CLINICAL STUDY PROTOCOL

EudraCT Number: 2020-001645-40
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Protocol Title: Adaptive phase 2/3, randomized, controlled multicenter study on the efficacy and safety of Reparixin in the treatment of hospitalized patients with COVID-19 pneumonia
Short Tile: Reparixin in COVID-19 pneumonia
Acronym: REPAVID-19

Protcol Number: 1.5
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Investigational compound: Reparixin

Study period	Projected starting date (first-patient-in):	April 2020
	Projected study end date (last-patient-last-visit):	June 2020

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1. PROTOCOL SUMMARY

Protocol Title: Adaptive phase 2/3, randomized, controlled, multicenter study on the efficacy and safety of Reparixin in the treatment of hospitalized patients with COVID-19 pneumonia

Short Title: Reparixin in COVID-19 pneumonia.

Investigational medicinal products: Reparixin oral tablets.
Rescue medication: At discretion of investigators in phase 2; Reparixin oral tablets or intravenous (IV) infusion in Phase 3.

Background and Rationale: see section 2.1 and 2.2

Phase of development: 2 and 3

Patient population: 48 patients will be enrolled in Phase 2 and an estimated total of 111 patients will be enrolled up to the end of Phase 3, with a randomization 2:1 Reparixin vs Control (Standard of care)

Study Objectives and Endpoints
- Phase 2 Study Objectives: efficacy and safety of Reparixin as compared to the control arm in adult patients with severe COVID-19 pneumonia
- Phase 3 Study Objectives: efficacy and safety of Reparixin treatment as compared to the control arm in adult patients with severe COVID-19 pneumonia
- **Phase 2 Primary Endpoint**: Composite endpoint of clinical events (the patient requires at least one of the following: supplemental oxygen requirement, mechanical ventilation use, admission to Intensive Care Unit (ICU), and use of a rescue medication for any reason)
- **Phase 3 Primary Endpoint**: Composite endpoint of death and of severe clinical events (the patient dies or requires mechanical ventilation use and/or admission to ICU)

- Other Phase 2/3 Study Endpoints:

- **Secondary Endpoints:**
  - Changes in clinical severity score (as recommended by the World Health Organization –WHO– for COVID studies)
  - Dyspnea severity (Liker scale and VAS scale)
  - Changes in body temperature
  - Changes in hematology test values
  - Duration and quantity of supplemental oxygen treatment
  - Incidence and duration of mechanical ventilation use
  - Incidence of intensive care unit (ICU) admission need
  - Lung damage extension changes from baseline (assessed by Chest CT or Rx)
  - Change from baseline in lung exudation degree (assessed by Chest CT or Rx)
  - PaO2
  - SpO2
  - Partial arterioral oxygen pressure (PaO2) to fraction of inspiration O2 (FiO2) ratio
  - CRP
  - Hs-CRP
- **Exploratory Endpoints (not mandatory):**
  - Cytokine profile

    - Concentration of Reparixin in serum over time
    - SARS-CoV-2 virologic counts
- **Safety Endpoints:**
    - Adverse events
    - Serious adverse events

Procedures and Assessments: Efficacy and Safety procedures and assessments for this study will include all the listed enpoints as well as the recording of concomitant medications' use.

Overall Study Design: this clinical trial is designed as an adaptive, randomized, controlled, multicentre study to evaluate efficacy and safety of Reparixin in hospitalized adult patients with severe COVID-19 pneumonia. In the phase 2 segment of this study, patients will be randomized 2:1 to Reparixin oral tablets 1200 mg (Group 1, active treatment) or standard of care (Group 2, control arm). In case of worsening (e.g. need of ICU and/or mechanical ventilation) after the first 24hrs, patients will be offered a rescue medication based on their physicians' judgement, without any constrain from the sponsor. The administration of rescue medications is allowed, without constituting a protocol deviation. In the phase 3 segment of this study, it is planned that patients will be randomized 2:1 to Reparixin or standard of care. In case of worsening after the first 24hrs, patients under the control arm will be offered a rescue medication with the active treatment (Reparixin 1200 mg oral tablets TID) or Reparixin 2.772 mg/kg body weight/hour IV infusion, if the oral route is unfeasible for the clinical condition. The Phase 3 design will be reassessed and decided based on the results of the Phase 2.

Population: Hospitalized adult (≥18 <90 years old) patients with severe COVID-19 pneumonia. No gender and/or ethnicity restrictions will apply.

Inclusion/exclusion criteria

- Phase 2 Inclusion Criteria:

1. Age 18 to 90.
2. Confirmed COVID-19 diagnosis
3. At least one of the following: ① Respiratory distress, RR ≥ 30 breaths/min without oxygen; ② Partial arterial oxygen pressure (PaO2) / Fraction of inspiration O2 (FiO2) >100 <300mmHg (1mmHg = 0.133kPa).
4. Chest imaging confirms lung involvement and inflammation.
5. Inflammatory status as documented by at least one of the following: Lactate dehydrogenase (LDH) > normal range, C-reactive protein (CRP) ≥ 100mg/L or IL-6 ≥ 40pg/mL, serum ferritin ≥ 900ng/mL, XDP >20mcg/mL.

- Phase 3 Inclusion Criteria: Same as above; other criteria TBD based on Phase 2 outcomes.

- Phase 2/3 Exclusion Criteria:

1. Cannot obtain informed consent.
2. Severe hepatic dysfunction (Child Pugh score ≥ C, or AST> 5 times the upper limit); Severe renal dysfunction (estimated glomerular filtration rate ≤ 30mL / min / 1.73 m2) or receive continuous renal replacement therapy, hemodialysis, or peritoneal dialysis.
3. Patients with hypersensitivity to ibuprofen or to more than one non steroidal anti-inflammatory drug or to more than one medication belonging to the class of sulfonamides (e.g.

sulfamethazine, sulfamethoxazole, sulfasalazine, nimesulide or celecoxib; hypersensitivity to sulphanilamide antibiotics alone, e.g. sulfamethoxazole, does not qualify for exclusion)

4. Severe, active bleeding such as hemoptysis, gastrointestinal bleeding, central nervous system bleeding, and nosebleeds within 1 month before enrollment.
5. Pregnant and lactating women and those planning to get pregnant.
6. Participated in other interventional clinical trials with investigational medicinal products, not considered suitable for this study by the researchers.
7. At the time of enrollment, patients not in a clinical condition compatible with the oral administration of the study drug.

Number of patients enrolled: It is planned that 48 patients will be enrolled in Phase 2 and a total of 111 patients will be enrolled up to completion of the Phase 3. However, the sample size for the Phase 3 segment of this study may be re-estimated based on the Phase 2 results. In the current sample size scenario, 32 patients will receive Reparixin in Phase 2.

Study duration: treatment duration will be up to a maximum of 21 days. Overall Phase 2 study duration will be maximum 1 month (including 21 days of treatment and up to 7±3 days of follow-up).

End of study (EOS) definition: EOS is defined as the last day the last patient completes the last study assessment in the hospital, or retracts the consent to participate in the study, or withdraws from the study, or is deceased or otherwise lost to follow-up.

Interventions: Patients who satisfy the predefined inclusion and exclusion criteria for this trial will be randomized 2:1 to one of the following treatment groups:

- **GROUP 1:** Reparixin oral tablets 1200 mg TID for 7 days. **In case of improvement**, treatment can be prolonged at discretion of the investigator up to a maximum of 21 days of treatment in total or live discharge from the hospital, whichever comes first.
- **GROUP 2 (CONTROL):** Standard of care.

RESCUE MEDICATION: In case of worsening (e.g. need of ICU and/or mechanical ventilation), patients will be offered after the first 24hrs, patients will be offered a rescue medication based on their physicians' judgement, without any constrain from the sponsor. In the phase 3 segment of this study, it is planned that patients will be randomized 2:1 to Reparixin or standard of care. In case of worsening after the first 24hrs, patients under the control arm will be offered a rescue medication with the active treatment (Reparixin 1200 mg oral tablets TID) or Reparixin 2.772 mg/kg body weight/hour IV infusion, if the oral route is unfeasible for the clinical condition. The Phase 3 design will be reassessed and decided based on the results of the Phase 2.

Statistical plan: The sample size for this trial will be adaptively determined through a phase 2/3 design. Enrollement is planned to last approximately 2 months. Each patient will be randomly allocated to Raparixin or Control according to a randomization ratio of 2:1 and he/she will be followed for the subsequent 21 days for collectioning the efficacy endpoints of each study phase.
For the Phase 2 portion of the study, the primary analysis will compare Reparixin versus Control in terms of time to a composite endpoint including the following events: supplemental oxygen requirement, mechanical ventilation use, admission to ICU, and any use of rescue medication. Expecting an 1.5 fold improvement of the median time freedom from composite event and assuming an

exponential distribution, a total sample size of 48 patients will allow to provide 80% power to show superiority of Reparixin compared to control using a log-rank test at a one-sided significance level of 0.025.

The sample size for the phase 3 is based on assessment of superiority of Reparixin compared to control arm in terms of freedom from a composite endpoint of severe events including: death, admission to the ICU, and mechanical ventilation.
Based on same assumptions of phase 2 and expecting a double median time freedom from severe composite endpoint for Reparixin compared to control (2-4 days), a total sample size of 111 patients will allow to achieve a power of 90% to show superiority of Reparixin over control using a log-rank test at a one-sided significance level of 0.025. No drop-out are expected to happen. The number of patients to be included may be revised based on considerations from Phase 2 data analysis.
Primary endpoint of phase 3 will be tested only if superiority of reparixin is shown in phase 2. Thus, the overall type I error of the study will be controlled at 0.025 via the hierarchical testing procedure.

Additional Statistical considerations: This trial's design will allow for adaptations based but not limited to the following:

- Interruption of an experimental treatment route of administration in favour of the other due to safety reasons;
- Confirmation or modification of the primary, secondary and exploratory endpoints;
- Confirmation or modification of the sample size for the Phase 3 segment;
- Early closure of the study for efficacy, futility or safety reasons.

Due to the open-label nature of this trial, an Indipendent Data Monitoring Committee (DMC) is foreseen to provide timely recommendations about the above mentioned early closures or modifications. This monitoring committee will include 2 to 4 indipendent physicians, supported by a statistician.

2. INTRODUCTION

2.1 BACKGROUND:

IN DECEMBER 2019, A NEW IDENTIFIED CORONAVIRUS (SARS-COV-2) OUTBREAK IN WUHAN, CAUSES PUBLIC HEALTH CRISIS IN CHINA AND SPREADS WORLDWIDE. On February 11, 2020, the World Health Organization officially named the disease caused by the new coronavirus "COVID-19". The Chinese Government takes stronger and harsher measures to control the progression of its outbreak. Meanwhile, five editions of "Diagnosis and Treatment for Novel Coronavirus-Infected Pneumonia" has been timely and continuously issued, which play extremely important roles in guiding the clinical management of COVID-19 nationwide in China.The symptoms of human infection with SARS-CoV-2 are generally fever, fatigue, dry cough and dyspnea. Noteworthy, a considerable percentage of COVID-19 cases have rapidly progressed to severe and critical type, among which acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are the most common complications, resulting in a large number of pneumonia hospitalized patients requiring supplemental oxygen, mechanical ventilation, or even ECMO.Pulmonary edema is a detrimental feature as well as a key causal factor of ALI/ARDS.

