I.R.C.C.S. Ospedale San Raffaele
Gruppo San Donato

EudraCT Number: **2020-001513-20**

ClinicalTrial.gov Identifier: NCT04335201

Protocol Title: **"Use of Defibrotide to reduce progression of acute respiratory failure rate in patients with COVID-19 pneumonia"**

Study code: **DEFI-VID19**

Protocol Version: **n. 3.0 – April 16, 2020**

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Principal Investigator:	Prof. Fabio Ciceri, Ospedale San Raffaele – Milano Tel: 02 26434289 e-mail: ciceri.clinicaltrials@hsr.it

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PROTOCOL AUTHORIZATION PAGE

Codice protocollo: DEFI-VID19

Version n.: 3.0. – April 16, 2020

EudraCT Number: 2020-001513-20

ClinicalTrial.gov Identifier: NCT04335201

I have read this study protocol and agree that it contains all the information required to conduct the study. I agree to conduct the study as set out in this protocol. In particular, I agree to adhere to the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki, the guidelines on Good Clinical Practice and the appropriate national laws.

Protocol Title: *"***Use of Defibrotide to reduce progression of acute respiratory failure rate in patients with COVID-19 pneumonia***"*

Titolo in italiano: "**Uso di Defibrotide in infusione intravenosa per il trattamento dei pazienti con polmonite da COVID-19"**

Local Investigator

_____ _____

Date

Trial Promoter Coordinating Centre

_____ 7 April 2020

_____

Date

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SYNOPSIS

Use of Defibrotide to reduce progression of acute respiratory failure rate in patients with COVID-19 pneumonia

DEFI-VID19 (**DEFI**brotide in Co**VID19**)

EudraCT: 2020-001513-20

Title Abbreviated

Defibrotide in COVID-19 pneumonia

Promotor

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Study design

Phase II, prospective, interventional, single-arm, multicentric, open label trial, with a parallel retrospective collection of data on not treated patients from IRCCS, San Raffaele Scientific Institute included in the institutional observational study (ClinicalTrials.gov Identifier: NCT04318366)

Study objective

Primary Objective

To demonstrate that the treatment with Defibrotide administered intravenously in addition to the best available therapy according to institutional guidelines (protease inhibitors antiviral treatment and hydroxychloroquine (HCQ), and if needed, metilprednisolone up to 1 mg/Kg body weight) is able to reduce the progression of acute respiratory failure, the need of mechanical ventilation, the transfer to the intensive care unit or death, in patients with severe COVID-19 pneumonia.

Secondary Objectives

- To evaluate the safety of Defibrotide in the cohort of patients treated (incidence of adverse events)
- To evaluate the duration of hospitalization for patients enrolled
- To evaluate the overall survival at day+28 after start treatment with Defibrotide
- To evaluate the change in biological features of systemic inflammation (PCR, LDH, ferritin, IL-10, IL-6, TNF-alpha, IFN-gamma, PTX3) at day +7 and +14 after start of treatment with Defibrotide

Primary Endpoint

The primary endpoint is the Respiratory-failure rate (RFR) defined according to the following criteria:

- Patients with a baseline PaO2/FiO2 >= 200 (Nava, Lancet 2009)): progression of respiratory failure is defined by 1) severe gas transfer deficit (PaO2/FiO2 < 200), 2) persistent respiratory distress while receiving oxygen (persistent marked dyspnea, use of accessory respiratory muscles, paradoxical respiratory movements), 3) transfer to the intensive care unit or 4) death. The rate will be calculated as the proportion of patients who experienced at least one of the events above by day+14 from treatment start.

Secondary Endpoints

- Overall Survival at day 28
- Days of hospitalization
- Frequency and incidence of adverse drug reactions (ADR) and SAE
- Variation of haematological parameters (RBC, Hb, Ht) and of systemic inflammation (PCR, LDH, serum ferritin, IL-10, IL-6, TNF-alpha, IFN-gamma, PTX3)

- Minor bleeding events
- Major bleeding events

Study population

Phase II, prospective, interventional, single-arm, multicentric, open label trial, with a parallel retrospective collection of data on not treated patients.

