance, Safety and Surveillance Department shall break the treatment code for subjects who experience a potential Suspected Unexpected Serious Adverse Reaction (SUSAR), in order to determine if the individual case requires expedited regulatory reporting and fulfills expedited regulatory reporting requirements. Unmasked information will not be disclosed to Investigators and other Sponsor staff.

With the exception of the above-mentioned episodes, the identity of the treatments will remain unknown to the subject, Investigator, site staff, CRO and Dompé's personnel until the study completion and formal unmasking. Only the Data Monitoring Committee (DMC) will have access to group-unblinded and/or fully unblinded DMC reports.

For analysis purposes, the randomization codes will be broken when the last enrolled patient has completed therapy, and once the database has been locked.

5.2. DOSE, ROUTE AND SCHEDULE OF IMP ADMINISTRATION

Reparixin will be administered through a nasogastric tube at the dose of 1200 mg (2 x 600 mg tablets) TID every 8 hours (6 tablets daily) for 14 days. If the patient becomes extubated prior to the end of treatment on day 14 and if swallowing ability has been restored as per clinical judgment, the IMP can be administered orally. In such cases the IMP may be administered either intact or crushed and mixed with vehicle as per speech swallow recommendation. The IMP may be administered for a total of 21 days if the patient is still intubated on Day 14. Placebo will be administered with the same treatment schedule.

Administration of the study drug through a nasogastric tube should follow this procedure: for each administration, disperse two reparixin 600 mg tablets in 25 mL of drinking water in a suitable container (e.g., conical tubes for 50 mL Falcon centrifuge). Disintegrate the tablets (shaking manually or with the aid of a

planetary shaker or a rocker) until obtaining a homogeneous milky suspension (time required 7–10 minutes). Keep the prepared suspension at room temperature and protected from light for up to 24 hours. Immediately before administration, manually shake the suspension again until complete and homogeneous resuspension, withdraw using a 50 mL needle-free syringe and administer to the patient through the nasogastric tube. After administration, flush the gastric tube with 25 ml of water.

5.3. CRITERIA FOR SCHEDULE ADJUSTMENT/DOSE-MODIFICATION OR DISCONTINUATION OF THE IMP

5.3.1. Criteria for schedule adjustment/dose-modification

No schedule adjustment and/or dose modification is foreseen, except for discontinuation of IMP as detailed below.

5.3.2. Criteria for permanent discontinuation of the IMP

The IMP must be discontinued in the case:

- Upon initiation of renal replacement therapy;
- Upon increase of ALT and/or AST to≥ 3x ULN and total bilirubin > 2x ULN or upon increase of ALT and/or AST to ≥ 5x ULN
- The patient is discharged home;
- Withdrawal of informed consent by the patient or his/her legal representative (in this case, the patient / representative will be asked whether they consent to usage of the data already collected in the study; if not, the data will be deleted);
- Pregnancy occurs;
- Development of Serious AE probably or possibly related to IMP, as per PI's judgment;
- Inadvertent missing of 3 or more consecutive doses of the IMP;
- Inability to resolve within 24 hours any medical condition/event that led to temporary withholding of the IMP (see also 5.3.3.);
- Recurrence of AE probably or possibly related to IMP upon re-introduction of the IMP.

Occurrence of renal or hepatic dysfunction will be monitored as part of the biochemistry data set through safety laboratory tests obtained at day 3±8h, 7±1, 14±2, and 21 (if the IMP is meant to be administered up to 21 days). The other criteria that qualify the patient to treatment discontinuation will be monitored daily.

If the IMP administration is prematurely discontinued, the primary reason for discontinuation must be recorded in the eCRF. Patients who discontinue the treatment with the IMP will NOT be withdrawn from the study by default and will be asked to complete safety and efficacy observations as per the protocol unless they withdraw their consent.

5.3.3. Criteria for temporary withholding of the IMP

The IMP can be temporarily withheld upon:

- Development of AE (excluding Serious AE) probably or possibly related to IMP, as per PI's judgment
- Any AE (including Serious AE) not thought to be related to IMP as per PI's judgment.
- Any medical condition that may threaten the safety of the patient if they continue to receive study treatment (as per investigator or Sponsor evaluation)

Temporary withholding of the IMP should not exceed 24 hours during which time every effort should be made to resolve/stabilize conditions that led to IMP withholding. If this is not resolved within the allotted 24 hours the

IMP should be permanently discontinued.

5.4. ACCOUNTABILITY OF THE IMP

All supplies will be maintained under adequate security by the designated member of site staff, until they are dispensed to the patients. The Investigator will ensure that study treatment is only dispensed by designated staff within the study site.

When the IMP is received at the site, the designated staff member will check for accurate delivery and acknowledge receipt by signing and dating the documentation provided by or on behalf of Dompé and returning it to Dompé or to the appointed CRO. A copy will be retained for the Investigator/Pharmacy file.

The dispensing of the IMP will be carefully recorded on the eCRF and appropriate drug accountability forms; an accurate accounting will be available for verification by the CRA at each monitoring visit.

Drug accountability records will include:

1. the confirmation of receipt of the IMP at the trial site,
2. the dispensing of the IMP to the patient,
3. the disposition of unused product(s),
4. accounts of any IMP accidentally or deliberately destroyed.

They should include dates, quantities, batch numbers, expiration dates (if applicable), and any unique code numbers assigned to the IMP and/or patients. Investigators should maintain records which document adequately that:

1. The patients were provided the doses specified by the protocol/amendment(s),
2. The IMP provided was fully reconciled at the site.

The administration of the IMP (date/time for each administration) will be recorded by the site staff in the eCRF. The CRA will review the drug accountability forms/eCRF and check all IMP (both unused and used) prior to deciding on their disposal.

IMP which has been dispensed to a patient and was not used will not be re-dispensed to a different patient. Unused IMP (tablets) must remain in the Patient Kit and must not be discarded or used for any purpose. Any remaining test material at the end of the trial will be returned to Dompé or disposed of, as determined by Dompé.

5.4.1. Assessment of compliance

The actual doses of the study drugs received by each patient during the trial will be recorded. A reconciliation will be made by the study monitor between expected and actual administrations over the time between the start and the end of treatment. Discrepancies will be documented and justified.

Compliance with the study product dosing schedule will be verified by the investigator and confirmed/checked by the delegated CRA during on-site monitoring visits, as per records in the specific study eCRF and actual tables remaining in the Patient Kits. Exposure, calculated on the 48h regimen, will be assessed based on the actual duration of treatment.

5.5. PRIOR AND CONCOMITANT MEDICATIONS

5.5.1. Reporting of prior and concomitant medications

Prior and concomitant therapy consists of any medication (e.g., prescription drugs, over-the-counter drugs, vaccines) used by a patient in addition to the IMP from 7 days before the screening to the end of the study. All such medications will be reported in the appropriate section of the eCRF.

All the details as per the eCRF fields (sequential number, drug name, indication, starting dose, start/stop date, route of administration) will be recorded.

5.5.2. Medications to be used with caution

From an in vitro study, reparixin was found to slightly inhibit human CYP3A4, CYP2C9 and, in minor extent, CY2C19 isoenzymes. However, the results of a clinical study, investigating the interaction of reparixin with probe drugs for CYP2C9 and CYP3A4 showed that the concomitant administration of reparixin does not have a significant effect on PK of drugs metabolized by the above isoenzymes. Reparixin, in human hepatocytes, did not show induction of the CYP2C8, CYP3A4 and CYP3A5 mRNA levels at the tested concentration range. Furthermore, reparixin is not an inhibitor of docetaxel metabolism and inhibits paclitaxel metabolism with a non-clinical relevant IC50 value > 100 μM. Therefore, for the purposes of this study and taken under consideration the intended patient population, the following medications with a potential to alter CYP2C9 activity may be used if the clinical benefit is considered to be higher than the perceived risk according to the PI's discretion:

1) CYP2C9 inducers (rifampin, phenytoin, carbamazepine, aprepitant, bosentan, phenobarbital)

2) CYP2C9 inhibitors (amiodarone, fluconazole, miconazole, oxandrolone, capecitabine, cotrimoxazole, etravirine, fluvastatin, fluvoxamine, metronidazole, sulfinpyrazone, tigecycline, voriconazole, zafirlukast).

6. STUDY PROCEDURE AND ASSESSMENT

During the course of the study, assessments will be performed as defined in the Schedule of Assessments (Section 2).

For all measurements, the actual date and time of assessment, including date of sampling, will be recorded in the Source Document and in the eCRFs.

6.1. STUDY VISITS AND STUDY EVENTS/PROCEDURES DETAILS

The study will consist of 4 study periods: screening, randomization and baseline assessments, treatment (up to 21 days), and follow-up (up to 60 days). Potential participants will be identified from those hospitalized at the participating clinical sites with a diagnosis of ARDS. Patients under evaluation at the Emergency Department with an expected admission to the ward will be considered hospitalized for study purposes.

6.1.1. Screening Procedures

Informed consent must be documented before any study-specific screening procedure. Patients should not be screened if, for any reason, there is a high probability that they will be transferred during the treatment phase to another institution not belonging to the study network.

Procedures included in the screening will include:

- Demographic data, past medical history, allergies to medications, disease-specific clinical information
- Concomitant medications
- History of side effects related to prior use of NSAIDs such as gastrointestinal (GI) bleeding or other form of bleeding, peptic ulcer disease)
- Blood sampling for measurement of hematology/biochemistry (Safety Laboratory Tests) and derived renal and hepatic function (unless already collected for clinical care)
- Pregnancy test (either urine or serum test is acceptable) in women of childbearing potential; positive urine test results will be confirmed with a serum pregnancy test;
- 12-lead ECG performed using local equipment (unless already collected for clinical care)
- PaO_2 and FIO_2
- Chest imaging (either CXR or CT scan). Imaging performed for diagnostic purposes prior to the informed consent process will be accepted if it is done within 24 hours from screening

The overall eligibility of the subject to participate in the study will be assessed once all screening values are available. If a subject fails screening, he/she will be recorded as a screen failure. In that case the subject may be rescreened once (immediately or later as long as randomization can be accomplished within the 48 hour window from ARDS diagnosis as per inclusion criteria) if deemed appropriate by the investigator. In that case, the subject must be re-consented. A new rescreening period will start, all screening procedures must be repeated.

6.1.2. Randomization and Baseline assessments

Patients meeting all inclusion and none of the exclusion criteria will be enrolled (eligibility confirmed) and randomized (see sections 4.1 and 4.2) at the latest within 48 hours from ARDS diagnosis as described in inclusion criteria. Consecutive randomization numbers will be given to the subjects upon their confirmed eligibility for randomization. Subjects will be assigned to their treatment according to their randomization number. Patients will be randomized in a 1:1 fashion between reparixin and placebo using a computer-generated randomization list generated in the study. Randomization will be performed through IRT. Investigators will have to remain blind throughout the whole study duration. Information for unblinding is provided in section 5.1. IMP will be administered TID for 14 days with the option of continuation to 21 days if the patient is still intubated on Day 14.

Before starting the administration of the IMP, the following baseline data will be collected:

- PaO_2, $PaCO_2$ and FIO_2
- Vital signs: respiratory rate (RR), heart rate (HR), mean arterial pressure (MAP)
- IMV parameters [tidal volume (TV), PEEP, mean airway pressure, plateau airway pressure)

- Use of neuromuscular blocking agents and inhaled vasodilators
- Prone positioning
- Vasoactive medications (drug and dose calculated in norepinephrine equivalents)
- Sequential organ failure assessment (SOFA) score
- CXR (if no more than 24 hours have elapsed from a screening CXR, baseline CXR will be deferred)
- Il-6, IL-8, PAI-1, TNFr-1, ICAM-1, RAGE

6.1.3. Treatment and post-treatment hospital stay up to day 60

The below data will be collected daily from day 1 until extubation:

- PaO_2, $PaCO_2$ and FIO_2
- Vital signs: RR, HR, MAP
- IMV parameters (TV, PEEP,, mean airway pressure, plateau airway pressure)
- Use of neuromuscular blocking agents and inhaled vasodilators
- Prone positioning
- Transition to ECMO
- Vasoactive medications (drug and dose calculated in norepinephrine equivalents)
- SOFA score
- Number of attempts at weaning defined as continuous positive airway pressure (CPAP) trial (CPAP 5 cmH_2O plus PEEP 5 cmH_2O) of 2 hours duration

If an initial extubation is followed by re-intubation the above variables will be collected daily for each subsequent intubation.