LUNG EDEMA, ENDOTHELIAL AND EPITHELIAL INJURY ARE ACCOMPANIED BY AN INFLUX OF NEUTROPHILS INTO THE INTERSTITIUM AND BRONCHEOALVEOLAR SPACE. Neutrophils are considered to play a key role in the progression of ALI and ARDS (1), as activation and transmigration of neutrophils is a hallmark event in the progression of ALI and ARDS. Proof for the importance of neutrophils in ALI comes from clinical data and animal models. In patients with ARDS, the concentration of neutrophils in the bronchoalveolar lavage (BAL) fluid correlates with severity of ARDS and outcome (2; 3), whereas the severity of lung injury has been reduced by neutrophil depletion in mice. Furthermore, after blocking interleukin-8 (IL-8), a major chemoattractant for neutrophils, rabbits have been protected from acid aspiration-induced lung injury. A multitude of experimental and clinical data point at the causative role of neutrophils in lung injury (1-57). Although neutrophil activation is vital for the host defense, overzealous activation leads to tissue damage by release of cytotoxic and immune cell–activating agents such as proteinases, cationic polypeptides, cytokines, and reactive oxygen species (ROS).

2.2 DRUG MECHANISM OF ACTION AND STUDY RATIONALE:

NUMEROUS STUDIES HAVE CONFIRMED A KEY ROLE OF CXCR1/CXCR2 RECEPTOR AS POTENTIAL THERAPEUTIC TARGET IN ACUTE LUNG INJURY (ALI) AND ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS). Neutrophil infiltration of the lung is controlled by a complex network of chemokines that are released by a variety of cell types. Alveolar macrophages are a major source of chemokines in the alveolar space and produce IL-8, growth-regulated oncogene (GRO)-related peptides and CXCL5 (also known as epithelial neutrophil-activating protein [ENA]-78) (58; 59) (60; 61). High concentrations of IL-8 in BAL fluid from ARDS patients are associated with increased neutrophil influx into the airspace (62; 63). Recent studies have revealed that IL-8 in BAL fluid is bound to IL-8 autoantibodies (anti-IL-8:IL-8 complexes) (64; 65) and BAL fluid concentrations of these complexes correlate with development and outcome of ALI (66; 67). In particular, this complex exhibits chemotactic and proinflammatory activity (68). Moreover, intratracheal application of IL-8 induces lung injury which can be attenuated by inhibition in different models of ALI (69). In rodents, the most relevant chemokines for neutrophil recruitment into the lung are keratinocyte-derived chemokine (KC, also named CXCL1) or cytokine-induced neutrophil chemoattractant (CINC; the rat homolog to KC) and macrophage inflammatory protein-2 (MIP-2, also named CXCL2). Similar to IL-8, CXCL1, CXCL2, lipopolysaccharide-induced CXC chemokine (LIX, also named CXCL5) and lungkine (CXCL15) bind to CXCR2. Inhibition or

knockout of CXCR2 receptor diminishes neutrophil influx into the lung (70-77). In contrast to the multiple CXC chemokines only two CXC chemokines receptors, CXCR1 and CXCR2, have been shown to mediate the response to CXC chemokines in human neutrophils. Whereas human CXCR1 binds to CXCL6 and CXCL8 (IL-8) with a high affinity, human CXCR2 binds also to CXCL6 and IL-8 as well as several CXC chemokines (GRO-α,GRO-β, GRO-γ, CXCL1, CXCL2, CXCL3), ENA-78 (CXCL5) and (CXCL7) (78).

REPARIXIN IS A POTENTIAL DRUG FOR THE TREATMENT OF SEVERE OR CRITICAL PATIENTS WITH COVID-19 PNEUMONIA. Reparixin (DF1681Y) is a specific inhibitor of CXC ligand 8 [CXCL8; formerly interleukin (IL)-8] biological activity, stemming from a program of drug design of molecules intended to modulate chemokine action. Reparixin is *in vitro* a potent and specific inhibitor of CXCL8 biological activity. *In vitro* chemotaxis experiments have shown that reparixin inhibits CXCL8-induced chemotaxis of human polymorphonuclear leukocytes (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. *In vivo*, reparixin prevented PMN infiltration into the transplanted kidney and reduced creatinin levels in a rat model of kidney transplantation. Similarly, in a rat model of lung transplantation, reparixin improved isolated graft oxygenation, decreased pulmonary oedema, and significantly reduced neutrophil infiltration into transplanted lungs. 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%. Efficacy was seen in all models at reparixin dose of 9.90 mg/kg.

REPARIXIN EFFECTS ON ACUTE LUNG INJURY (ALI) MODELS. The therapeutic potential of reparixin in murine models of LPS-induced pulmonary inflammation and acid-induced ALI was studied. Reparixin (15 μg g−1) reduced neutrophil recruitment in the lung by approximately 50% in an in vivo model of LPS-induced ALI.Reparixin also reduced accumulation of neutrophils in the interstitial compartment and vascular permeability in LPS-induced ALI. 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 (70). In the CDE (cat dander extract) single challenge model (SCM), administration of RPX (15 mg/kg) suppressed neutrophil recruitment into the lungs. In the CDE Multiple Challenge Model, RPX inhibited eosinophil, neutrophils, and total cell numbers in BALF, serum levels of total IgEand CDE specific IgE, airway epithelial mucin secretion, levels of Th2 inflammation-associated genes periostinand muc5ac, and the BALF levels of IL-4, IL-13, IL-33, and TSLP in BALF.

REPARIXIN SUPPRESSES ALLERGEN CHALLENGE INDUCED NEUTROPHILIC INFLAMMATION AND ALLERGIC AIRWAY INFLAMMATION. In the CDE (cat dander extract) single challenge model (SCM), administration of reparixin (15 mg/kg) suppressed neutrophil recruitment into the lungs. In the CDE Multiple Challenge Model, reparixin inhibited eosinophil, neutrophils, and total cell numbers in BALF, serum levels of total IgEand CDE specific IgE, airway epithelial mucin secretion, levels of Th2 inflammation-associated genes periostin and muc5ac, and the BALF levels of IL-4, IL-13, IL-33, and TSLP in BALF. Pharmacological inhibition of CXCR1/2-axis by administration of reparixin inhibits allergen induced innate and allergic airway inflammation in mice (79).

REPARIXIN AMELIORATES THE INCREASED SEVERITY OF PULMONARY FIBROSIS CAUSED BY PARTICULATE MATTER

In a murine model of bleomycin-induced pulmonary fibrosis, pharmaceutical inhibition of CXCR2 with reparixin ameliorated Particulate Matter-induced increased severity of pulmonary fibrosis. Co-treatment with reparixin in mice receiving Particulate Matter and bleomycin reduced neutrophil number and neutrophil elastase concentration of day 2-BALF. Moreover, reparixin improved lung function and ameliorated pulmonary fibrosis as assayed by total collagen content and histochemical stains of fibrosis markers on day 14-lung tissues. (80)

REPARIXIN ANALOGUES RESULTED A VALID THERAPEUTIC STRATEGY FOR TREATING LUNG INFECTIONS CAUSED BY INFLUENZA A VIRUS OR STREPTOCOCCUS PNUEMONIAE.

The role of CXCR1/2 during influenza, pneumococcal, and post-influenza pneumococcal infections was investigated. Mice were infected with influenza A virus (IAV) or Streptococcus pneumoniae and then treated daily with the CXCR1/2 antagonist DF2162. To study secondary pneumococcal infection, mice were infected with a sublethal inoculum of IAV then infected with S. pneumoniae 14 days later. DF2162 was given in a therapeutic schedule from days 3 to 6 after influenza infection. Lethality, weight loss, inflammation, virus/bacteria counts, and lung injury were assessed. CXCL1 and CXCL2 were produced at high levels during IAV infection. DF2162 treatment decreased morbidity and this was associated with decreased infiltration of neutrophils in the lungs and reduced pulmonary damage and viral titers. During S. pneumoniae infection, DF2162 treatment decreased neutrophil recruitment, pulmonary damage, and lethality rates, without affecting bacteria burden. Therapeutic treatment with DF2162 during sublethal IAV infection reduced the morbidity associated with virus infection and also decreased the magnitude of inflammation, lung damage, and number of bacteria in the blood of mice subsequently infected with S. pneumoniae. These data suggested that modulation of the inflammatory response by blocking CXCR1/2 improves disease outcome during respiratory influenza and pneumococcal infections, without compromising the ability of the murine host to deal with infection (81).

DF2162, a Reparixin analogue, belong to the same family of non competitive-allosteric inhibitors of CXCR1 and CXCR2 widely characterized in our research labs in terms of structure activity relationship and mechanism of action.

The two molecules belong to the chemical class of 2-(R)- phenyl propionamide derivatives thus sharing the same chemical moiety.

Reparixin and DF2162 exhibit similar potency in the inhibition of the target receptors CXCR1 and CXCR2 (IC50s in CXCL8 induced-chemotaxis in the range of 1 nM). The molecular mechanism of action has been deeply characterized by point-mutagenesis studies on CXCR1 and CXCR2 showing that Reparixin and DF2162 bind the receptors in the same allosteric site in the Trans-Membrane region, highly conserved in the two receptor subtypes.

[Moriconi et.al ACS Med Chem Lett. 2011 Aug 7;2(10):768-73.; Moriconi et al. J Med Chem. 2007 Aug 23;50(17):3984-4002.; Allegretti M et al J Med Chem. 2005 Jun 30;48(13):4312-31.; Bertini R et al; Proc Natl Acad Sci U S A. 2004 Aug 10;101(32):11791-6.].

The different pharmacokinetic profile of the two molecules account for a bid (15mg/Kg) oral administration of DF2162 as compared a tid (15 mg/Kg) administration (on continuous infusion) for reparixin.

For this reason, DF2162 has been used to assess the role of CXCR1/2 during influenza (IAV), pneumococcal, and post-influenza pneumococcal infections in the mice models. Even though the extensive characterization work was conducted with DF2162, preliminary experiments showed a similar behaviour using reparixin in the single IAV model leading to comparable results.