Inclusion criteria

- Documented COVID-19 pneumonia: defined as upper respiratory tract specimen (nasopharyngeal swab (NPS) or viral throat swab) positive for COVID-19 and/or imaging at computed tomography scan suggestive of COVID-19 pneumonia
- Oxygen saturation (SaO2) of 92% or less without oxygen support, or reduction of 3% from basal value of SaO2, or a ratio of the partial pressure of oxygen (PaO2) to the fraction of inspired oxygen (FiO2) (PaO2/FiO2) below 300.
- Any gender
- Age >= 18 years
- Written informed consent or as per Ethical Committee indication in case of patients not able to express written informed consent

Exclusion criteria

- Onset of COVID-19 pneumonia >14 days
- Orotracheal intubation
- Uncontrolled systemic infection (other than COVID-19)
- Concomitant use of thrombolytic therapy Concomitant systemic anticoagulant therapy (e.g. heparin, warfarin, direct thrombin inhibitors and direct factor Xa inhibitors)
- Haemodynamic instability, defined as inability to maintain mean arterial pressure with single pressor support
- Hypersensitivity to the active substance or to any of the excipients of the experimental drug
- Patients who, based on the investigator's clinical judgement, are not able to receive the treatment
- Pregnancy or breastfeeding patient

The study will also include a parallel retrospective group of temporally concomitant patients who suffered from pulmonary involvement of COVID-19 /or presenting CT scan evaluation suggestive of pulmonary involvement who were admitted in the hospital for the COVID-19 outbreak before trial initiation, who did not receive an experimental treatment and who are enrolled in an already IRB approved observational study (CLINICALTRIAL.GOV NR NCT04318366). Inclusion and exclusion criteria for the parallel retrospective control

group will be the same as for the study group as detailed above. This group of patients will be the comparative group after verification of the clinical and baseline characteristics.

Treatment Plan

Patients will be treated according to the standard institutional procedures and will receive the best available treatment as per institutional guidelines in association with the experimental drug: Defibrotide 25 mg/kg body weight total dose in 2 hours duration infusion each, every 6 hours (Defibrotide 6.25 mg/kg body weight each dose)

Treatment duration = 14 days

Sample Size

A sample of 50 patients with COVID-19 pneumonia will allow to detect an absolute reduction in the rate of Respiratory-failure at day+14 after treatment of 20%, assuming that the actual rate of failure in the corresponding not treated patients is 70% (alpha=5%, power=90%, two-sided test). The baseline has been calculated based on the published reported data (Yang et al. Lancet 2020). The software PASS15 was used for calculations.

The study will also include a parallel retrospective group of temporally concomitant patients from IRCCS, San Raffaele Scientific Institute, who did not receive an experimental treatment and who are enrolled in an already IRB approved observational study (CLINICALTRIAL.GOV NR NCT04318366). This group of patients will be the comparative group after verification of the clinical and baseline characteristics.

Data Safety Monitoring Board

The Data Safety Monitoring Board (DSMB) will ensure the safety of patients enrolled in the study and the quality of the study management and analysis.

Keywords

COVID-19, respiratory failure, Defibrotide, mechanical ventilation

Protocol Schedule

Procedure	Baseline before the first infusion of the first dose of Defitelio	Treatment and hospitalization period (daily)	Day +7 and day +14 after treatment	Day +28 after treatment
Informed consent	X			
Inclusion and exclusion criteria	X			
Demography	X			
Full physical examination including height and weight	X			
Medical history (includes past and current medical conditions)	X			
Arterial Blood Gas (ABG) Analysis	X	X	X	
Respiratory assistance assessment	X	X	X	
Laboratory assessments 1	X	X	X	X
IL-6, IL10, TNF alpha, IFN gamma, CRP, LDH, Serum ferritin,	X		X	X
12-lead ECG	X			X
Vital signs	X	X	X	X
Thoracic CT scan or Chest XR or lung ultrasound	X		X	
AE review	X	X	X	X
Concomitant medication review	X	X	X	X
Survival follow-up	X	X	X	X

1. Complete blood count (CBC), serum biochemical tests (including renal and liver function), albumin, total protein, coagulation profile and D-dimer, BetaHCG, myocardial enzymes, lactate dehydrogenase (LDH), C-reactive protein, serum ferritin, IL-6, IL-10, TNF alpha, IFN gamma, PTX3, biobank.
   Only at baseline: BetaHCG, TBC quantiferon, HBV, HCV, HIV serology