At days 2(±8h), 3(±8h), 7 (±1 day), and 14 (±2 day) a CXR will be performed (CXR performed for routine clinical care in ICU patients will be accepted)

At days 3±8h, 7±1, 14±2 blood samples will be obtained for measurement of the following plasma biomarkers: IL-6, IL-8, PAI-1, TNFr-1, ICAM-1, RAGE

Between extubation and day 28 or hospital discharge (if it occurs before day 28) the following data are collected every 48±8 hours:

- Vital signs: RR, HR, MAP
- PaO_2 (or SpO_2) and FIO_2
- Type of oxygen supportive therapies used if any
- Need for reintubation (after initial extubation)

At day 28 and hospital discharge, the following data will be collected:

- PaO_2 (or SpO_2) and FIO_2
- Type of oxygen supportive therapies used, if any
- Duration of IMV
- ICU length of stay
- Need for reintubation (after initial extubation)
- Hospital length of stay
- Performance of tracheostomy
- Transfer to long term acute care (LTAC*) facility

The above information, together with mortality, may be collected by phone follow-up.

***LTAC**: An LTAC is a Long Term Acute Care Hospital, i.e., a facility that specializes in the treatment of patients with serious medical conditions, including patients with ongoing needs for mechanical ventilation, but who no longer require intensive care or extensive diagnostic procedures. The patients in LTAC are transferred there directly from the intensive care unit because they require more care than they can receive in a rehabilitation center, skilled care facility or at home.

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Safety: The following safety assessments will be performed on day 3±8h, 7±1, 14±2, 21±2 (if reparixin is still administered), 28±2 and at hospital discharge

- Safety Laboratory Tests (Haematology/biochemistry)
- Estimated Glomerular Filtration rate (eGFR) evaluation
- Hepatic function evaluation up to day 14 or 21, depending on end of treatment
- 12 lead ECG and rhythm analysis

Post-hospital discharge follow-up: on day-60 the patients will be contacted by phone for evaluation of vital status and AEs. Patients will be considered lost to follow up when unable to be contacted upon 3 attempts made on 3 separate days and at 3 separate times.

6.2. EARLY PATIENT WITHDRAWAL

6.2.1. Withdrawal criteria

Patients will be informed that they have the right to withdraw from the study at any time (withdrawal of consent), without prejudice to their medical care, and are not obliged to state their reasons.

The primary, actual, reason for withdrawal should be recorded, especially if there is suspicion of withdrawal due to adverse events ("withdrawal of consent" is a definition to be used if the patient does not give reasons). Safety laboratory tests should be performed whenever possible at patient withdrawal.

Patients who discontinue the treatment with the IMP (Section 5.3.2) will not be withdrawn from the study but will be asked to complete observations as per the protocol, unless they withdraw their consent. It is important that any randomized patient remains in the study and is followed for both efficacy and safety outcomes, regardless of whether he/she has completed or discontinued the study treatment.

Investigators will be trained on the importance of patient retention through the duration of the trial.

In case of pregnancy, the patient will be withdrawn from the study, but she will be monitored for safety and pregnancy outcomes, unless she withdraws her consent.

Any withdrawals must be fully documented in the eCRF.

6.2.2. Replacement procedures

There are no plans to replace discontinued subjects.

6.3. END OF STUDY

For the purpose of this trial, the End of Study is defined as the date of the last assessment of the last patient.

6.4. PATIENT MANAGEMENT AFTER STUDY COMPLETION OR TERMINATION

After completion of the 28-day assessment or at study termination (for any other reason), patients will receive post-study care as prescribed by their non-study health care provider. No post-study or post study-termination treatment will be provided by the study team or Dompé.

6.5. ASSESSMENTS AND CLINICAL DEFINITIONS

Assessment type	Parameters to be analyzed (units)
Demographic data	- age (years); - date of birth (or only year of birth if full date of birth cannot be recorded, for local regulations); - sex (M/F); - self-reported race/ethnicity (Black / African American, White, Asian, Hispanic / Latino, multiple / other) - height (cm), also self-reported; - body weight (kg)
Medical history	- all relevant past and ongoing diseases and surgeries - allergies, with a particular focus on IMP-related allergy/intolerance (such as ibuprofen hypersensitivity, lactase deficiency, galactosemia, glucose-galactose malabsorption) - past (no / yes) or current tobacco use (no / yes, < or ≥ 10 cigarettes daily or equivalent) - past (no / yes) or current alcohol consumption (no / yes, < or ≥ one liter of wine daily, or equivalent) - recent test for influenza (no / yes, positive / negative) - vaccination for COVID-19 (no / yes: number of doses) - date of hospital/emergency department admission
Chest imaging	CXR/CT scan Findings to be recorded (multiple answers allowed): ● Consolidation ● Ground-glass opacities ● Air bronchograms ● Pleural and Interlobular septal thickening
Safety Laboratory Tests	Hematology ● RBC count (n x10^6/μL), hematocrit (%), hemoglobin (g/dL) ● WBC count and differential count: neutrophils, eosinophils, basophils, monocytes, lymphocytes (n x10^3/μL or percentages of WBC) ● platelet count (n x10^3/μL) Biochemistry ● albumin (mmol/L or g/dL) ● AST (nkat/L or U/L) ● ALT (nkat/L or U/L) ● total and direct bilirubin (e.g., mg/dl or μmol/L) ● creatinine (μmol/L or mg/dL) ● estimated Glomerular Filtration Rate (eGFR; 2021 CKD-EPI formula) ● serum glucose (mmol/L or mg/dL); specify if fasting or random ● sodium (mmol/L or mEq/L), potassium (mmol/L or mEq/L), chloride (mmol/L or mEq/L), calcium (mmol/L or mg/dL) (units may change as per local lab standard)

Inflammatory markers	● IL-6 (pg/ml) ● IL-8 (pg/ml) ● TNFr1 (pg/ml) ● PAI-1 (ng/ml) ● ICAM-1 (ng/ml) ● RAGE (pg/ml)
Prior and Concomitant medications	At screening: recording of drug name, indication, total daily dose, dose unit, route, start and end dates During hospital stay: recording of any change of already recorded, or new concomitant medications
Lung function	- Peripheral arterial oxygen saturation (SpO_2; %) - Partial pressure of oxygen (PaO_2; mmHg) - Partial pressure of carbon dioxide ($PaCO_2$; mmHg) - Mean airway pressure (cm H_2O) - Plateau airway pressure (cm H_2O) - PEEP (cm H_2O) - Tidal volume (mL) - Fraction of inspired O_2 (FIO_2; 0.21 to 1) - PaO_2/FIO_2 (mmHg); SpO_2 may be used in place of PaO_2 if arterial sample is not available
IMV/ECMO	- Endotracheal intubation - Tracheostomy tube Extracorporeal Membrane Oxygenation Duration of IMV or ECMO to be recorded (days)
SECONDARY INFECTIONS	Defined as: 1) New (occurring after the first IMP intake) infection in a previously known to be sterile site, including blood, body fluid or tissue. 2) New pathogen isolated from cultures of biological samples known to be previously infected
ECG	ECG after the subject has been lying quietly for 5 minutes. The following to be recorded: - overall assessment for the presence of alterations (no / yes), - presence of: arrhythmia, conduction abnormalities; ST-segment elevation / depression; T-wave alterations; - HR (bpm) - PQ interval (msec); - QT/QTc (Bazett formula; msec) Clinically significant abnormalities should be marked in the ECG-CRF (no / yes, specify) and also reported as an AE

7. STUDY ENDPOINTS

7.1. EFFICACY ENDPOINTS

Primary endpoint:

- Change in oxygenation index (OI) from baseline to day 7 of treatment. The OI is defined as: % mean airway pressure x FIO_2/PaO_2
- Ventilator free days (VFD) at day 28 (see 14.4.11)

Secondary endpoints:

- Change in OI from baseline to day 4
- Acute lung injury score [composite of PaO_2/FIO_2 ratio, PEEP, lung compliance (plateau airway pressure minus PEEP/TV) and extent of pulmonary infiltrates] at 2, 3, 7, 14 days (if still intubated)
- SOFA scores at 2, 3, 7, 14 days (if still intubated)
- Ventilatory ratio (product of minute ventilation and $PaCO_2$) at 2, 3, 7, 14 days (if still intubated)
- Incidence of ECMO at day 14
- Use of vasoactive medications at day 14
- CXR assessment of pulmonary edema by "radiographic assessment of lung edema" (RALE) score at 2, 3, 7, 14 days
- Percentage of patients achieving pressure support ventilation equal to 5 cm H_20 with PEEP equal to 5 cm H_20 for 2 hours (measure of weaning) by day 28 and hospital discharge
- ICU-free days by day 28 and hospital discharge
- Hospital-free days by day 28 and hospital discharge
- Incidence of tracheostomies by day 28 and hospital discharge
- Incidence of LTAC facility by day 28 and hospital discharge
- All-cause mortality by day 28
- Hospital discharge by day 28
- All-cause mortality by day 60
- Change from baseline to day 3, 7 and 14 in plasma levels of Il-6, IL-8, PAI-1, Plasma TNFr-1, ICAM-1 RAGE

Footnote: day 1 is the day when the IMP is first administered. Hour 1 of day 1 starts when the first dose of the IMP is administered. Subsequent days are measured in 24-hour intervals from hour 1. Therefore, day 2(±8h) signifies 24 hours ±8 hours from hour 1.

7.2. SAFETY ENDPOINTS

- Hematology (RBC, hematocrit, hemoglobin, WBC, neutrophils and lymphocytes absolute count, platelets count) and biochemistry (sodium, potassium, chloride, calcium, glucose, creatinine, eGFR albumin, AST, ALT, total and direct bilirubin). Change from screening to day 3±8h, 7±1, 14±2, 21±2 (if still receiving reparixin, or end of treatment), 28±2 and at hospital discharge
- eGFR, absolute value and change from screening to day 3±8h, 7±1, 14±2, 21±2 (if still receiving reparixin, or end of treatment), 28±2 and hospital discharge
- ECG. Change from screening to day 3±8h, 7±1, 14±2, 21±2 (if still receiving reparixin, or end of treatment), 28±2 and at hospital discharge.
- Incidence of secondary infections defined as new (occurring after the first IMP intake) infection in a previously known to be sterile site, including blood, body fluid or tissue, or new pathogen isolated from cultures of biological samples known to be previously infected by day 28±2
- Incidence of TEAEs and TESAEs from the beginning of IMP administration up to the end of study participation

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8. ADVERSE EVENTS

8.1. DEFINITIONS

Adverse event

An Adverse Event **(**AE**)** is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Adverse Drug Reaction

An Adverse Drug Reaction **(**ADR**)** is defined as an adverse event, which is reasonably likely to have been caused by the IMP. The definition also covers medication errors and use of the IMP outside what is foreseen in the protocol, including misuse and abuse of the product. For the purposes of IND safety reporting in the U.S., "reasonable possibility" means there are facts (evidence) or arguments to suggest a causal relationship between the drug and the adverse event.

Serious Adverse Event/Reaction

A Serious Adverse Event **(**SAE**)**/Reaction is defined as any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (i.e., the patient is at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or prolongation of existing hospitalization

NOTE: In general, hospitalization means that the individual remained at the hospital or emergency ward for observation and/or treatment (usually involving an overnight stay) that would not have been appropriate in the physician's office or an out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred, the event should be considered serious.

- results in persistent or significant disability/incapacity

NOTE: This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, or accidental trauma (e.g., sprained ankle, back pain) which may interfere or prevent everyday life functions but does not constitute a substantial disruption.

- is a congenital anomaly/birth defect
- is a medically significant or important medical condition, i.e., an important medical event as determined by appropriate medical judgment, which may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

NOTE: An important medical condition is an event that may not result in death, be life-threatening, or require hospitalization but may be considered a SAE when, based upon appropriate medical judgment, it may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in patient hospitalization, or the development of drug dependency or drug abuse.

Pre-planned hospitalization or hospitalization for routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, are not considered to be serious events. These events must be recorded in the AE section (except for hospitalization for study procedures) of the eCRF where a variable will be ticked to indicate that they are not SAEs.

Death shall always be reported as SAE, and cause of death shall always be specified, when known. If unknown, "death NOS" (not otherwise specified) shall be reported as an event.