To investigate the role of CXCR1/2 during influenza infection, mice were infected with 1 × 104 PFU of IAV and then treated three times a day (from day 0—at the time of the infection—to day 5 post-infection)

with reparixin at 15 mg/Kg. Similarly to DF2162, treatment with reparixin decreased morbidity, as seen by the reduction of weight loss, reduced leukocytes infiltration into the airways, including neutrophils, and the levels of the pro-inflammatory cytokines TNF-α and CXCL1 and reduced the lung injury associated with IAV infection measure by histopathological score.

REPARIXIN IMPROVED SURVIVAL AFTER LUNG TRANSPLANTATION. In a US-Canada phase 2 study [*REP0104*], 101 patients (46 on reparixin, 55 on placebo) undergoing single or bilateral lung transplant were trated with reparixin. The patients were randomized to receive 48h i.v. continuous infusion (loading: 4.488 mg/kg/h for 30 min, maintenance: 2.772 mg/kg/h for 47.5hrs) of either reparixin or placebo starting a few hours before the transplant. The study showed a statistically significant difference in patient survival at Month 12 post-transplant between the placebo (7 deaths) and reparixin (no deaths) groups (p-value = 0.0111 [Log-Rank]).

IRCCS OSPEDALE SAN RAFFAELE COORDINATED A PHASE 3 INTERNATIONAL TRIAL WITH REPARIXIN IN PANCREATIC ISLET TRANSPLANTATION. A phase 3, multicenter, randomized, double-blind, parallel-assignment study (NCT01817959) was conducted and coordinated by OSR in recipients of islet allo-transplants randomized (2:1) to reparixin or placebo in addition to immunosuppression. Patients received either reparixin at a dose of 2.772 mg/kg body weight/h or matched (flow rate/length of infusion) placebo according to their randomization number. Study drugs were administered on top of immunosuppression by continuous infusion through a high-flow vein for 7 days starting 12 h before each islet infusion. No clear differences between treatment groups were observed for rates, severity, and distribution of AEs or SAEs. Analysis of patient subsets showed a trend for a higher percentage of subjects retaining insulin independence for 1 year after a single islet infusion in patients receiving reparixin, as compared with patients receiving placebo (26.7% vs. 0%, P = 0.09) when antithymocyte globulin was used as induction immunosuppression (82).

IRCCS OSPEDALE SAN RAFFAELE IS A REFERENCE CENTER IN THE MANAGEMENT OF THE COVID-19 PANDEMIC EMERGENCY, AND HAS RECENTLY TREATED WITH REPARIXIN (UNDER A COMPASSIONATE USE APPLICATION) FOUR PATIENTS WITH SEVERE COVID-19 PNEUMONIA. THE SPONSOR PROVIDES HERE THE OUTCOMES OF SUCH TREATMENTS AS PROVIDED BY THE SAN RAFFAELE HOSPITAL PHYSICIANS: Four patients with ARDS caused by COVID-19 pneumonia with the clinical indication for intubation and mechanical ventilation were treated with Reparixin IV infusion (2.772 mg/kg body weight/hour) into a high-flow central vein for 5 days at the San Raffaele Hospital of Milan, Italy. The first patient started treatment on 24 March 2020 and the last patient on 31 March 2020. As of the date of preparation of the this Protocol (Version 1.3 dated 9 April 2020), all patients are still alive. Specifically, two of the four patients were never intubated, and two of them are currently intubated: from the report of their ICU physician one is currently in stable conditions, and one is improving. Therefore, looking at this preliminary results on a limited number of patients who had an indication for intubation and mechanical ventilation in ICU care (both events being in the proposed primary endpoint of this study) before starting treatment, the improvement observed on the expected clinical outcome in 2 out of 4 patients is aligned with the assumptions we used for sample size calculations of this trial.

From a hematochemical standpoint, during treatment with Reparixin an improvement or at least stabilization of the inflammatory markers (C-reactive protein, procalcitonin, ferritin) and of the tissue damage markers (LDH, AST, ALT) was also observed during this compassionate use experience. A list

of the patients' characteristics is provided below in tabular format as provided by the San Raffaele Hospital physicians

PT ID	Sex	Age	Comorbidity	Basal treatment	Symptoms before hospital admission	Treatment start	Treatment duration
Pt1	M	63	Diabetes	Insulin	14 d, Fever-Cough	24/03/2020 12:00	5 days
Pt2	M	57	Hypertension, dyslipidemia	ARB, Statin	7 d, Fever-Cough- diarrea-anosmia-disgneneusia	30/03/2020 23:30	5 days
Pt3	M	62	Hypertension, dyslipidemia, Obstructive sleep apnea	ASA, ARB, Statin	10d, Fever	31/03/2020 19:20	5 days
Pt4	F	47	Diabetes, CVD, Mood disorder	Insulin, ASA, nitrate	7d, Fever	31/03/2020 19:30	5 days

As of today no other compassionate use cases for the use of Reparixin in patients with Covid-19 pneumonia have been activated at the San Raffaele Hospital or in other sites.

3 OBJECTIVES AND ENDPOINTS

- Phase 2 Study Objectives: efficacy and safety of of Reparixin treatment as compared to the control arm in adult patients with severe COVID-19 pneumonia

- Phase 3 Study Objectives: efficacy and safety of Reparixin treatment as compared to the control arm in adult patients with moderate or severe COVID-19 pneumonia

- **Phase 2/3 Study Endpoints:**
  - **Primary Endpoint for Phase 2:** Composite endpoint of clinical events (the patient requires at least one of the following: supplemental oxygen requirement, mechanical ventilation use, admission to ICU, and use of a rescue medication for any reason)
  - **Primary Endpoint for Phase 3:** Composite endpoint of death and clinical severe events (the patient dies or requires mechanical ventilation use and/or admission to ICU)
  - **Secondary Endpoints:**
    - Changes in clinical severity score (as recommended by WHO for COVID studies), defined as the time to clinical improvement of two points from the time of randomization on a seven-category ordinal scale or live discharge from the hospital, whichever comes first. The seven-category ordinal scale consists of the following: 1) not hospitalized, with resumption of normal activities; 2) not hospitalized, but unable to resume normal activities; 3) hospitalized, not requiring supplemental oxygen; 4) hospitalized, requiring supplemental oxygen; 5) hospitalized, requiring high-flow oxygen therapy, noninvasive mechanical ventilation, or both; 6) hospitalized, requiring ECMO, invasive mechanical ventilation, or both; and 7) death.
    - Dyspnea severity (Liker scale and VAS scale)
    - Changes in body temperature
    - Changes hematology test values
    - Duration and quantity of supplemental oxygen treatment
    - Incidence and duration of mechanical ventilation use
    - Incidence of intensive care unit (ICU) admission need
    - Lung damage extension changes from baseline (assessed by Chest CT or Rx)
    - Change from baseline in lung exudation degree (assessed by Chest CT or Rx)
    - PaO2
    - SpO2
    - Partial arterioral oxygen pressure (PaO2) to fraction of inspiration O2 (FiO2) ratio
    - CRP
    - Hs-CRP
  - **Exploratory Endpoints (not mandatory):**
    - Cytokine profile
    - Concentration of Reparixin in serum over time
    - SARS-CoV-2 virologic counts
  - **Safety Endpoints:**
    - Serious adverse events
    - Adverse events (AEs) coded by System Organ Class (SOC) and preferred Term (PT), using the Medical Dictionary for Regulatory Activities (MedDRA).
    - The number and percentage of patients with any AE and the number of TEAEs, tabulated by SOC and PT, seriousness, relationship to treatment and severity.

4 STUDY DESIGN AND METHODOLOGY

4.1 Phase 2 Study design

The study is designed for the investigational use of a CXCR1 and CXCR2 allosteric antagonist named Reparixin. It will involve 48 adult patients with severe COVID-19 pneumonia in accord with any of the following:

1. Age 18 to 90.

2. Confirmed COVID-19 diagnosis

3. At least one of the following: ① Respiratory distress, RR ≥ 30 breaths/min without oxygen; ② Partial arterial oxygen pressure (PaO2) / Fraction of inspiration O2 (FiO2) >100 <300mmHg (1mmHg = 0.133kPa).

4. Chest imaging confirms lung involvement and inflammation.

5. Inflammatory status as documented by at least one of the following: Lactate dehydrogenase (LDH) > normal range, C-reactive protein (CRP) ≥ 100mg/L or IL-6 ≥ 40pg/mL, serum ferritin ≥ 900ng/mL, XDP >20mcg/mL.

Patients randomized to the active treatment will receive an oral dose of reparixin (1200 mg tablets TID) for 7 days. In case of improvement, treatment can be prolonged at discretion of the investigator up to a total of 21 days of treatment. Patients randomized to the both arms will be offered, in case of worsening (e.g. need of ICU and/or mechanical ventilation), a rescue medication based on their physicians' judgement, without any constrain from the sponsor.

4.2 Phase 3 Study design

In the phase 3 segment of this study, it is planned that patients will be randomized 2:1 to Reparixin or standard of care. Patients randomized to the control arm will be offered a rescue medication with the active treatment (Reparixin 1200 mg oral tablets TID) or Reparixin 2.772 mg/kg body weight/hour IV infusion, if the oral route is unfeasible for the clinical condition. The Phase 3 design will be reassessed and pending re-evaluation based on Phase 2 study results.

Phase 2 Study Design and Methodology: Randomized 2:1, controlled, multicentre clinical trial in 48 adult patients with severe COVID-19 pneumonia

Phase 3 Study Design and Methodology: Randomized 2:1, controlled, multicentre clinical trial in 111 adult patients with severe COVID-19 pneumonia, to be re-assessed based on Phase 2 results.

5 POPULATION

5.1 Number of patients

A maximum of 48 patients with moderate-to-severe COVID-19 pneumonia will be enrolled in the Phase 2 study. Enrollment can be interrupted at any given time for futility or clear superiority reasons of the oral tablet vs IV infusion, or both vs standard of care, to proceed into Phase 3 or interrupt the clinical study.

The estimated number of 111 patients to be enrolled until the end of Phase 3 will be re-estimated based on the Phase 2 results.

5.2 Inclusion/exclusion criteria

Phase 2 Inclusion Criteria:

1. Age 18 to 90.
2. Confirmed COVID-19 diagnosis
3. At least one of the following: ① Respiratory distress, RR ≥ 30 breaths/min without oxygen; ② Partial arterial oxygen pressure (PaO2) / Fraction of inspiration O2 (FiO2) >100 <300mmHg (1mmHg = 0.133kPa).
4. Chest imaging confirms lung involvement and inflammation.
5. Inflammatory status as documented by at least one of the following: Lactate dehydrogenase (LDH) > normal range, C-reactive protein (CRP) ≥ 100mg/L or IL-6 ≥ 40pg/mL, serum ferritin ≥ 900ng/mL, XDP >20mcg/mL.

Phase 3 Inclusion Criteria: same as above; other criteria TBD based on Phase 2 outcomes.