2. BACKGROUND AND RATIONALE

As of March 12, 2020, coronavirus disease 2019 (COVID-19) has been confirmed in 125 048 people

worldwide, carrying a mortality of approximately 3·7%, 1 compared with a mortality rate of less than 1% from influenza. There is an urgent need for effective treatment. The 2019 novel Coronavirus (2019-nCoV; severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)) has spread rapidly since its recent identification in patients with severe pneumonia in Wuhan, China. The clinical spectrum of COVID-19 varies from asymptomatic or pauci-symptomatic forms to clinical conditions characterized by respiratory failure that necessitates mechanical ventilation and support in an intensive care unit (ICU), to multiorgan and systemic manifestations in terms of sepsis, septic shock, and multiple organ dysfunction syndromes (MODS). Current management of COVID-19 is supportive, and respiratory failure from acute respiratory distress syndrome (ARDS) is the leading cause of mortality.

The cytokine profile of COVID-19 patients supports that the fatality is driven by a virally-triggered, self-propagating, hyper-inflammatory state (Mehta et al, 2020). Elevated ferritin (mean 1297·6 ng/ml in non-survivors *vs* 614·0 ng/ml in survivors; p<0·001) and IL-6 (p<0·0001) have been found to be independent predictors of fatality in COVID-19 patients in China (*Ruan et al, 2020*).

VOD, known as Veno-Occlusive-Disease or Synusoidal Obstruction Syndrome, is the most characterized of a spectrum of post bone marrow transplantation (BMT) syndromes (including ELS, IPS or aGVHD) characterized by reactive endothelial activation and damage, endothelial-driven paracrine signaling and a pro-inflammatory and pro-coagulant state. Severe VOD frequently progresses to Multi Organ Failure and is characterized by high mortality (>80%), characterized mostly by lung and kidney failure.

High levels (elevated early in the course of the disease) of IL-6, IL-8 and TNF-alpha characterize VOD and the other post-BMT endothelial diseases (*Schots et al, 2003, Gugliotta et al 1994, Remberger et al 1997, Symington et al, 1992*). In detail, IL-8 may be involved in the respiratory failure following ARDS, a frequent and fatal outcome of sVOD patients. Intriguingly, high ferritin levels are a well recognized risk factor for the development of VOD in both adult and pediatric settings (*Morado et al, 2000: lee et al, 2010*).

Furthermore, the histopathological examination of lung lesions in VOD syndromes show early alveolar epithelial and lung endothelial injury, resulting in accumulation of protein- and fibrin-rich inflammatory edematous fluid in the alveolar space and progression to interstitial fibrosis, at later stages. (*Bunte et al, 2008 : Mandel et al, 2000*).

These patterns are reminiscent of what observed in the only three cases of autoptic examination of lung tissue so far obtained from COVID-19 infected individuals: two from putatively early phase (*Sufang et al, 2020*) and one from a late phase (*Zhang et al, 2020*) of the disease: such as, early diffuse alveolar damage with proteinaceous exudates, and chronical inflammation with intra alveolar deposition of fibrin and interstitial fibrosis, respectively.

Thus, we suggest here that the pattern of circulating cytokines, similar histopathological findings in infected patients and hyper-ferritinemia represent some hallmarks common to both COVID-19 induced pathology and

VOD/SOS (and other endothelial damage syndromes), possibly underlying common mechanisms of progression.

Defibrotide, a polydisperse mixture of predominantly single-stranded polydeoxyribonucleotides, is currently the only therapy approved to treat hepatic VOD/SOS with pulmonary/renal dysfunction (ie, multiorgan dysfunction/multiorgan failure [MOD/MOF]) following HSCT in the United States and to treat severe hepatic VOD/SOS post-HSCT in the European Union. In preclinical and human studies, defibrotide has demonstrated profibrinolytic, antithrombotic, anti-inflammatory, and angio-protective actions, thus promoting an anticoagulant phenotype of the endothelium that protects and stabilizes the function of endothelial cells. In a phase 3, historically controlled, multicenter trial in adults and children with VOD/SOS and MOD/MOF (defibrotide: n = 102; controls treated before defibrotide availability: n = 32), defibrotide resulted in significantly greater day +100 survival following HSCT (38.2%) vs controls (25.0%; propensity analysis-estimated between-group difference: 23%; P = .0109). Importantly, a posthoc analysis of a conspicuous number of defibrotide-treated patients (n=651) has recently shown that defibrotide treatment also to critically ill patients, such as those with ventilator dependence at study entry, could highly benefit from defibrotide treatment (*Richardson et al, 2020*).