Unexpected Adverse Events

An AE or ADR is considered unexpected if it is not listed in the Investigator Brochure (Reference Safety Information section). An event is unexpected also when it is not listed at the specificity or severity that has been observed and listed in the Investigator Brochure. Events that are mentioned in the Investigator Brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation are considered unexpected. The determination of expectedness shall be made on the basis of the IB Reference Safety Information (RSI) section.

According to the Investigator Brochure (Reference to Safety Information, section 6.4), for the purpose of this study and considering the current early clinical development status of the IMP for the indication investigated, each adverse event will be carefully evaluated and no Serious Adverse Reaction will be considered expected by the sponsor for the purpose of expedited reporting of SUSARs and the identification of SUSARs in the "Cumulative summary tabulation of serious adverse reactions" in the Development Safety Update Report on the IMP.

Suspected serious unexpected adverse reaction

A suspected serious unexpected adverse reaction (SUSAR) is defined as an adverse reaction that is both unexpected (not consistent with the applicable product information) and also meets the definition of a Serious Adverse Reaction.

8.2. MONITORING FOR ADVERSE EVENTS

Following study informed consent form signature, the Investigator or appropriate designee should inquire/monitor daily about the occurrence of an AEs.

AEs should be reported for any clinically relevant untoward (unfavorable and unintended) change in a patient's medical condition. Changes in any protocol-specific systemic parameter evaluated during the study are to be reviewed by the Investigator. Any untoward (unfavorable and unintended) change in a protocol-specific parameter or questionnaire response that is clinically relevant is to be reported as an AE. These clinically relevant changes will be reported regardless of causality.

8.3. RECORDING OF ADVERSE EVENTS

All AEs (serious and non-serious) which occur from signature of the informed consent through patient participation in the study (last planned visit or early withdrawal date) will be collected and recorded in the eCRF. It is important that the AE dedicated section of the eCRF includes the duration of the AE (onset/resolution dates), the relationship to the drug, the severity, the outcome, the action(s) taken and relevant concomitant treatments dispensed. When possible, signs and symptoms indicating a common underlying pathology should be documented as one comprehensive event.

All AEs should be followed-up to determine the outcome of the reaction. The Investigator should follow up the event until resolution or stabilization of the condition. It is the Investigator's responsibility to assure that the patients experiencing an AE receive definite treatment for any AE, if required.

Medical conditions/diseases present before starting study treatment shall be documented in the medical history section of the eCRF; these conditions are considered AEs only if they increase either in frequency or severity once informed consent has been signed.

8.3.1. Follow-up of patients with AEs

The Investigator is responsible for adequate and safe medical care of patients during the trial and for ensuring that appropriate medical care and relevant follow-up procedures are maintained after the trial. All AEs should be followed-up to determine the outcome of the reaction. The Investigator should follow up the event until resolution or stabilization of the condition. It is the Investigator's responsibility to assure that the patients experiencing AEs receive definite treatment for any AE, if required.

If a patient's conditions worsen due to a SAE, the Investigator will provide supporting documentation, as well as results of any relevant laboratory tests and redacted section of medical records may be provided to

the Sponsor and/or delegate, if relevant for the SAE. In case of death, a copy of the autopsy report, if performed, should also be provided.

The Investigator shall inform the Sponsor with an appropriate written communication, whenever he becomes aware of new available information regarding the SAE, once the condition is resolved or stabilized and when no more information about the event is expected. Follow-up SAE information should be processed as initial SAE notification (see Sections 8.4, 8.5).

For pharmacovigilance purposes, all SAEs should be followed-up in order to clarify as completely as possible their nature and/or causality and until all queries have been resolved. All SAEs will be followed up until the events resolve or the events or sequelae stabilize, or it is unlikely that any additional information can be obtained after demonstration of due diligence with follow-up efforts (i.e. patient or Investigator is unable to provide additional information, or the patient is lost to follow up), unless patient has withdrawn his/her consent.

8.3.2. Relationship of AEs to the Investigational Medicinal Product

The Investigator will assess the causal relationship between the AE and the IMP (either reparixin or placebo), according to the criteria in the Table below:

Relationship of the AEs to the IMP

None (Intercurrent Event)	An event that is not and cannot be related to the Investigational Product, e.g. a surgical intervention for nevus removal performed during the study, but planned before patient enrolment into the study
Unlikely (remote)	Relationship is not likely e.g. a clinical event including laboratory test abnormality without temporal relationship to drug administration which makes a causal relationship improbable and in which other drugs, chemicals or underlying disease provide more plausible explanations
Possible	Relationship may exist, but could have been produced by the patient's condition or treatment or other cause
Probable	Relationship is likely, the AE abates upon discontinuation of Investigational Product and cannot be due to the patient's condition
Highly Probable	Strong relationship, the event abates upon discontinuation of Investigational Product and, if applicable, re-appears upon repeat exposure

Any AE reported in the study having a possible, probable or highly probable relationship to the study drug will be considered as an ADR. On the other hand, AEs marked with relationship none or unlikely, will not be considered as ADR.

8.3.3. Severity of adverse events

The Investigator will grade the severity of any AE using the definitions in the Table below. For each episode, the highest severity grade attained should be reported.

Severity of the Adverse Event

Mild	Grade 1 - Does not interfere with the patient's usual function (awareness of symptoms or signs, but easily tolerated [acceptable]).
Moderate	Grade 2 - Interferes to some extent with the patient's usual function (enough discomfort to interfere with usual activity [disturbing]).
Severe	Grade 3 - Interferes significantly with the patient's usual function (incapacity to work or to do usual activities [unacceptable])

8.4. SERIOUS ADVERSE EVENT REPORTING

8.4.1. Reporting Procedure to Dompé/CRO

The Investigator must report all SAEs occurring during patient participation in the study, regardless of presumed causal relationship, to the appropriate Sponsor and CRO Pharmacovigilance contact **within 24 hours** of learning of the event. Contact details for SAE reporting by the Investigator are provided in the section "Contact Information" (See Page 2 of this Protocol). SAE form notification will be performed on a paper based or electronically using a validated e-system according to Sponsor indication and upon proper training on the e-system. Paper form can be used as back-up in case of unavailability of the eDC and after study termination.

The Investigator should also report information on SAEs that continue after the patient has completed his/her participation in the study (whether study completion or withdrawal) unless the patient has withdrawn his/her consent.

In line with CT3 Detailed Guidance and ICH E2A provisions, although the Investigator does not usually need to actively monitor patients for AEs once the trial has ended, if the Investigator becomes aware of a SAE occurring to a patient after that patient has ended his/her participation in the study (whether study completion of withdrawal), the SAE should be reported by the Investigator directly to the Dompé Global Pharmacovigilance Safety and Surveillance Department, even after the termination of the study. Such "post-study cases" should be regarded for expedited reporting purposes as though they were study reports. Therefore, a causality assessment and determination of expectedness are needed for a decision on whether or not expedited reporting is required.

Information on SAEs will be recorded on a specific SAE form. Both electronic and blank paper copies will be included in the Investigator's Site File. Follow-up reports (as many as required) should be completed and emailed/faxed following the same procedure above.

Whenever more than one SAE is observed, the Investigator should identify which is the primary adverse event, i.e., the most relevant one. If other events are listed in the same report, the Investigator should identify, along with their relatedness to the IMP, which adverse events are serious, and which are non-serious. In all cases, the Investigator is requested to record his/her opinion about the relatedness of the observed event(s) with the IMP.

8.4.2. Conditions that should not be reported as serious adverse events

Not applicable.

8.4.3. Adverse events exemption

Not applicable. There is no event to be considered routinely associated with any clinical study procedure, therefore requiring neither recording nor reporting.

8.4.4. Reporting Procedure to IEC and to Regulatory Authorities in the European Union

Reporting of Suspected Unexpected Serious Adverse Reaction

The Investigator must report all SAEs to the Sponsor/CRO immediately, within 24 hours (see Section 8.4.1).

Dompé Global Pharmacovigilance, Safety and Surveillance Department, with the support of the CRO as appropriate, shall report any SUSAR to the concerned IEC which approved the protocol and the Regulatory Authority (via the EudraVigilance Clinical Trial module) as soon as possible, and in no event later than:

- seven calendar days after becoming aware of the information if the event is fatal or life threatening; to be followed by any relevant information within eight days.
- fifteen calendar days after becoming aware of the information if the event is neither fatal nor life threatening.

Treatment will be unblinded by Dompé Global Pharmacovigilance, Safety and Surveillance Department prior to regulatory submission of a SUSAR to Regulatory Authorities and IEC, and only cases referred to as active treatment will be considered expeditable for regulatory reporting, in line with law requirements.

If the results of an investigation show that an AE not initially determined to be reportable is reclassified as reportable, Dompé shall notify such SUSAR in a written safety report as soon as possible, but in no event later than 7/15 calendar days after the determination is made.

Copies of all correspondence relating to reporting of any SAEs to the IEC should be maintained in the Investigator's Files.

Periodical Reporting to EU Regulatory Authorities and Investigators

Dompé Global Pharmacovigilance, Safety and Surveillance Department will prepare and submit (via the CRO as applicable) to Investigators appropriate periodic safety updates as per applicable EU and local requirements and regulations. Dompé Global Pharmacovigilance, Safety and Surveillance Department shall also be responsible to prepare and submit annual safety reports (Development Safety Update Report – DSUR) to relevant Regulatory Authorities and to IECs.

8.4.5. Reporting Procedures to IRB and to the FDA in the United States

Reporting of Suspected Unexpected Serious Adverse Reaction

The Investigator must report all SAEs to the Sponsor/CRO immediately, within 24 hours (see Section 8.4.1).

In line with provisions set forth in 21CFR312, Dompé Global Pharmacovigilance, Safety and Surveillance Department, with the support of the CRO as appropriate, shall notify the Investigators and the FDA in an IND safety report of any SUSAR and of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than:

- seven calendar days after becoming aware of the information if the event is fatal or life threatening; to be followed by any relevant information within eight days.
- fifteen calendar days after becoming aware of the information if the event is neither fatal nor life threatening.

The Investigators in turn shall notify their IRB. Investigators are required to promptly report "to the IRB all unanticipated problems involving risk to human patients or others," including adverse events that should be considered unanticipated problems (21 CFR 312.66).

The blind should ordinarily be broken for IND safety reports submitted to FDA and all participating investigators. Treatment will be unblinded by Dompé Global Pharmacovigilance, Safety and Surveillance Department prior to regulatory submission of a SUSAR to FDA and IRB and only cases referred to as active treatment will be considered expedited for regulatory reporting, in line with law requirements.

If the results of an investigation show that an AE not initially determined to be reportable is reclassified as reportable, Dompé Global Pharmacovigilance, Safety and Surveillance Department shall notify such SUSAR in a written safety report as soon as possible, but in no event later than 7/15 calendar days after the determination is made.

Copies of all correspondence relating to reporting of any SAEs to the IRB should be maintained in the Investigator's Files.

Potential serious risks arising from clinical trials or any other source, to be reported to FDA and to the Investigators, include:

- Any SUSAR. Dompé must report an adverse event as a suspected adverse reaction only if there is evidence to suggest to the Sponsor a causal relationship between the drug and the adverse event.
- Findings from other studies that suggest a significant risk in humans exposed to the drug. Such a finding would result in a safety-related change in the overall conduct of the clinical investigation.
- Findings from animal or *in vitro* testing that suggest a significant risk in humans exposed to the drug.
- Increased rate of occurrence of serious suspected adverse reactions.

Periodical Reporting to US Regulatory Authorities

Based on the specific Investigator's site requirements, Dompé Global Pharmacovigilance, Safety and Surveillance Department (via the CRO as applicable) will submit to IRBs and Investigators periodic safety updates, as per applicable local requirements and regulations.

Dompé Global Pharmacovigilance, Safety and Surveillance Department (via the CRO as applicable) shall also be responsible to prepare and submit annual safety reports (Development Safety Update Report – DSUR) to FDA and IRBs, as applicable.