Phase 2/3 Exclusion Criteria:

1. Cannot obtain informed consent.
2. Severe hepatic dysfunction (Child Pugh score ≥ C, or AST> 5 times the upper limit); Severe renal dysfunction (estimated glomerular filtration rate ≤ 30mL / min / 1.73 m2) or receive continuous renal replacement therapy, hemodialysis, or peritoneal dialysis. Serum albumin <2.5 g/dL.
3. Patients with hypersensitivity to ibuprofen or to more than one non steroidal anti-inflammatory drug or to more than one medication belonging to the class of sulfonamides (e.g. sulfamethazine, sulfamethoxazole, sulfasalazine, nimesulide or celecoxib; hypersensitivity to sulphanilamide antibiotics alone, e.g. sulfamethoxazole, does not qualify for exclusion)
4. Severe, active bleeding such as hemoptysis, gastrointestinal bleeding, central nervous system bleeding, and nosebleeds within 1 month before enrollment.
5. Pregnant and lactating women and those planning to get pregnant.
6. Participated in other interventional clinical trials with investigational medicinal products, not considered suitable for this study by the researchers.
7. At the time of enrollment, patients not in a clinical condition compatible with the oral administration of the study drug.

6 INTERVENTIONS

6.1 Interventional treatments

Phase 2 Investigational treatment
Reparixin in oral pharmaceutical form (1200 mg tablets TID), as follows:
GROUP 1: Reparixin oral tablets 1200 mg TID for 7 days.

- **In case of improvement**, treatment can be prolonged at discretion of the investigator up to a - maximum of 21 days of treatment in total.

Phase 3 Investigational treatment
Reparixin in oral pharmaceutical form (1200 mg tablets TID). Treatment duration to be decided based on Phase 2 study results.

6.2 Control treatment

Phase 2/3 Control treatment
Control group in both the phase 2 and 3 segments will be standard of care, which due to the nature of the COVID-19 pandemic is expected to evolve over time and, thus, cannot be prespecified. This group will be populated in a prospective manner and offered a rescue medication in case of worsening (e.g. need of ICU and/or mechanical ventilation). Due to the evolving understanding of novel treatments being currently tested during the COVID-19 pandemic, the Sponsor will not put any restriction on the selection of the rescue medication during phase 2. In the phase 3, Reparixin will be offered as rescue medication, as oral tablets or as IV infusion in case of unfeasibility of the oral administration due to the clinical conditions (e.g. intubated patients in the ICU).

6.3 Preparation, Handling, Storage and Accountability of the study drug

Study Drug (REPARIXIN tablets)

FORMULATION

The investigational product is in the form of oral immediate release 600 mg tablets containing the active ingredient reparixin. Reparixin 600 mg immediate release tablets are white oblong tablets.

Refer to the following table for Reparixin tablets Description and Composition.

NAMES OF INGREDIENTS	AMOUNT PER TABLET	FUNCTION OF INGREDIENT	REFERENCE TO QUALITY STANDARDS
Reparixin (DF 1681Y)	600.0 mg	Drug substance	Internal monograph
Cellulose Microcrystalline	141.48 mg	Diluent/ Disintegrant	Eur. Ph.
Lactose monohydrate	94.77 mg	Diluent	Eur. Ph.
Croscaramellose Sodium	36.00 mg	Disintegrant	Eur. Ph.
Hydroxypropyl cellulose	20.07 mg	Binder	Eur. Ph.

Silica, colloidal anhydrous	3.15 mg	Glidant	Eur. Ph.
Magnesium stearate	4.50 mg	Lubricant	Eur. Ph.
Total	900 mg	-	-

Manufacturing, Packaging and Labelling of Investigational Product

Reparixin tablets will be packaged in white PVDC/PE/PVC/Aluminum blister packages and should be stored at temperatures not higher than 30°C.

The drug is manufactured by Monteresearch according to current Good Manufacturing Practice requirements.

Medication labels will comply with the Competent Authority requirements and will be printed in a Multilanguage format where needed. Refer to the Appendix 4 for reparixin packaging and labeling details.

Reparixin tablets are packaged in white PVDC/PE//PVC/Aluminum blisters in the form of patient kits, numbered to maintain blinding and should be stored at temperature not higher than 30°C.

The Investigator (or pharmacist in those countries where it is required that shipment is made directly to the hospital pharmacy) is responsible for receipt, proper storage and usage of study drug. Partially used or unused study drug boxes should be destroyed on site (and documentation of destruction provided to Dompé farmaceutici s.p.a.) or returned to Dompé farmaceutici s.p.a., at the end of the study. The Investigator, who will keep a cumulative inventory and dispensing records, will maintain all supplies under adequate security. Adequate record of receipt, use or loss of drug will be retained.

Pharmacists will be provided with the 'Instructions to the Pharmacy'.

Dose and Route of Administration

- **Oral tablets:** For patients able to comply with the oral treatment, it is recommended to take for each administration two Reparixin 600 mg tablets, for the total of three daily administrations. It is advisable to take the tablets with a glass of water to facilitate swallowing.
  For patients who are unwilling or unable, in the opinion of the investigator, to comply with the oral tablets treatment, is possible to administer the medicine through a naso-gastric tube, following 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). Disgregate 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 using a naso-gastric tube. After administration, run 25 ml of drinking water through the gastric tube.

Study Drug (REPARIXIN iv)

FORMULATION

The investigational product is in the form of concentrate for solution for i.v. infusion packaged into 250 mL clear Glass Vials with the following composition per single (250 mL) unit.

Refer to the following table for Reparixin i.v. 33mg/mL Description and Composition

NAME OF INGREDIENT	PER-UNIT FORMULA	FUNCTION OF INGREDIENT	REFERENCE TO QUALITY STANDARDS
Reparixin (DF1681Y)	8.25 g	Drug substance	Internal monograph
Sodium Dihydrogen Phosphate Dihydrate	1.96 g	Buffer	Eur. Ph.
L-lysine monohydrate	4.78 g	Solubilizer	German Pharmacopoeia - current edition
Sodium hydroxide	qs to pH 8.0	Buffer	Eur. Ph.
Water for injections	qs to 250 mL	Solvent	Eur. Ph.

Manufacturing, Packaging and Labelling of Investigational Product

Reparixin will be manufactured by Patheon (Italy) according to current Good Manufacturing Practice requirements.

The Reparixin iv is packaged into 250 mL clear Glass Vials.

The Investigational Product must be kept at a temperature not exceeding 30°C and must not be frozen

Medication labels will comply with the Competent Authority requirements and will be printed in a Multilanguage format where needed. Refer to the Appendix 4 for reparixin packaging and labeling details.

The Investigator (or pharmacist in those countries where it is required that shipment is made directly to the hospital pharmacy) is responsible for receipt, proper storage and usage of study drug. Partially used or unused study drug boxes should be destroyed on site (and documentation of destruction provided to Dompé farmaceutici s.p.a.) or returned to Dompé farmaceutici s.p.a., at the end of the study. The Investigator, who will keep a cumulative inventory and dispensing records, will maintain all supplies under adequate security. Adequate record of receipt, use or loss of drug will be retained.

Pharmacists will be provided with the 'Instructions to the Pharmacy'.

Dose and Route of Administration

IV infusion: In the Phase 3, for patients randomized to the control arm who have worsened after 24hrs and within 14 days from randomization, Reparixin will be offered as rescue medication also as a continuous i.v. infusion into a central vein or dedicated peripherally inserted central catheter at a dose of 2.772 mg/kg body weight/hour for up to 7 days (120hrs).

The Reparixin solution for IV infusion will be provided by the Sponsor.
The dosing solution for infusion will be prepared at the designated Pharmacy or authorized location within each centre according to local guidelines for sterile re-consitution of i.v. injectable solutions.
For each 750 mL dosing solution volume, the content of a Vial (250 mL) will be diluted with 500 mL of 0.9% sterile saline to dispense reparixin as 11.00 mg/mL solution. The dosing solution will be placed in a 1000 mL sterile empty Infusion Bag. Dosing solutions will be prepared and used within 72 hours from preparation, unless the site has more restrictive rules

6.4 Compliance to Treatment

The effective doses of study drugs received by each patient during the trial will be recorded.
Treatments received by the patients in the control (standard of care) group will be recorded.
Any concomitant medication that the participant receives during the participation in this study will also be recorded. Specifically, dose, posology, frequency of administration, start and end date and reason of use will be required and collected.
There is no contraindication to the use of concomitant supportive and prophylactic care medications as deemed necessary by the treating physicians, unless incompatible with the study drug. Since Reparixin is metabolized by CYP2C9 there could be a theoretic effect on drug availability by any medication that is a known inhibitor or inducer of CYP2C9. These medications are not considered incompatible with Reparixin, however, a list of such medications is provided below for the consideration of the investigators:

- CYP2C9 Inducers: rifampin, carbamezapine, aprepitant, bosentan, phenobarbital, St. John's Wort;

- CYP2C9 Inhibitors: amiodarone, fluconazole, miconazole, oxandrolone, capecitabine, cotrimoxazole, etravirine, fluvastatin, fluvoxamine, metronidazole, sulfinpyrazone, tigecycline, voriconazole, zafirlukast.

7 ASSESSMENTS AND PROCEDURES

7.1 Procedures

Potential study patients with confirmed COVID-19 diagnosis will be identified from those referring to the participating clinical sites for diagnosis and/or management. Screening will be performed and completed in consented patients and potential study patients will be treated. Each patient will be involved in the study for the entire duration of the hospital treatment and for a maximum of 31 days (one month) including the follow-up.

7.2 Efficacy assessments

Clinical parameters to assess the efficacy of the experimental treatment will be assessed according to the Statistical Analysis Plan (SAP) and the Schedule of Evaluations.
All the laboratory and clinical parameters, including radiography imaging assessments, will be collected based on the local procedures of the participating clinical sites.
The degree of dyspnea will be collected using the “Liker” and Visual Analogues (VAS) scales.
The Liker scale is used as follows: the patient grades his current breathing compared to when he first started the drug (from -3 to 3). "0" = no change, "1" =minimally better, "2" =moderately better, "3" =markedly better, "-1" =minimally worse, "-2" =moderately worse, "-3" =markedly worse.
The VAS scale is used as follows: the patient draws a horizontal line on an axial graph (from 0 to 100) to show the degree of how he feels about breathing. The number "0" equals the worst breathing the patient has ever felt and the number "100" equals the best he has ever felt.
All the clinical endpoints are listed in Section 3 of this protocol but may be implemented considering the adaptive nature of this clinical trial, at the end of phase 2 of at any given time following the advice of the Data Monitoring Committee (DMC). The SAP will be modified accordingly.

7.3 EVALUATION OF ADVERSE EVENTS AND SAFETY INFORMATION

7.3.1 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 unfavourable 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 experience which is a reasonably likely to have been caused by the drug. Adverse events are to be considered unsuspected if the relationship to the study drug as described in the table in section 7.3.4 is none or unlikely; whereas any AE reported in the study having a possible, probable or highly probable relationship to study drug will be considered as an ADR. The definition covers also medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product.