Defibrotide has, overall, demonstrated endothelial-protective properties, with pro-fibrinolytic, anti-thrombotic, anti-ischemic, anti-inflammatory, and antiadhesive activities, but no significant systemic anticoagulant effects. Defibrotide appears to exert several anti-inflammatory and antioxidant effects through interaction with the EC membrane, as shown in an endothelial cell line of hepatic origin.

At least part of defibrotide's lifesaving activity is due to downregulation of circulating cytokines, chiefly IL-6 and TNF-alpha and by reducing PAI-1 levels, NFKB activation and expression of MHCI and MHCII molecules. As compared to other agents, targeting a specific cytokines or a specific factor, defibrotide's pleiotropic mechanism of action may underlie its effectiveness in both early and late progressed states of MOF and in prophylactic settings as well (*for a review, Richardson et al, 2018, Richardson et al, 2020*).

Patients with clinical and radiological evidence consistent with idiopathic pneumonia syndrome, all showed underlying disease processes which can be interpreted as associated with endothelial cell activation injury. Idiopathic pneumonia syndrome is a rare complication following hematopoietic stem cell transplantation (HSCT), defined by diffuse lung injury with no identified etiology, with an incidence of 2–12%.; it shows histological evidence of type II endothelial cell activation, with display of ICAM-1 and/or VCAM-1, and endothelial injury, with endothelial upregulation of eNOS. This, together with histological findings of intra-alveolar fibrin and pulmonary hypertension suggests that endothelial cell activation injury may be a causative factor underlying idiopathic pneumonia syndrome Defibrotide may prove to be useful in the treatment of patients with idiopathic pneumonia syndrome, although stratifying which patients will benefit from this treatment requires further study (*Altmann T et al, 2018*).

A potential beneficial effect from defibrotide in treating pulmonary veno-occlusive disease (PVOD) has been suggested. Its action is probably due to the drug's ability to selectively increase prostaglandin I2 and E2 levels and to increase tissue plasminogen activator and decrease plasminogen activator inhibitor function (*Pescador et al 1996, Willems et al 2009*).

The use of defibrotide does not change the pathway of care, therefore the only changes to the budget are drug acquisition costs and cost avoidance as a result of reduced ICU/HDU use. Defibrotide reduces the length of stay avoiding extended use of ICU or HDU beds (*NHS England Clinical Commissioning Policy: Use of defibrotide in severe veno-occlusive disease following stem cell transplant https://www.england.nhs.uk/wp-content/uploads/2018/07/Defibrotide-in-severe-veno-occlusive-disease.pdf)*

3. STUDY OBJECTIVES AND ENDPOINTS

Primary Objective

To demonstrate that the treatment with Defibrotide administered intravenously in addition to the best available therapy according to institutional guidelines (protease inhibitors antiviral treatment and hydroxychloroquine (HCQ), and if needed, metilprednisolone up to 1 mg/Kg body weight) is able to reduce the progression of acute respiratory failure, the need of mechanical ventilation, the transfer to the intensive care unit or death, in patients with severe COVID-19 pneumonia.

Secondary Objectives

- To evaluate the safety of Defibrotide in the cohort of patients treated (incidence of adverse events)
- To evaluate the duration of hospitalization for patients enrolled
- To evaluate the overall survival at day+28 after start treatment with Defibrotide
- To evaluate the change in biological features of systemic inflammation (PCR, LDH, ferritin, IL-10, IL-6, TNF-alpha, IFN-gamma, PTX3) at day +7 and +14 after start of treatment with Defibrotide

Primary endpoint

The primary endpoint is the Respiratory-failure rate (RFR) defined according to the following criteria:

- Patients with a baseline PaO2/FiO2 >= 200 (Nava, Lancet 2009)): progression of respiratory failure is defined by 1) severe gas transfer deficit (PaO2/FiO2 < 200), 2) persistent respiratory distress while receiving oxygen (persistent marked dyspnea, use of accessory respiratory muscles, paradoxical respiratory movements), 3) transfer to the intensive care unit or 4) death. The rate will be calculated as the proportion of patients who experienced at least one of the events above by day+14 from treatment start.