8.5. EXPOSURE TO IMP DURING PREGNANCY

Women of childbearing potential are defined as all women physiologically capable of becoming pregnant. As the study will be conducted in hospitalized critically ill patients, we consider the possibility of pregnancy during the use of IMP to be highly unlikely. However, female patients of childbearing potential and their partners will be advised of the importance of avoiding pregnancy for at least 30 days after the end of IMP administration and of the potential risks associated with an unintentional pregnancy. The Investigator must report every pregnancy on a Pregnancy Report Form as soon as possible (within 24 hours of learning of the pregnancy) to the Pharmacovigilance Contacts specified in the section "Contact Information", even if no AE has occurred, and follow it to term. If, however, the pregnancy is associated with an SAE (e g, if the mother is hospitalized for dehydration), in addition to the Pregnancy Report Form, a separate SAE report form must be filed as described in Section 8.6, with the appropriate serious criterion indicated on the SAE report form. Miscarriage, stillbirth and any malformation/disease must be reported as a SAE.

Any pregnancy leads to the immediate withdrawal of the study patient from the trial.

8.6. ADVERSE EVENTS CAUSING TREATMENT DISCONTINUATION

If a patient is withdrawn from the study as a consequence of an AE, this must be recorded and reasoned in the eCRF, and the patient must be followed up until the resolution of the AE or as instructed by the medical expert.

8.7. OVERDOSE

Accidental or intentional overdose, which may or may not result in serious adverse reactions, is to be reported to CRO Pharmacovigilance, Dompé Global Pharmacovigilance, Safety and Surveillance Department and to Dompé Medical Expert, following the same procedure for SAE, within 24 hours from the Investigator's knowledge of its occurrence. This includes reports related to drug intake through different routes (e.g. ingestion) or with suicidal intentions and consequent drug overdose.

An overdose of reparixin is defined as the administration of 3 or more additional tablets on any given treatment day.

The Investigator shall provide in the SAE form information about symptoms, corrective treatment, and outcome of overdose. The Medical Expert should be contacted to discuss corrective treatment, if necessary.

9. STATISTICAL CONSIDERATIONS

9.1. SAMPLE SIZE

The sample size of the study is calculated based on results from literature[32-35]. Evaluation of the efficacy for this study is being accomplished through estimation of a 95% confidence interval. No hypotheses are specified for this study and no p-values are being calculated but for descriptive purposes, therefore no level of significance is specified.

Considering a randomization ratio 1:1 (reparixin: placebo), a sample size of 60 evaluable subjects is considered adequate for assessing the difference between groups in primary endpoints, i.e., change from baseline in OI at day 7 and VFD at day 28.

The given sample size will provide a reasonable precision in the estimation of group differences in primary endpoints to an expected margin of error for a 95% one-sided confidence interval of at most 46.5% the standard deviation, whatever it is:

- Assuming a standard deviation of 11.5 points for OI, the 95% one-sided confidence interval for the difference between groups will have a width of 5.35 points;
- Assuming a standard deviation of 8 days for VFD, the 95% one-sided confidence interval for the difference between groups will have a width of 3.75 days.

If 10% of enrolled patients will not be evaluable for primary analysis, the total number of patients to be enrolled will be approximately 66.

9.2. RANDOMIZATION

Enrolled patients will be randomized in a 1:1 ratio to either reparixin or placebo according to the randomization list. Dropouts after randomization will not be replaced.

The permuted block randomization list will be generated with a computer procedure by a CRO independent statistician not involved in the conduct of the study and will be provided to Dompé in a sealed envelope to prevent unblinding. The facility responsible for IMP packaging/labeling will also receive appropriate randomization codes for the purpose of IMP preparation.

Randomization will be performed through the IRS. Each Patient Kit number will be randomly associated with a treatment group. Access to individual patient treatment codes will be allowed only in the event of a medical emergency where the knowledge of patient treatment is required to provide the patient with appropriate care. The investigator and Dompé Pharmacovigilance will have access to the randomization code for a specific patient in case of a medical emergency or for safety reasons. Unblinding events will be recorded and reported in the Clinical Study Report (CSR). The treatment assignment information will be kept confidential and will not be disclosed to any other.

Once the study has been completed and the database has been locked, the treatment assignment information will be accessible to the study biostatistician(s) who will perform the statistical analyses and will generate reports.

9.3. OVERVIEW OF PLANNED STATISTICAL ANALYSIS

The study plans for the following statistical analyses:

- Analyses for the Data Monitoring Committee: these analyses will be produced periodically according to the DMC Charter.
- Final analysis: this analysis will be conducted when all enrolled subjects have completed the study and the study database has been locked and unblinded.

9.4. ANALYSIS POPULATION

The following populations will be defined:

- The Safety (SAF) population will consist of all randomized patients who received at least one dose of the IMP. The SAF population will be analyzed according to the actual treatment received. The SAF population will be used to present results on safety data.
- The Full Analysis Set (FAS) population will consist of all randomized patients who received at least one dose of the IMP. FAS population will be analyzed according to the intention-to-treat (ITT) principle, i.e. by treatment allocation. The FAS population will be used for the primary analysis of the study and to present results on efficacy data.

9.5. STATISTICAL METHODOLOGY

Statistical analysis will be performed by the CRO appointed by Dompé.

Appropriate descriptive statistics will be produced by treatment arms according to the nature of the variable. For continuous data, the number of observations, mean, standard deviation, median and range (minimum and maximum) will be presented. For qualitative data, frequency distributions and percentages per category will be presented. Two-sided 95% confidence intervals around the mean or the proportions (Wilson method) will be presented. The number of subjects with missing data will be presented under the "Missing" category. Missing values will be included in the denominator count when computing percentages. When continuous data will be summarized, only the non-missing values will be evaluated for computing summary statistics. Any exception will be declared.

For time-to-event variables, cumulative freedom from events will be evaluated using Kaplan-Meier (KM) method. The degree of uncertainty will be expressed with 95% confidence limits (calculated per the method proposed by Greenwood). Comparison of curves among arms will be performed with the log-rank test. KM graphs will be presented along with the number of patient-at-risk at exact time points. Subjects who are free from events at the time of DB lock will be censored at the DB lock date. Reasons for discontinuation will be incorporated into the analysis for determining censoring and failure status. Specifically, study discontinuation for Adverse Event, Death, Lost to follow-up or other negative outcomes will be considered as failure events. Subjects who have discontinued for other reasons without an event will be censored at the date of discontinuation.

Unless otherwise specified, the significance level used for other statistical testing (for descriptive purposes) will be 0.05 and two-sided tests will be used. All patient data collected on the CRF will be listed by patient and center.

The Statistical Analysis Plan (SAP) will be issued before database lock with more technical and detailed elaboration of the principal features of statistical analyses. Additional post-hoc analysis may be produced to further allow comparison between treatment and control, according to the results obtained. Any deviations from the original statistical plan (including unplanned analyses) will be documented in the Clinical Study Report.

9.5.1. Analysis of efficacy variables

9.5.1.1. Primary analyses

Primary endpoints will be analyzed by means of regression models adjusting by pre-defined baseline factors (details will be provided in the SAP). Since patients who discontinue the IMP will not be withdrawn from the study but will be asked to complete safety and efficacy assessments as per the protocol, missing data due to intercurrent events will be addressed by using multiple imputation based on retrieved dropouts information. Retrieved dropout patients are defined as patients who discontinue study treatment and decide to remain in the study by following the schedule of assessments and continuing to adhere to protocol requirements.

If not enough data was retrieved after study treatment discontinuation for assure the convergence of the MI-RD regression model (the final decision will be done at the time of the analysis and reported in the CSR), the same multiple imputation model will be fit using data from subjects of control group, washing-out the effect of treatment. This approach does not assume benefits for reparixin in case of discontinuation and limits a post-discontinuation clinical effect to that of placebo.

The adjusted estimated treatment differences between reparixin and placebo will be displayed together with

the corresponding 90% confidence intervals (to reflect one-sided 95% confidence intervals). Assumption of Normal distribution will be assessed graphically. If required, a log transformation might be applied to meet normality assumption.

9.5.1.2. Secondary analyses

Descriptive in nature analyses will be performed on all secondary endpoints at each available time point by means of descriptive statistics and by appropriate parametric tests depending on the nature of the variable and its distribution. Data transformation might be used in order to satisfy the assumption of normality requested by parametric statistical tests. In case such assumptions are not met, non-parametric counterpart tests will be used. Details will be provided in the SAP. Change from baseline value (for continuous variables) and shift tables versus baseline (for categorical variables) may also be summarized for all post-baseline visits.

9.5.2. Analysis of safety variables

TEAEs and TESAEs will be presented by treatment arms in terms of number of TEAEs and TESAEs and incidence by System Organ Class and Preferred Term using MedDRA. Analyses will be provided also by severity and relationship to the study drug.

Vital signs, hematology and biochemistry , eGFR and ECG parameters will be summarized by treatment at each available time point by means of descriptive statistics.

9.5.3. Specification of subgroups for analysis

In the presence of congruous numbers, subgroup analyses of primary and key secondary endpoints will be performed on the following subgroups defined by baseline characteristics:

- Age class,
- Gender,
- Race,
- Ethnicity.

Statistical details and potential new subgroups definitions will be reported in the SAP.

9.5.4. Intermediate analyses for the DMC

DMC meetings will be performed during the trial to monitor the safety of patients and to protect study subjects from undue harm. Information on accrual, eligibility, concomitant medications, medical history, concomitant diseases, discontinuation, adverse events and other safety assessments will be analyzed at each DMC meeting. Access to unblinded information on the efficacy analyses is allowed on DMC request to balance patient safety risk against a possible gain in efficacy.

Further details will be provided in Section 12.6 and in the DMC Charter, where all roles and responsibilities will be defined.

The DMC will consider the appropriateness of trial continuation if there is emerging evidence that reparixin is harmful. Since the DMC does not monitor primary endpoints for early efficacy termination, no Type I error adjustment is necessary.

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9.6. Missing data

All reasonable efforts will be made to reduce the rate of missing data. Investigators will be trained about the importance of patient retention and full data capture. Also, any reasonable attempts should be made by the Investigators to emphasize continued patient's participation for the full duration of the trial.

Details on how missing data will be handled in the primary analysis are reported in section 9.5.1.1.

10. ETHICAL CONSIDERATIONS

10.1. INDEPENDENT ETHICS COMMITTEE (IEC) / INSTITUTIONAL REVIEW BOARD (IRB)

It is the responsibility of the CRO appointed by Dompé or of the Study PI to obtain approval of the trial protocol/amendments from the appropriate IEC/IRB.

Prior to the initiation of the study, the followings will be submitted to the IEC/IRB for approval:

- the study protocol,
- the Informed Consent Form (ICF),
- the current version of the Investigator's Brochure,
- Investigator's current curriculum vitae (CV) as well as the current CVs of all key study personnel,
- Insurance certificate,
- any other IEC/IRB requested document(s).

A copy of the IRB approval will be sent to Dompé along with relevant correspondence with the IEC/IRB, a roster of IEC/IRB members or the US Department of Health and Human Services (DHHS) general assurance number.

The study will not be started until full written approval has been obtained from the appropriate IEC/IRB. The letter of approval should be dated, and should specify the type (e.g. protocol number) and the date of the documents which were reviewed and approved.

The CRO appointed by Dompé or the PI will submit any future amendment to the protocol to the IEC/IRB which granted the original approval. Any amendment will be implemented only when full approval has been obtained from the appropriate IEC/IRB, except for those amendments which involve only logistical or administrative aspects of the study.

The CRO appointed by Dompé or the PI will send to the IEC/IRB any updated Investigator's Brochure.

The CRO appointed by Dompé or the PI will also submit to the IEC/IRB which approved the protocol, at least annually, any required progress reports and study update, and will inform the IEC/ IRB of the termination of the study.

The CRO appointed by Dompé or the PI will report to the IEC/IRB any serious ADRs, life-threatening problems or deaths occurred at other sites participating in this clinical trial and/or in other clinical studies conducted with reparixin.

10.2. ETHICAL CONDUCT OF THE STUDY

This study will be conducted in compliance with the protocol and current GCP, adopting the principles of the Declaration of Helsinki, and all applicable regulatory requirements (ICH E6, 45CFR46, and FDA 21 CFR sections 11, 50, 56, 312).

Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by the IEC/IRB. Any amendments to the protocol or consent materials must also be approved before they are implemented.

10.3. SUBJECT INFORMATION AND CONSENT

No study-related procedures (including non-invasive and diagnostic procedures) will be undertaken prior to completion of the consenting process.

When an eligible subject is identified, informed consent will be pursued. Consent will be sought from the subjects themselves, if this is possible. However, it is recognized that the vast majority of subjects will be unable to give written informed consent themselves due to alteration in their level of consciousness caused by therapeutic sedation. Hence, legally authorized representative consent will be required for most subjects.