Serious Adverse Event

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

- results in death,

- is life-threatening (i.e. the patient was 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) which may interfere or prevent everyday life functions, but do not constitute a substantial disruption.

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

  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.

Death shall always be reported as SAE: anyway, death due to progression of disease would not have a causal relationship to the product, based on Investigator's assessment. The investigator should report the event immediately to the sponsor, but the sponsor will not report the event as expedited to regulatory authority. Cause of death shall always be specified when known.

Unexpected Adverse Events

An AE or ADR is considered unexpected if it is not listed in the Investigator Brochure (Reference Safety Information section) or in the applicable authorised Summary of Product Characteristics. 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.

Suspected Serious Unexpected Adverse Reaction (SUSAR)

A suspected serious unexpected adverse reaction 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.

7.3.2 MONITORING FOR ADVERSE EVENTS

During the study, the subject shall have the opportunity to spontaneously mention any problems and anyway the Investigator or appropriate designee should inquire about AEs by asking the standard questions:

• "Have you had any health problems since your last study visit?"

• "Have there been any changes in the medicines you take since your last study visit?"

AEs should be reported for any clinically relevant change in concomitant condition(s) that is the result of an untoward (unfavorable and unintended) change in a subject's medical health, regardless of causality. Changes in any protocol-specific systemic parameter evaluated during the study are to be reviewed by the Investigator.

7.3.3 RECORDING

AEs will be collected and recorded for any untoward event that occurs in a patient from the time he or she signs the Informed Consent for the trial until the end of the follow up period. Thus, any untoward medical occurrences or unfavorable and unintended signs, symptoms, or diseases that occur in the pretreatment, in treatment, or post treatment period are to be considered AEs and/or SAEs, and consequently recorded and reported as such. Should a non-serious AE become serious, the Investigator will then follow the same reporting procedures as for SAEs.

Each AE will be described by:

- Its duration (start and stop dates).
- Its seriousness.
- Its relationship to the study drug (suspected/unsuspected).
- Action(s) taken.
- Outcome.

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

7.3.4 RELATIONSHIP AND SEVERITY OF AES TO THE INVESTIGATIONAL PRODUCT

The Investigator will assess the possible relationship between the AE and the investigational medication, according to the criteria in Table below:

Relationship of the Adverse Event to the IMP

None (Intercurrent Event)	An event that is not and cannot be related to the Investigational Product, e.g. patient is a passenger in a road traffic accident.
Unlikely (remote)	Relationship is not likely e.g. a clinical event including laboratory test abnormality with 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

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 patient's usual function (awareness of symptoms or signs, but easily tolerated [acceptable]).
Moderate	Grade 2 - Interferes to some extent with patient's usual function (enough discomfort to interfere with usual activity [disturbing]).
Severe	Grade 3 - Interferes significantly with patient's usual function (incapacity to work or to do usual activities [unacceptable])

7.3.5 FOLLOW-UP OF PATIENTS WITH ADVERSE EVENTS

The Investigator is responsible for adequate and safe medical care of subjects 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 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 ensure that the subjects experiencing AEs receive definite treatment for any AE, if required.

If subject was hospitalized due to a SAE, a copy of the discharge summary is to be forwarded to the Sponsor as soon as it becomes available.

In addition, a letter from the Investigator that summarizes the events related to the case as well as results of any relevant laboratory tests also may be requested. Further, depending upon the nature of the SAE, Dompé may request copies of applicable segments of the patient's medical records. 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 Par. 7.3.6).
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. subject or Investigator is unable to provide additional information, or the subject is lost to follow up), unless subject has withdrawn his/her consent.

7.3.6 SERIOUS ADVERSE EVENT REPORTING

Reporting Procedure for Investigators

The Investigator must report all SAEs, regardless of presumed causal relationship, to Dompé Pharmacovigilance Department, preferably by e-mail (to farmacovigilanza@dompe.com) or fax (02 36026913) within 24 hours of learning of the event. Contact details for SAE reporting are provided in the section "Contact Information".
The investigator should also report information on SAEs that continue after patient has completed his/her participation in the study (whether study completion or withdrawal) unless patient has withdrawn his/her consent.
Information on SAEs will be recorded on the SAE form. Follow-up reports (as many as required) should be completed and e-mailed /faxed following the same procedure above, marking the SAE form as "follow up Number XX".
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, along with their relatedness to the Investigational Product, should identify which adverse events are serious and which are non-serious. In any case, the Investigator is requested to record his/her opinion about the relatedness of the observed event(s) with the investigational medication.
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 or withdrawal), the SAE should be reported by the Investigator to Dompé Pharmacovigilance. Such "post-study cases" should be regarded for expedited reporting purposes by Dompé, 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.

The following situation shall not be considered SAE:

- Abnormal lab values or test results that do not induce clinical signs and/or symptoms and require intervention/therapy, i.e. are clinically significant.

7.3.7 REPORTING PROCEDURE TO ETHIC COMMITTEES (EC) AND TO REGULATORY AUTHORITIES

The Investigator shall report all serious adverse events immediately to Dompé Pharmacovigilance.
The Investigator shall notify SAE to his/her EC as applicable; in addition, for reported deaths of a subject, the Investigator shall supply Dompé Pharmacovigilance and the Ethics Committee with any additional information requested. Copies of all correspondence relating to reporting of any SAEs to the EC should be maintained in the Investigator's Files.

Dompé Pharmacovigilance shall submit any serious unexpected ADR (SUSAR) to the concerned EC and Regulatory Authority (via Eudravigilance) which approved the study, 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 serious event is neither fatal nor life threatening.

Dompé Pharmacovigilance shall report any relevant updated follow-up safety information as soon as available.
If the results of an investigation show that an ADR not initially determined to be reportable is reclassified as reportable, Dompé Pharmacovigilance shall report such reaction in a written safety report as soon as possible, within the timeframes defined by current law requirements.

Dompé shall be responsible to prepare and submit annual safety reports (Development Safety Update Report – DSUR) to relevant Regulatory Authorities, as applicable. In addition, Investigator will receive from Dompé Pharmacovigilance appropriate periodic safety updates, as per applicable local requirements and regulations.

7.3.8 EXPOSURE TO INVESTIGATIONAL PRODUCT DURING PREGNANCY

Women of childbearing potential are not excluded from the study as long as adequate birth control methods are being utilized. Women of childbearing potential are defined as all women physiologically capable of becoming pregnant. Prior to enrolment in the clinical trial, female patients of childbearing potential and their partners must be advised of the importance of avoiding pregnancy during the entire course of the study treatment and for the 30 days after the study treatment period ends and of the potential risks associated with an unintentional pregnancy. During the trial, female patients are to be instructed to contact the Investigator immediately if they suspect they might be pregnant. In the same way, male patients who become aware that the partner might be pregnant, are to be instructed to contact the Investigator immediately.
The Investigator must report every pregnancy on a pregnancy report form as soon as possible (within 24 hours of learning of the pregnancy to Dompé 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 (eg, 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 7.3.6, with the appropriate serious criterion (eg, hospitalization) indicated on the SAE report form. Miscarriage, stillbirth and any malformation/disease must be reported as a SAE.
Any pregnancy leads to the immediate exclusion from the trial.

7.3.9 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 CRF, and the patient must be followed up until the resolution of the AE or as instructed by the medical monitor.

7.3.10 OVERDOSE

Cases of overdose (accidental or intentional) which may or may not result in serious adverse reactions are to be reported to Dompé Pharmacovigilance and Dompé Medical Expert, following the same

procedure for SAE, within 24 hours from the Investigator's knowledge of its occurrence. The Medical Expert should be contacted to discuss corrective treatment, if necessary.
An overdose of the study drug is defined as:

- Tablets: The administration of 3 or more additional tablets on any given treatment day
- IV formulation: The administration of more than 50% of the daily dose.

Overdose includes reports related to drug intake through different routes (e.g. ingestion) or with suicidal intentions and consequent drug overdose.
The Investigator shall provide in the SAE form information about symptoms, corrective treatment and outcome of overdose.

7.4 Study Procedures and Randomization

Potential study patients with confirmed COVID-19 diagnosis will be identified from those hospitalized at the participating clinical sites for management of their COVID-19 associated severe pneumonia. Screening will be performed and completed in consented patients and potential study patients will be treated. All the subjects who sign the informed consent form for the present study and will be screened will be coded with "unique subject identifiers" (see section 10.5).
Each patient will be involved in the study for the entire duration of the hospital treatment and for a maximum of 31 days (one month) including the follow-up.

All the laboratory and clinical parameters, including radiography imaging assessments, will be collected based on the local procedures of the participating clinical sites.
The degree of dyspnea will be collected using the "Liker" and Visual Analogues (VAS) scales.
The Liker scale is used as follows: the patient grades his current breathing compared to when he first started the drug (from -3 to 3). "0" = no change, "1" =minimally better, "2" =moderately better, "3" =markedly better, "-1" =minimally worse, "-2" =moderately worse, "-3" =markedly worse.
The VAS scale is used as follows: the patient draws a horizontal line on an axial graph (from 0 to 100) to show the degree of how he feels about breathing. The number "0" equals the worst breathing the patient has ever felt and the number "100" equals the best he has ever felt.

Before randomization, and any study related procedures, at study entry all patients must have given a written informed consent for the study and must be assigned a screening number. In order to request randomization to the study, all the pre-treatment evaluations must be completed and all the inclusion and exclusion criteria satisfied.
Consecutive randomisation numbers will be given to the subjects upon their definitive enrolment in the study. Subjects will be assigned to their treatment according to their randomisation number.
Patients will be randomized in a 2:1 fashion between Reparixin and standard treatment using a computer generated Randomization List generated by the Sponsor.

8 END OF STUDY, DISCONTINUATION AND WITHDRAWAL

8.1 End of study (EOS):

The EOS is defined as the last day the last patient completes the last study assessment in the hospital, or retracts the consent to participate in the study, or withdraws from the study, or is deceased or otherwise lost to follow-up.

8.2 Discontinuation and Withdrawal

A patient has the right to withdraw from this clinical trial at any time and for any reason, without any repercussion.

The investigators also have the right to withdraw a patient from the study at any time if in their opinion it is no longer in the best interest of the patient to remain in the study.

As no withdrawals have been anticipated in the sample size calculations, patients who are withdrawn before completing the minimum of 24hrs treatment with the oral tablets in the active group can be replaced to reach the predefined samples size of the Phase 2 segment of the study. Participants who whitdraw from the study cannot be re-randomized.

Considerations on withdrawals for the Phase 3 segment of the study will be reassessed.

All discontinuations must be recorded by the investigators and reported to the Sponsor and, if possibile, an early discontinuation visit should be conducted and data recorded.