Secondary endpoints

Overall Survival at day 28

Days of hospitalization

Frequency and incidence of adverse drug reactions (ADR) and SAE

Variation of haematological parameters (RBC, Hb, Ht) and of systemic inflammation (PCR, LDH, serum ferritin, IL-10, IL-6, TNF-alpha, IFN-gamma, PTX3)

Minor bleeding events

Major bleeding events

4. **STUDY DESIGN**

Phase II, prospective, interventional, single-arm, multicentric, open label trial, with a parallel retrospective collection of data on not treated patients from IRCCS, San Raffaele Scientific Institute, included in the institutional observational study (ClinicalTrials.gov Identifier: NCT04318366).

5. STUDY POPULATION

The study will enroll all consecutive hospitalized patients with COVID-19 acute pneumonia with and without ARDS/CPAP dependency

Inclusion criteria

Documented COVID-19 pneumonia: defined as upper respiratory tract specimen (nasopharyngeal swab (NPS) or viral throat swab) positive for COVID-19 and/or imaging at computed tomography scan suggestive of COVID-19 pneumonia

Oxygen saturation (SaO2) of 92% or less without oxygen support, or reduction of 3% from basal value of SaO2, or a ratio of the partial pressure of oxygen (PaO2) to the fraction of inspired oxygen (FiO2) (PaO2/FiO2) below 300.

Any gender

Age >= 18 years

Written informed consent or as per Ethical Committee indication in case of patients not able to express written informed consent

Exclusion criteria

Onset of COVID-19 pneumonia >14 days

Orotracheal intubation

Uncontrolled systemic infection (other than COVID-19)

Concomitant use of thrombolytic therapy

Concomitant systemic anticoagulant therapy (e.g. heparin, warfarin, direct thrombin inhibitors and direct factor Xa inhibitors)

Haemodynamic instability, defined as inability to maintain mean arterial pressure with single pressor support

Hypersensitivity to the active substance or to any of the excipients of the experimental drug

Patients who, based on the investigator's clinical judgement, are not able to receive the treatment

Pregnancy or breastfeeding patient

The study will also include a parallel retrospective group of temporally concomitant patients from IRCCS, San Raffaele Scientific Institute, who suffered from pulmonary involvement of COVID-19 /or presenting CT scan evaluation suggestive of pulmonary involvement who were admitted in the hospital for the COVID-19 outbreak before trial initiation, who did not receive an experimental treatment and who are enrolled in an already IRB approved observational study (CLINICALTRIAL.GOV NR NCT04318366). Inclusion and exclusion criteria for the parallel retrospective control group will be the same as for the study group as detailed above. This group of patients will be the comparative group after verification of the clinical and baseline characteristics.

6. TREATMENT PLAN

Patients will be treated according to the standard institutional procedures and will receive the best available treatment as per institutional guidelines in association with the experimental drug: Defibrotide 25 mg/kg body weight total dose in 2 hours duration infusion each, every 6 hours (Defibrotide 6.25 mg/kg body weight each dose)

Treatment duration = 14 days

Concomitant Therapy

Any medication that the participant is receiving at the time of enrollment or receives during the study must be recorded indicating the reason for use, the dates of administration including start and end dates and dosage information including dose and frequency

7. STUDY ASSESSMENT

Pre-treatment screening

Informed consent, demography, complete blood count (CBC), serum biochemical tests (including renal and liver function), albumin, total protein, coagulation profile and D-dimer, myocardial enzymes, lactate dehydrogenase (LDH), C-reactive protein, serum ferritin, IL-6, IL-10, TNF alpha, IFN gamma, PTX3, biobank, quantiferon, HBV, HCV, HIV serology, arterial Blood Gas (ABG), beta-HCG. Biobanking for patients from IRCCS, San Raffaele Scientific Institute will be performed according to institutional guidelines.

PaO2 / FiO2 ratio or P/F ratio, 12-lead ECG Vital signs (respiratory rate, pulse, blood pressure and temperature).

Thoracic CT scan or Chest XR or lung ultrasound (if clinically indicated).

Tests during hospitalization

Blood test will be performed daily (complete blood count, bilirubin, AST, ALT, creatinine, CRP, PT, PTT, LDH, D-dimer, ABG

PaO2 / FiO2 ratio or P/F ratio 12-lead ECG,

Vital signs (respiratory rate, pulse, blood pressure, and temperature)

Thoracic CT scan or Chest XR or lung ultrasound (if clinically indicated).