The Investigator or delegate personnel, according to site procedures, will explain the study fully to subjects and/or their legally authorized representatives sing the ICF. The Investigator is responsible for ensuring that subjects and/or their legally authorized representatives understands the risks and benefits of participating in the study and answering any questions they may have throughout the study and sharing any new information in a timely manner that may be relevant to subjects' and/or their legally authorized representatives' willingness to continue with study participation. Although subjects and/or their legally authorized representatives will be informed that the consent could be withdrawn at any time, the Investigator will also emphasize that missing data diminish the scientific value of all subjects' contributions. Similarly, subjects and/or their legally authorized representatives will be informed that safety data might have to be collected after their participation in the study has been completed. If subjects and/or their legally authorized representatives are willing to participate in the study, they will be requested to give written informed consent after being given sufficient time to consider their participation and the opportunity to ask for further details. The ICF will be signed and personally dated by **both** the subjects and/or their legally authorized representatives as well as the Investigator or delegate personnel according to site procedures. A copy of the signed form will be provided to subjects and/or their legally authorized representatives, and the original signed ICF will be retained and filed in the Investigator Site File. Subject consent will be documented in the hospital records.

Individual (i.e. site specific; local language) ICFs will be provided to the site once approved by the IEC/IRB. Any changes requested by the IEC/IRB must be approved by Dompé prior to the documents being used.

10.3.1. IDENTIFICATION OF LEGALLY AUTHORIZED REPRESENTATIVES

As stated before, due to their critical illness, most subjects will not be able to provide informed consent. Accordingly, written informed consent will be sought from a legally authorized representative. As this is an international study, the appropriate local country guidance and laws will be followed for each country. A legally authorized representative may be a Personal Legal Representative or Professional Legal Representative.

The Personal Legal Representative may be a relative, partner or close friend. Any country specific guidance on who may act as a Personal Legal Representative will be followed. Once the Personal Legal Representative has been informed about the study, he/she will be given a copy of the ICF and will be asked to give an opinion as to whether the subject would object to taking part in the study. After being given an adequate amount of time to consider the information, if the Personal Legal Representative decides that the subject would have no objection to participating in the study then he/she will be asked to sign two copies of the ICF.

If no Personal Legal Representative is available, then a doctor at the investigational site who is not connected with the conduct of the study may act as a Professional Legal Representative. Any country-specific guidance on who may act as a Professional Legal Representative will be followed. After being informed about the study, the Professional Legal Representative will be given a copy of the ICF. If the Professional Legal Representative decides that the subject is suitable for entry into the study, then he/she will be asked to sign two copies of the ICF.

10.3.2 RETROSPECTIVE SUBJECT INFORMED CONSENT

Subjects for whom consent was given by a legally authorized representative will be informed of their participation in the study by the Investigator. This process will take place once the subject has regained the capacity to understand the details of the study. The timing of this process will thus vary between subjects. However, every attempt to obtain retrospective consent must be made when the subject's condition is appropriate. Once informed, subjects will be given an adequate amount of time to consider their decision to consent to continued participation in the study and to sign an ICF.

If the consent has been given by the legally authorized representative, the subject should be informed about their participation in the study and should have the opportunity to confirm or revoke their consent. If the subject does not give retrospective consent, then the subject will be asked if the data collected to that time point can be analyzed. If the subject does not allow any of their data to be used, then the data collected from the subject until that time point will not be entered into the analyses and no further data will be collected; the

subject will be considered as withdrawn and the reason for withdrawal will be "retrospective consent not given".

10.4. CONFIDENTIALITY

All information obtained during the conduct of the study will be regarded as confidential. An agreement of disclosure will be obtained in writing by the patient and will be included in the ICF. Patient's data collected during (or after completion of) the study will be handled in accordance with applicable USA data protection laws, HIPAA regulations, and European data protection Regulation (EU) No. 679/2016 of the European Parliament and of the European Council regarding the protection of natural person's personal data and the free circulation of said data (hereinafter GDPR EU No. 679/2016) and according the standards of Good Clinical Practice.

On the eCRF patients will be identified ONLY by the assigned patient number. If patient names are included on copies of documents submitted to Dompé or the CRO appointed by Dompé, the names will be obliterated or masked and the assigned patient number added to the document.

The Investigator should keep a separate identification log for patients screened in the trial.

10.5. COMPENSATION FOR MEDICINE-INDUCED INJURY AND INDEMNIFICATION

Before the trial formally starts, Dompé will take out a study-specific insurance covering the amount requested by the respective national laws for patients/Investigators/Institutions participating in the clinical trial.

In case of questions about medical care, cost for medical care or insurance, patients can talk to their Investigator. Contact details will be given in the ICF.

Insurance and any updates will be provided to the Investigator before trial commencement for filing into the Investigator Site File.

11. DATA HANDLING AND RECORD-KEEPING

11.1. CASE REPORT FORM (CRF)

All data relating to the study will be recorded on the eCRF to be provided by the CRO, through an electronic data capture system. eCRF should not be made available in any form to third parties, except for authorized Dompé designees or representatives of appropriate Health/Regulatory Authorities, without written permission from Dompé.

An eCRF is required and should be completed for each consented patient, regardless of actual enrollment. All entries must be written in English. Source documents should be available to support all the data recorded in the eCRF; location of source documents will be specified and listed at the center Initiation Visit.

The eCRF must be available for review by designated Dompé representatives at each scheduled monitoring/audit visit. The PI is responsible for verifying that all data entries in the eCRFs are accurate and correct. The PI must sign the completed eCRF before database lock and its submission to the Sponsor.

11.2. DATA MANAGEMENT

Data management will be performed by the CRO appointed by Dompé. Main Data Management activities and procedures will be accurately described in the DMP, created by the CRO and approved by Dompé.

Data collection will involve the use of an EDC system, to which only authorized personnel will have access. In addition to periodic monitoring occurring within the system by Sponsor/CRO Monitors, programmatic edit checks will be used to review the data for completeness, logic, and adherence to study protocol (following the Data Validation Plan). As a result of this monitoring and these checks, queries may be electronically issued to the study centers and electronically closed by those study centers. The identifying information (assigned username, date, and time) for both the originator of the query (if created during the monitoring process) and the originator of the data change (if applicable), as well as the PI's approval of all changes performed on his or her patients' data, will be collected. Reconciliation of study data and SAEs between Clinical and Drug Safety databases will be performed on an ongoing basis and before database lock. Procedure will be detailed in the DMP.

Encoding of specific data will be carried out. For this trial, Medical History, Adverse Events and Concomitant Medication will be coded; Medical Dictionary for Regulatory Activities (MedDRA) and World Health Organisation (WHO)-DRUG Enhanced dictionaries will be used, and version number of each dictionary will be documented in the DMP. Dictionary version numbers will not be changed during the study.

All data collected in the context of this study will be stored and evaluated per regulatory requirements and applicable guidelines for electronic records. Also, data will be stored and evaluated in such a way as to guarantee patient confidentiality and in accordance with relevant legal stipulations. Study records (e.g., copies of eCRFs, regulatory documents) will be retained at the study center, along with adequate source documentation, according to Competent Authority and ICH requirements. All study records must be available for audit by Dompé, its authorized representatives, or Regulatory Authorities.

The investigator/institution should maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trial subjects. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained, if necessary, via an audit trail.

11.3. DOCUMENTATION REQUIRED PRIOR TO INITIATION OF, AND DURING THE STUDY

The following documents will be required from the Investigator prior to the initiation visit (and during the course of the study in case of any update):

- Current, signed and dated Curriculum Vitae of the PI any Sub-Investigators/co-workers. Updates should be provided at least every two years.
- Confidential disclosure agreement Form in accordance also with European data protection

Regulation (EU) No. 679/2016 (GDPR).

- Normal ranges of all laboratory tests to be performed at the study site and a recent certification or accreditation of established quality control (or other documentation of established quality control or external quality assessment or other validation). Updates should be provided as soon as any reference value has changed.
- A signed page of the final clinical protocol and any amendments.
- IEC/IRB approval documentation, IEC/IRB-approved ICFs and study materials. Documentation of continuing IEC/IRB review and annual renewals.
- A signed copy of the study Financial Agreement/Clinical Study Agreement with Dompé (or CRO appointed by Dompé), including all study specific costs.
- List and any updates of delegated responsibility (Study Team Signature List / Delegation of Responsibilities form).
- FDA Form 1572 and financial disclosure form 3455 from all the persons listed on the 1572. If applicable, the PI will provide an updated financial disclosure agreement to the Sponsor 1 year after the completion of the study.

11.4 ESSENTIAL DOCUMENT RETENTION

The Investigator will retain copies of all the essential documents (as defined by ICH-GCP) until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or at least 2 years have elapsed since the formal discontinuation of clinical development of the Investigational Product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements. The Investigator should take measures to prevent accidental or premature destruction of these documents.

The essential documents include, but are not limited to: the signed protocol, copies of the completed eCRF, and Diary, signed Patient Informed Consent Forms from all patients who consented, hospital records and other source documents, and all other documentation included in the Investigator Site File and Pharmacy/Dispensing File.

The Investigator will inform Dompé (or designee) of the storage location of these essential documents and must contact Dompé before disposing of any. If the Investigator wishes to assign the files to someone else or to remove them to another location, he/she should consult with Dompé about this change.

Dompé will inform the Investigator in writing when these documents no longer need to be retained.

12 STUDY MANAGEMENT

The study will be performed in accordance with the protocol, the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, October 2013) and ICH Harmonised Tripartite Guideline for Good Clinical Practice (*ICH-GCP*) and any local regulations.

12.1 REGULATORY BODY OF APPROVAL

The CRO appointed by Dompé will obtain the necessary approval from the Competent Authorities, as needed, prior to initiation of the study. In the US, Dompé or other consultant appointed by Dompé will submit to the FDA this protocol under a new ARDS-specific IND, according to 21 CFR Part 312.30.

The study will not be started until written approval from the relevant Competent Authorities (or no objection within the timeframe set by the local regulation, as applicable) has been received by Dompé.

12.2. SERIOUS BREACHES MANAGEMENT

Process (as defined under art. 52 of European Regulation 536/2014):

Institutions, CRO, and investigators involved in the clinical trial management must notify the Sponsor about any Serious Breaches and suspected Serious Breach within 24 hours from the identification of such Serious Breach.

If not otherwise specified in relevant study plans and agreements with the contracted CRO, the Sponsor will manage the event according to the internal process for the management and notification to the relevant competent authority.

Definitions:
Serious Breach: a breach likely to affect to a significant degree the safety and rights of a subject, or the reliability and robustness of the data generated in the clinical trial.
Suspected Serious Breach: an incident, which at the time of communication from the investigators or from the service providers to the Sponsor, has not yet been assessed by the Sponsor to be a Serious Breach.

12.3 MONITORING

Monitoring will be carried out by the monitor of the designated CRO.

The purpose of the monitoring is to verify that the rights and the wellbeing of the patient are protected, that the reported data are accurate, complete, and verifiable from source documents and that the conduct of the trial complies with the currently approved protocol and any amendments, with ICH GCP, and with regulatory requirements.

Prior to study start, the Investigator will be informed of the anticipated frequency of the monitoring visits. (S)He will also receive a notification prior to each monitoring visit during the study. It is expected that the PI and/or his/her Sub-Investigator(s) and other appropriate staff will be available on the day of the visit to discuss study conduct and to cooperate with the sponsor representative to ensure that any problems detected during these monitoring visits are resolved.

12.4 ACCESS TO RECORD

The Investigator will allow designated Dompé representatives, including staff from the appointed CRO, and Regulatory/Ethics Bodies, to have direct access to the source documents to verify the data reported in the eCRF. Source documents are the originals of any documents used by the Investigator or hospital/institution that allow verification of the existence of the patient and substantiate the integrity of the data collected during the trial. The investigator/institution should maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trial patients. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained, if necessary, via an audit trail.

All study records must be available for audit by Dompé, its authorized representatives, and Regulatory

Inspection by Regulatory Authority.

12.5 AUDIT AND INSPECTION

In addition to the institutional IEC/IRB(s), audit activities will be performed by the Dompé Quality Assurance Unit or any other third party delegated by Dompé, as appropriate.

12.6 PROTOCOL AMENDMENTS

Changes to the Study Protocol will be implemented only when written amendments have been signed by all individuals who signed the Protocol.