Temporary discontinuation or drug discontinuation is not allowed since treatment with the medication is planned for continuous use throughout the treatment phase.

Patients will be followed-up according to the study procedures set forth in this clinical trial protocol and until the scheduled last day of follow-up. The only exception will be the collection and reporting of AEs that have occurred during the clinical trial and have not resolved by the time of the end of follow-up: in this case AEs will be followed until resolution or stabilization.

9 STATISTICAL CONSIDERATIONS

9.1 Sample size

The sample size for this trial will be adaptively determined through a phase 2/3 design. Enrollment is planned to last approximately 2 months. Each patient will be randomly allocated to Reparixin or Control according to a randomization ratio of 2:1 and he/she will be followed for the entire treatment and follow-up duration for collecting the efficacy endpoints of each study phase.

For the Phase 2 portion of the study, the primary analysis will compare Reparixin versus Control in terms of time to a composite endpoint (primary endpoint of phase 2) including the following events: requirement of supplemental oxygen treatment, mechanical ventilation use, admission to ICU, and any requirement of rescue medication. Based on clinical experience, an improvement of the median time freedom from composite event of 1.5 times is expected. Assuming an exponential distribution, a total sample size of 48 patients will allow to provide 80% power to show superiority of Reparixin compared to control using a log-rank test at a one-sided significance level of 0.025.

The sample size for the phase 3 is based on assessment of superiority of Reparixin compared to control arm in terms of freedom from a composite endpoint (primary endpoint of phase 3) of severe events including death, admission to the ICU, requirement of mechanical ventilation.
Based on same assumptions of phase 2 regarding randomization (2:1) and expecting to double the median time freedom from severe composite event using Reparixin compared to control (from 2-4 to 4-8 days), a total sample size of 111 patients will allow to achieve a power of 90% to show superiority of Reparixin over control using a log-rank test at a one-sided significance level of 0.025. No drop-out are expected to happen. The number of patients to be included may be revised based on considerations from Phase 2 data analysis.
Primary endpoint of phase 3 will be tested only if superiority of Reparixin is shown in phase 2. Thus, the overall type I error of the study will be controlled at 0.025 via the hierarchical testing procedure.

9.2 Overview of planned statistical analyses

The study plans for the following statistical analyses:

- Phase II analysis: this analysis will be conducted when all planned enrolled subjects for phase II have reached the primary composite endpoint of phase II and the study database has been (interim) locked.
- Phase III analysis: in case of success of phase II, this analysis will be conducted when all planned enrolled subjects for phase III have reached the primary composite endpoint of phase III and the study database has been locked.
- Analyses for the Data Monitoring Committee: these analyses will be produced periodically

9.3 Analysis Population

The following population will be defined:

- The Safety (SAF) population will consist of all randomized patients who received at least one dose of the investigational product (either Reparixin or Standard of Care). Safety 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 investigational product (either Reparixin or Standard of Care). FAS population will be analyzed according to ITT principle, i.e. by treatment allocation. The FAS population will be used for the primary analyses of the study and to present results on efficacy data.
- The Per Protocol (PP) population will consist of all randomized patients who received at least one dose of the investigational product and do not have Major Protocol Deviations. The PP population will be used for sensitivity analyses.

9.4 Statistical Methodology

9.4.1 General Considerations

This trial's design will allow for adaptations based but not limited to the following:

- Interruption of an experimental treatment route of administration in favour of the other due to safety reasons;
- Confirmation or modification of the primary, secondary and exploratory endpoints;
- Confirmation or modification of the sample size for the Phase 3 segment;
- Early closure of the study for efficacy, futility or safety reasons.

All study adaptation will be carefully detailed in the Statistical Analysis Plan and in the Clinical Study Report.

Appropriate descriptive statistics will be produced by treatment arms and by route of administration of Reparixin, according to the nature of the variable. For continuous data, 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. If appropriate, confidence intervals around the mean or the proportions will be presented. The number of subjects with missing data will be presented under the "Missing" category. Missing values will not 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 event will be evaluated using Kaplan-Meier 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. Kaplan-Meier graphs will be presented along with the number of patient-at-risk at exact time points. Subjects ongoing and who are free from event at the analysis cut-off date will be censored at the analysis cut-off date. Subjects who have discontinued without an event will be censored at the date of discontinuation.

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

The Statistical Analysis Plan will be issued before the 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.

9.4.2 Analysis of efficacy variables

9.4.2.1 Primary analysis of phase II

In phase II, the primary endpoint to assess the efficacy of Reparixin versus the Control is the time freedom from a composite endpoint of events including supplemental oxygen treatment, mechanical ventilation use, admission to ICU, and any use of rescue medication. The time to the first event (any one of the components defined in the phase II composite endpoint) will be used for analysis. In case of no events, the rules for censoring defined in section 9.4.1 will be used.

The primary endpoint will be evaluated by means of Kaplan-Meier estimates and log-rank test.
The null hypothesis $H_{0phaseII}$ is that the freedom form events in Reparixin ($T_{REPARIXIN}$) is lower or equal than control ($T_{CONTROL}$):

$H_{0phaseII}$: $T_{REPARIXIN} \leq T_{CONTROL}$
$H_{1phaseII}$: $T_{REPARIXIN} > T_{CONTROL}$

where $T_{REPARIXIN}$ and $T_{CONTROL}$ are the freedoms from event for Reparixin and Control, respectively.
The null hypothesis $H_{0phaseII}$ will be rejected, and superiority of Reparixin concluded (alternative hypothesis $H_{1phaseII}$) if log-rank p-value will be lower than pre-specified threshold, depending at which analysis (interim or final) the test is performed. Thresholds are calculated according to O'Brien-Fleming spending function boundaries and tare reported in Table 1.

9.4.2.2 Primary analysis of phase III

In case of rejection of the null hypothesis $H_{0phaseII}$, the primary endpoint to assess the efficacy of Reparixin versus the Control in phase III is the time freedom from a severe composite endpoint of events including death, admission to the ICU, mechanical ventilation. The time to the first severe event (any one of the components defined in the phase III composite endpoint) will be used for analysis. In case of no events, the rules for censoring defined in section 9.4.1 will be used.

The primary endpoint will be evaluated by means of Kaplan-Meier estimates and log-rank test.
The null hypothesis $H_{0phaseIII}$ is that the freedom form severe events in Reparixin ($\Pi_{REPARIXIN}$) is lower or equal than control ($\Pi_{CONTROL}$):

$H_{0phaseIII}$: $\Pi_{REPARIXIN} \leq \Pi_{CONTROL}$
$H_{1phaseIII}$: $\Pi_{REPARIXIN} > \Pi_{CONTROL}$

where $\Pi_{REPARIXIN}$ and $\Pi_{CONTROL}$ are the freedoms from severe event for Reparixin and Control, respectively.
The null hypothesis $H_{0phaseIII}$ will be rejected, and superiority of Reparixin concluded (alternative hypothesis $H_{1phaseIII}$) if log-rank p-value will be lower than pre-specified threshold, depending at which analysis (interim or final) the test is performed. Thresholds are calculated according to O'Brien-Fleming spending function boundaries and tare reported in Table 2.

Primary endpoint of phase 3 will be tested only if superiority of Reparixin is shown in phase 2.

Both null hypotheses $H_{0phaseII}$ and $H_{0phaseIII}$ must be rejected in order to claim superiority on Reparixin over Standard of Care. Consequently, no multiplicity correction of type I error will be applied on primary endpoints.

9.4.2.3 Handling of 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 subject's participation for the full duration of the trial.

9.4.2.4 Sensitivity analyses

The following sensitivity analyses are defined to assess the robustness of results on primary endpoints versus adherence to protocol and presence of confouning factors:
Comparison between treatment and control will be performed in the PP population (see section 9.3 for definition) instad of FAS;
Comparison between treatment and control will be performed by means of a Cox regression model, using pre-specified baseline charateristics as covariates (details will be provided in the SAP).
The above sensistivity assessemnts will be performed at the time of phase III analysis.
Additional sensitivity analyses may be added in the SAP.

9.4.2.5 Secondary Efficacy Analyses

Each component of the primary composite endpoints will be evaluated separately, and their 95% C.I. will be reported. In addition to descriptive statistics, all secondary endpoints will be analyzed 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) will also be summarized for all post-baseline visits.

9.4.3 Safety Analysis

Treatment-Emergent Adverse Events (TEAEs), Adverse Drug Reactions (ADRs) and Serious Adverse Events (SAEs) will be presented treatment arm (and route of administration if required) in terms of number of AEs and their incidence by System Organ Class (SOC) and Preferred Terms (PT) using MedDRA. Analyses will be provided also by severity and relationship to the treatment.
Vital signs and laboratory tests will be presented using descriptive statistics at each available visit. Additionally, the frequency of subjects reporting an abnormal or abnormal clinically significant laboratory value at each available visit will be presented for each laboratory parameter. Shift tables versus baseline will compare abnormal laboratory findings at each post-baseline visit.

9.4.4 Analysis of exploratory variables

In addition to descriptive statistics at each available timepoint, explorative variables will be analyzed by means of inferential tests depending on their nature and distribution (all confidence intervals and statistical tests on explorative endpoints are of descriptive nature).

9.4.5 Intermediate analyses for DMC

Due to the open-label nature of this trial, an indipendent data monitoring committee is foreseen to provide timely recommendations about the early closures or modifications. This monitoring committee will include 2 to 4 indipendent physicians, supported by a statistician.

Safety and efficacy data will be reviewed on an ongoing basis by a DMC.
The DMC will give careful consideration to the appropriateness of trial continuation if there is emerging evidence that Reparixin is harmful. Similarly, DMC may suggest to interrupt one of the routes of administration of Reparixin. In making any recommendations about termination for safety reasons, the totality of data will be considered, including the number of unexpected deaths in the control group, and the available evidence about efficacy and the overall safety profile.
Early termination would be considered if the rate of clinically significant post-surgical complications that require reoperation reliably exceeds the rate expected currently in standard practice settings.
The DMC will also consider early termination if the quality of conduct of the trial is such that the trial will not be able to provide a timely and reliable answer to the questions it was designed to address.

Primary endpoint data will be evaluated by DMC for identification of early superiority of Reparixin (efficacy) or for an early stop of the trial for futility (nonbinding). During phase II it has been planned to have 1 interim analysis, while 2 interim analyses will be performed during phase III. O'Brien-Fleming spending functions will be used to control the type I and II errors for analyses of primary endpoints. P-values boundaries for efficacy and futility at interim and final analyses for phase 2 and phase 3 are reported in Table 1 and Table 2, respectively. Unplanned DMC interim analyses might be performed. In that case, only safety data will be evaluated.