At day +7 and day +14 after treatment patients will be tested for complete blood count (CBC), serum biochemical tests (including renal and liver function), coagulation profile and D-dimer, lactate dehydrogenase (LDH), C-reactive protein, serum ferritin, IL-6, IL-10, TNF alpha, IFN gamma, PTX3, biobank.

Adverse Events

All adverse events recorded from treatment, throughout the treatment and observation period up to 28 days following registration, have to be reported in the toxicity case report form, graded according to the corresponding CTCAE term (Version 5.0). The Investigator must immediately report to the promoter all serious adverse events. The report should be made using the SAE report form immediately and not exceeding 24 hours following knowledge of the event. During the course of the study all AEs and SAEs should be proactively followed up for each subject. Every effort should be made to obtain a resolution for all events, even if the events continue after discontinuation/study completion. The investigator is responsible for following all SAEs until resolution, until the subject returns to baseline status, or until the condition has stabilized with the expectation that it will remain chronic, even if this extends beyond study participation.

Definitions of Adverse Events

According to Regulation (EU) No 536/2014 of the European Parliament and of the council of 16 April 2014 for definition of adverse events, adverse reactions and reporting including causality.

For the purpose of this protocol adverse events are classified into the following categories:

Adverse Event (AE): Adverse event' means any untoward medical occurrence in a subject to whom a medicinal product is administered and which does not necessarily have a causal relationship with this treatment;

Adverse Drug Reaction (ADR): is "a response to a medicine which is noxious and unintended, and which occurs at doses normally used in man".

In this description it is of importance that it concerns the response of a patient, in which individual factors may play an important role, and that the phenomenon is noxious (an unexpected therapeutic response, for example, may be a side effect but not an adverse reaction).

Serious Adverse Event (SAE): Serious adverse event' means any untoward medical occurrence that at any dose requires inpatient hospitalization or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, results in a congenital anomaly or birth defect, is life-threatening, or results in death;

Unexpected Serious Adverse Event (USAE): means a serious adverse reaction, the nature, severity or outcome of which is not consistent with the reference safety information;

Unexpected Adverse Event: An unexpected adverse event is an event, the nature or severity of which is not

consistent with applicable product information. During study follow up SAEs and AEs will be documented using CTC AE v4.

8. STATISTICAL PLAN AND DATA ANALYSIS

SAMPLE SIZE

A sample of 50 patients with COVID-19 pneumonia will allow to detect an absolute reduction in the rate of Respiratory-failure at day+14 after treatment of 20%, assuming that the actual rate of failure in the corresponding not treated patients is 70% (alpha=5%, power=90%, two-sided test). The baseline has been calculated based on the published reported data (Yang et al. Lancet 2020). The evaluation of the baseline estimation will be updated according to the availability of new results in the COVID-19 affected population. The software PASS15 was used for calculations.

STATISTICAL ANALYSIS

Primary endpoint

The percentage of subjects with respiratory rate failure at day+14 will be estimated and will be reported along with the corresponding 95% Confidence Intervals (CI).

Secondary endpoints

The time to event endpoints will be described using the Kaplan-Meier approach and estimates at pre-defined time points will be obtained along with 95%Cis. Patients will be censored at study closure, withdrawn of consent or loss to follow-up.

Continuous variables will be summarized with indices of location (i.e. mean or median) and dispersion (i.e. standard deviation or interquartile range), as appropriate. All relevant estimates will be reported with the corresponding 95% Confidence Intervals (CI).

As for safety analysis, the number of ADR (expected/unexpected) and SAEs (expected/unexpected and/or related/not related) and the percentage of subjects experiencing ADR and SAEs in the 3 months of observation of the study will be summarized by severity and within body system involved. Narratives will also be presented. The rate of occurrence of these events will also be estimated. The same approach will be used for the analysis of infections.

Subgroup analyses and regression models (i.e. logistic model on proportions and Cox model on time to event outcomes) will be performed considering age, sex, biological features and Defibrotide cumulative dose.

Any changes in the planned statistical methods will be documented and justified in the study report.