Any amendment will be sent to the IEC/IRB and Competent Authority / FDA, as appropriate. No deviations from or changes to the protocol will be implemented without documented approval of an amendment from the IEC/IRB which granted the original approval, except where necessary to eliminate an immediate hazard(s) to trial patients, or when the change(s) involves only logistical or administrative aspects of the trial. The deviations from or changes to the protocol implemented to eliminate an immediate hazard to the trial patient and the proposed amendment, if appropriate, should be submitted to the IEC/IRB for review and approval as soon as possible.

Any other deviation from the protocol that has not been approved by Dompé and the IEC/IRB could result in a discontinuation from the study at the center involved.

Any written amendment will be sent to all recipients of the protocol.

12.7 DATA MONITORING COMMITTEE

An independent DMC will be appointed with the responsibility of safeguarding the interests of trial participants, assessing the safety and efficacy of the interventions during the trial, and monitoring the overall conduct of the clinical trial. These tasks will be accomplished on an ongoing basis throughout the trial. The DMC will provide recommendations about stopping or continuing the trial. In order to contribute to enhancing the integrity of the trial, the DMC can also formulate recommendations relating to the selection/recruitment/retention of participants, their management, improving adherence to protocol-specified regimens and retention of participants, and the procedures for data management and quality control.

The DMC will be advisory to the clinical trial leadership group (the Scientific Committee of the study). The Scientific Committee comprised the sponsor's study team and lead study investigators, who jointly have responsibility for the design, conduct and analysis of the clinical trial. The Scientific Committee is responsible for promptly reviewing the DMC recommendations, to decide whether to continue or terminate the trial, and to determine whether amendments to the protocol or changes in the study conduct are required.

12.8. DISCONTINUATION OF THE STUDY

Dompé reserves the right to stop the study at any time on the basis of new information regarding safety or efficacy, or if study progress is unsatisfactory, or for other valid administrative reasons.

After such a decision is made, the Investigator must inform all relevant persons e.g. study staff, patients etc. within 2 weeks. All delivered study materials must be collected and all eCRF completed to the extent possible.

Study discontinuation will be notified to the IEC and Competent Authority/FDA within 15 days from decision. The Investigator will inform his/her IRB within the same timeframe.

12.9. PUBLICATIONS

As this study is part of a multicenter trial, publications derived from this study will be planned and agreed with the participating Study Investigators. Publications will include input from the Investigators, his/her colleagues, other investigators in this trial and Dompé personnel. Such input will be reflected in publication authorship. All PIs contributing patients to the study are eligible for inclusion in publications derived from this study. The first authorship will be offered (upon agreement of all involved) to the PI with the greatest number of enrolled patients whereas the senior authorship will be offered to the lead PI (s). Authorship order

can be adjusted as deemed necessary and with the agreement of all PIs and Dompé personnel.

Any manuscript, abstract or other publication or presentation of results or information arising in connection with the study must be prepared in conjunction with Dompé and must be submitted to Dompé for review and comment at least 45 days prior to submission for publication or presentation. The Sponsor reviews proposed manuscripts prior to submission within a reasonable period (30-90 business days in relation with the complexity of the work). If such draft contains confidential patentable information, the Investigator will refrain from publishing any such information for a period not exceeding 180 days, to enable Dompé to file for the protection of any intellectual or proprietary property interest.

The Sponsor agrees that the study results (including negative and inconclusive as well as positive results) can be made publicly available by the Investigator in the form of: publications in peer reviewed journals; presentation of the results at scientific meetings; and posts of the results on internet-based public registers and databases.

Regardless, study results will be communicated in full to the Competent/authority/FDA by the submission of a complete CSR.

The Investigator(s) will also be provided by the Sponsor with the CSR and the results of any additional analysis, tables, figures, etc. undertaken for the purposes of the article, in order to take responsibility for the content of the publication(s).

On an exceptional basis, the Sponsor may temporarily delay registration of certain data elements (e.g. compound, name, outcome, measures etc.) to seek necessary intellectual property protection. This is because early disclosure of such data could, in some circumstances, prevent or negatively impact patentability.

13. REFERENCES

1. Allegretti M, Cesta MC, Bizzarri C, et al. 2-Arylpropionic CXC chemokine receptor 1 (CXCR1) ligands as novel noncompetitive CXCL8 inhibitors. J Med Chem. 2005 Jun 30;48(13):4312-31;
2. Bertini R, Allegretti M, Bizzarri C, et al. A new class of non-competitive allosteric inhibitors of the inflammatory chemokine receptors CXCR1 and CXCR2: prevention of reperfusion injury. PNAS 2004; 101: 11791-6
3. Zarbock A, Allegretti M, Ley K. Therapeutic inhibition of CXCR2 by Reparixin attenuates acute lung injury in mice. Br J Pharmacology 2008; 155: 357–364
4. Hosoki K, Rajarathnam K, Sur S. Attenuation of murine allergic airway inflammation with a CXCR1/CXCR2 chemokine receptor inhibitor. Clin Exp Allergy 2019; 49:130-132
5. Cheng IY, Liu CC, Lin JH, et al. Particulate Matter Increases the Severity of Bleomycin-Induced Pulmonary Fibrosis through KC-Mediated Neutrophil Chemotaxis. Int J Mol Sci 2020; 21:227
6. Tavares LP, Garcia CC, Machado MG, et al. CXCR1/2 Antagonism Is Protective during Influenza and Post-Influenza Pneumococcal Infection. Front Immunol 2017; 8:1799;
7. Kaiser R, Leunig A, Pekayvaz K et al. Self-sustaining interleukin-8 loops drive a prothrombotic neutrophil phenotype in severe COVID-19. JCI Insight 2021; 6:e150862
8. Ware LB, Matthay MA. The acute respiratory distress syndrome. N Engl J Med. 2000 May 4;342(18):1334-49
9. Ranieri VM, Rubenfeld GD, Thompson BT, et al. Acute respiratory distress syndrome: the Berlin definition. *JAMA* 2012; 307: 2526–33
10. Meyer NJ, Gattinoni L, Calfee CS. Acute respiratory distress syndrome. Lancet 2021; 398:622-637
11. Chen GY, Nuñez G. Sterile inflammation: sensing and reacting to damage. Nat Rev Immunol 2010;10:826-837.
12. Vénéreau E, Ceriotti C, Bianchi ME. DAMPs from cell death to new life. Front Immunol 2015;6:422.
13. Frevert CW, Huang S, Danaee H, et al. Functional characterization of the rat chemokine KC and its importance in neutrophil recruitment in a rat model of pulmonary inflammation. J Immunol. 1995; 154:335–44
14. Goodman RB, Strieter RM, Martin DP, et al. Inflammatory cytokines in patients with persistence of the acute respiratory distress syndrome. Am J Respir Crit Care Med. 1996; 154(Pt 1):602–11.
15. Steinberg KP, Milberg JA, Martin TR, et al. Evolution of bronchoalveolar cell populations in the adult respiratory distress syndrome. Am J Respir Crit Care Med. 1994; 150:113–22
16. Zemans RL, Matthay MA. What drives neutrophils to the alveoli in ARDS? Thorax 2017;72:1-3
17. Donnelly SC, Strieter RM, Kunkel SL, et al. Interleukin-8 and development of adult respiratory distress syndrome in at-risk patient groups. Lancet. 1993; 341:643–7
18. Groeneveld AB, Raijmakers PG, Hack CE, et al. Interleukin 8-related neutrophil elastase and the severity of the adult respiratory distress syndrome. Cytokine. 1995; 7:746–52
19. Miller EJ, Cohen AB, Nagao S, et al. Elevated levels of NAP-1/interleukin-8 are present in the airspaces of patients with the adult respiratory distress syndrome and are associated with increased mortality. Am Rev Respir Dis. 1992; 146:427–32
20. Bikfalvi A, Billottet C. The CC and CXC chemokines: major regulators of tumor progression and the tumor microenvironment. Am J Physiol Cell Physiol. 2020;318:C542-C554
21. Stillie R, Farooq SM, Gordon JR, et al. The functional significance behind expressing two IL-8 receptor types on PMN. J Leukoc Biol 2009; 86:529-543
22. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. Science. 2004; 303:1532–5.
23. Camicia G, Pozner R, de Larrañaga G. Neutrophil Extracellular Traps in Sepsis. Shock. 2014; 42:286–94
24. Caudrillier A, Kessenbrock K, Gilliss BM, et al. Platelets induce neutrophil extracellular traps in transfusion related acute lung injury. J Clin Invest. 2012; 122:2661–71.
25. Liu S, Su X, Pan P, et al. Neutrophil extracellular traps are indirectly triggered by lipopolysaccharide and contribute to acute lung injury. Sci Rep. 2016; 6:37252.
26. Li H, Zhou X, Tan H, et al. Neutrophil extracellular traps contribute to the pathogenesis of acid-aspiration-induced ALI/ARDS. Oncotarget 2018;9:1772-1784.
27. Bao Z, Ye Q, Gong W et al. Humanized monoclonal antibody against the chemokine CXCL-8 (IL-8) effectively prevents acute lung injury. Int Immunopharmacol 2010;10:259-63.

28. Bellani G, Laffey JG, Pham T, et al. Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries. JAMA 2016; 315: 788–800.
29. Calfee CS, Delucchi K, Parsons PE; NHLBI ARDS Network.

Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomized controlled trials. Lancet Respir Med. 2014 Aug;2(8):611-20
30. Delucchi K, Famous KR , Ware LW. Stability of ARDS subphenotypes over time in two randomized controlled trials. Thorax 2018 May;73(5):439-445.
31. Famous KR, Delucchi K, Ware LB. Acute respiratory distress syndrome subphenotypes respond differently to randomized fluid management strategy. Am J Respir Crit Care Med 2017;195(3):331-338
32. Go L, Budinger GR, Kwasny MJ, Peng J, Forel JM, Papazian L, Jain M. Failure to Improve the Oxygenation Index Is a Useful Predictor of Therapy Failure in Acute Respiratory Distress Syndrome Clinical Trials. Crit Care Med. 2016 Jan;44(1):e40-4.
33. Brower RG, Matthay MA, Morris A, Schoenfeld D, Thompson BT, Wheeler A. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. N Engl J Med. 2000; 342:1301–8.
34. Brower RG, Lanken PN, MacIntyre N, MAtthay MA, et al. Higher versus lower positive end-expiratory pressures in patients with the acute respiratory syndrome. N Engl J Med 2004;351:327-36
35. Papazian L, Forel J, Gacouin A et al. Neuromuscular blockers in early acute respiratory distress syndrome. N Engl J Med 2010;363: 1107-1116
36. Yehya N, et al. Reappraisal of Ventilator-Free Days in Critical Care Research. Am J Respir Crit Care Med. 2019 Oct 1;200(7):828-836

14. APPENDICES

14.1. APPENDIX 1 - SPONSOR APPROVAL PAGE

Sponsor Medical Expert: _ PPD

Sponsor Clinical Trial Manager PPD

Sponsor Development Directo PPD

14.2. APPENDIX 2 - INVESTIGATOR'S SIGNATURE PAGE

Investigator's Statement

I have read study protocol REP0122 *(Phase II, proof-of-concept, randomized, double-blinded, placebo-controlled, multicenter study to assess efficacy and safety of reparixin as add-on therapy to standard of care in adult patients with Acute Respiratory Distress Syndrome*) and agree to conduct the study as outlined in the protocol, and in accordance with the Declaration of Helsinki, ICH-GCP and any local regulations, being responsible for personally supervise the study conduct and ensure study staff complies with protocol requirement.

Principal Investigator: Name (block letters) _____

Signature: _____ Date: _____/_____/_____

14.3. APPENDIX 3 - PACKAGING AND LABELING DETAILS

A Patient Kit will be prepared for each patient for a [redacted].

Sample label content is summarized below and will be adjusted to meet local regulatory requirements.

NOTE:

Kit No. XXYY according to the randomization list (and IRS system drug dispensation number)

Patient No. _____ to be filled by Investigator according to Patient Randomization Number

Investigator: _____ to be filled by Investigator according to PI name

The following represents a template of the minimal details to be presented in labels.