Table 1: O'Brien-Fleming spending functions boundaries for primary analysis in phase II

Analysis	Sample Size	Boundaries for primary endpoint (C-peptide)	
		Efficacy	Futility
Interim #1	~27	p-value <0.00258	p-value ≥0.23349
Final	48	p-value <0.02400	p-value ≥0.02400

Table 2: O'Brien-Fleming spending functions boundaries for primary analysis in phase III

Analysis	Sample Size	Boundaries for primary endpoint (C-peptide)	
		Efficacy	Futility
Interim #1	~60	p-value <0.00296	p-value ≥0.26674
Interim #2	~81	p-value <0.00892	p-value ≥0.09841
Final	111	p-value <0.02151	p-value ≥0.02151

10 SUPPORTING DOCUMENTATION

10.1 Clinical and non clinical pharmacology

Reparixin (DF1681Y) is a specific inhibitor of CXC ligand 8 [CXCL8; formerly interleukin (IL)-8] biological activity, stemming from a program of drug design of molecules intended to modulate chemokine action. Reparixin is the first low molecular weight blocker of CXCL8 biological activity in clinical development. Relevant pre-clinical, toxicological and phase 1 to 3 clinical data are summarized below. It is anticipated that data on human exposition to oral 1200 mg TID, as proposed in this trial, is available from the study REP0111 (Phase 1b pilot study to evaluate reparixin in combination with chemotherapy with weekly paclitaxel in patients with HER-2 negative metastatic breast cancer). In particular from the pharmacokinetics data at the 21st day of treatment with 1200 mg reparixin oral tablets (i.e. no paclitaxel from day 15th) sufficient elements are available to estimate the drug concentration (mean) at the steady state. The Css at steady state (after repeated oral administration) can calculated form the following formula: Css = AUC/τ where AUC is the area under curve (mg*h/ml) and τ is the dose interval (h). The mean AUC 0-8h(τ) at 21st day is 191 mcg*h/ml, the interval of dose is 8 hours; consequently the mean Css is 23,88 mcg/ml, which is comparable to the Css obtained by IV continuous infusion. Please also refer to the Investigator's Brochure for more detailed information.
Reparixin (formerly repertaxin) was granted orphan drug designation for the "prevention of delayed graft function after (solid) organ transplantations" by the European Commission of Orphan Medicinal Products in September 2001 and by the Food and Drug Administration in January 2003. Recently orphan drug designation has been grated in the EU for "prevention of graft rejection in pancreatic islet transplantation". In the 4th quarter of 2011, Dompé has received Scientific Advice from the European Medicines Agency (EMA) for the development of reparixin in pancreatic islet transplantation.

10.1.1 RELEVANT NON-CLINICAL PHARMACOLOGY

Reparixin is *in vitro* a potent and specific inhibitor of CXCL8 biological activity. *In vitro* chemotaxis experiments have shown that reparixin inhibits CXCL8-induced chemotaxis of human polymorphonuclear leukocytes (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 [*Bertini, 2004; Allegretti, 2005*]. *In vivo*, reparixin prevented PMN infiltration into the transplanted kidney and reduced creatinin levels in a rat model of kidney transplantation. Similarly, in a rat model of lung transplantation, reparixin improved isolated graft oxygenation, decreased pulmonary oedema, and significantly reduced neutrophil infiltration into transplanted lungs. 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%. Efficacy was seen in all models at reparixin dose of 9.90 mg/kg [*Bertini, 2004; Cugini, 2005; Souza, 2004; Cavalieri, 2005; Garau, 2005; Villa, 2007; Gorio, 2007*]. More recently, reparixin lysine salt was evaluated in different models of intrahepatic pancreatic islet transplantation in mice, which include syngeneic and allogeneic settings. Reparixin was administered by s.c. continuous infusion for 7 or 14 days starting from day -1 of islet transplantation. A dose of 5.28 mg/kg/hour was administered in all experiments. Reparixin was able to significantly improve islet engraftment, as demonstrated by its ability to increase the likelihood of and to reduce the time to gain non-fasting blood glucose levels less than 200 mg/dl (normo-glycaemia) in marginal mass syngeneic islet transplantation model. In the fully mismatched allogeneic model, reparixin not only protected islets

from early graft failure, but was also able to increase the time to rejection, as shown by post-transplant prolongation of normo-glycaemia. Graft function was indefinitely prolonged in 20/30% of mice treated with reparixin and rapamycin, suggesting possible tolerance induction. In parallel, reparixin treatment reduced intrahepatic infiltration of PMNs, macrophages, T helper and dendritic cells.

10.1.2 TOXICOLOGY DATA

Reparixin was tested for toxicity in rodent and non-rodent animal species after single and repeated i.v. doses. The repeated dose administration studies were conducted by i.v. continuous infusion, according to the intended human administration route. The general toxicological profile of i.v. reparixin, in the studies conducted to date, is characterized by a low toxicity after single or repeated dose administrations in rats (LD50 = 229.68 mg/kg i.v.; 660.00 mg/kg/day as No Observed Adverse Effect Level from 4 week studies) and mice (401.94 mg/kg i.v.). Continuous i.v. administration to dogs for 2 weeks resulted in a safe dose of 39.60 mg/kg/day. Continuous i.v. infusion of reparixin to the male and female rat at dose levels of up to 660.00 mg/kg/day 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 to be used 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 4.95 mg/mL (1 mL/kg) infused over a 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 that those reached in man, as may occur during the treatment of patients receiving kidney transplantation.

10.1.3 PHARMACOKINETICS AND PRODUCT METABOLISM

PK studies by i.v. injection revealed that reparixin is very rapidly eliminated in rats and humans (t1/2 0.5-3hrs and 1.0-1.5hrs, respectively) whereas elimination is slower in dogs (12-28hrs). 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 catalysed 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. Exposure to ibuprofen after administration of reparixin 2.77 mg/kg/h for 48hrs (the highest dose tested in humans) was similar or lower than that obtained after a standard therapeutic single dose of ibuprofen (300mg). Preliminary PK data obtained in a few patients undergoing islet transplantation shows that plasma levels of reparixin (total and unbound) and its major metabolite DF2243Y appears to be within the expected range according to the dose administered. Due to extensive metabolism, unchanged reparixin was poorly or not excreted into the urine of rat, dogs and humans so 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 holyday 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 protein bound 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 intersubject variability. The $t_{1/2}$ of all three metabolites appeared to remain about the same from day 1 to day 21.
To investigate the PK/PD characteristics of reparixin, we assessed the effect of the drug in inhibiting IL-8-mediated hPMN NETs release in whole blood of healthy volunteers, 500 µL/sample of whole blood were pre-incubated with vehicle or different concentrations of reparixin for 15 min at 37°C and next placed onto 13-mm polylysine coated glass circular coverslips and incubated for 30 min at 37°C in a 5% CO_2 atmosphere to allow for cell adherence. Post-incubation, samples were stimulated with vehicle or IL-8 (100 ng/mL) for 4h (37°C, 5% CO_2), after which the supernatant was removed and coverslips washed one time with HBSS. Next, samples were stained with 40 nM Sytox Green for 10 min. Finally, cells were fixed in 2% paraformaldehyde and NETs formation determined by confocal microscopy.
Reparixin blocked IL-8-induced NETs formation in a concentration dependent manner being the inhibition statistically significant at 5 µg/mL (40% of inhibition) and reaching the almost complete inhibition (about 90%) at 25 µg/mL thus confirming that the total blood concentration of 25 µg/mL (corresponding to a free unbound concentration of 100 nM) that is reached at the steady state by iv infusion or by repeated oral administration (Css) is coherent with the objective to reach the drug exposure necessary to maximize the potential clinical efficacy of the compound.
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 concentration 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 remote. 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.

10.1.4 SAFETY DATA

A total of 448 subjects have been exposed to reparixin in the clinical studies completed to date, of whom 337 and 112 received i.v. formulation and oral tablets, respectively. The patient population exposed to i.v. 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 transplant (31M/17F), 22 undergoing liver transplant (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 i.v. infusion up to 10.6 mg/kg over 30min or 133 mg/kg over 48h and, in pancreatic islet and liver transplantation studies, 2.772 mg/kg body weight/hour i.v. 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 (SAEs) or Adverse Event (AE)-related withdrawals were reported. The majority of AEs reported were of mild intensity. All subjects had recovered completely or had ongoing adverse events of mild intensity when they were discharged. The safety of reparixin was confirmed also 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 2 and 3 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 twice in several patients. Most frequent ADRs were nausea, headache, and vomiting; great majority of these were mild to moderate in nature and none required discontinuation of the Investigational Product. Tachycardia occurred in one

patient from Days 5 to 38 after 1st islet infusion was judjed probable in relation to Investigational Product. Vomiting, nausea and headache on Day 5 and 6 after 2nd islet infusion in one patient and erythema, nausea and headache on Days 2 to 6 after 1st islet infusion in another patient were judged highly probable in relation to the Investigational Product. Nausea, vomiting and severe gastrointestinal bleeding associated with anaemia developed in a female patient early after the beginning of reparixin infusion because the patient 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.
Overall, the most frequent (>10%) ADRs observed in the phase 1 to phase 3 I.V. studies were: Gastrointestinal disorders (about 26% of the total number of reports), including abdominal pain lower, abdominal pain NOS, abdominal pain upper, constipation, diarrhea, dyspepsia, flatulence, gastroesophageal reflux disease, gastrointestinal haemorrhage, intra-abdominal haemorrhage, nausea and vomiting.
Nervous system disorders (about 19%), including headache, dizziness, hypoaesthesia, somnolence.
General disorders and administration site conditions (about 16%), including cannula site reaction, fatigue, implant site haemorrhage, injection site thrombosis, infusion site oedema, lethargy, malaise, oedema, oedema peripheral, and pyrexia
The patient population exposed to reparixin oral tablets consists of 111 female receiving either single agent reparixin (REP0210, operable breast cancer: 20 patients) or the combination of reparixin and weekly paclitaxel in metastatic breast cancer (REP0111, phase 1b: 30 patients; REP0114, randomized phase 2: 61 patients).

In the studies completed so far, 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 (REP0114). 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 three studies were:

Gastrointestinal disorders (31.8%), including nausea, vomiting, abdominal pain, discomfort or distension, dyspepsia, flatulence, constipation.
General disorders and administration site conditions (16.4%), including fatigue and peripheral oedema.

10.2 Study documentation and record keeping

The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor in the source documents and in all required reports.
The investigator must keep source documents for each subject in the study.
The investigator and the sponsor should maintain the study documents as specified in the “Essential Documents for the Conduct of a Clinical Trial” chapter 8 of ICH-GCP and as required by the applicable regulatory requirement(s).
These are documents which individually and collectively permit evaluation of a study and the quality of the data produced and include groups of documents, generated before the study commences, during the clinical study, and after termination of the study and include but are not limited to, study protocol, amendments, submission and approval of Ethics Committee (EC), raw data of subjects, insurance contracts, certificate of analysis of the IMP(s), drug accountability records, signed informed consent forms, confidential subjects identification code.
The investigator and the sponsor should take measures to prevent accidental or premature destruction of these documents.