9. ETHICAL AND LEGAL ASPECTS

The study will be conducted according to the principles of Good Clinical Practice (GCP) as reported in current Italian and European legislation. The responsible investigator will ensure that this study is conducted in agreement with the declaration of Helsinki and the Italians laws and regulations, whichever provides the

greatest protection of the patient. The protocol has been written and the study will be conducted according to the ICH Harmonized Tripartite Guideline for GCP, issued by the European Union. The responsible Local Ethical Committee approval must be obtained before starting the trial. A copy of the patient informed consent form must be submitted to the appropriate authority or committee, together with the protocol for written approval. Written approval of the protocol and informed consent by the responsible and appropriate authority or committee must be obtained prior to recruitment of patients to the study.

The investigator must inform the appropriate authority or committee of subsequent protocol amendments, which must be approved by this one.

Informed Consents

Before a patient's participation in this prospective data collection, the investigator is responsible for obtaining written informed consent and information forms from the patient, or legally acceptable representative, after adequate explanation of the aims and methods. A legally acceptable representative is an individual or other body authorized under applicable law to consent, on behalf of a prospective patient, to the patient's participation in the clinical study.

The acquisition of informed consents and information forms should be documented in the patient's medical records, as required by ICH GCP, and the information and informed consent forms should be signed and personally dated by the patient, or a legally acceptable representative, and by the physician who conducted the information and informed consent discussion. The original signed information and informed consent forms should be retained in accordance with institutional policy, and a copy of the signed forms should be provided to the patient or legally acceptable representative.

All patients will be informed of the aims of the study, the potential benefit, the possible adverse events, the procedures and possible hazards to which he/she will be exposed, and the mechanism of treatment. It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol, whenever he/she wants. This will not prejudice the patient's subsequent care. They will be informed as to the strict confidentiality of their data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician. This must be done in accordance with the local regulatory requirement. Witnessed written informed consent must be obtained for all patients included in the study or their legally authorized guardian/representative, before they are enrolled. Record the name of the witness and the date that informed consent is obtained in the patient's hospital notes.

For patients unable to express consent (i.e. unconscious or obnubilated patients), and for whom a legal guardian is not available, investigators will refer to the Authorization Gen. N. 9/2016 of the Italian Data Protection Authority, point 5 (Requirements related to the processing of personal data for scientific research purposes) of which the essential excerpt is reported: "If it is not possible to obtain the consent of the interested parties, the investigators must document, in the research project, the existence of exceptional

reasons, for which informing the interested parties is impossible or implies a disproportionate effort, or prejudice seriously the achievement of the research".

Subject confidentiality

Regulatory authorities and/or IEC/IRB may request access to all source documents, data capture records, and other study documentation for one-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities.

The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations (Low n. 675/1996 and amendments) and Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation).

An identification number will be automatically attributed to each patient enrolled in the trial. This number will identify the patient and must be included on all case report forms. In order to avoid identification errors, date of birth will also be reported on forms.

10. DATA SAFETY MONITORING BOARD

The Data Safety Monitoring Board (DSMB) will be nominated to ensure the safety of patients enrolled in the study and the quality of the study management and analysis. The DSMB will be responsible for reviewing activity and safety data through progress reports produced by the promoter and recommending modifications in case of unexpected or unexpectedly severe toxicities for study treatment, or in case of preliminary data suggesting inactivity or surprisingly positive efficacy in specific subgroups of patients; and for evaluating the effect on the study of possible changes in scientific evidence, such as results of other studies, and recommending modifications as above on the basis of such external data.

11. DATA COLLECTION

Investigators must enter the information required by the protocol into the Patient Data Collection Forms (CRFs) of the protocol in Ospedale San Raffaele through email at defi-vid19@hsr.it and trinca.stefania@hsr.it or by paper CRF to be sent to Trinca Stefania, IRCCS, San Raffaele Scientific Institute, Via Olgettina 60, 20132–Milan**,** as soon as possible after completion.

12. ADMINISTRATIVE ASPECTS

This is a non-profit investigator initiated trial. In this trial, the experimental drug Defitelio will be provided at no cost by the manufacturer (Jazz).

Study protocol, patient information, and informed consent at beginning and at each required amendment

will be submitted to the appropriate Ethical Committee for approval. After the first approval the study will be started at each Italian centre requiring to participate and such participation will be notified together with the approved protocol to the local Institutional Ethical Committee.

Coverage for any damage resulting from the participation of the subjects in the clinical trial is included in the general insurance of the individual participating clinical centers.

13. REFERENCES

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