Content of the Label for each Patient Kit

STUDY REP0122 **Sponsor** Dompé farmaceutici s.p.a.; Via Santa Lucia 6, Milan – Italy

PPD

INVESTIGATOR: _____

KIT No. XXYY * **PATIENT No. _____**

INVESTIGATIONAL PRODUCT: reparixin (600 mg) or placebo oral tablets

CONTAINS: [redacted]

coded BATCH No. coded EXPIRY DATE mm/yyyy **DO NOT STORE AT >30°C DO NOT FREEZE**

DIRECTIONS: [redacted]. Administration of the drug must be through a naso-gastric tube and/or oral use after extubation. Refer to the protocol for the tablet disintegration procedure and use after extubation.

For clinical trial use only. Caution: New Drug-Limited by Federal (or United States) law to investigational use**

* The number of KIT will be reported according to the randomization list that will be generated

**For US labels only

Content of the Label for the PPD

STUDY REP0122 **Sponsor** Dompé farmaceutici s.p.a.

INVESTIGATOR: _____

KIT No. XXYY* **PATIENT No. _____**

INVESTIGATIONAL PRODUCT: reparixin (600 mg) or placebo oral tablets

CONTAINS: [redacted]

coded BATCH No. coded EXPIRY DATE mm/yyyy **DO NOT STORE AT >30°C DO NOT FREEZE**

DIRECTIONS: Administration of the drug must be through a naso-gastric tube three times a day (2 tablets administered approximately every 8 hours) and/or oral use after extubation. Refer to the protocol for the tablet disintegration procedure and use after extubation.

For clinical trial use only. Caution: New Drug-Limited by Federal (or United States) law to investigational use**

* The number of KIT will be reported according to the randomization list that will be generated

**For US labels only

14.4. APPENDIX 4 - METHODOLOGICAL DETAILS

14.4.1. Calculation of Oxygenation Index

$$OI = \frac{FiO_2 \times Paw}{PaO_2}$$

OI: oxygenation index

FIO_2: fractional inspired oxygen

Paw: mean airway resistance

PaO_2: arterial partial oxygen pressure

The Paw is calculated from the following formula:

Formula:

Paw = ((Inspiratory Time x Frequency) / 60) x (PIP – PEEP) +PEEP

PIP: peak inspiratory pressure

PEEP: plateau end-expiratory pressure

From: Durand M et al. Oxygenation index in patients with meconium aspiration: conventional and extracorporeal membrane oxygenation therapy. *Critical Care Med*. 18(4):373-7, 1990

The investigators are encouraged to record the Paw as depicted on the ventilator display screen. If this information is not available the PI can use the formula presented above to calculate the Paw.

14.4.2. SOFA score

SOFA	0	1	2	3	4
Respiration PaO_2/FiO_2 (mmHg)	≥400	<400	<300	<200	<100
Coagulation Platelets x 10^3 mm^3	≥150	<150	<100	<50	<20
Liver Bilirubin (mg/dL)	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	>12.0
Cardiovascular Hypotension	MAP ≥ 70 mmHg	MAP < 70 mmHg	dopamine <5 or dobutamine (any dose)	dopamine 5.1-15 or epinephrine ≤0.1 or norepinephrine ≤0.1	dopamine >15 or epinephrine >0.1 or norepinephrine >0.1
CNS Glascow Coma Score	15	13-14	10-12	6-9	<6
Renal Creatinine (mg/dL) or Urine output (mL/d)	<1.2	1.2-1.9	2.0-3.4	3.5-4.9 <500	>5.0 <200

SOFA score is calculated every 24 hours using (for each organ system) the worst variable recorded within the same 24 hours. The best possible score corresponds to 0 whereas the worst score corresponds to 24.

MAP, mean arterial pressure; CNS, central nervous system; SaO_2, peripheral arterial oxygen saturation. PaO_2/FIO_2 ratio was used preferentially. If not available, the SaO_2/FIO_2 ratio is used vasoactive medications administered for at least 1 hr (dopamine and norepinephrine μmg/kg/min).

Adapted from: Jones AE, Trzeciak S, Kline JA. The Sequential Organ Failure Assessment Score for predicting outcome in patients with severe sepsis and evidence of hypoperfusion at the time of emergency department presentation. Crit Care Med 2009; 37: 1649-1654

14.4.3. RALE score

Consolidation[a]

Consolidation Score	Extent of alveolar opacities
0	None
1	< 25 %
2	25 – 50 %
3	50 – 75 %
4	> 75 %

Density[b]

Density Score	Density of alveolar opacities
1	Hazy
2	Moderate
3	Dense

Final RALE Score[c]

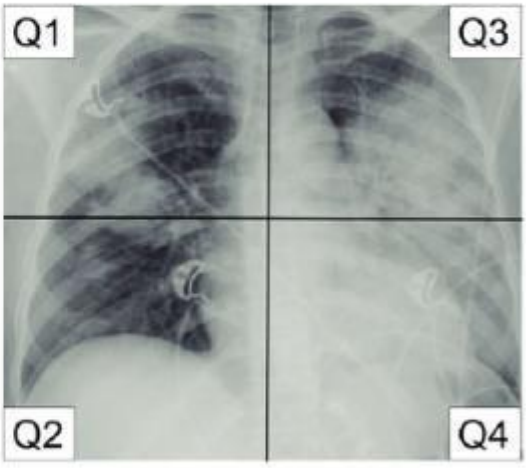
Right Lung	Left Quadrant
Upper Quadrant	Upper Quadrant
Cons x Den = Q1 Score	Cons x Den = Q3 Score
Lower Quadrant	Lower Quadrant
Cons x Den = Q2 Score	Cons x Den = Q4 Score

Total RALE = Q1 + Q2 + Q3 + Q4

[a] Consolidation is scored for each quadrant
[b] Density is scored for each quadrant having a consolidation > 0
[c] If Quadrant consolidation Score is 0 than Quadrant score il 0

Calculation of the RALE score for radiograph

Score	Q1	Q2	Q3	Q4	Total
Consolidation	2	1	3	4	
Density	3	3	3	3	
Quadrant Score	2 x 3 = 6	1 x 3 = 3	3 x 3 = 9	4 x 3 = 12	30



From: Zimatore et al. Accuracy of the radiographic assessment of lung edema score for the diagnosis of ARDS. Front Physiol 2021, http://dx.doi.org/10.3389/fphys.2021.672823

14.4.4. Ventilatory ratio (VR)

VR=[minute ventilation (ml/min) × $Paco_2$ (mm Hg)]/[predicted body weight × 100 (ml/min) × 37.5 (mm Hg)]

From: Sinha P et al. Physiologic analysis and clinical performance of the ventilatory ratio in acute respiratory distress syndrome. Am J Respir Crit Care Med 2019; 199: 333-341

14.4.5. Acute lung Injury Score

Component	0	1	2	3	4
Chest X-ray (Alveolar infiltrates)	No	1 quadrant	2 quadrants	3 quadrants	4 quadrants
Hypoxaemia (PaO_2/FiO_2, mmHg)	≥300	225–299	175–224	100–174	≤100
PEEP (PEEP-setting, cmH_2O	≤5	6–8	9–11	12–14	≥15
Compliance (Static, mL/cmH_2O)	≥80	60–79	40–59	30–39	≤29

From: Murray JF et al. An expanded definition of the adult respiratory distress syndrome. Am Rev Respir Dis 1988;138:720-3

The final score is obtained by dividing the collective score by the number of components that were used.

14.4.6. Norepinephrine equivalents

Vasoactive Agent	Dose	NEE
Epinephrine	0.1	0.1
Norepinephrine	0.1	0.1
Dopamine	15	0.1
Phenylephrine	1	0.1
Vasopressin	0.04	0.1

All doses in mcg/kg/min except vasopressin, which is U/min. NEE norepinephrine equivalents

From: Vallabhajosyula S. Development and performance of a novel vasopressor-driven mortality prediction model in septic shock. Ann. Intensive Care 2018;8:112

14.4.7. Suggested ventilation protocol

- Ventilator mode: volume assist–control
- Initial tidal volume: 6–8 ml/kg of predicted body weight
- Plateau pressure: ≤32 cm of water
- Oxygenation goal: PaO2 of 55–80 mm Hg or SpO2 of 88–95%
- Permitted combinations of FiO2 and PEEP, respectively (cm of water): 0.3 and 5, 0.4 and 5, 0.4 and 8, 0.5 and 8, 0.5 and 10, 0.6 and 10, 0.7 and 10, 0.7 and 12, 0.7 and 14, 0.8 and 14, 0.9 and 14, 0.9 and 16, 0.9 and 18, 1.0 and 18, 1.0 and 20, 1.0 and 22, and 1.0 and 24
- pH goal: 7.20–7.45
- Procedure when oxygenation goal not achieved despite adjustments to FiO2 and PEEP: use inhaled nitric oxide, almitrine mesylate, prone positioning, or any combination thereof
- Procedure when plateau pressure is >32 cm of water for at least 10 min (in the following order, as needed): increase sedation, reduce tidal volume to 4 ml/kg, decrease PEEP by decrements of 2 cm of water, and perform injection of cisatracurium in a bolus of 20 mg (not to be given again if plateau pressure decreased by <2 cm of water because further doses would

probably be futile, but permitted if the drug had its intended effect)

- Procedure to correct hypercapnia when pH is <7.20 (in the following order, as needed): connect Y-piece directly to endotracheal tube, increase respiratory rate to a maximum of 35 cycles per min, and increase tidal volume to a maximum of 8 ml/kg
- Weaning attempt: starting on day 3, if FiO2 ≤0.6
- Goals during weaning procedure: SpO2 ≥88% and respiratory rate 26–35 cycles per min
- Weaning procedure: decrease PEEP over 20–30 min to 5 cm of water Pressure-support ventilation levels used during weaning procedure: 20, 15, 10, and 5 cm of water
- If weaning procedure fails at a pressure-support ventilation level of 20 cm of water, switch to volume assist–control mode of ventilation
- After at least 2 hr of successful pressure-support ventilation at a level of 5 cm of water, disconnect patient from the ventilator

Adopted from: Papazian L. et al. Neuromuscular blockers in early Acute Respiratory Distress Syndrome. N. Eng. J. Med 2010;363: 1107-1116

14.4.8. Formula for calculation of predicted body weight:

predicted body weight (PBW):
Males = 50 + 2.3 [height (inches) – 60]
Females = 45.5 + 2.3 [height (inches) – 60]

14.4.9. Formula for calculation of eGFR:

Renal function will be evaluated by estimated Glomerular Filtration Rate (eGFR), calculated using the 2021 Chronic Kidney Disease Epidemiology Collaboration (2021 CKD-EPI) as per the following formula:

$eGFR_{cr} = 142 \times \min(S_{cr}/\kappa, 1)^{\alpha} \times \max(S_{cr}/\kappa, 1)^{-1.200} \times 0.9938^{Age} \times 1.012$ [if female]

where:

S_{cr} = standardized serum creatinine in mg/dL

$\kappa = 0.7$ (females) or 0.9 (males)

$\alpha = -0.241$ (female) or -0.302 (male)

$\min(S_{cr}/\kappa, 1)$ is the minimum of S_{cr}/κ or 1.0

$\max(S_{cr}/\kappa, 1)$ is the maximum of S_{cr}/κ or 1.0

Age (years)

From: CKD-EPI Creatinine Equation (2021) | National Kidney Foundation

14.4.10. Handling of samples for assays

Local laboratories will perform hematology/biochemistry tests (Safety Laboratory Tests).

CCI measurement of inflammatory markers (Il-6, IL-8, PAI-1, TNFr1, ICAM-1, RAGE) will be performed by a central laboratory selected by Dompé.

All steps will be tracked to ensure correct data reporting.

All samples submitted to these labs will be destroyed after the CSR has been issued or after the patient has withdrawn his/her consent.

14.4.11 Ventilator-free days (VFDs)

VFDs is a well-known endpoint, often used in adult and pediatric ARDS trials; 28-day is the most common time frame for VFDs in ARDS.

VFDs at 28 days is defined as follows[36]:

- VFDs = 0 if the patients die within 28 days
- VFDs = 28 − x if successfully liberated from ventilation x days after initiation
- VFDs = 0 if the patients are mechanically ventilated for >28 days

14.5. APPENDIX 14.5 - CLINICAL TRIALS SUBJECT TO TRANSITION FROM EUROPEAN DIRECTIVE 2001/20/EC TO CLINICAL TRIAL REGULATION EU NO 536/2014

REP0122 (EudraCT 2022-001612-25) constitutes a Consolidated Protocol and the minor differences between the nationally authorized trials is summarized in the following table:

EU Member State	**Minor Difference of trial conduct**
Germany	Chest imaging has to be performed as per local clinical practice/needs.