Study documents must be retained by the investigator and the sponsor as long as needed to comply with ICH-GCP, national and international regulations. By signing the protocol, the investigator and the sponsor agree to adhere to these requirements.

10.3 Ethical considerations, quality assurance and monitoring

The procedures outlined in this clinical trial protocol are designed to ensure that the Sponsor and the Investigators from the Clinical Sites perform their activities throughout the set-up, conduct, evaluation, documentation and analysis of the study, in accordance to the principles of the Good Clinical Practice (GCP) guidelines of the International Conference on Harmonization (ICH) and the Declaration of Helsinki. The study will be carried out adhering to local legal requirements and the applicable national laws, whichever represents the greater protection for the individuals.
Study protocol, patient information and informed consent will be submitted to the appropriate Ethics Committee for approval. The Sponsor will be responsible to inform in a timely manner the appropriate Ethics Committee about any changes in the study protocol which could interfere with the patient's safety.
Due to the constrains and risks of the ongoing COVID-19 pandemia, no on-site monitoring activities will be carried out. Remote monitoring will be available periodically or at the investigators' request via web or phone communications with the adequate Sponsor personnel or their approved representatives.

10.4 Informed consent

The investigators or sub-investigators involved in this clinical trial who are responsible for treating the hospitalized patient for COVID-19 are responsible for providing all necessary information about the participation in the study to their patients, and consequently to obtain the written Informed Consent. The same procedure applies to the information of the patient and providing of consent to the processing of personal data according to the European Regulation n. 679/2016 on the Protection of Personal Data, the Personal Data Protection Code (Legislative Decree 196/03) and subsequent amendments and additions, and to the provisions, guidelines and general authorizations of the National Guarantor for personal Data Protection.

10.5 Data collection

The investigator must ensure that the clinical data required by the study protocol are carefully reported in the subject's source documents detailing the unique identification number and the date and time of the study procedures performed. Any correction to the source data entries must be carried out by the investigator or a designated member of staff. Incorrect entries must not be covered with correcting fluid, or obliterated, or made illegible in any way. A single stroke must be drawn through the original entry. Corrections have to be dated and initialled. The investigator must provide a reasonable explanation for all missing data. The source documents will be completed, signed by the investigator, the sensitive data will be obscured (i.e. only randomization number will be clearly legible) and the source document will be made available to the Sponsor for data management procedures.

10.6 Confidentiality and data protection

By signing this protocol, the investigator agrees to keep all the information provided by the sponsor in strict confidentiality and to request the same confidentiality from his/her staff. Study documents provided by the sponsor (protocols, randomization list and other materials) will be stored appropriately to ensure confidentiality. The information provided by the sponsor to the investigator cannot be disclosed to others without direct written authorisation from the sponsor, except for the extent necessary to obtain the informed consent from the subjects wishing to participate in the study.

Data on subjects collected in the source documents during the study will be transferred to the Sponsor in an anonymized way. If, as an exception, for safety or regulatory reasons identification of a subject becomes necessary, the sponsor and the investigator will be bound to keep this information confidential.

10.7 Unique subject identifier

All the subjects who sign the informed consent form for the present study will be coded with "unique subject identifiers". The unique subject identifier consists of the sponsor study code (i.e. REPAVID-19), the 3-digit site number (i.e. 001), the 4-digit screening number (e.g. S001, S002, etc.) and, if applicable, the 3-digit subject randomisation number (e.g. 001, 002, etc.).
Study code, site number, screening number and subject randomisation number are separated by slashes ("/").

10.8 Database management

The Sponsor will provide a double data entry with total re-entry of data and discrepancy resolution by a second data entrist and will update and verify the database and create the final data sets.

10.9 Coding dictionaries

Medical/surgical history and underlying diseases and AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA™). Previous and concomitant medications will be coded using the WHO Drug Dictionary Enhanced (WHODDE). The version of the coding dictionaries will be stated in the study report.

10.10 Publication policy

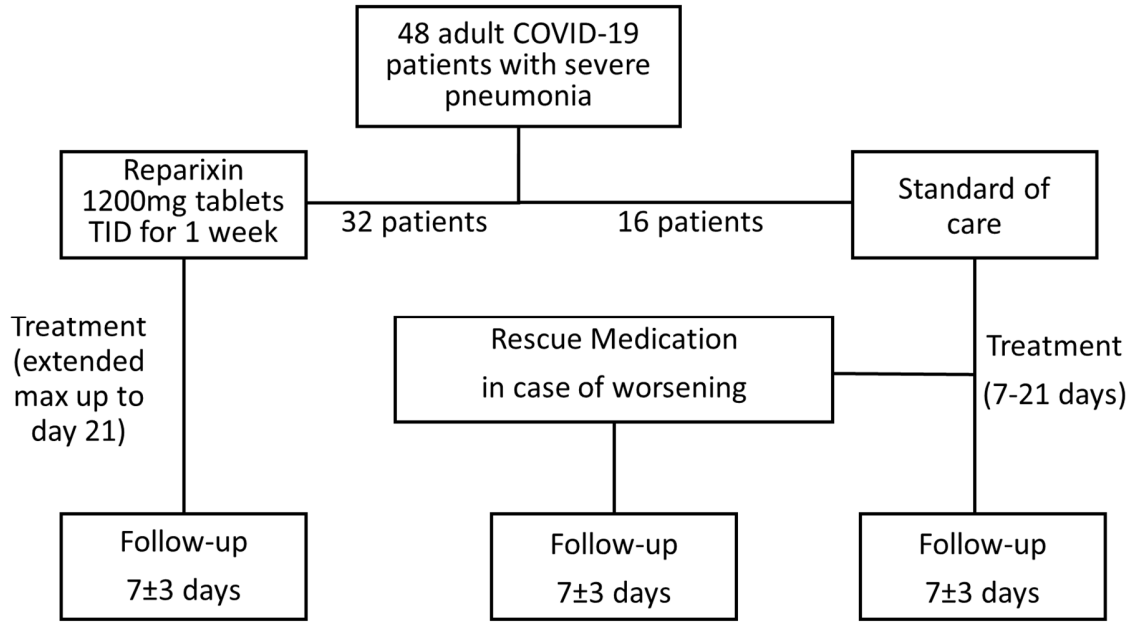
Study results will be communicated in full to the competent Health Authorities by the submission of a complete clinical study report.
The sponsor agrees that the study results may be published by the investigator, and the investigator agrees to submit any manuscript (abstract, publication, paper, etc.) to the sponsor before any public disclosure.
This will be done in order to ensure that clinical study results are reported in an objective, accurate and balanced manner. The sponsor reviews the proposed manuscripts, before submission, within a reasonable period of time (30-90 days in relation with the complexity of the work).
The investigator will also be provided by the sponsor with the clinical study report 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.

10.11 Administrative aspects

The investigational medicinal products required for the conduct of this study will be provided free of charge by the Sponsor to the participating clinical sites.
Coverage for any damage resulting from the participation of the subjects in the clinical trial is warrented. In addition to the general insurance of the individual participating clinical centers, an insurance cover will be issued in favour of the subjects participating in this clinical study. The insurance is in compliance with the local regulation and with the requirements of the Health Authorities.

11 TABLES AND FIGURES

11.1 Study outline



Phase 2 study to continue with Phase 3. In fase 3 Reparixin will be offered as rescue medication. Final design of Phase 3 to be adapted based on Phase 2 results.

11.2 Schedule of Evaluations

The following schedule reports the relative timeframes and all the evaluations and assessments that are performed during the study:

Study procedures	Screening	Baseline	Day 1	Day 2	Week 1	End of treatment	End of follow up
Informed Consent	X						
Inclusion/Exclusion Criteria	X	X					
Pregnancy Test	X						X
Randomization		X					
Demographics	X						
Medical History	X						
Previous And Concomitant Medications	X	X	X	X	X	X	X
Clinical severity score	X	X	X	X	X	X	X
Liker scale	X	X	X	X	X	X	X
Dyspnea VAS scale	X	X	X	X	X	X	X
Body temperature	X	X	X	X	X	X	X
Hematology evaluations*	X	X	X	X	X	X	X
Oxigen treatment (time and quantity)	X	X	X	X	X	X	X
Mechanical ventilation (Yes/No)	X	X	X	X	X	X	X
ICU admission (Yes/no)	X	X	X	X	X	X	X
Chest radiologic imaging	X	X[§]	X[§]	X[§]	X[§]	X	X[§]
PaO2, SpO2, FiO2	X	X	X	X	X	X	X
CRP, Hs-CRP	X	X	X	X	X	X	X
Cytokine profile **	X	X	X	X	X	X	X
Reparixin blood concentration **			X	X	X	X	X
SARS-CoV-2 virologic counts **		X			X	X	X
Study drug dispensation		X					
Verify study medication dosing compliance			X	X	X	X	
Record AEs / SAEs	X	X	X	X	X	X	X

* Including routine hematology as for local laboratory procedures and predefined hematochemical evaluations required for this study; ** Not mandatory; [§] When deemed appropriate by the investigators.

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Insulin Secretion After Pancreatic Islet Transplantation: A Phase 3, Double-Blind, Randomized, Placebo-Controlled Trial in Type 1 Diabetes. Diabetes Care 2020;

APPENDICES

Appendix 1-INVESTIGATOR'S SIGNATURE PAGE

Investigator's Statement

I have read study protocol REPAVID-19 from the title *"Adaptive phase 2/3, randomized, controlled multicenter study on the efficacy and safety of Reparixin in the treatment of hospitalized patients with COVID-19 pneumonia*" and agree to conduct the study as outlined in the protocol, and in accordance with the Declaration of Helsinki, ICH-GCP E6 (R2) and any local regulations, being responsible for personally supervise the study conduct and ensure study staff complies with protocol requirement.

Name of Principal Investigator (block letters):_____

Signature: _____ Date: _____

Appendix 2-SPONSOR SIGNATURE PAGE

Adaptive phase 2/3, randomized, controlled multicenter study on the efficacy and safety of Reparixin in the treatment of hospitalized patients with COVID-19 pneumonia

Sponsor Medical Expert: _____Date: _____/____/____

Flavio Mantelli, Chief Medical Officer

Sponsor Research & Development Director _____Date: _____/____/____

Marcello Allegretti, Chief Scientific Officer

Appendix 3. Investigator's Brocure (IB)
Please refer to dedicated document

Appendix 4. Investigational Drug Labels
Please refer to dedicated document

Appendix 5. Severe Adverse Event (SAE) reporting form and compltion guideline
Please refer to dedicated document

Appendix 6. Pregnancy reporting form and completion guideline
Please refer to dedicated document

Appendix 7. Informed consent form
Please refer to dedicated document