14.6. **SUMMARY OF CHANGES**

Miscellaneous: Typos/editing have been corrected throughout the entire document.

REASON: CCI [redacted].

1. SYNOPSIS

Randomization and Baseline assessments, changed **from:**
Patients successfully completing the screening process and confirmed eligible for the study will be randomized to either reparixin or placebo at the latest within 48 hours from screening assessment.

To:

Randomization and Baseline assessments: Patients successfully completing the screening process and confirmed eligible for the study will be randomized to either reparixin or placebo within 24 hours and as soon as possible from screening assessment.

REASON: CCI [redacted]

Inclusion criteria

- **Inclusion Criterion No. 6** changed **from:**

≤48 hours from fulfilling above ARDS criteria

To:

≤48 hours from fulfilling above ARDS criteria (if a patient is transferred from a non-participating hospital to a participating site a 12-hour period beyond the 48 hours is allowed)

REASON: CCI [redacted]

- **Inclusion Criterion No. 7 (≤ days from hospital admission)** was removed

REASON: CCI [redacted]

- **Inclusion Criterion No. 8 (currently No. 7)** changed **from:**

Females of child-bearing potential who are sexually active must be willing not to get pregnant within 30 days after the last Investigational Medicinal Product (IMP) dose and must agree to at least one of the following reliable methods of contraception:

a. Hormonal contraception, systemic, implantable, transdermal, or injectable contraceptives from at least 2 months before the screening visit until 30 days after the last IMP dose;
b. A sterile sexual partner;

c. Abstinence.

Female participants of non-child-bearing potential or in post- menopausal status for at least 1 year will be admitted. For all female subjects with child-bearing potential, pregnancy test result must be negative before first drug intake.

To:

Females of child-bearing potential who are sexually active must be willing not to get pregnant within 30 days after the last Investigational Medicinal Product (IMP) dose and must agree to at least one of the following reliable methods of contraception:

a. Hormonal contraception, systemic, implantable, transdermal, or injectable contraceptives from at least 2 months before the screening visit until 30 days after the last IMP dose;
b. A sterile sexual partner;
c. Abstinence.

In patients non able to personally consent to above due to complications of acute illness and/or its treatment assurances for the above must be given by LR and reiterated by patient when/if she is able to do so.

Female participants of non-child-bearing potential or in post- menopausal status for at least 1 year will be admitted. For all female subjects with child-bearing potential, pregnancy test result must be negative before first drug intake.

REASON: CCI [redacted]

Exclusion criteria

- **Exclusion Criterion No. 1** changed **from:**

Moderate-Severe chronic hepatic disease (as verified by relevant history, imaging, if pre-existent, and Child-Pugh Score B and C)

To:

Moderate-Severe chronic hepatic disease (as verified by a previously known Child-Pugh score ≥7). If baseline Child-Pugh score is not known it should not be calculated while the patient is acutely ill. In that case the patient is excluded on the basis of: ALT/AST ≥ 3x ULN and total bilirubin > 2x ULN or ALT/AST ≥ 5x ULN

REASON: CCI [redacted]

- **Exclusion Criterion No. 2** changed **from:**

Severe chronic renal dysfunction: eGFR (MDRD) < 30 mL/min/1.73m^2

To:

Severe chronic renal dysfunction: eGFR (2021 CKD-EPI) < 30 mL/min/1.73m^2. If baseline (chronic) renal function is not known the patient is only excluded if in need of acute renal replacement therapy (currently on RRT or to be imminently placed on RRT)

REASON: CCI [redacted]

CCI [redacted]

- **Exclusion Criterion No. 5** was removed

REASON: CCI [redacted]

- **Exclusion Criterion No. 7 (currently No. 6)** changed **from:**

Anticipated extubation within 24 hours of enrollment.

To:

Anticipated extubation within 24 hours of screening. (In such cases re-screening is allowed if the patient is within the enrollment window).

REASON: CCI [redacted]

- **Exclusion Criterion No. 8** was removed

REASON: CCI [redacted]

- **Exclusion Criterion No. 9** was removed

REASON: CCI [redacted]

- **Exclusion Criterion No. 10 (currently No. 7)** changed **from:**

Evidence of GI dysmotility e.g. due to acute pancreatitis or immediate post op state, as demonstrated by persistent gastric distention, enteral feeding intolerability and/or persistent gastric residuals >500 ml).

To:

Evidence of GI dysmotility as demonstrated by presence of all the following: persistent gastric distention and enteral feeding intolerability and persistent gastric residuals >500 ml).

REASON: CCI [redacted]

- **Exclusion Criterion No. 11 (currently No. 8)** changed **from:**

Anticipated discharge from the hospital or transfer to another hospital within 72 hours of screening.

To:

Anticipated transfer to a hospital not participating in the trial within 72 hours of screening.

REASON: CCI [redacted]

- **Exclusion criterion No. 13 (currently No. 10)** changed **from:**

History of:

a) Documented allergy/hypersensitivity to more than one medication belonging to the class of sulfonamides such as sulfamethazine, sulfamethoxazole, sulfasalazine, nimesulide or celecoxib (hypersensitivity to sulphanilamide antibioitcs alone , e.g. sulfamethoxazole does not qualify for exclusion) and to the study product and/or its excipients
b) Lactase deficiency, galactosemia or glucose-galactose malabsorption.
c) History of GI bleeding or perforation due to previous NSAID therapy or recurrent peptic ulcer/haemorrhage
d) Hypersensitivity to ibuprofen

To:

History of:

a) Documented allergy/hypersensitivity to sulfonamides, ibuprofen and other COX-1 and 2 inhibitors, and to the study product and/or its excipients.
b) Lactase deficiency, galactosemia or glucose-galactose malabsorption.
c) History of peptic ulcer, GI bleeding or perforation due to previous NSAID therapy.

REASON: CCI [redacted]

- **Exclusion Criterion No. 14 (currently No. 11) changed from:**

Active bleeding (excluding menses) or bleeding diathesis including patients on chronically high doses of NSAIDs

To:

Active bleeding (excluding menses) from uncontrolled site that cannot be definitively resolved prior to enrollment.

REASON: CCI [redacted]

- **Exclusion Criterion No. 16 (currently No. 13)** changed **from:**

Women of childbearing potential and fertile men who do not agree to use at least one primary form of contraception during the study and up to 30 days after the last IMP dose

To:

Women of childbearing potential and fertile men who do not agree to use at least one primary form of contraception during the study and up to 30 days after the last IMP dose. For patients non able to personally consent to above due to complications of acute illness and/or its treatment assurances for the above must be given by LR and reiterated by patient when/if he/she is able to do so.

REASON: [redacted]

<u>Test Product, Dosage and Mode of Administration</u> changed **from**

Reparixin 600 mg tablets, administered crushed through nasogastric tube at the dose of 1200 mg TID (2 tablets TID) as add-on to the standard of care

To:

Reparixin 600 mg tablets, administered crushed through nasogastric tube at the dose of 1200 mg TID (2 tablets TID) as add-on to the standard of care. <u>After extubation and if the patient can swallow, reparixin may be administered orally</u>

REASON: [redacted]

<u>Reference product, Dosage and Mode of Administration</u> changed from

Placebo tablets. Administered crushed through nasogastric tube with the same schedule as reparixin as add-on to the standard of care

To:

Placebo tablets. Administered crushed through nasogastric tube with the same schedule as reparixin as add-on to the standard of care. <u>After extubation and if the patient can swallow placebo may be administered orally</u>

REASON: [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

2.5. SUMMARY OF CLINICAL DATA

Updated [redacted]

2.5.2. Safety

ADRs from IV formulation and oral formulation in the oncologic studies as well as the respiratory studies updated CCI [redacted]

2.6. DISEASE REVIEW AND STUDY RATIONALE

CCI [redacted]

[redacted]

3. OVERALL STUDY DESIGN AND INVESTIGATIONAL PLAN

3.3. OVERALL STUDY DESIGN

The following sentence **was added:** The IMP may be administered orally after extubation and between extubation and day 14 should the patient be able to swallow. In such cases the IMP may be administered either intact or crushed and mixed with a vehicle as per speech swallow evaluation.

3.4. STUDY TIME TABLE was updated

4. SELECTION OF STUDY POPULATION

4.1. INCLUSION CRITERIA were updated CCI [redacted]

4.2. EXCLUSION CRITERIA were updated CCI [redacted]

5. STUDY MEDICATION

5.1.1. Presentation of the Investigational Medicinal Product changed **from**

In this study the IMP will be either reparixin or matched placebo, which will be provided in the form of tablets to be disintegrated for naso-gastric tube administration

To:

In this study the IMP will be either reparixin or matched placebo, which will be provided in the form of tablets to be disintegrated for naso-gastric tube administration or administered orally after extubation.

REASON: CCI [redacted]

5.2. DOSE, ROUTE AND SCHEDULE OF IMP ADMINISTRATION the following sentences **were added:** " If the patient becomes extubated prior to the end of treatment on day 14 and if swallowing ability has been restored as per clinical judgment, the IMP can be administered orally. In such cases the IMP may be administered either intact or crushed and mixed with vehicle as per speech swallow recommendation."

REASON: CCI [redacted]

5.3. CRITERIA FOR SCHEDULE ADJUSTMENT/DOSE-MODIFICATION OR DISCONTINUATION OF THE IMP

5.3.2. Criteria for discontinuation of the IMP changed **from:**

The IMP must be discontinued in the case:

- The patient develops renal (eGFR < 30 mL/min) or hepatic (increased ALT/AST ≥ 3x ULN + total bilirubin > 2x ULN or ALT/AST ≥ 5x ULN) dysfunction;
- The patient is discharged home;
- Any medical condition that may threaten the safety of the patient if they continue to receive study treatment (as per investigator or Sponsor evaluation);
- Withdrawal of informed consent by the patient or his/her legal representative (in this case, the patient / representative will be asked whether they consent to usage of the data already collected in the study; if not, the data will be deleted);
- Pregnancy occurs.

To:

The IMP must be discontinued in the case:

- Upon initiation of renal replacement therapy;
- Upon increase of ALT and/or AST to≥ 3x ULN and total bilirubin > 2x ULN or upon increase of ALT and/or AST to ≥ 5x ULN
- The patient is discharged home;
- Withdrawal of informed consent by the patient or his/her legal representative (in this case, the patient / representative will be asked whether they consent to usage of the data already collected in the study; if not, the data will be deleted);
- Pregnancy occurs;
- Development of Serious AE probably or possibly related to IMP, as per PI's judgment;
- Inadvertent missing of 3 or more consecutive doses of the IMP;
- Inability to resolve within 24 hours any medical condition/event that led to temporary withholding of the IMP (see also 5.3.3.);
- Recurrence of AE probably or possibly related to IMP upon re-introduction of the IMP.

Added**: 5.3.3 Criteria for temporary withholding of the IMP**

The IMP can be temporarily withheld upon:

- Development of AE (excluding Serious AE) probably or possibly related to IMP, as per PI's judgment
- Any AE (including Serious AE) not thought to be related to IMP as per PI's judgment.
- Any medical condition that may threaten the safety of the patient if they continue to receive study treatment (as per investigator or Sponsor evaluation)

REASON: CCI [redacted]

5.5.2. Medications to be used with caution: language was updated CCI [redacted]

Added**: 12.2 SERIOUS BREACHES MANAGEMENT**

APPENDIX 14.3 - PACKAGING AND LABELING DETAILS

Kits and Blister labels have been updated as follows:

- **SPONSOR Fax number** has been removed
- **DIRECTIONS** has been changed **from:**

Administration of the drug must be through a naso-gastric tube three times per day (2 tablets for each administration at a time about every 8 hours). Refer to the protocol for the tablet disintegration procedure

To:

Administration of the drug must be through a naso-gastric tube three times a day (2 tablets **administered approximately** ~~for each administration at a time about~~ every 8 hours) **and/or oral use after extubation**. Refer to the protocol for the tablet disintegration procedure **and use after extubation**.

REASON: CCI [redacted].

APPENDIX 14.5-CLINICAL TRIALS SUBJECT TO TRANSITION FROM EUROPEAN DIRECTIVE 2001/20/EC TO CLINICAL TRIAL REGULATION EU NO 536/2014 was added.